Neuregulin-1/ErbB activity in hippocampal plasticity and psychopathology

Ian Mahar

McGill Group for Suicide Studies, Douglas Mental Health University Institute

Integrated Program in Neuroscience

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of PhD.

Submitted: September 2015.

Table of contents

i. Foreword and general acknowledgements

Seven years ago, I took on a position with Dr. Naguib Mechawar to examine whether a particular peptide spurred the proliferation of new hippocampal cells. Instead, I have spent most of the last seven years attempting to study the absolute depths of human misery. The subsequent pages will attempt to catalogue the transition from the examination of the effects of a particular neurotrophic factor on a specific cellular phenomenon, to the speculative establishment of a neurochemical affective regulation pathway and its involvement in psychopathology.

To whatever extent I have supported these preliminary conclusions with my experiments to date, I owe this evidence to the support of my research group, the McGill Group for Suicide Studies, especially those in the Mechawar lab, and with a particular debt of gratitude to my supervisor, Dr. Naguib Mechawar, who was been a phenomenal mentor to an extent that is difficult to sufficiently convey in the current context. I would also like to acknowledge my sources of funding: FRQS, RQRS, the McGill Integrated Program in Neuroscience, and the Steven S. Zalcman Memorial Award. I am deeply indebted to my family, without whose support this research would not have been possible. Finally, for you reading this, either through obligation or an overzealous but admirable sense of curiosity, you have my appreciation as well.

ii. Bibliography of publications produced or published during the thesis (items in bold are included in this document)

***Mahar, I.*, Labonte, B., Lopez, J.P., Yogendran, S., Isingrini, E., Perret, L., Davoli, M.A., Qiang, C., Rachalski, A., Giros, B., Turecki, G., Mechawar, N. Disrupted hippocampal neuregulin-1/ErbB3 signaling and dentate gyrus granule cell alterations in suicide. (In preparation).**

***Mahar, I.*, MacIsaac, A., Kim, J.J., Turecki, G., Mechawar, N. (2015). Effects of neuregulin-1 administration on neurogenesis in the adult mouse hippocampus and characterization of immature neurons along the septotemporal axis. (In preparation).**

Juster, R.P., Seeman, T., McEwan, B.S., Picard, M., *Mahar, I.*, Mechawar, N., Sindi, S., Smith, N.G., Souza-Talarico, J., Sarnyai, Z., Lanoix, D., Plusquellec, P., Ouellet-Morin, I., Lupien, S.J. (2015). Social Inequalities and the Road to Allostatic Load: From Vulnerability to Resilience. In Dante Cicchetti (Ed.), Developmental Psychopathology (3rd Edition). Hoboken, NJ: Wiley. (Accepted)

Albuquerque MS*, *Mahar I**, Davoli MA, Chabot J-G, Mechawar N, Quirion R and Krantic S (2015). Regional and sub-regional differences in hippocampal GABAergic neuronal vulnerability in the TgCRND8 mouse model of Alzheimer's disease. Front. Aging Neurosci. 7:30. (*Equal contribution.)

***Mahar, I.**, Bambico, F.R.*, Mechawar, N., Nobrega, J.N. (2014). Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. Neuroscience & Biobehavioral Reviews 38: 173-192. (*Equal contribution.)**

Mahar, I., Bagot, R.C., Davoli, M.A., Miksys, S., Tyndale, R.F., Walker, C.D., Maheu, M., Huang, S-H., Wong, T.P., Mechawar, N. (2012). Developmental Hippocampal Neuroplasticity in a Model of Nicotine Replacement Therapy during Pregnancy and Breastfeeding. PLoS ONE 7(5): e37219.

Labonte, B., Suderman, M., Maussion, G., Navaro, L., Yerko, V., *Mahar, I.*, Bureau, A., Mechawar, N., Szyf, M., Meaney, M.J., Turecki, G. (2012). Genome-Wide Epigenetic Regulation by Early-Life Trauma. Archives of General Psychiatry 69(7): 722-731.

***Mahar, I.*, Tan, S., Davoli, M.A., Dominguez-Lopez, S., Qiang, C., Rachalski, A., Turecki, G., Mechawar, N. (2011). Peripheral neuregulin-1 administration increases hippocampal neurogenesis and induces antidepressant effects. PLoS ONE 6(10):e26610.**

Dominguez-Lopez, S., *Mahar, I.*, Bambico, F.R., Labonte, B, Leyton, M., Gobbi, G. (2011). Short term effects of melatonin and pinealectomy on serotonergic neuronal activity across the light-dark cycle. Journal of Psychopharmacology 26(6): 830-44.

iii. Author contributions

Disrupted hippocampal neuregulin-1/ErbB3 signaling and dentate gyrus granule cell alterations in suicide. Conceived and designed the experiments: IM, BL, JPL, EI, LP, MAD, AR, BG, GT, NM. Performed the experiments: IM, BL, JPL, SY, EI, LP, CQ. Analyzed the data: IM, BL, JPL, EI, LP. Wrote the paper: IM, JPL, MAD, BG, NM.

Effects of neuregulin-1 administration on neurogenesis in the adult mouse hippocampus and characterization of immature neurons along the septotemporal axis. Conceived and designed

the experiments: IM, JJK, NM. Performed the experiments: IM, AM, JJK. Analyzed the data: IM, AM, JJK. Contributed additional resources: GT. Wrote the paper: IM, NM.

Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects: Wrote the paper: IM, FB, NM, JN.

Peripheral neuregulin-1 administration increases hippocampal neurogenesis and induces antidepressant effects: Conceived and designed the experiments: IM, NM. Performed the experiments: IM, ST, MAD, CQ, AR. Analyzed the data: IM, ST, MAD, SDL, CQ, AR, GT, NM. Wrote the paper: IM, GT, NM.

All other content produced by IM.

iv. General outline

The format of this document will be as follows:

- Abridged research overview
- Overview of the relevant concepts
- Existing theories for depressive etiology, antidepressant mechanisms, and the current research's contextual role
- Identification of NRG1 as pro-neurogenic and anti-depressant, and ErbB3 as a candidate mechanistic receptor
- Additional characterization of the neurogenic effects of NRG1, and identification of factors distinguishing immature dorsal and ventral dentate gyrus neurons

- Investigation of the role of NRG1/ErbB3 in psychopathology and related suicidal endophenotypes

v. Abstract

We initially sought to examine the effects of neuregulin-1 (NRG1) administration on adult hippocampal neuroplasticity, and determined that this paradigm increases cell proliferation and neurogenesis in the ventral (but not dorsal) hippocampus. This neurogenic increase was accompanied by antidepressant-like behaviour that was present when these cells became functional neurons, but not acutely after administration. We also identified ErbB3 as a candidate mechanistic receptor in this phenomenon. Next we completed our characterization of the neurogenic effects of NRG1, and determined that they are limited to proliferation and neurogenesis in the ventral hippocampus. We also discovered morphological differences between immature neurons in the dorsal and ventral dentate gyrus (DG). Having determined that NRG1-ErbB3 signaling has antidepressant-like properties, we then sought to investigate whether the converse was also true, in that psychopathological samples and animal models might show deficits in NRG1-ErbB3 signaling. As hypothesized, we found decreased hippocampal ErbB3 expression in suicide completers, as well as deficits in anterior DG granule neurons (which we and others show ubiquitously express ErbB3), with the latter phenotype being reversed with antidepressant treatment, as well as cell body hypertrophy in the posterior DG granule cell layer. Together these results identify a pathway that is putatively involved in both psychopathology and its amelioration, ostensibly through its effects in the DG.

Nous avons d'abord cherché à examiner les effets d'une administration de la NRG1 sur la neuroplasticité hippocampique et déterminé que ce traitement augmente la prolifération cellulaire ainsi que la neurogenèse au sein de l'hippocampe ventral (mais non dorsal). Cette neurogenèse accrue fut accompagnée, dès lors que les cellules devinrent des neurones fonctionnels, d'un comportement typique d'un effet antidépresseur. Nous avons également identifié le récepteur ErbB3 comme étant le récepteur pouvant médier ces effets. Nous avons par la suite complété la caractérisation des effets neurogéniques de NRG1 et déterminé ainsi que ces effets se limitent à la prolifération et à la neurogenèse au sein de l'hippocampe ventral. Nous avons aussi découvert des différences morphologiques entre les neurones immatures localisés dans l'hippocampe ventral et ceux de l'hippocampe dorsal. Ayant déterminé que la signalisation NRG1-ErbB3 présente des propriétés antidépressives, nous avons ensuite cherché à savoir si des déficits dans cette signalisation étaient détectables au sein d'échantillons cérébraux humains et de modèles animaux de psychopathologies. Notre hypothèse fut vérifiée par la démonstration d'une forte régulation à la baisse de l'espression d'ErbB3 dans l'hippocampe de suicidés, ainsi que de déficits dramatiques au niveau des neurones granulaires du gyrus denté antérieur (phénomène renversé par le traitement aux antidépreseurs), connus pour leur expression de ce récepteur. De plus, nous avons observé une hypertrophie du corps cellulaire dans la couche de cellules granulaires du gyrus denté postérieur. Dans leur ensemble, ces résultats identifient une nouvelle voie pouvant être impliquée, par ses actions au sein du gyrus denté, dans la psychopathologie ainsi que dans son traitement.

vi. List of abbreviations

5-HT: Serotonin (5-hydroxytryptamine)

5-HTTLPR: 5-HT transporter-linked promoter region

ADAM: A disintegrin and metalloproteinase domain-containing protein

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

BACE1: Beta-secretase 1

BBB: Blood-brain barrier

BDNF: Brain-derived neurotrophic factor

BNST: Bed nucleus of the stria terminalis

BrdU: Bromodeoxyuridine

CDK: Cyclin-dependent kinase

CE: Coefficient of error

CUS: Chronic unpredictable stress

DCX: Doublecortin

ddH2O: Double-distilled water

DG: Dentate gyrus

DR: Dorsal raphe

EGF: Epidermal growth factor

EGFR: Epidermal growth factor receptor

ErbB: Avian erythroblastosis oncogene B

ERK: Extracellular signal-regulated kinase

FST: Forced swim test

GABA: γ-aminobutyric acid

GFP: Green fluorescent protein

HPA: Hypothalamic-pituitary-adrenal

Ig: Immunoglobulin

IGF: Insulin-like growth factor

IR: Immunoreactive

MDD: Major depressive disorder

miRNA: MicroRNA

mPFC: Medial prefrontal cortex

mRNA: Messenger RNA

NAc: Nucleus accumbens

NGS: Normal goat serum

NMDA: N-methyl-D-aspartate

NRG: Neuregulin

PBS: Phosphate-buffered saline

PBS-T: PBS containing 0.2% Triton X-100

PC12: Pheochromocytoma cell line 12

PFC: Prefrontal cortex

PI3K: Phosphoinositide 3-kinase

PKA: Protein kinase A

PKC: Protein kinase C

PPI: Prepulse inhibition

PSD-95: Postsynaptic density protein 95

PVN: Paraventricular nucleus of the hypothalamus

qRT-PCR: Quantitative RT-PCR

SERT: Serotonin transporter

SGZ/GCL: Subgranular zone and granule cell layer

SSRI: Selective serotonin reuptake inhibitor

SVZ: Subventricular zone

TrK: Tyrosine receptor kinase

VEGF: Vascular endothelial growth factor

1.1 INTRODUCTION

1.1.1 NEUREGULIN-1 AND ERBB RECEPTORS

Research interest in neuregulins, a group of epidermal growth factor- (EGF-) related signaling proteins, and ErbB (avian erythroblastosis oncogene B) receptor tyrosine kinases, has historically come from four distinct research domains: 1) oncological research, due to their roles in particular cancers; 2) developmental neuroscience, due to their critical role in early development; 3) psychiatric research, based initially on the identification (and subsequent validation) of neuregulin-1 (NRG1) as a schizophrenia candidate gene, and more recently the potential involvement of NRG1 and ErbBs in a broader psychopathological role; and 4) cardiac research, based initially on the strong expression of NRG1 in cardiac tissue and subsequently on its apparent therapeutic potential in ameliorating heart conditions. However, although these fields may seem disparate, the findings in this section relating to NRG1-ErbB signaling converge on a central thesis driving our initial experiments (**Figure 1**). Specifically, they first supported the hypothesis that NRG1 could plausibly induce proliferation of new neurons in the hippocampus, as it has been shown to stimulate proliferation in oncological contexts. Given its prominent role in neurodevelopment, NRG1 would likely affect adult brain activity, including hippocampal plasticity in the form of adult neurogenesis. As both NRG1-ErbB signaling and neurogenesis have been associated with affective regulation and psychopathology, we were driven to examine whether NRG1 administration could also influence affective behaviour in animals in which neurogenesis had been augmented. Finally, the potential therapeutic utility of any positive effects of NRG1-ErbB signaling examined throughout this document is bolstered by cardiac research indicating its suitability as a novel treatment in psychopathological contexts.

NRG1 binds to ErbB receptor homo- or heterodimers. ErbB3 and ErbB4 are capable of binding NRG1 directly, and the extracellular domain (domain IV) of these receptors hinges toward domains II and III (Franco-Gonzalez et al., 2013). ErbB3 and ErbB2 commonly heterodimerize, primarily at domain II and (to a lesser extent) domain IV (Franco-Gonzalez et al., 2013). ErbB1 also shows a weak affinity for heterodimerizing with ErbB3, typically preferring to homodimerize in the presence of the latter receptor, but ErbB1 does form heterodimers with ErbB4 (Macdonald-Obermann et al., 2013; Mei and Xiong, 2008) (for a visual representation of NRG1/ErbB structure and signaling, see Falls, 2003; Mei and Nave, 2014; Mei and Xiong, 2008).

Expression of NRG1 (and its receptors) is widespread throughout the body, including the heart, in blood, and at neuromuscular junctions (Camprecios et al., 2011; Fischbach and Rosen, 1997; Zhang et al., 2008). Numerous studies have indicated that NRG1 crosses the blood-brain barrier (BBB) to affect brain processes. Kastin et al. (2004) showed that this occurs readily, through receptor-mediated transport. Additional studies have also shown that peripherally-administered NRG1 crosses the BBB, modulates brain activity and behaviour, increases ErbB phosphorylation in the brain, and acts as a neurotrophic factor (Abe et al., 2011; Carlsson et al., 2011; Cui et al., 2013; Depboylu et al., 2015; Engel et al., 2014; Kato et al., 2015; Kato et al., 2011; Rosler et al., 2011).

One complicating factor in the discussion of neuregulins and ErbB receptors is their large taxonomical variety and confounding nomenclature, instances of which have subsequently been established as misnomers. NRG1 isoforms have been known variously as glial growth factor (GGF), neu differentiating factor (NDF), heregulin (HRG), acetylcholine receptor inducing-activity (ARIA), and sensory and motor neuron differentiating factor (SMDF). NRG receptors

have similar nominal variability, with ErbB1-4 being known as Her1-4, ErbB1 referred to as EGFR (EGF receptor), ErbB2 known originally as Neu, and so on. For the purposes of this document, I will refer to their most common designations, with ErbB1 referred to as EGFR, and the neuregulins and other ErbB receptors referred to as such.

Although at least four NRG classes (1-4) are known to exist, comparatively little is known about NRG2-4 (Falls, 2003), and so this introduction will focus predominately on NRG1. The transcriptional products of the NRG1 gene are remarkably (one hesitates to say 'infuriatingly') diverse, due to alternative splicing, including at least 31 known isoforms (Mei and Xiong, 2008). I will attempt to summarize this variance in as painless a manner as possible. Suffice it to say, in general the varieties of NRG1 isoforms are distinguished by their EGF-like domains, their N-terminal sequences, and their membranous status.

The EGF-like domain of NRG1 isoforms serves as their active signaling domain; in fact, this domain alone is sufficient for signaling, and is often used in isolation in NRG1 administration paradigms (Falls, 2003). The immunoglobulin- (Ig)-like domain, in contrast, is dispensable for signaling. The EGF-like domain is present in one of two forms, α or β, with the β variant having higher binding potency {Falls, 2003 #1086}.

The N-terminal sequence of NRG1 isoforms divides them into six categories; I (Ig-like domain, short N-terminal), II (Ig-like domain, long N-terminal), and III (cysteine-rich domain), with two transmembrane domains (Fleck et al., 2013) are the most widely studied, whereas IV-VI are generally much less expressed or absent in the brain {Falls, 2003 #1086;Liu, 2011 #1231}.

Their method of release prior to signaling further distinguishes NRG1 isoforms. NRG1s may be released from a transmembrane conformation by proteolytic processing, transcribed as a secreted

cytokine, or may signal through a juxtacrine conformation, with the latter being restricted to type-III NRG1 isoforms. Paracrine signaling by transmembrane NRG1 is attained through proteolytic processing by beta-secretase 1 (BACE1), a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), ADAM17, and ADAM19 (Falls, 2003; Fleck et al., 2013; Luo et al., 2011; Mei and Xiong, 2008). Notably, BACE1 knockdown increases hippocampal doublecortin (DCX) expression (Modarresi et al., 2011). Backward or reverse signaling is also possible, in which membrane-anchored NRG1 acts as a receptor for soluble or membrane-bound ErbBs. This has been shown to regulate promoter activity of postsynaptic density protein 95 (PSD-95) through the Eos transcription factor (Bao et al., 2004; Mei and Xiong, 2008). Finally, he isoforms are also distinguished (though not commonly) by their cytoplasmic tails at the C-terminus into a- or b-type.

As β, a-type tail, and type-I NRG1 isoforms are among the most highly expressed in the nervous system, the NRG1-βIa (β EGF-like domain, type-I N-terminal, a-type tail) is likely one of the most prominent isoforms in the central nervous system (Falls, 2003; Liu et al., 2011), and was thus our primary focus during our initial experiments, as described subsequently.

1.1.2 Oncological studies of NRG/ErbBs

The naming in full of ErbB receptors reflects their initial oncological discovery. Clinical oncological examination of NRG/ErbBs began with the finding that ErbB2 was upregulated in a subset of breast cancer patients, resulting in interest in neuregulins as ErbB2 ligands (Montero et al., 2008). These findings led to the development of ErbB antagonists lapatinib, trastuzumab, and pertuzumab for the treatment of ErbB-expressing tumors. NRG1-ErbB3 signaling in particular induces stem cell characteristics in breast tumor cells (Jeong et al., 2014). Expression of NRG1 or ErbBs has been identified in a multitude of tumors in addition to breast cancers (Montero et

al., 2008). While seemingly distal from my own research topics, these studies offer critical support for the role of NRG/ErbBs in cell proliferation, which I identify with respect to neural progenitor cell proliferation later in Chapter 2, and in identifying the signaling cascades in this phenomenon. In addition, concerted industrial efforts to develop both broad and selective ErbB antagonists in order to treat ErbB-related cancers, while generally not commercially available at the time of this writing, offer future avenues of research that could validate and refine the findings and models discussed herein.

1.1.3 Role of NRG1/ErbBs in neural development

The vast majority of neurological research into NRG1/ErbBs to date has focused on its pivotal role in early neurodevelopment. Indeed, research into the role of this signaling pathway in the function of the adult brain has almost exclusively occurred in the last few years with few exceptions, providing an impetus for our research into this topic. Consequently, multiple developmental roles for NRG1 and ErbBs have been discovered, including neuronal migration, neurite development, synapse formation, myelination, oligodendrocyte development, and neuromuscular junction formation (Garcia et al., 2013; Mei and Xiong, 2008).

Insight into the onset of NRG1 expression influencing nervous system development has come from knockout studies, in which Schwann cell precursor alterations are seen in overall NRG1 knockouts as early as E10.5, at which point the embryo does not survive due to heart failure (Falls, 2003). Similarly, NRG1 type-III knockouts show neuromuscular synapse disruption at birth (Falls, 2003). Finally, expression of NRG1 transcripts in both the rodent and human cortex decrease from early life to adulthood (Liu et al., 2011).

NRG1 is involved in the migration of a wide variety of cells in the developing brain, including γ-aminobutyric acid- (GABA-)ergic and glutamatergic neurons. This may be NRG1-ErbB4 signaling-dependent, as ErbB4 inhibition impairs interneuronal migration, and inhibition of ErbB4 activity specifically in glial cells prevents radial migration of glutamatergic neurons along glial fibers (Flames et al., 2004; Mei and Xiong, 2008; Rio et al., 1997). Neural crest cell migration is also altered in mice lacking ErbB4 (Golding et al., 2000).

ErbB receptors are also involved in neurite development. Pheochromocytoma cell line 12 (PC12) cells treated with medium from sciatic nerve and a tyrosine receptor kinase/protein kinase C (TrK/PKC) inhibitor leads to elongated neurites, Na+ current elicitation, increased neuronal differentiation, and increased expression of EGFR, ErbB2, and (in particular) ErbB3; pan-ErbB inhibitor administration prevented neuritogenesis, differentiation and Na+ current effects. These effects may be mediated by inhibition of PKC, and ErbB receptors and PKC modulation may regulate neuronal differentiation, Na+ current initiation and neuritogenesis, at least in PC12 cells (Garcia et al., 2013). Finally, NRG1 acts as a distal chemoattractant for thalamocortical axons (Lopez-Bendito et al., 2006).

The developmental influence of NRG1 on synapse formation in the brain has been demonstrated for acetylcholinergic, GABAergic, and glutamatergic receptors, with animals in which NRG1-ErbB4 signaling is inhibited showing disrupted N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Li et al., 2007). Postsynaptic ErbB4 may regulate excitatory synapses in an activity-dependent manner that requires its recruitment by PSD-95 (Li et al., 2007; Liu et al., 2001; Mei and Xiong, 2008; Rieff et al., 1999; Yang et al., 1998). In particular, NRG1 has been shown to activate NMDA receptor subunit NR2B in immature cortical neurons (Pandya and Pillai, 2014), and NRG1 increases spine

density, spine size, and AMPA receptor GluA1 subunit surface expression *in vitro* in cortical pyramidal neurons (Cahill et al., 2013).

The involvement of NRG1 in oligodendrocyte function and brain development has not been as thoroughly investigated as with Schwann cells and the peripheral nervous system (Mei and Xiong, 2008). However, it has been shown that NRG1 regulates oligodendrocyte proliferation, differentiation, and survival, as well as the migration of oligodendrocyte precursor cells (Canoll et al., 1996; Flores et al., 2000; Kim et al., 2003b; Ortega et al., 2012; Vartanian et al., 1994). Additionally, ErbB4 hypomorphs display reduced numbers of oligodendrocytes, altered oligodendrocyte morphology, and disrupted myelination (Roy et al., 2007).

Together these results suggest that NRG1 has wide-ranging effects on peripheral and central nervous system development. However, the influence of NRG1 on adult brain function has not been as thoroughly studied, and the examinations to date have largely focused on its regulation of neuronal firing and synaptic plasticity.

1.1.4 NRG1 in adult central nervous system neuronal activity

In the central nervous system, NRG1 is predominately expressed in neurons (Fleck et al., 2013), and NRG1 affects multiple ion channels in its influence on neuronal firing, although studies to date have predominately focused on NRG1-ErbB4 signaling-modulated activity in particular (as with brain development studies). NRG1 acts through Kv1.1 channels to regulate fast-spiking neuronal excitability in parvalbumin-expressing interneurons (Li et al., 2012a), and also regulates outward Kv4.2 current through Akt and mTor signaling in immature neurons during development (Yao et al., 2013). In the substantia nigra, NRG1 potentiates mGluR1-activated currents through phosphoinositide 3-kinase (PI3K)/Akt/mTor signaling, and NRG1

administration increases dopamine release in this region as well as in the hippocampus, in which NRG1 depotentiates long-term potentiation at glutamatergic synapses (Kwon et al., 2008; Ledonne et al., 2015). Similarly, NRG1β causes D4 receptor activation-dependent increases in gamma oscillation in the hippocampus (Andersson et al., 2012). The role of NRG1-ErbB signaling in depotentiation may be ErbB4-dependent, as ErbB4 hypomorphic mice do not display reversal of long-term potentiation after stimulation, and show (along with NRG1 hypomorphic mice) increased hippocampal long-term potentiation (Shamir et al., 2012). However, NRG1 type-I overexpression does not affect hippocampal long-term potentiation, although spatial memory performance is impaired in aged mice (Deakin et al., 2012). NRG1 also reduces excitability in voltage-gated sodium currents in hippocampal interneurons by increasing firing threshold, in an ErbB-dependent manner (Janssen et al., 2012).

Thus NRG1 has been shown to regulate synaptic plasticity, including in the hippocampus. However, whether NRG1 affects adult hippocampal neurogenesis, an additional form of synaptic plasticity and largely a recapitulation of early brain neurogenesis (Kempermann, 2006), was unknown at the outset of our studies. This was made especially intriguing by previous studies showing increased hippocampal neurogenesis after administration with other neurotrophic factors {Aberg, 2000 #308;Jin, 2002 #307;Scharfman, 2005 #293}, as discussed later in this Chapter. Thus we determined the effects of NRG1 administration on adult hippocampal neurogenesis, in order to address this gap in understanding. However, in order to hypothesize whether any NRG1-induced neurogenic changes would induce neurogenesis-related alterations in affective behaviour, it is important to examine whether NRG1 is disrupted in psychopathological conditions, as has been reported in studies in humans.

1.1.5 NRG1 and ErbBs in human studies

Studies of NRG1 in human psychopathological and healthy contexts largely began with the discovery of an association between NRG1 and schizophrenia (Stefansson et al., 2002), but rapidly proliferated subsequently. Understandably, a large proportion of the human NRG1 studies following this seminal paper sought to validate this association, with many supporting the initial finding. Genetic associations between NRG1 and schizophrenia have been found in Icelandic (Stefansson et al., 2002), Scottish (Stefansson et al., 2003), Pakistani (Naz et al., 2011), Indian (Kukshal et al., 2013), and Korean (Yang, 2012) populations. There is also genetic linkage in schizophrenia patients between NRG1-related genes (Hatzimanolis et al., 2013), NRG1 polymorphisms have been associated with a schizophrenia-related endophenotype (Kang et al., 2012), and NRG1-induced downstream Akt signaling is impaired in first-episode schizophrenia, with decreased hippocampal volume correlating with Akt signaling levels (Szamosi et al., 2012).

Similarly, NRG1 genetic variation is associated with structural alterations in multiple brain regions in healthy individuals, bipolar patients, and schizophrenia patients (Barnes et al., 2012; Cannon et al., 2012; Douet et al., 2014; Gruber et al., 2008; Nickl-Jockschat et al., 2014; Tosato et al., 2012). ErbB4 genotype has similarly been associated with structural alterations (Douet et al., 2015), as well as cortical GABA levels (Marenco et al., 2011).

NRG1 has also been associated with other pathological phenotypes. Depressed patients show reduced numbers of NRG1-expressing neurons in the prefrontal cortex {Bertram, 2007 #527}. NRG1 has been shown to have a genetic association with bipolar disorder (Cao et al., 2014). Additionally, decreased levels of NRG1 in blood are associated with increased risk of subsequent appearance of psychosis in high-risk individuals (Kiss et al., 2012).

Finally, NRG1 genotype has been associated with ubiquitous phenotypes, such as sociality (Yoo et al., 2015) and creativity (Keri, 2009).

In addition to NRG1, ErbB4 has been associated with depression, bipolar disorder, and schizophrenia, and peripheral ErbB3 has also been associated with major depressive disorder (MDD) and antidepressant response, subsequent to our identification of its putative involvement in antidepressant-like behaviour (Chen et al., 2012; Chong et al., 2008; Mahar et al., 2011; Milanesi et al., 2012).

Together, these studies suggest that NRG1 and ErbB disruptions may be etiologically related to psychopathology. This includes depression, and as discussed at length later in this chapter, decreases in neurogenesis or its end-product, granule cell neurons, have been associated with depression or depression-like behaviour (Mahar et al., 2014). Similarly, increases in neurogenesis have been associated with antidepressant usage and antidepressant-like behaviour (Mahar et al., 2014). Thus, we examined whether any increases in neurogenesis induced by NRG1 administration would affect antidepressant-like behaviour.

1.1.6 Cardiac NRG/ErbB research

Cardiac research into NRG/ErbBs grew from the discovery that knockouts for NRG1, ADAM17, ErbB2, and ErbB4 are all embryonically lethal and include cardiac developmental defects (Yin et al., 2015). Additionally, ErbB2 inhibition by trastuzumab has been shown to induce cardiotoxicity (Gao et al., 2010; Guarneri et al., 2006). Preclinical studies established that NRG1 has cardioprotective effects in rodent models of chronic heart failure, potentially through increased proliferation, growth, and survival of cardiac myocytes (Liu et al., 2006; Zhao et al., 1998). This led rapidly to human trials, several of which are still in progress (Gao et al., 2010;

Jabbour et al., 2011). Again, while this research arm may seem distal to the aims of my thesis, multiple relevant points can be gleaned. In particular, NRG1's cardioprotective effects seemed to be via cardiac reverse remodeling, due to downstream signaling changes, epigenetic modifications, and cell metabolism effects (Yin et al., 2015), and perhaps more relevantly, NRG1 treatment in humans has been shown to be tolerated well, with minimal side effects (Gao et al., 2010; Jabbour et al., 2011). This latter point suggests that NRG1 treatment for neuropsychiatric contexts may be a viable alternative or adjunct therapeutic, as I propose in subsequent Chapters based on our experimental findings.

1.1.7 NRG1-ErbB signaling in adult hippocampal neurogenesis, affective behaviour, and psychopathology

As is hopefully clear at this point, NRG1-ErbB signaling has been demonstrated to have cytogenic properties, plays a crucial role in neurodevelopment and mature brain function, and is linked with psychopathology, but had not been studied in the context of adult hippocampal neurogenesis at the outset of our studies, providing the initial impetus for our research. The original thesis behind the subsequent experiments was predicated upon the fact that NRG1 might be involved in adult brain functioning, particularly with respect to hippocampal plasticity and psychopathology. Along these lines, the subsequent sections discuss not only existing theories of depressive and suicidal etiology from a conventional standpoint of the predominant monoaminergic theory of depression, but also discuss hippocampal neurogenesis in particular and presents a model by which these two leading etiological elements of depressive psychopathology might be integrated, with particular relevance to the original studies discussed herein.

1.2 Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects

Published in Neuroscience & Biobehavioral Reviews

Ian Mahar[1,2,*,#], Francis Rodriguez Bambico[3,#], Naguib Mechawar[1,2,4], José N. Nobrega[3,5,6,7]

[#] Equivalent contribution.

[1]McGill Group for Suicide Studies, Douglas Mental Health University Institute, Verdun, QC, Canada

[2]Integrated Program in Neuroscience, McGill University, Montreal, QC, Canada

[3]Behavioural Neurobiology Laboratory, Centre for Addiction and Mental Health, Toronto, ON, Canada

[4]Department of Psychiatry, McGill University, Montreal, QC, Canada

[5]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada

[6]Department of Psychiatry, University of Toronto, Toronto, ON, Canada

[7]Department of Psychology, University of Toronto, Toronto, ON, Canada

*Corresponding author

ABSTRACT

Chronic stressful life events are risk factors for developing depression, the pathophysiology of which is strongly linked to impairments in serotonin (5-hydroxytryptamine, 5-HT) neurotransmission. Exposure to chronic unpredictable stress (CUS) has been found to induce depressive-like behaviours, including passive behavioural coping and anhedonia in animal models, along with many other affective, cognitive, and behavioural symptoms. The heterogeneity of these symptoms represents the plurality of corticolimbic structures involved in mood regulation that are adversely affected in the disorder. Chronic stress has also been shown to negatively regulate adult hippocampal neurogenesis, a phenomenon that is involved in antidepressant effects and regulates subsequent stress responses. Although there exists an enormous body of data on stress-induced alterations of 5-HT activity, there has not been extensive exploration of 5-HT adaptations occurring presynaptically or at the level of the raphe nuclei after exposure to CUS. Similarly, although hippocampal neurogenesis is known to be negatively regulated by stress and positively regulated by antidepressant treatment, the role of neurogenesis in mediating affective behaviour in the context of stress remains an active area of investigation. The goal of this review is to link the serotonergic and neurogenic hypotheses of depression and antidepressant effects in the context of stress. Specifically, chronic stress significantly attenuates 5-HT neurotransmission and 5-HT_{1A} autoreceptor sensitivity, and this effect could represent an endophenotypic hallmark for mood disorders. In addition, by decreasing neurogenesis, CUS decreases hippocampal inhibition of the hypothalamic-pituitary-adrenal (HPA) axis, exacerbating stress axis overactivity. Similarly, we discuss the possibility that adult hippocampal neurogenesis mediates antidepressant effects via the ventral (in rodents; anterior in humans) hippocampus' influence on the HPA axis, and mechanisms by which

antidepressants may reverse chronic stress-induced 5-HT and neurogenic changes. Although data are as yet equivocal, antidepressant modulation of 5-HT neurotransmission may well serve as one of the factors that could drive neurogenesis-dependent antidepressant effects through these stress regulation-related mechanisms.

Highlights:

- The central 5-HT system regulates affect and stress
- Acute and chronic stress paradigms have divergent neurobiological effects on the 5-HT system and neurogenesis
- Hippocampal neurogenesis is influenced by endogenous and exogenous 5-HT modulation
- Neurogenesis is related to stress response, affect, and antidepressant effects
- Ventral (in rodents; anterior in humans) hippocampal neurogenesis may modulate affect and stress via the HPA axis

Keywords: Stress, neurogenesis, serotonin, hypothalamic-pituitary-adrenal axis, chronic unpredictable stress, depression, neurotrophic factors, antidepressant

1.2.1 Introduction

MDD is a recurrent and debilitating mental disorder with a lifetime prevalence of up to 20% in the general population, among the highest for psychiatric disorders (Kessler et al., 2003). Its diagnosis is based upon the presence of persisting affective, cognitive and behavioural symptoms (see **Table 1**), with a depressive episode requiring at least five of these symptoms (including depressed mood or anhedonia) persisting for at least two weeks to meet diagnostic

criteria (American Psychiatric Association, 2013). Despite advancements in the development of therapeutics, current treatment options have not reached optimal efficacy. For instance, pharmacological antidepressant treatments typically require several weeks of treatment before improvement of symptoms can be observed (Jacobs et al., 2000). In the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, the largest prospective, randomized antidepressant trial to date, 16% of participants dropped out due to tolerability issues, only 36.8 % achieved remission after a first level treatment with an antidepressant, and 40% of remittances subsequently led to relapses (McGrath et al., 2008). A large proportion of remitted patients also suffer from residual symptoms that may fluctuate over time (Judd et al., 1998). These clinical limitations owe in part to gaps in knowledge about depressive pathophysiology, which is increasingly being recognized as involving multiple levels, encompassing disturbances in molecular signaling and in modulatory network function.

The etiology of depression is still largely unknown, and theories vary widely in scope and perspective. For example, depression has been proposed to be an evolutionary adaptation to social environments (Allen and Badcock, 2006), or as a consequence of deleterious social environment (Billings et al., 1983). Cognitive theories span from earlier psychoanalytical perspectives of depression as a product of subconscious libidinal drive (Silverman, 1976) to more modern and supported cognitive theories concerning negative and irrational cognitive distortions regarding the self as well as ruminative cognitive patterns (Possel, 2011), and other psychosocial theories suggest particular personality factors such as introversion and pessimism might predispose to depression (Akiskal et al., 1983). More recent theoretical focus has largely been on biological factors, including a wealth of information supporting stress as a causal factor in depression, largely concerning chronic stress-related HPA dysregulation and toxicity from

excessive glucocorticoid release (Lupien et al., 2009), though other theories posit that a downregulation of hippocampal neurogenesis underlies the disorder (Kempermann & Kronenberg, 2003), or suggest genetic or epigenetic factors for developing depression (Karg et al., 2011; Menke et al., 2012). The diathesis-stress model accounts for the interaction of a number of factors as crucial for the etiology of depression. In particular, it posits that a depressive episode is triggered by a combination of a biological predisposition or intrinsic vulnerability (the diathesis) and a precipitating stressful event that may occur much later in life (Monroe and Simons, 1991). The diathesis may stem from genetic liabilities impacting different neurobiological systems involved in stress adaptation and affective processing (as discussed in later sections) or from postnatal or periadolescent events such as child abuse, which directly impact early development of the nervous system (Kendler et al., 2002, 2006; Kendler et al., 2004). The effect of stress, on the other hand, can be modulated by many other factors such as personality, intra-psychic conflict, and presence (or absence) of social support, which could affect how stressful events are perceived in terms of controllability and agency. These environmental and stress factors could in turn influence biological systems, such as causing excessive glucocorticoid release or other HPA dysregulation, particularly in tandem with genetic polymorphisms influencing physiological response to stress, leading to changes in limbic and cortical brain areas as well as depressive (including cognitive) symptoms. Thus theories of depression to date involve evolutionary, social, environmental, interpersonal, psychoanalytical, cognitive, personality, behavioural, endocrine, cellular, and genetic and epigenetic factors and levels of analysis.

However, at the neurochemical level, the most widely accepted hypothesis concerns the depletion of monoamines, most notably of the indoleamine serotonin (5-hydroxytryptamine, 5-

HT), in the brains of depressed patients. Indeed, conventional antidepressants that enhance 5-HT transmission, such as inhibitors of 5-HT reuptake, are the primary choice for first-line pharmacotherapy (Bambico et al., 2009a; Bambico and Gobbi, 2008). However, it is important to point out that some findings do not entirely support a simplistic explanation of depression as purely arising from a serotonergic deficit. For example, a decrease in 5-HT tone does not precipitate a full-blown clinical depressive phenotype in healthy individuals, although this has been shown to occur in some individuals with a history of depressive episodes. Moreover, not all depressed patients respond substantially to treatment with 5-HT agonists (Albert et al., 2012; Neumeister et al., 2002). In addition, although typically chronic treatment with antidepressants is necessary for therapeutic efficacy in treating depressive symptoms in patients or reversing depression-like endophenotypes in animal models (Jacobs et al., 2000), extracellular 5-HT levels increase rapidly after administration (Hervas and Artigas, 1998), suggesting that restoration of 5-HT activity is not immediately sufficient for depressive amelioration and that additional longer-term mechanisms are likely involved. As such, other potentially contributing neurobiological etiological factors underlying depression (and its treatment) have been proposed, including deficits in other neurotransmitters and in neurotrophic factors such as brain-derived neurotrophic factor (BDNF), changes in hippocampal neurogenesis, HPA dysregulation, and circadian rhythm disruption (Hasler, 2010; Jacobs et al., 2000). Notably, these factors do not exist in isolation and frequently influence each other; for example (as discussed later in this article, in sections **4.2** and **4.4**), BDNF and 5-HT positively modulate hippocampal neurogenesis, which in turn regulates HPA function and response to stress.

5-HT is produced from the essential amino acid L-tryptophan in neurons of midbrain raphe nuclei, primarily by the dorsal raphe (DR), which contains about half of the mammalian

nervous system's 5-HT neurons and represents a major source of 5-HT projections in the central nervous system, including the spinal cord (Dahlstroem and Fuxe, 1964; Descarries et al., 1982). Virtually all corticolimbic structures that are involved in mood regulation and the stress response, and which express receptors for 5-HT, are extensively innervated by DR-originating axons. These include the prefrontal cortex (PFC), amygdala, hippocampus and nucleus accumbens (NAc) (Holmes, 2008; Steinbusch, 1981). Not surprisingly, depressive symptomatology is extremely complex and overlaps with that of other neuropsychiatric disorders, such as the negative symptoms seen in schizophrenia. It has been hypothesized that disturbances in 5-HT activity in these postsynaptic targets underlie the wide range of emotional, cognitive, vegetative, and endocrine symptoms found in depression (Blier and de Montigny, 1999; Ressler and Nemeroff, 2000). Genetic (Caspi et al., 2003; Lesch, 2004; Levinson, 2006), brain imaging (Rosa-Neto et al., 2004), tryptophan depletion (Delgado et al., 1999; Leyton et al., 2000), and post-mortem studies (Mann et al., 2000) have provided further evidence in support of this view. The origin of 5-HT impairment in depression is multifaceted and is likely due to the interaction of many intrinsic (e.g. genetic predisposition, gender, and personality factors) and extrinsic factors (e.g. drug use, insufficient social support, and stress) (Jans et al., 2007). Among environmental elements, stress has been given considerable attention as one of the most potent precipitating factors for depression. The emergence of depressive symptoms does indeed proceed in many cases from an experience with a stressful stimulus of one form or another, with which the organism is incapable of coping (Jans et al., 2007). Furthermore, the impact of genetic factors has been shown to be modulated by stress (Kendler et al., 1995; Pucilowski et al., 1993; Silberg et al., 1999). Under normal conditions, the stress response is integral to survival and proper biological and psychological functioning. However, an individual subjected repeatedly to stress,

especially where it finds itself unable to neutralize the source of stress, may eventually succumb to despair. In this case, the accumulated psychological and physical demands (allostatic load) of the stressful experiences will have become detrimental to the central nervous system. These consequences are conducive in potentiating one's vulnerability to depression and other neuropsychiatric disorders. The neural transmission of monoamine transmitter systems has been examined in response to the depressogenic nature of stressful stimuli. It is gradually being recognized from neurochemical and electrophysiological studies that 5-HT neural excitability is greatly influenced by stress and other depressogenic factors, as well as manipulations or agents possessing antidepressant activity. This impact of stress on 5-HT activity could influence the spontaneous and evoked single-spiking and burst-firing activity of 5-HT neurons, as well as the function of their presynaptic and postsynaptic receptors. Stress could also affect different levels of the monoamine metabolic pathway (synthesis, intracellular trafficking, degradation, and re-uptake), which could in turn influence electrochemical signaling (Holmes, 2008). Notably, environmental context may also play a role in the interaction between stress and monoaminergic antidepressant effects. A recent study has shown that an enriched environmental context during treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine leads to behavioural, glucocorticoid, and hippocampal and hypothalamic BDNF recovery from chronic stress, whereas fluoxetine treatment in a stressful context (after enriched housing) exacerbates these consequences of chronic stress (Branchi et al., 2013). Future studies may further elucidate whether environmental context, among other as yet unconsidered factors, modulates response to antidepressants and stress.

Chronic stress has also been shown to affect several aspects of hippocampal neuroplasticity. In particular, it potently decreases adult hippocampal neurogenesis (Dranovsky

and Hen, 2006), the process by which new granule cell neurons are added throughout life to the dentate gyrus (DG) of the hippocampus (Altman and Das, 1965; Kempermann, 2011). Given that hippocampal neurogenesis can regulate the hypothalamic-pituitary-adrenal (HPA) axis (Schloesser et al., 2009; Snyder et al., 2011b), this consequence of chronic stress may exacerbate affective and behavioural responses to stress (Raison and Miller, 2003) in addition to predisposing an individual to subsequent depressive episodes in response to stress. Notably, treatments with antidepressant efficacy have been shown to increase hippocampal neurogenesis, including antidepressant medication (serotonergic as well as non-serotonergic (Banasr et al., 2006; Dranovsky and Hen, 2006)) and non-pharmacological interventions such as electroconvulsive shock (Perera et al., 2007), transcranial magnetic stimulation (Ueyama et al., 2011), and exercise (Glasper et al., 2010; Kiuchi et al., 2012); the neurogenic efficacy of non-serotonergic therapies suggests that antidepressant-stimulated neurogenesis may also be regulated by non-serotonergic pathways. In fact, hippocampal neurogenesis may be required for the actions of some antidepressant agents, particularly serotonergic medications (Airan et al., 2007; Jiang et al., 2005; Santarelli et al., 2003; Surget et al., 2008), and this requirement may involve inhibition of the HPA axis by adult-born neurons in the hippocampus (Snyder et al., 2011b).

This review considers the effects of short-term and chronic stress on 5-HT and neurogenesis-related neurophysiology, primarily within the context of stress-related animal models of depression. The central 5-HT system will be extensively discussed, particularly in relation to the influences of acute (section **1.2.2**) and chronic (**1.2.3**) stress on 5-HT activity, as well as hedonic and motivational behaviour (**1.2.3.1**). The role of presynaptic and postsynaptic 5-HT1A receptors in the control of 5-HT activity, which have also been exhaustively studied in the

past regarding receptor mechanisms of antidepressant action and stress adaptation, will be also be examined (**1.2.3.2**). Finally, the potential role of hippocampal neurogenesis (**1.2.4**) in depression and antidepressant effects (**1.2.4.1**), its regulation by monoamines and neurotrophic factors (**1.2.4.2**), its relationship with antidepressant response (**1.2.4.3**), and the particular function of ventral (in rodents) or anterior (in primates) hippocampal neurogenesis in mediating response to stress and antidepressant effects (**1.2.4.4**) will be discussed, along with a timeline of important stages in hippocampal neurogenesis and when neurogenic manipulations at various stages should have functional consequences on behaviour (**1.2.4.5**). 5-HT and neurogenic responses to stress and influences on affective behaviour explored throughout this review are summarized in **Fig. 2**. We also propose a link between these two systems by suggesting that stress-induced neurogenic deficits contribute to 5-HT dysfunction through HPA dysregulation, and that antidepressants acting on 5-HT may drive hippocampal neurogenesis-dependent antidepressant effects through stress regulation.

1.2.2 Acute effects of stress on serotonergic neuronal activity

Under normal conditions, stress sets into motion a cascade of physiological mechanisms (i.e. autonomic and endocrine), paralleled by cognitive and affective processes, which rapidly prepare for the mobilization of energy (glycogenolysis). This equips the organism with an arsenal of defensive behaviours, such as escape, aggression, and avoidance, to address the source of danger. Acute stress exposure modulates the activity of the central 5-HT system as an important neurophysiological component of the stress response. Acute or short-term exposure to various noxious (physical) stimuli and psychological (situational) stressors have been found to influence the synaptic transmission of monoamines in varying degrees (Lanfumey et al., 2008;

Sapolsky, 2003; Schultz, 2007). These responses are rather rapid, transient (may last for several seconds beyond stimulus offset) electrochemical changes that aim to re-establish homeostasis in the organism, and are generally commensurate to the demand imposed by the stressful situation (i.e. allostatic load) (Lanfumey et al., 2008; Sapolsky, 2003; Schultz, 2007).

Many electrophysiological studies have examined how various external stimuli and events might influence central 5-HT neural activity. These entail monitoring changes in the electrical discharge pattern of 5-HT neurons while awake animals are being exposed to the stimuli or are under anaesthesia immediately following exposure to stimuli. In freely moving cats, DR 5-HT neurons are resistant to any effect of acute physical or psychological stress on action potential firing, such as following exposure to restraint, loud (100 dB) white noise, confrontation with a dog, or injection of formalin into the extremities (Shima et al., 1986; Wilkinson and Jacobs, 1988). Similarly in anesthetized rats, DR 5-HT neurons are unaffected by repeated light flashes (Mosko and Jacobs, 1974). In anaesthetized rats, prior exposure to 30 min of restraint produces at most a slight non-significant increase in the mean firing activity of DR 5-HT neurons (Bambico et al., 2009b). However, some aversive stimuli, such as a foot shock (Schweimer and Ungless, 2010) or defensive encounter with an intruding conspecific in the home cage or experimenter (Walletschek and Raab, 1982), as well as innocuous (non-noxious) stimuli such as clicks (auditory) and light flashes (visual), are able to increase the firing rate of these neurons in awake cats and tree shrews or anesthetized rats (Heym et al., 1982; Trulson and Jacobs, 1979; Trulson and Preussler, 1984; Walletschek and Raab, 1982). In studies with anesthetized rats investigating the response of DR 5-HT neurons to prior restraint, interspike interval (ISI) histograms showed a clear disruption in the rhythmicity of firing in about half of 5-HT neurons monitored, compared to an otherwise Gaussian profile normally exhibited under

non-stress conditions. Additionally, the probability of encountering 5-HT neurons that fired in burst was markedly increased by stress exposure (about 50% in comparison to 18% under control, non-stress conditions) (Bambico et al., 2009b). Crawford et al. (2010) proposed that the excitability of these 5-HT neurons in response to acute stress, particularly exhibited by 5-HT neurons in the lateral wings and the medial extent of the DR, is consistent with the their unique intrinsic active and passive membrane properties (greater membrane resistance, hyperpolarized spike threshold, smaller activation gap, larger tau, long afterhyperpolarization duration, increase in current-induced firing rate). These characteristics are argued to contribute to greater converging input and consequent output, necessary for a role for stress adaptation. In agreement with these electrophysiological studies, several neurobiological studies have detected an increase in immediate-early gene expression (such as c-fos) in the DR, indicating enhanced activation of 5-HT neurons in this midbrain nucleus, following acute stress exposure (Amat et al., 2005; Commons, 2008; Crawford et al., 2010; Grahn et al., 1999; Hale et al., 2008; Takase et al., 2004). Corroborating stress-induced activation of DR 5-HT neurons, *in vivo* microdialysis has also correlated acute stress exposure with significant increases in the extracellular/tissue levels or 5-HT efflux, detected within the DR, medial PFC (mPFC), amygdala and hippocampus, which could be related to the rapid increase in amount of transmitter released per electrical impulse (Bambico et al., 2007; Gartside et al., 2000; Holmes, 2008; Mo et al., 2008; Sheikh et al., 2007). This has been observed following a wide range of different stimuli (such as electric shock, tail pinch, exposure to an elevated platform or predator, social defeat, restraint/immobilization), but not for all forms of aversive stimuli and contexts (Amat et al., 2005; Holmes, 2008; Maier and Watkins, 2005). The exact synaptic and neurophysiological mechanisms that drive increases in tonic or burst-firing activity in DR 5-HT neurons by acute stress have not been completely

clarified. A number of hypotheses have been proposed, including a role for excitatory glutamatergic feedback (Crawford et al., 2010; Crespi, 2009, 2010; Rouchet et al., 2008) and alterations in the function of small-conductance calcium-activated potassium (SK) channels (Crespi, 2009, 2010; Rouchet et al., 2008) that are expressed in midline and lateral wing DR 5-HT neurons. The role of glutamatergic inputs from the mPFC to the DR has been the subject of recent investigations regarding the DR 5-HT-activating consequences of acute stress; this has been explained in relation to the ability of the mPFC to communicate synaptic information to DR 5-HT neurons about the controllability of acute stressors (Amat et al., 2010; Amat et al., 2005; Amat et al., 2008; Maier et al., 2006; Maier and Watkins, 2005), as will be discussed in the following sections. Amat and colleagues (2005) have determined through measurements of c-fos expression that inescapable stressors possess the capacity to activate 5-HT neurons, particularly those located in the caudal region of the DR, and that this activation is associated with the behavioural consequences of acute stress. Such activation can be driven by modifications in mPFC-DR glutamatergic excitatory input to GABAergic and 5-HT neurons in the DR (Amat et al., 2005; Maier et al., 2006). Other stress-associated events may act in concert with these mechanisms in modulating 5-HT activity. These include enhancement in tryptophan hydroxylase (TPH)2-mediated intracellular synthesis, vesicular monoamine transporter (VMAT)2-mediated vesicular packaging of transmitter, and reduction in 5-HT cleavage by monoamine oxidase-A and in reuptake by the 5-HT transporter (SERT). In an extensive review, Holmes (2008) suggested that these are also subject to genetic variability (strain and species-dependence in animal studies), and are likewise sensitive to conventional and putative antidepressants. More investigations are warranted to clearly understand how properties of stressors, such as severity,

chronicity, controllability, and aversiveness interact to affect 5-HT activity, and to determine whether and which discrete 5-HT neuronal subgroups are influenced by stress.

1.2.3 Effects of chronic stress on behaviour and 5-HT activity

1.2.3.1 Chronic stress and other models of depression

As depression is a complex, symptomatically heterogenous disorder, existing animal models may be limited in reproducing all of the pathological dimensions of depression, and face a particular obstacle in demonstrating face validity. Nevertheless, these models can be very useful testing tools, as they reproduce particular endophenotypes related to specific components of depression symptomatology. **Fig. 3** illustrates some of these animal models of depression and tests for depressive-like behaviour.

Vulnerability to a depressive-like state can be precipitated by a number of validated experimental manipulations that mimic early life adversity, genetic liabilities, stress, and other predisposing factors (Monroe and Simons, 1991). The maternal separation model of early life adversity involves periodic separation of neonates from the dam (Leussis et al., 2012). Genetic manipulations, such as with SERT knockout mice (Olivier et al., 2008) and cannabinoid CB1 receptor knockout mice (Valverde and Torrens, 2012), have shown depressive-like phenotypes. Similarly, congenital learned helplessness rats are selectively bred for learned helplessness behaviour (Weiss et al., 1998), and Flinders sensitive line rats are selectively bred for increased responses to cholinesterase inhibitors (Overstreet, 1993). Olfactory bulbectomy (complete ablation of the olfactory bulb) has been used to model depression (Song and Leonard, 2005), and causes widespread neuronal degernation and remodelling in limbic, raphe, and other brain regions (Harkin et al., 2003). Pharmacological models are also used, including psychostimulant

(such as amphetamine) withdrawal (Barr et al., 2002) and clomipramine treatment in neonates (Vogel et al., 1990). Finally, multiple models involve repeated exposure to stress or stress hormones. These include chronic administration of corticosterone (Rainer et al., 2011), chronic restraint stress (Chiba et al., 2012), chronic social defeat by a dominant conspecific (Avgustinovich et al., 2005), prolonged social isolation (Brenes Saenz et al., 2006), repeated foot shock (Swiergiel et al., 2008), and chronic unpredictable mild stress (CUS) exposure (Willner et al., 1992), which have been shown to increase glucocorticoid levels and induce depressive-like phenotypes.

Although the neurobiological foundations of these models may be argued to be distinct from that of depression, these models are typically validated by behavioural tasks with quite remarkable predictive validities in screening for antidepressants. Among these are the forced swim test (FST), tail suspension test, and learned helplessness test measuring stress coping impairment or behavioural despair, the sucrose preference test measuring anhedonia-like reactivity, the novelty-suppressed feeding test examining anxiety-like behaviour, and the social interaction test examining sociality (Cryan and Holmes, 2005; Dulawa and Hen, 2005; Lucki, 1997; McArthur and Borsini, 2006; Nestler et al., 2002; Willner, 1990).

Of these models, CUS merits particular examination due to its widespread use and validation (Willner, 1997, 2005). In this model, multiple varied non-debilitating inescapable and uncontrollable physical, psychological, and circadian stressors are applied in an unpredictable (occurring at any time) and randomized fashion for several weeks, whereas control (unstressed) animals are not exposed to any of the stressors and may be housed in pairs throughout the course of the experiment (Bambico et al., 2009b; Bekris et al., 2005; Moreau et al., 1992; Moreau et al., 1995; Willner et al., 1992). **Table 2** shows some of the commonly used micro-stressors, whose

combinatory and cumulative effects are sufficient to produce depressive-like behaviours. CUS exposure delays or prevents habituation, has increased physical and psychological demand, progressively increases corrective behavioural/physiological reactivity, and adversely alters neural systems involved in subsequent stress appraisal (Fairbank et al., 1991; Roman et al., 2004), effects than could otherwise be more effectively overcome with short-term exposure and with one kind of stressor (homotypic). CUS can therefore lead to the eventual exhaustion of physical and cognitive resources, which eventually translates to depression-relevant behavioural responses. These depressive-like consequences develop only after an extended duration (Bambico et al., 2009b; Banasr and Duman, 2008; Grippo et al., 2006; Grippo et al., 2005; Kim et al., 2003a), with the exact time of onset varying, likely due to differences in the CUS protocol (intensity, duration, types and combination of stressors), animal strain, and handling procedures used across different laboratories. Depressive-like features cover a large spectrum that includes alterations in emotional and hedonic reactivity, cognitive function, motivational states, grooming behaviour, and body weight, features analogous to those found in depressed patients, and which could accordingly be prevented or reversed by chronic treatment with antidepressants (Moreau et al., 1992; Moreau et al., 1995; Willner, 1997, 2005; Willner et al., 1992; Willner et al., 1987).

CUS possesses face (symptom profile, including anhedonia), construct (theoretical background), predictive (treatment profile), and etiological (causation) validity as a model of depression (Willner, 2005). Moreover, its validity may surpass that of other models (including those that also involve repeated stress), many of which fail to show validity in each of these three domains or to show the same depression-relevant chronicity of behavioural endophenotype duration (Willner, 1997). The efficacy of long-term, but not short-term, antidepressant treatment in reversing behavioural and biochemical sequelae associated with CUS reliably mirrors the

temporal delay of antidepressant treatment effect in depressed patients (predictive validity) (Willner, 1997, 2005; Willner et al., 1987).

CUS also mimics from the human milieu the role of stress in the etiology of depressive disorders. Epidemiological studies have indicated that chronic exposure to stressful life events in the human population is considered a major risk factor for developing depression (Firk and Markus, 2007; Kendler et al., 2004). Interestingly, the distinct pattern of depressive symptoms has been found to be dependent upon the characteristics (e.g. the kind and severity) of the stressful event(s) experienced (Keller et al., 2007). This association of stress and depression has also been supported by experiments among healthy individuals, such as those demonstrating that a stressful experience and associated changes in cortisol responses greatly magnify self-reported depressive symptoms and the effects of mood-impairing manipulations including tryptophan depletion (Pruessner et al., 2003; Richell et al., 2005). In recent years, evidence for the strong link between stress and genetics as an essential factor in the pathogenesis of depressive disorders has been rapidly mounting (El Hage et al., 2009; Jans et al., 2007). Polymorphisms in genes encoding glucocorticoids and attendant receptors or other elements of the HPA axis, such as the CRF-R1 and NR3C1 genes (for review, see El Hage et al., 2009), in the 5-HT transporter-linked promoter region (5-HTTLPR) of the 5-HT transporter-encoding SLC6A4 gene (chromosome 17, 11 in mouse), or of the Htr1A promoter region on the 5-HT1A-encoding gene on chromosome 5 (13 in mouse) confer greater tendency to develop depression or depressive symptoms after stressful life events, such as childhood trauma (Caspi et al., 2003; Jacobs et al., 2006; Kaufman et al., 2004; Keller et al., 2007; Kendler et al., 2004; Kendler et al., 2005; Le Francois et al., 2008; Zalsman et al., 2006). In particular, the 5-HTTLPR s/s genotype, associated with reduced SERT expression, and the G(-1019) variant of the Htr1A promoter region, which is associated

with reduced 5-HT1A receptor expression, have been found to impair affective regulation (Caspi et al., 2010; Le Francois et al., 2008; Lesch et al., 1996). Moreover, CUS exposure in both humans and animals is argued to profoundly influence epigenetic regulation that may produce gradual but stable behavioural and neurobiological adaptations seen in depression (for review, see Tsankova et al., 2007; Zhang and Meaney, 2010). In particular, chronic but not acute exposure to stress has been determined to decrease the function of the enzyme histone deacetylase in the mouse NAc, leading to increased histone acetylation and transcription of target genes and hypersensitivity to the behavioural effects of chronic stress (Renthal et al., 2007). Furthermore, CUS increases hippocampal histone deacetylase activity and decreases histone acetylation (Ferland and Schrader, 2011). Finally, resilience or susceptibility to stress is also a feature of both chronic stress animal models of depression and depressive etiology in humans (Russo et al., 2012). Specifically, the subset of animals submitted to CUS that are susceptible to developing anhedonia also show decreased hippocampal expression of synapse-related genes, increased thymopoietin, and increased glucocorticoid levels, similar to findings in depressed patients (Brown et al., 2004; Christiansen et al., 2012; Duric et al., 2013; Goldstein et al., 2000; Henningsen et al., 2012).

1.2.3.2 Chronic unpredictable stress alters serotonergic neural activity

The underlying neurobiological underpinnings of CUS-induced depressive-like behaviours are likely multi-factorial and may include neurodegenerative, morphological, and immunological processes, as well as impairments in intracellular signalling and hippocampal neurogenesis. Indeed, CUS results in the attenuation of cell proliferation and neurogenesis (Jayatissa et al., 2009; Mineur et al., 2007), extracellular signal-regulated kinase (ERK)

phosphorylation (Qi et al., 2006), vascular endothelial growth factor (VEGF) expression (Bergstrom et al., 2008), and an increase in long-term depression (Holderbach et al., 2007) in key limbic structures, notably the hippocampus and the PFC. It also enhances pro-inflammatory cytokines and immunosuppressant processes (Pitychoutis et al., 2009; Rasheed et al., 2008). At the neurochemical level, CUS can directly impact the 5-HT system, the impairments of which correlate with several dimensions of depressive symptomatology.

1.2.3.2.1 Effect of CUS on DR serotonergic neural activity

Following CUS, spectrophotometric and neurochemical analyses have found an increase in monoamine oxidase-A (Bhutani et al., 2009) and a global depletion of 5-HT (Ahmad et al., 2010; Bhutani et al., 2009; Dang et al., 2009a; Dang et al., 2009b; Rasheed et al., 2008). There have also been reports of reductions in 5-HT release, tissue concentration, and synaptic activity in key corticolimbic structures, such as the hippocampus and NAc (Bekris et al., 2005; Kang et al., 2005; Luo et al., 2008; Yi et al., 2008). These changes are consistent with those observed in a subgroup of depressed patients who display reductions of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (van Praag, 1996), although some studies on 5-HT metabolites in depressed patients provide mixed results (Belmaker and Agam, 2008). Moreover, experimentally-induced depletion of 5-HT through a diet lacking the 5-HT precursor tryptophan produces greater depressed mood among healthy subjects with a family history of depression (Benkelfat et al., 1994; Klaassen et al., 1999a; Klaassen et al., 1999b), and triggers a relapse among remitted depressive patients (Delgado et al., 1999), likely involving a direct attenuating effect on 5-HT action potential firing. CUS has been shown to induce a significant decline of about 35% in the mean spontaneous neural firing activity of 5-HT neurons

recorded from the DR, a significant decrease in the number of spontaneously active neurons, anhedonia-like reductions in the preference for and intake (absolute and relative) of sucrose in the sucrose preference test (Bambico et al., 2009b), which is a depression-related endophenotype thought to be instigated by dysregulation in dopaminergic neurotransmission (Willner, 2005), and enhanced immobility (passive behavioural coping or behavioural despair) in the FST (Banasr and Duman, 2008). Conversely, the observed enhancement in 5-HT neurotransmission is widely considered to be a benchmark of antidepressant-like activity (Bell et al., 2001; De Montigny, 1981).

1.2.3.2.2 CUS downregulates presynaptic 5-HT$_{1A}$ autoreceptor function

Among the numerous subtypes of 5-HT receptors, the 5-HT$_{1A}$, a Gi/Go-protein-coupled receptor, is one of the most commonly studied in relation to the neurobiology underlying mood disorders and antidepressant response (Holmes, 2008). The activity of DR 5-HT neurons is negatively regulated by these receptors, which may be localized on somatodendritic compartments (presynaptic auto-inhibitory receptors) of 5-HT neurons or postsynaptic targets of 5-HT terminals (Hall et al., 1997; Pazos et al., 1987). Postsynaptic 5-HT$_{1A}$ receptors, such as those in the ventral (infralimbic) regions of the mPFC, exert inhibitory and excitatory control over DR 5-HT neuronal activity (Hajos et al., 1999; Martin-Ruiz and Ugedo, 2001) by modulating glutamatergic excitatory input to DR GABAergic and 5-HT neurons (Amat et al., 2005; Bambico et al., 2007), as discussed in the next section. For this position to influence 5-HT neural activity, presynaptic and postsynaptic 5-HT$_{1A}$ receptors are implicated in the therapeutic mechanisms of antidepressants and are putative antidepressant targets (Blier et al., 1998).

Alterations in the function and levels of 5-HT_{1A} receptors in depressive pathology are not as consistently reported in humans as in animal models. As shown in **Table 3**, human brain imaging and post-mortem studies on depressed patients have obtained divergent findings (van Praag, 2004). These equivocal findings could arise from the difficulty of delineating depression-specific changes from those associated with comorbidities, including other neuropsychiatric conditions and history of drug use, as well as from those incurred by medications. However, animal models have consistently shown reductions in somatodendritic 5-HT_{1A} autoreceptor sensitivity in the DR. This has been demonstrated in *ex vivo* and *in vivo* electrophysiological experiments on CUS-exposed rats or mice by a diminution in the inhibitory response of DR 5-HT neurons to a local application of the 5-HT_{1A} agonist/partial agonist 8-OH-DPAT or ipsapirone (**Table 3**). Interestingly, CUS-induced 5-HT_{1A} desensitization was accompanied by a reduction in spontaneous 5-HT firing activity (Bambico et al., 2009b), effects that were recapitulated in SERT knockout mutant mice (Fabre et al., 2000; Gobbi et al., 2001; Li et al., 2000; Lira et al., 2003) and neonatal clomipramine treatment models of depression (Kinney et al., 1997; Maudhuit et al., 1995, 1996). These observations are striking given that chronic SSRI administration decreases 5-HT_{1A} autoreceptor binding in depressed patients (Gray et al., 2013). In rodent electrophysiology models, SSRIs acutely decrease 5-HT neuronal firing activity, and following chronic administration desensitize 5-HT_{1A} autoreceptors to restore normal 5-HT neuronal firing activity (Bambico et al., 2009a; Bambico and Gobbi, 2008; Blier and de Montigny, 1999). As SSRI-mediated and CUS-induced 5-HT_{1A} autoreceptor desensitization are associated with opposing behavioural profiles, it is likely that these progress via somewhat unrelated mechanisms. SSRI-induced 5-HT_{1A} autoreceptor desensitization appears to determine the gradual restorative increase in DR 5-HT firing activity with chronic SSRI treatment. A more

complex process might be at play though, as acute and not chronic SSRI treatment has been reported to internalize DR 5-HT$_{1A}$ autoreceptors (Riad et al., 2008; Riad et al., 2004). CUS-induced desensitization, however, is paralleled by a lower basal 5-HT neuronal firing tone (Bambico et al., 2009b). It can therefore be hypothesized as a compensatory response to the primary CUS-induced decrease in 5-HT tone, possibly among a presumed subpopulation of 5-HT neurons (about 16%) with low or null expression of these 5-HT$_{1A}$ autoreceptors (Kiyasova et al., 2013).

There is a long-standing view that 5-HT$_{1A}$ autoreceptor desensitization is essential for the therapeutic mechanism of SSRIs. 5-HT$_{1A}$ autoreceptors are desensitized by chronic treatment with SSRIs, whereas postsynaptic 5-HT$_{1A}$ receptors, particularly those in the hippocampus, are sensitized by chronic tricyclic antidepressant treatment and electroconvulsive shock administration (for review, see (Bambico et al., 2009a; Bambico and Gobbi, 2008; Hill et al., 2009; Sharp et al., 2007). It is recognized that the desensitizing action of SSRIs occurs to disengage negative regulation of 5-HT neuronal activity, but may also tap into other 5-HT$_{1A}$-related intracellular processes. However, SSRI treatment has been shown to reverse CUS-induced behavioural deficits (for review, see Willner, 1997, 2005), suggesting that the occurrence of 5-HT$_{1A}$ desensitization with CUS does not completely abrogate the therapeutic action of SSRIs. This has been similarly observed in CB1 (Aso et al., 2008; Aso et al., 2009; Steiner et al., 2008b) and 5-HT$_{1A}$ (Santarelli et al., 2003) knockout mice, which are responsive to the antidepressant effects of SSRIs (paroxetine) and tricyclic antidepressants (imipramine and desipramine), respectively, despite the absence or desensitization of 5-HT$_{1A}$ autoreceptors. More recently, Richardson-Jones and colleagues generated mice with high and low 5-HT$_{1A}$ autoreceptor expression in the DR, without affecting postsynaptic 5-HT$_{1A}$ receptors, by

genetically engineering the Htr1A gene that encodes 5-HT$_{1A}$ (Richardson-Jones et al., 2010). They found that high-DR 5-HT$_{1A}$ mice showed a blunted physiological response to acute stress, increased passive behavioural coping and a non-response to fluoxetine despite displaying gradual desensitization of 5-HT$_{1A}$ autoreceptors. Conversely, low-DR 5-HT$_{1A}$ mice were more responsive. It appears from these studies that SSRIs may still be therapeutically active in the presence of desensitized 5-HT$_{1A}$ autoreceptors, but not in their complete absence, and that the interplay between the sensitivity and expression of these receptors may more reliably determine the severity of the depressive-like state and the response to SSRIs. This notion corroborates some clinical studies (Rabiner et al., 2004) but not others (Lan et al., 2013; Miller et al., 2013), and therefore warrants further investigation on the differential role of 5-HT$_{1A}$ receptor expression, internalization, and sensitivity in the partial or lack of response to SSRIs among a subgroup of patients.

1.2.3.2.3 CUS alters postsynaptic 5-HT$_{1A}$ heteroreceptor function: possible influence on medial prefrontal glutamatergic feedback

In addition to the effects of CUS on presynaptic 5-HT$_{1A}$ autoreceptors, an impact on postsynaptic 5-HT$_{1A}$ heteroreceptors is likely to contribute to the behavioural and neurophysiological impairments found in CUS-exposed animals, including impairments in midbrain 5-HT neuronal activity. Postsynaptic 5-HT$_{1A}$ heteroreceptors located in different corticolimbic regions, such as the PFC, amygdala, and hypothalamus, have been suggested to indirectly modulate DR 5-HT activity (Bosker et al., 1997; Holmes, 2008). Of these, the mPFC is of particular interest as it abundantly expresses 5-HT$_{1A}$ and serves as the origin of most cortical projections to the DR (Gabbott et al., 2005; Hajos et al., 1999; Peyron et al., 1998). 5-HT$_{1A}$

receptors in the mPFC, like 5-HT_{1A} autoreceptors in the DR, exert regulatory feedback onto DR 5-HT neurons via a long-range loop (Celada et al., 2001; Gabbott et al., 2005; Hajos et al., 1999; Martin-Ruiz and Ugedo, 2001; Peyron et al., 1998). Application of 8-OH-DPAT directly into the mPFC inhibits the spontaneous and glutamate-mediated neuronal firing of the major projection neurons therein (Araneda and Andrade, 1991; Ashby et al., 1994; Borsini et al., 1995; Cai et al., 2002). This could, in turn, inhibit DR 5-HT neuronal firing activity, as has been demonstrated under conditions where DR somatodendritic 5-HT_{1A} autoreceptor activity was inhibited by pertussis toxin blocking intracellular signaling of Gi/Go proteins (Martin-Ruiz and Ugedo, 2001). Interestingly, following CUS it was observed that a systemic injection of 8-OH-DPAT that could activate these mPFC 5-HT_{1A} receptors failed to attenuate DR 5-HT neuronal firing activity (Bambico et al., 2009b). As DR somatodendritic 5-HT_{1A} autoreceptors were desensitized in these CUS-exposed animals, this decrease in the ability of 8-OH-DPAT to inhibit 5-HT neurons suggests that mPFC 5-HT_{1A} receptors themselves were desensitized by CUS, and as 5-HT_{1A} receptors are inhibitory, their desensitization in the mPFC would translate into a hyperactivation of mPFC glutamatergic projections to the midbrain. The desensitization of mPFC 5-HT_{1A} receptors following CUS concurs with observations from other animal models, as well as with the significant reduction of PFC 5-HT_{1A} receptor binding and 5-HT_{1A} sensitivity found in treated and untreated depressive patients (Drevets et al., 2007). However, contradictory findings have also been reported, such as reports of an increase in postsynaptic 5-HT_{1A} receptor density (Vicentic et al., 2006; Ziabreva et al., 2003), messenger RNA (mRNA) levels (Neumaier et al., 2002), or sensitivity (Arborelius et al., 2004) in the rodent model of maternal separation and congenital learned helplessness. Nevertheless, data from brain imaging studies on depressed patients appear to converge at abnormal hyperactivation in cortical regions such as the anterior

cingulate and the subgenual cortex (Price and Drevets, 2010), structures that are considered analogous to the mPFC in the rodent. Indeed, the most parsimonious hypothesis that could explain this hyperactivity is a desensitization or downregulation of inhibitory 5-HT_{1A} receptors in the PFC.

The mPFC-DR circuit is an important locus for cognitive appraisal of stressful situations, and likely subserves some of the cognitive, executive, and memory-related dysfunctions associated with depression, such as disturbance in concentration and learning, negative and irrational cognitive distortions regarding the self, increased rumination, and despair (Amat et al., 2005; Celada et al., 2001; Price and Drevets, 2010). The prelimbic/infralimbic region of the mPFC is postulated to be the cortical detector and processor of stress controllability. It functions to inhibit the stress-induced activation of 5-HT neurons of the caudal DR when the stimulus is perceived to be controllable or escapable (Amat et al., 2010; Amat et al., 2005; Amat et al., 2008). This could well explain the opposing effects of aggressiveness/offensiveness-(controllable) and defensiveness-related (uncontrollable) stimuli on DR 5-HT neuronal activity observed in tree shrews made to confront a conspecific intruder or an experimenter (Walletschek and Raab, 1982). Electrical stimulation of the mPFC also elicits antidepressant-like effects, enhances DR 5-HT activity, and increases the postsynaptic release of monoamines, notably 5-HT (Hamani et al., 2010a; Hamani et al., 2010b; Juckel et al., 1999), which could provide the neurochemical basis for the efficacy of subgenual cingulate (Cg25) deep brain stimulation as an antidepressant treatment for intractable depression (Mayberg et al., 2005). As the mPFC forms a reciprocal feedback circuit with the DR (Celada et al., 2001; Martin-Ruiz et al., 2001), repeated experience of loss of control and unpredictability, as well as the associated allostatic load observed with CUS, could progressively impair both the mPFC and the DR, as well as their

interaction. Functionally, this could translate to alterations in neural excitability in the mPFC, as has been demonstrated (Wilber et al., 2011), and to decreases in spontaneous single-spiking and burst-firing activity of 5-HT DR neurons (Bambico et al., 2009b). Behaviourally, these effects could translate into passive behavioural coping, anhedonia, and other depression-related markers. Interestingly, Maier and colleagues have shown that prior experience to an otherwise controllable (escapable) aversive stimulus (such as tail shock) potently blocks the behavioural and neurochemical alterations produced by subsequent uncontrollable (inescapable) stressor (tail shock) (Amat et al., 2010; Amat et al., 2005; Amat et al., 2008; Maier et al., 2006; Maier and Watkins, 2005), and possibly the progressive cascade of adverse consequences of CUS. Similarly, predictable chronic mild stress may have antidepressant, anxiolytic, and pro-neurogenic effects in the hippocampus (Parihar et al., 2011). These results indicate that controllable or predictable stress exposure may inoculate against the deleterious effects of stress seen in paradigms such as CUS, and suggest an interaction between the processes involved in neural responses to controllable and uncontrollable stress.

Thus chronic stress induces changes to 5-HT systems in the brain, including the hippocampus, associated with depression and depressive-like phenotypes, which can be reversed by antidepressant treatment. However, chronic stress also impacts hippocampal neurogenesis, which is itself regulated by 5-HT, and the effects of stress on neurogenesis can also be reversed by monoaminergic antidepressant treatment. In the following section we discuss how this regulation of neurogenesis occurs, and how neurogenesis interacts with ADs, 5-HT, and stress to mediate the opposing effects of stress and antidepressant treatment on depression-related pheonotypes.

1.2.4 The role of hippocampal neurogenesis in mediating affect, antidepressant action, and stress response

1.2.4.1 Neurogenesis in depression

Soon after the discovery of adult hippocampal neurogenesis in humans (Eriksson et al., 1998), it was theorized that basal deficits in hippocampal neurogenesis may underlie symptoms of psychiatric disorders, particularly depression (Jacobs et al., 2000; Kempermann, 2002). This theory was largely based on findings indicating decreased hippocampal volume in depressed patients and increased neurogenesis with administration of antidepressant medication and therapies (Kempermann, 2002; Kempermann and Kronenberg, 2003), supported by recent studies indicating decreased numbers of granule cells and decreased granule cell layer volume in the anterior and mid-DG in unmedicated depressed patients relative to controls, as well as increased hippocampal neurogenesis and increased granule cell layer volume in depressed patients who had taken antidepressants relative to unmedicated patients (Boldrini et al., 2012; Boldrini et al., 2013; Boldrini et al., 2009). A particularly exciting recent report by Spalding et al. has revealed that adult hippocampal neurogenesis in humans is substantial and that neuronal turnover is high in comparison to rodents, supporting the possibility that human hippocampal neurogenesis is sufficient to support a role in affective and cognitive phenomena such as depression and response to stress and antidepressants (Spalding et al., 2013). In addition, the delay in efficacy of antidepressants (in both humans and animal models) appears to mirror the time required for newly proliferated neurons to become functional and hyperplastic (Ge et al., 2007; Jacobs et al., 2000).

However, in presenting a balanced view of the role of hippocampal neurogenesis in depression and antidepressant effects, it is important to consider evidence that fails to support or

contradicts this view. Recent studies have brought into question whether decreased neurogenesis underlies depression (Eisch and Petrik, 2012; Hanson et al., 2011; Petrik et al., 2012). Whether ablating neurogenesis in animals is sufficient to induce a depressive phenotype remains controversial (Perera et al., 2011; Schloesser et al., 2009; Snyder et al., 2011b; Surget et al., 2008; Vollmayr et al., 2003; Wang et al., 2008). Further disputing the role of hippocampal neurogenesis deficits in depressive etiology is the fact that depressed patients have not been demonstrated to have significantly reduced hippocampal neurogenesis (Boldrini et al., 2009), although granule cells and granule cell layer volume are reduced (Boldrini et al., 2013), leaving the neurogenic hypothesis of depression in doubt (Hanson et al., 2011; Petrik et al., 2012). Consequently, a more supported hypothesis has emerged: that adult hippocampal neurogenesis is involved in mediating response to antidepressant treatments (Hanson et al., 2011). This is supported by studies showing that increases in hippocampal neurogenesis are associated with antidepressant effects mirroring the developmental latency of new neurons (Mahar et al., 2011), that antidepressant treatments increase hippocampal neurogenesis (Boldrini et al., 2009; Malberg et al., 2000), and that the behavioural effects of antidepressants may require intact neurogenesis (Perera et al., 2011; Santarelli et al., 2003; Wang et al., 2008). However, in understanding the importance of adult hippocampal neurogenesis in the mechanism of exogenous monoamine-related agents, it is important to discuss the regulation of neurogenesis by endogenous monoaminergic systems.

1.2.4.2 Monoaminergic and neurotrophic factor regulation of neurogenesis

Hippocampal neurogenesis has been shown to be regulated by a variety of monoamines and neurotrophic factors, as shown in **Table 4**. The hippocampus receives dense innervation by 5-HT fibers (Gage and Thompson, 1980; Gasbarri et al., 1994). Ablation of 5-HT neurons in the DR and median raphe decreases neurogenesis, and these effects are reversed by 5-HT re-innervation (Brezun and Daszuta, 2000a, b). Regarding 5-HT receptors, effects of 5-HT-mediated signalling on hippocampal neurogenesis appear to be receptor subtype-specific. Pharmacological studies suggest that 5-HT_1 stimulation is pro-neurogenic, 5-HT_{1B} stimulation does not produce an effect, 5-$HT_{2A/C}$ antagonism decreases proliferation, 5-HT_{2B} stimulation increases neurogenesis (and has antidepressant-like effects; notably, blocking activity of this receptor ablates behavioural and neurogenic efficacy of SSRIs), and 5-HT_{2C} stimulation does not affect neurogenesis (Banasr et al., 2004; Diaz et al., 2012). In addition, suppression of SERT expression increases hippocampal neurogenesis and causes antidepressant-like behaviour (Ferres-Coy et al., 2013).

Apart from the influence of the 5-HT system, the dopaminergic and norepinephrinergic systems have also been shown to influence neurogenesis, although the findings are not as clear as those regarding 5-HT. Dopamine depletion reduces hippocampal cytogenesis, an effect that is reversed by ropinirole administration (Hoglinger et al., 2004), although in other studies a transient increase is observed after depletion (Park and Enikolopov, 2010). D2 receptor activation by quinpirole increases subgranular zone proliferation (Yang et al., 2008), and D3 knockout or blockade also increases proliferation (Egeland et al., 2012), suggesting a receptor-specific regulation. However, the effects of dopaminergic antagonists (e.g. haloperidol) on hippocampal neurogenesis are mixed (Veena et al., 2011), and merit further experimental investigation. Although dopamine transporter-immunoreactive (-IR) fibers have been identified

near neurogenic cells in the subgranular zone (Hoglinger et al., 2004), dopaminergic subgranular zone innervation is thought to be limited (Kempermann, 2011). This suggests that the regulation of hippocampal neurogenesis by dopamine may be due to an indirect mechanism, potentially involving reciprocal interactions with the 5-HT and norepinephrinergic systems (Guiard et al., 2008a; Guiard et al., 2008b).

Norepinephrine depletion decreases hippocampal progenitor proliferation (Kulkarni et al., 2002). Norepinephrine has been shown to positively modulate hippocampal neural precursor cell proliferation in a β-adrenergic receptor-dependent (but not α1- or α2-dependent) manner (Jhaveri et al., 2010; Masuda et al., 2012), although in other studies α2-receptor blockade increases cell survival and dendritic arborisation (Rizk et al., 2006). These effects may result from direct norepinephrine input onto neurogenic cells (Rizk et al., 2006).

Neurotrophic factors have also been shown to increase adult hippocampal neurogenesis and modulate antidepressant-related behaviours, particularly BDNF, insulin-like growth factor (IGF), VEGF, and neuregulin-1 (NRG1). Notably, many of these factors can be administered peripherally to induce these effects (Aberg et al., 2000; Duman et al., 2009; Mahar et al., 2011; Schmidt and Duman, 2010), suggesting that peripheral levels of neurotrophic factors may modulate hippocampal neurogenesis and mood.

Infusion of BDNF, both peripherally and centrally, increases neuronal survival and overall hippocampal neurogenesis, and also produces antidepressant-like effects (Scharfman et al., 2005; Schmidt and Duman, 2010; Shirayama et al., 2002). Decreases in BDNF are predominantly detrimental to neurogenesis, although results are mixed. Proliferation is largely unchanged in BDNF+/- mice (Rossi et al., 2006; Waterhouse et al., 2012) (though one study reports an increase in proliferation in these animals (Sairanen et al., 2005) and another reports

increased proliferation but decreased neuronal differentiation in mice largely lacking dendritic BDNF (Waterhouse et al., 2012)), but these animals show decreased survival (Sairanen et al., 2005) and a lack of effectiveness for environmental enrichment on increasing neurogenesis (Rossi et al., 2006). DG-specific knockdown of BDNF leads to decreased neurogenesis through decreased neuronal differentiation (Taliaz et al., 2010). BDNF-hypomorphic mice also show depression-like behaviour and behavioural resistance to antidepressants (Adachi et al., 2008; Taliaz et al., 2010). Notably, BDNF may contribute to the modulation of neurogenesis in response to both stress and antidepressants, as hippocampal BDNF levels decrease in response to chronic stress (Larsen et al., 2010; Shi et al., 2010b; Smith et al., 1995a; Smith et al., 1995b) and increase in response to antidepressant treatments (Altar et al., 2003; Czubak et al., 2009; Gersner et al., 2010; Hanson et al., 2011; Musazzi et al., 2009; Nibuya et al., 1995).

Peripherally administered IGF increases hippocampal neurogenesis by stimulating DG proliferation and neuronal differentiation (Aberg et al., 2000), and also produces antidepressant effects (Duman et al., 2009), whereas IGF-knockout mice display decreased numbers of hippocampal granule cells (Beck et al., 1995). Blocking IGF activity decreases proliferation in both sedentary and exercised animals (Glasper et al., 2010).

VEGF administration increases hippocampal neurogenesis by promoting cell proliferation (Jin et al., 2002). In contrast, VEGF-knockout mice display decreased numbers of immature neurons and reduced proliferation (Sun et al., 2006). Hippocampal VEGF levels increase after antidepressant treatment, and VEGF (through signalling with Flk-1) may also be necessary for antidepressant-induced proliferation and antidepressant-like behavioural effects (Warner-Schmidt and Duman, 2007). Stress-induced depressive-like behaviour and decreases in

DG cell survival are reversed by exercise, but this effect is abolished with VEGF antagonists (Kiuchi et al., 2012), similar to effects seen with IGF antagonists (Glasper et al., 2010).

NRG1 has been shown to cross the blood-brain barrier (Carlsson et al., 2011; Kastin et al., 2004), and peripheral administration of NRG1 increases ventral hippocampal neurogenesis by rapidly increasing proliferation, possibly through direct stimulation of ErbB3 receptors in DG cells, leading to antidepressant-like effects four weeks after administration (Mahar et al., 2011). This delayed effect mirrors the latency for newborn cells to have differentiated into hyperplastic neurons (Ge et al., 2007). It remains to be determined whether other EGF-related proteins have similar effects, or whether NRG1 antagonism leads to neurogenic deficits.

Thus monoamines (including 5-HT) and neurotrophic factors have been shown to modulate hippocampal neurogenesis, and this represents one avenue by which stress-induced 5-HT system changes can affect hippocampal neurogenesis and its functions. Overall, the neurogenic influence of these factors is primarily positive for 5-HT and norepinephrine as well as neurotrophic factors, and positive to mixed for dopamine, suggesting that further studies into endogenous regulation of neurogenesis by monoamines should investigate the complex (and potentially indirect) modulation of hippocampal neurogenesis by dopamine, including various dopaminergic receptors and receptor-specific antagonists. In contrast, the relationship between hippocampal neurogenesis and antidepressants (primarily exogenous monoaminergic signalling-related agents), as well as behavioural antidepressant effects, has been more thoroughly characterized.

1.2.4.3 Relationship between neurogenesis, antidepressants, and antidepressant effects

Chronic antidepressant treatment has been shown to increase neurogenesis (see (Dranovsky and Hen, 2006) for review) and decrease anhedonia and learned helplessness behaviour (David et al., 2009; Holick et al., 2008; Jayatissa et al., 2006; Jayatissa et al., 2008; Malberg and Duman, 2003; Pechnick et al., 2011; Perera et al., 2011). These effects have been observed in rodents (David et al., 2009; Jayatissa et al., 2006; Jayatissa et al., 2008; Malberg and Duman, 2003), non-human primates (Perera et al., 2011), and humans (Boldrini et al., 2012; Boldrini et al., 2009). A recent report by Sahay et al. has also shown that increasing hippocampal neurogenesis through genetic manipulation in unstressed mice is not sufficient to induce some of the anxiolytic and antidepressant-like effects seen after antidepressant treatment (Sahay et al., 2011). In addition to suggesting that an increased number of new neurons alone might not be responsible for all of the behavioural effects of antidepressants, these findings also bring to light the possibility that the involvement of increased numbers of new neurons in certain behavioural responses to antidepressants may require the presence of the monoaminergic modulation induced by these treatments, or may exist only in the presence of stress in some paradigms. It is important to note that some agents with antidepressant properties, such as nicotine, do not increase hippocampal neurogenesis (Abrous et al., 2002; Mahar et al., 2012; Mudo et al., 2007; Vazquez-Palacios et al., 2005), and in some studies chronic antidepressant treatment did not increase neurogenesis (Holick et al., 2008; Huang et al., 2008; Navailles et al., 2008).

Antidepressants can also reverse stress-induced effects on affective behaviour and neurogenesis (Li et al., 2004; Surget et al., 2008), and in some studies stress or stress hormones are required for these effects of antidepressants (Anacker et al., 2011; Dagyte et al., 2010). In fact, antidepressants may regulate neurogenesis through glucocorticoid receptors and cyclin-dependent kinase (CDK) inhibitors. The SSRI sertraline has been found to affect glucocorticoid

receptor phosphorylation via protein kinase A (PKA), and blocking PKA or glucocorticoid receptor activity blocks antidepressant-induced increases in cell proliferation (Anacker et al., 2011). Similarly, antidepressants act to differentially increase expression of the GR-dependent CDK inhibitors p27Kip1 and p57Kip2, which regulate exit from the cell cycle and increase neuronal differentiation (Lee et al., 2006; Shin et al., 2009; Ye et al., 2009), and decrease expression of p21Cip1, which prevents proliferation of neuronal progenitors (Anacker et al., 2011; Pechnick et al., 2011). The involvement of glucocorticoid receptor stimulation in proliferation is supported by the finding that antidepressants may only increase proliferation in the presence of glucocorticoids (Anacker et al., 2011; David et al., 2009). Together, these results show that antidepressants may regulate hippocampal neurogenesis through glucocorticoid receptor phosphorylation via PKA, leading to altered expression of CDK inhibitors to increase proliferation and neuronal differentiation. However, the CDK inhibitor regulation effects may be antidepressant-specific, given that antidepressants have differing effects on proliferation and later stages of neurogenesis, corresponding to differing effects on CDK inhibitor expression changes (Anacker et al., 2011; Pechnick et al., 2011). Notably, antidepressants may also regulate hippocampal plasticity by causing the dematuration of existing granule cells to a more plastic immature state. In particular, chronic fluoxetine acting through 5-HT4 receptors causes mature granule cells in the DG to lose calbindin expression and to display increased excitability, mirroring features of immature granule cells (Kobayashi et al., 2010).

Most importantly with respect to neurogenesis and antidepressants/antidepressant effects, neurogenesis seems to be required for antidepressant effects in some paradigms (Airan et al., 2007; Jiang et al., 2005; Perera et al., 2011; Pollak et al., 2008; Santarelli et al., 2003; Surget et al., 2008). Ablating neurogenesis appears to abolish the effects of antidepressants in the novelty-

suppressed feeding task, as well as their effects on coat state in response to stress (David et al., 2009; Santarelli et al., 2003; Wang et al., 2008). Sucrose consumption is also decreased after ablation of neurogenesis (Snyder et al., 2011b). The role of neurogenesis in mediating antidepressant-like behaviour in the FST is somewhat controversial. David and colleagues showed that fluoxetine reduces FST immobility in the absence of neurogenesis (David et al., 2009), and Holick and colleagues showed that reduced neurogenesis did not affect response to antidepressants in the FST (Holick et al., 2008). However, other studies have provided conflicting results, in that increases in neurogenesis (but not proliferation) are associated with antidepressant-like effects in the FST at a time when the increased numbers of new neurons are especially hyperplastic (Ge et al., 2007; Mahar et al., 2011), and that ablating neurogenesis prevented an antidepressant-induced decrease in FST immobility (Airan et al., 2007; Jiang et al., 2005; Snyder et al., 2011b). Overall, the evidence suggests that antidepressant-mediated recovery from depression and depression-like phenotypes involves not only changes to 5-HT function but also changes to hippocampal neurogenesis.

Hippocampal neurogenesis also appears to be required for HPA axis regulation (Schloesser et al., 2009; Snyder et al., 2011b), and is necessary for antidepressants to restore inhibition of the HPA axis by the hippocampus following chronic stress (Surget et al., 2011). To understand how this regulation of HPA axis function by neurogenesis may occur, and how neurogenic deficits could result in dysregulated responses to stress contributing to stress-induced 5-HT dysfunction and depression-related phenotypes, it is important to consider the functional anatomy of the hippocampus.

1.2.4.4 Stress and the subregional localization of neurogenic function along the septotemporal axis

CUS has repeatedly been shown to induce depressive-like behaviour (Elizalde et al., 2010; Jayatissa et al., 2009; Schmidt and Duman, 2010; Valente et al., 2012), and to negatively affect multiple stages of hippocampal neurogenesis, including proliferation (Elizalde et al., 2010; Jayatissa et al., 2009) and survival (Dagyte et al., 2011; Mineur et al., 2007) (although see Hanson et al. (2011) for a discussion of studies failing to show an effect of CUS). High doses of cortisol *in vitro* decrease proliferation and neuronal differentiation of human neural progenitor cells in a glucocorticoid-dependent manner, potentially via decreased TGFβ-SMAD2/3 and Hedgehog signalling and increased SGK1 activity, with increased SGK1 transcription also being observed in the blood of depressed patients and hippocampi of rats exposed to CUS, providing a putative molecular mechanism for CUS-induced decreases in hippocampal neurogenesis (Anacker et al., 2013a; Anacker et al., 2013b). In addition, other chronic stress paradigms have also been shown to decrease neurogenesis and induce depression-related behaviour (Brummelte and Galea, 2010; Perera et al., 2011; Veena et al., 2009; Wong and Herbert, 2004, 2006). In one study, escitalopram treatment following CUS reversed anhedonic behaviour only in animals who also displayed restored hippocampal cytogenesis; notably, this neurogenic reduction, recovery, and putative requirement for antidepressant-like effects was restricted to the ventral hippocampus (Jayatissa et al., 2006).

In fact, the relationship between neurogenesis and response to stress may be specific to particular hippocampal subregions. The hippocampus as a whole has been shown to be neither structurally nor functionally homogenous along the septotemporal axis (Bannerman et al., 2004; Fanselow and Dong, 2010; van Strien et al., 2009). The dorsal hippocampus in rodents

(analogous to the posterior hippocampus in humans) appears to be more involved in learning and memory functions, whereas the ventral hippocampus (analogous to the anterior hippocampus in humans (Fanselow and Dong, 2010)) seems to be involved in emotional modulation (Bannerman et al., 2004). Lesions of the dorsal hippocampus lead to spatial memory deficits (Broadbent et al., 2010), whereas adult ventral hippocampal lesions are anxiolytic and can affect stress response (Bannerman et al., 2004; Fanselow and Dong, 2010; Hobin et al., 2006; Kjelstrup et al., 2002; Pentkowski et al., 2006). These ventral hippocampus effects may be a result of the functional connectivity of the ventral hippocampus. The ventral hippocampus has increased serotonergic, norepinephrinergic, and mesolimbic dopaminergic innervation in comparison to the dorsal hippocampus (Bjarkam et al., 2003; Gage and Thompson, 1980; Gasbarri et al., 1994; Wilson and Molliver, 1991). In addition, this structure projects to brain areas involved in emotional regulation, including the mPFC, amygdala, bed nucleus of the stria terminalis (BNST), and paraventricular nucleus of the hypothalamus (PVN) (Bannerman et al., 2004; Fanselow and Dong, 2010; van Strien et al., 2009). Notably, ventral hippocampal lesions specific to areas projecting to the neuroendocrine hypothalamus alter response to stress (Nettles et al., 2000).

The functional distinction is also reflected at the level of the DG itself, in both the temporal dynamics of neuronal maturation along the septotemporal axis (Snyder et al., 2012) and functionality, with the dorsal DG's implication in learning and memory function, and the ventral DG's implication in anxiety and depressive/antidepressant behavioural phenotypes (Deng et al., 2010; Kheirbek and Hen, 2011). More specifically, these behaviours have been associated specifically with the birth of new neurons in this structure, and many studies examining behavioural consequences of altered neurogenesis have revealed functional discrepancies between dorsal and ventral hippocampal neurogenesis. Chronic stress has been shown to cause

ventral DG-specific decreases in neurogenesis (Elizalde et al., 2010; Jayatissa et al., 2006; Jayatissa et al., 2008), antidepressants have been shown to increase neurogenesis selectively in the ventral DG (Banasr et al., 2006; Paizanis et al., 2010), and increases in neurogenesis restricted to the ventral DG have been associated with antidepressant effects (Mahar et al., 2011). A noteworthy recent study by Tanti et al. specifically investigating the distinction between dorsal and ventral DG consequences of stress and antidepressant effects on different neurogenic stages found that CUS reduced the number of neural progenitors specifically in the ventral DG and decreased the number of early immature neurons in the dorsal and ventral DG, with both effects reversed by fluoxetine treatment (Tanti et al., 2013).

The influence of ventral hippocampal neurogenesis on affect and antidepressant activity could be related to its connection to the mPFC, in that hippocampal plasticity could modulate cognitive plasticity, including helplessness-related cognition (Feldmann et al., 2007), cognitive distortions, or ruminative cognition. However, the hypothalamic projection of the ventral hippocampus may be a more likely candidate, as recent research suggests that the mood-regulating actions of the ventral hippocampus (and ventral hippocampal neurogenesis) may be due to its regulation of stress. Ablation of hippocampal neurogenesis causes aberrant HPA axis activity, including increased glucocorticoid response to stress, increased HPA recovery latency following stress, and decreased HPA suppression by dexamethasone (Schloesser et al., 2014; Schloesser et al., 2009; Snyder et al., 2011b). Antiglucocorticoids, which mimic the effects of increased neurogenesis with respect to HPA inhibition, have antidepressant properties (Fitzsimons et al., 2009). Thus changes in ventral hippocampal neurogenesis likely affect the HPA axis via the BNST/PVN, modulating the effects of stress on depression- and antidepressant-

related behaviour. Modulation of the HPA axis by neurogenesis would thus also affect 5-HT activity, given the regulation of 5-HT systems by stress, as described earlier.

1.2.4.5 Timeline of neurogenic effects and synopsis of neurogenic role in affective modulation

Several of the aforementioned findings are best examined from a temporal perspective. Specifically: (i) antidepressants typically take several weeks to attain clinical efficacy (Jacobs et al., 2000); (ii) chronic stress can have depressogenic effects whereas acute stress often fails to (Hanson et al., 2011); (iii) neurogenesis is required for certain antidepressant-related effects (Airan et al., 2007; Jiang et al., 2005; Perera et al., 2011; Santarelli et al., 2003; Surget et al., 2008); and (iv) short-term administration of glucocorticoid receptor antagonists can produce acute antidepressant and antidepressant-like effects in humans and animal models, respectively (Belanoff et al., 2002; Flores et al., 2006; Veldhuis et al., 1985).

To understand how neurogenesis might mediate these effects, it is important to take into consideration the time course of adult hippocampal neurogenesis (**Fig. 4**). Proliferation of new cells proceeds rapidly in the hours following pro- or anti-proliferative stimuli. Following this stage, new cells differentiate into neuronal progenitors, extend dendrites into the molecular layer surrounding the DG and form spines, undergo apoptosis or survive to a functional state, and experience a brief period of hyperplasticity at approximately 4-6 weeks of age (Ge et al., 2007), after which these new neurons are functionally indistinguishable from older mature granule cells. Notably, the timing of this plastic window at 4-6 weeks of age may be specific to rodents, as the timing and duration of this window may differ in primates (Kohler et al., 2011; Perera et al., 2011); this possibility emphasizes the importance of increasing our understanding of the

maturation of adultborn neurons in addition to their proliferation and survival. It seems that the hyperplastic stage has particular relevance to the function of hippocampal neurogenesis, as prior to this stage these cells are not functional, and immediately following this stage the loss of neurons that proliferated in the presence of a particular stimulus would represent a relatively negligible change in the function of a mature granule cell population that is relatively quiescent (Aimone et al., 2010; Alme et al., 2010). Thus modulation of hippocampal neurogenesis should produce changes in affective behaviour if the overall number of immature hyperplastic ventral (in rodents; anterior in humans) DG neurons is affected at the precise time of behavioural testing or clinical assessment (c.f. Jayatissa et al., 2009; Mahar et al., 2011) (**Fig. 4**). This would explain why chronic but not acute antidepressant treatment (initially increasing proliferation of new cells that weeks later become immature neurons) has clinical efficacy, why chronic but not acute stress is depressogenic and anti-neurogenic, and why anti-glucocorticoid treatments (mimicking the end result of increased neurogenesis in inhibiting HPA function) have comparatively rapid antidepressant effects.

1.2.5 Concluding remarks

Multiple theories of depression and antidepressant action have been put forward in recent years. Here we have attempted to review recent research involving two of these theories, the monoaminergic and neurogenic hypotheses, and present a view linking these theories in the context of stress and HPA axis activity. To summarize, chronic stress causes depression and depression-related behaviour through monoaminergic changes in several brain regions as well as suppression of hippocampal neurogenesis, leading to altered activity in cognition- and emotion-related brain regions, as well as HPA axis dysfunction that itself exacerbates the effects of stress, including its effects on 5-HT activity. Some of these effects are reversed by antidepressant

treatment, which may act by increasing hippocampal neurogenesis (possibly by increasing monoaminergic neurotransmission), leading to restoration of HPA activity and stress responsivity, ameliorating deleterious stress-induced 5-HT changes. Future studies involving the interaction between monoamines and neurogenesis may further elucidate this relationship. In addition, linking particular changes in brain function to particular depressive symptoms will prove useful in understanding the etiology of depression. Finally, future clinical and post-mortem studies in human patients may further characterize this interaction in the context of depression and antidepressant effects.

Acknowledgements: IM receives funding from the Fonds de recherche du Québec - Santé, and would like to acknowledge the family of Dr. Steven S. Zalcman for their memorial award. FRB receives funding from the Canadian Institutes of Health Research (CIHR). NM is a CIHR New Investigator and Bell Senior Fellow in Mental Health. JNN receives funding from the Ontario Mental Health Foundation.

FIGURES:

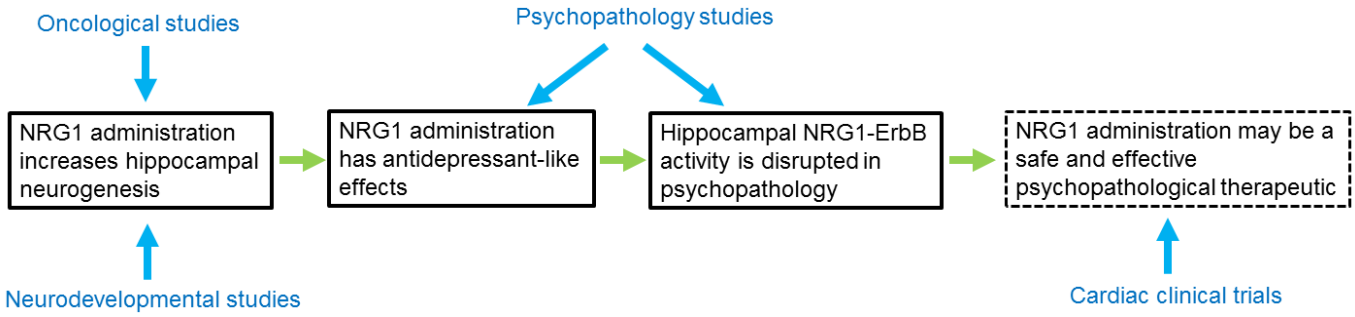


Figure 1. Brief synopsis of some of our main hypotheses (**black boxes**) and some of their experimental support from previous studies (**blue**) and our own findings (**green**). Oncological studies suggesting that neuregulin-1 (NRG1) has proliferative effects, as well as findings from neurodevelopmental studies, led us to hypothesize that NRG1 administration might increase hippocampal neurogenesis. Findings from human post-mortem psychopathology studies, as well as the influence of hippocampal neurogenesis on affective behaviour, suggested that increases in hippocampal neurogenesis might be accompanied by antidepressant-like effects. Our animal studies, as well as previous findings associating disrupted NRG1-ErbB activity with psychopathology, led us to investigate whether hippocampal NRG1-ErbB signaling was aberrant in specific psychopathology-related contexts. Together, our results suggested that NRG1 may have therapeutic benefit in particular psychopathological conditions, and may be safe and well-tolerated by patients based on clinical trials for NRG1 administration for cardiac conditions. The dotted line represents that this final hypothesis has yet to be tested directly.

Depressed mood most of the day, nearly every day
Compromised ability to experience pleasure (anhedonia) or interest in activities most of the day, nearly every day
Feelings of worthlessness or unreasonable guilt nearly every day
Sleep disturbance (insomnia or hypersomnia) nearly every day
Fluctuations in weight or appetite changes nearly every day
Psychomotor agitation or retardation nearly every day
Fatigue nearly every day
Diminished ability to think or concentrate nearly every day
Recurrent thoughts of death or suicidal ideation

Table 1. Symptoms of a depressive episode, at least five of which must persist for at least two weeks to meet diagnostic criteria, with depressed mood or anhedonia requisite (DSM-V; American Psychiatric Association, 2013).

Lighting conditions and disturbance in the circadian cycle
frequent, intermittent on-off switching of lights
abrupt reversal of the light/dark cycle
stroboscopic lights
Food and water availability
restricted food supply
restricted water supply
water deprivation
food deprivation
Housing conditions
isolation
group housing
cage tilt
used mouse cage exposure
damp bedding
Ecological challenges
foreign object exposure
cold room
noise
predator odor
restraint
forced swim

Table 2. Stressors used in the chronic unpredictable stress (CUS) model of depression.

	5-HT_{1A} Changes	Reference (species)
Animal models		
$SERT^{-/-}$ mice	↓ (desensitized) presynaptic	Fabre et al., 2000; Gobbi et al., 2001; Li et al., 2000; Lira et al., 2003
$CB1^{-/-}$ mice	↓ presynaptic	Aso et al., 2008; Aso et al., 2009
Chronic stress	↓ presynaptic	Bambico et al., 2009b (rat); Froger et al., 2004 (mouse); Lanfumey et al., 1999 (mouse)
Chronic corticosterone treatment	↓ presynaptic	Rainer et al., 2011 (mouse)
Maternal separation	↓ presynaptic	Gartside et al., 2003 (rat)
Neonatal clomipramine treatment	↓ presynaptic	Kinney et al., 1997 (rat); Maudhuit et al., 1995 (rat), 1996 (rat)
Acute stress exposure	↔ presynaptic	Laaris et al., 1997 (rat); Steciuk et al., 2000 (rat)
Chronic SSRI treatment	↓ presynaptic	Le Poul et al., 1997 (rat)
Chronic TCA treatment	↑ postsynaptic (hippocampus)	Bijak et al., 1996 (rat)
Depressed patients (5-HT_{1A} mRNA, 5-HT_{1A} receptor density and function)	↑ presynaptic	Stockmeier et al., 1998
	↓ presynaptic, also distribution volume	Arango et al., 2001; Meltzer et al., 2004; Rabiner et al., 2004
	↔ presynaptic	Bhagwagar et al., 2004
	↓ postsynaptic, medicated and unmedicated patients	Drevets et al., 2007; Hirvonen et al., 2008; Neumeister et al., 2004; Sargent et al., 2000
	↔ postsynaptic, midbrain	Stockmeier et al., 1998
	↓ neuroendocrine response to agonist	Pitchot et al., 2005
	↓ hypothermic response to agonist	Cowen et al., 1994

Table 3. Evidence of disrupted 5-HT_{1A} receptor function in depressed patients and animal models of depression, and related effects of antidepressants. Note that some findings are equivocal in the literature. $SERT^{-/-}$: serotonin transporter knockout; $CB1^{-/-}$: cannabiniod receptor knockout; SSRI: selective 5-HT reuptake inhibitor; TCA: tricyclic antidepressant.

	Agonism	Antagonism	References (species)
Monoamines			
Serotonin	↑	↓	Banasr et al., 2004 (rat); Diaz et al., 2012 (mouse)
Norepinephrine	↑	↓	Jhaveri et al., 2010 (mouse and rat); Kulkarni et al., 2002 (rat); Masuda et al., 2012 (rat)
Dopamine	↑	↕**?**	Hoglinger et al., 2004 (mouse); Veena et al., 2011 (rat and mouse); Yang et al., 2008 (mouse)
Neurotrophic factors			
BDNF	↑	↕	Sairanen et al., 2005 (mouse); Schmidt and Duman, 2010 (mouse); Waterhouse et al., 2012 (mouse)
IGF	↑	↓	Aberg et al., 2000 (rat); Beck et al., 1995 (mouse); Glasper et al., 2010 (mouse)
VEGF	↑	↓	Jin et al., 2002 (rat); Sun et al., 2006 (mouse)
NRG1	↑	**?**	Mahar et al., 2011 (mouse)

Table 4. Summary of findings regarding direction of influence of monoamines and neurotrophic factors on hippocampal neurogenesis. Serotonin and norepinephrine, as well as neurotrophic factors, appear to positively regulate hippocampal neurogenesis, although transgenic BDNF reduction may increase proliferation but decrease survival and neuronal differentiation, whereas findings on the influence of dopamine are mixed (particularly regarding the effects of pharmacological antagonism). BDNF: brain-derived neurotrophic factor; IGF: insulin-like growth factor; NRG1: neuregulin-1; VEGF: vascular endothelial growth factor.

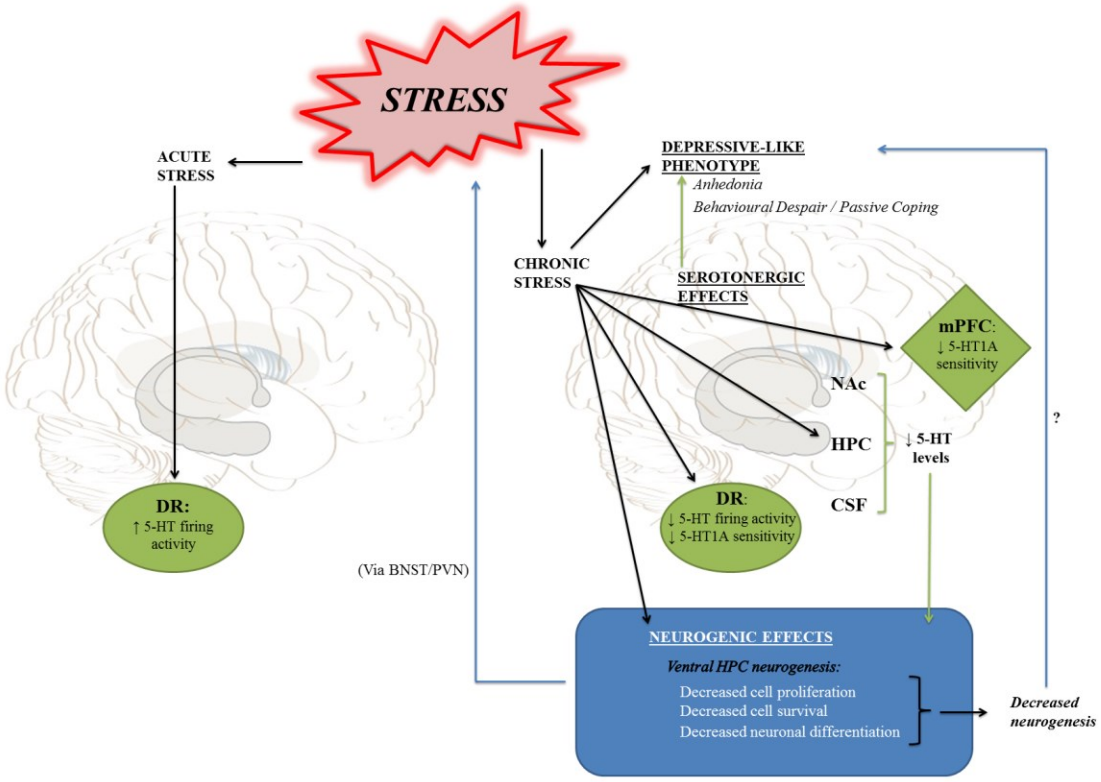


Figure 2. Model of serotonergic (5-HT; green arrow), neurogenic (blue arrow), and behavioural effects of stress. Acute stress (dotted box) increases dorsal raphe (DR) 5-HT firing. Chronic stress leads to monoaminergic changes (solid box), decreased neurogenesis (blue box), and depressive symptoms or endophenotypes. Decreases in ventral hippocampal (in rodents; anterior hippocampal in humans) neurogenesis, which may underlie depressive/depression-like phenotypes, impairs regulation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to HPA dysregulation and resulting monoaminergic and behavioural effects of chronic stress. Restoration of ventral (in rodents; anterior in humans) hippocampal neurogenesis (e.g. through serotonergic antidepressant treatment) reinstates hippocampal regulation of HPA activity and reverses depression-like behavioural phenotypes. BNST: bed nucleus of the stria terminalis; CSF: cerebrospinal fluid; HPC: hippocampus; mPFC: medial prefrontal cortex; NAc: nucleus accumbens; PVN: paraventricular nucleus of the hypothalamus.

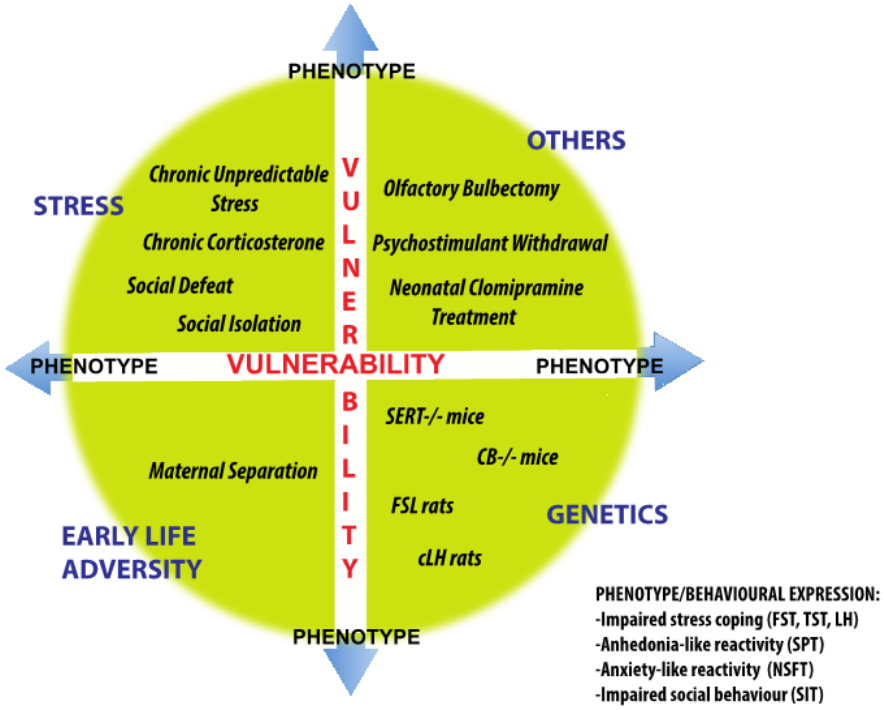


Figure 3. Animal models of depression vulnerability and depressive-like reactivity. Vulnerability to a depressive-like state can be precipitated by a number of experimental manipulations that mimic early life adversity, genetic liabilities, stress, and other predisposing factors (within the circle). Following development of depression vulnerability, enhanced expression of depressive-like phenotypes/behavioural reactivity can be assayed by the forced swim test (FST), tail suspension test (TST) and learned helplessness test (LH) that measure impairment in stress coping; by the sucrose preference test (SPT) for anhedonia-like reactivity; by the novelty-suppressed feeding test (NSFT) for anxiety-like reactivity; and by the social interaction test (SIT) for perturbed sociality. Other behavioural and physiological measures not shown here include exploratory behaviour in an open field, intracranial self-stimulation for reward sensitivity, changes in sleep architecture, and modifications in neuroendocrine response (corticosterone assays and dexamethasone challenge). CB1-/-: cannabinoid CB1 receptor knockout; cLH: congenital learned helplessness; FSL: Flinders sensitive line; SERT-/-: serotonin transporter knockout.

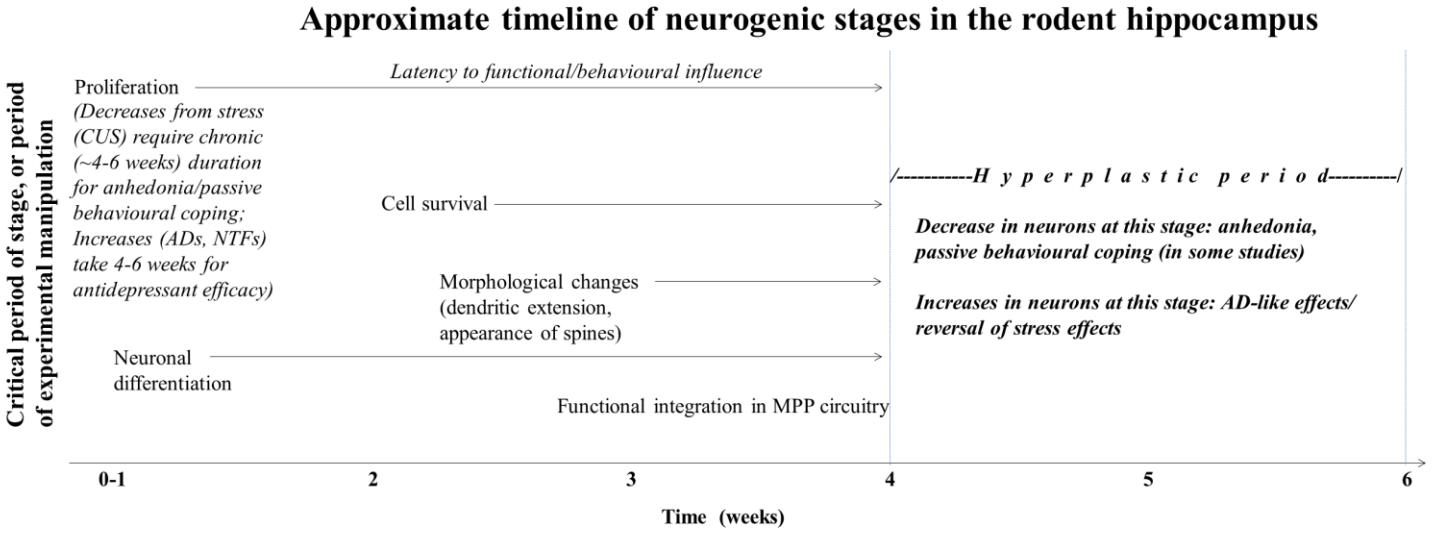


Figure 4. Temporal dynamics of neurogenic effects. Timing of stages as shown is roughly approximated and applies particularly to rodents. We suggest that the relevant feature of hippocampal neurogenesis with respect to depression-/antidepressant-related behaviour is the number of hyperplastic immature functional neurons (4-6 week-old neurons in mice) present in the ventral DG at behavioural assessment. Therefore, latency between proliferation effects and affective behaviour should be approximately 4-6 weeks (with potential variation by species); for changes in neuronal differentiation should be slightly sooner; and for changes in survival assessed in 2 week-old cells should be 2-4 weeks. If immature neurons can be ablated directly, or late-stage functional integration affected, effects should be apparent acutely. AD: antidepressant; CUS: chronic unpredictable stress; MPP: medial perforant path; NTF: neurotrophic factors.

1.2.6 ADDENDUM

Although the preceding section was published relatively recently (2014), the areas of research described therein are especially rapidly-advancing, and so it is worth updating with the most

recent studies published afterward, as well as adding topics relevant to subsequent aims and experiments.

Perhaps the largest omission of the preceding Chapter in the context of this thesis is regarding suicide, as a significant topic of our later experiments. A comprehensive review of the neuroscience of suicidality could easily fill more than the full volume of this (and many other) theses; however, I will attempt to summarize, as concisely as possible, our current knowledge of this topic and what our studies may contribute.

For obvious reasons, the vast majority of studies on the etiology of suicide have involved post-mortem tissue analyses. Consequently, the proliferation of brain banks worldwide in recent years has led to rapid growth in this aspect of suicide research, with much promise in the near future.

Suicidal etiology may be modeled upon two independent axes; a temporal axis, wherein both temporally proximal and distal events contribute to suicidality (Turecki, 2014), and a biological axis, in which etiology is represented on a continuum from the most microscopic (molecular, genetic, and epigenetic changes in the brain) to the anatomical (brain structural abnormalities in post-mortem suicidal individuals, brain imaging studies in living suicide attempters or relatives of suicide completers) and the most macroscopic (behavioural or interpersonal) levels.

Regarding genetic and epigenetic associations with suicide, high-throughput or genome-wide studies have often provided conflicting or unvalidated results; however, some moderate consensus has been reached with respect to the putative involvement of the hippocampus, stress, and HPA regulation (Turecki, 2014), strikingly analogous to the depressive model presented previously in this Chapter. These associations likely represent temporally distal factors to a suicidal event (Turecki, 2014), such as early life adversity-related epigenetic events affecting

predisposition toward suicide (Labonte et al., 2012; McGowan et al., 2009), as opposed to proximal factors leading acutely to a suicidal crisis such as substance abuse issues.

Anatomical and morphological post-mortem research, though proliferating, is still sparse in contrast with other research into psychiatric contexts. In the white matter of the anterior cingulate, fibrous astrocytes are hypertrophic, and a higher ratio of microglia are in a primed state, for depressed suicide completers (Torres-Platas et al., 2014; Torres-Platas et al., 2011), whereas anterior cingulate pyramidal neurons have fewer third-order dendritic branches (Hercher et al., 2010). Astrocytic hypertrophy has also been observed in the thalamus, caudate, and dorsolateral PFC (Steiner et al., 2008a; Torres-Platas et al., 2015). Along the subventricular zone-olfactory bulb neurogenic pathway, the number of DCX-expressing cells (and the volume of processes in these cells) was increased in the olfactory tract in suicides, and DCX expression was increased in the subventricular zone and olfactory bulb for suicides, with the former phenotype reversed by antidepressant treatment (Maheu et al., 2015b). However, despite its importance in affective and stress regulation, the hippocampus has not yet been studied anatomically in post-mortem suicide samples, apart from a report indicating a tendency for microglial activation in suicide completers (Steiner et al., 2008a).

Proteomic studies of suicide completers have been limited primarily to 5-HT1A, and these have produced mixed findings (Furczyk et al., 2013). However, of particular interest is a study examining DCX and polysialylated neural cell adhesion molecule (PSA-NCAM) as markers of neuroplasticity in the basolateral amygdala, which were found to be increased in depressed non-suicides but not depressed suicides, suggesting a potential failure of limbic neuroplasticity-related compensation in suicide completers (Maheu et al., 2013). Interestingly, chronic fluoxetine treatment has been shown to increase PSA-NCAM expression in the rat basolateral

amygdala, as well as in multiple hippocampal layers and the dorsal cingulate cortex (Guirado et al., 2012).

Regarding neurotrophic factors, depressed suicides show reduced levels of glial cell line-derived neurotrophic factor (GDNF) family receptor alpha 1a (GFRα1a) in the amygdala, likely due to increases in microRNA- (miRNA-)511 (Maheu et al., 2015a). Neurotrophin-3 levels are reduced in the hippocampi of unmedicated (but not medicated) suicide completers (Karege et al., 2005). Similarly, neurotrophin-4 levels in cerebrospinal fluid are reduced in depressed patients who had attempted suicide, but not in other depressed patients or controls (Kimata, 2005).

Research into BDNF in this context has been more extensive. The BDNF gene is hypermethylated in the Wernicke area of suicide completers (Keller et al., 2010). BDNF levels are reduced in the hippocampi and ventromedial PFCs of unmedicated (but not medicated) suicide completers (Karege et al., 2005). This hippocampal finding was partially validated by a study showing decreased hippocampal BDNF expression in male (but not female) depressed suicide completers, as well as decreased expression in the frontopolar PFC in female (but not male) suicide completers (Hayley et al., 2015). The Val66Met BDNF single nucleotide polymorphism in particular has repeatedly been associated with suicidality (Iga et al., 2007; Pregelj et al., 2011; Ratta-Apha et al., 2013; Sarchiapone et al., 2008; Zai et al., 2012), with additional BDNF single nucleotide polymorphisms associated with suicidality as a haplotype (Ropret et al., 2015). Levels of BDNF in blood, including BDNF overflow from the brain at the internal jugular vein, were decreased in suicidal individuals, relative to non-suicidal patients and controls (Dawood et al., 2007; Grah et al., 2014; Kim et al., 2007; Pinheiro et al., 2012). This may be due to changes in peripheral BDNF methylation, which is associated with suicidality in depressed patients (Kang et al., 2013). BDNF receptor TrkB-T1 is expressed in lower levels in

the frontal cortex of suicide completers, possibly due to altered methylation of the TrkB-T1 gene (Ernst et al., 2009) or increased expression of miRNA-185* (Maussion et al., 2012).

Together these neurotrophic factor studies have indicated that neurotrophic factors in particular, and brain plasticity by extension, are involved in suicidal etiology, predominately in the hippocampus. However, the neurotrophic factor NRG1 and its receptors, as well as the neuroanatomy of the hippocampal DG, had not been studied in the context of suicide.

Thus, research on suicide has not extended to several key areas, specifically the investigation of hippocampal plasticity in suicidality, the potential etiological and treatment role of NRG1/ErbB signaling in suicidality (in line with its association with psychopathology, and also as a neurotrophic factor), and potential alterations in the DG and neurogenesis. In Chapter 5, we attempt to augment existing suicide research with studies into these areas.

Of lesser relevance to this thesis, but of interest regarding the discussion of depressive or suicidal etiology in general, the preceding Chapter largely omitted discussing one of the more prominent psychopathological theories, specifically that of neurological inflammation. As with the topic of suicidality, this topic in itself extends far beyond the scope of the current document. However, suffice it to say that there is intriguing evidence of hyper-inflammation underlying depression and suicidality (Black and Miller, 2015; Zunszain et al., 2013). This model of depression is not incongruent with the findings discussed within this document, as glucocorticoid and inflammatory processes are linked (Horowitz et al., 2013), and as NRG1 ameliorates the inflammatory response of cells exposed to IL-1β (Wu et al., 2015).

Regarding additions to the general literature for monoaminergic theories of depression, it was quite recently found that methylation of the SERT gene is increased with early childhood trauma,

and is inversely associated with hippocampal volume (Booij et al., 2015). It was also recently found that 5-HT3 agonism is pro-neurogenic, and that 5-HT3 is required for exercise-induced neurogenesis and potentially the resulting behavioural effects (Kondo et al., 2014). Similarly, our discussion of neurotrophic factors involved in hippocampal neurogenesis also requires updating, as an additional neurotrophic factor, neurotrophic factor-α1 (NF-α1) has also been shown to be associated with stress, depression, and hippocampal neurogenesis (Cheng et al., 2014).

We omitted discussing sex-specific distinctions of neurogenesis in the preceding review, such as findings that spatial training spurs neurogenesis differently between male and female rats, neurogenic ablation disrupts behaviour more severely in female than male mice, oxidative stress differentially affects neurogenesis between sexes, and chronic restraint stress in rats also differentially affects neurogenic stages between sexes, whereas male rats had increased numbers of immature DG neurons at baseline (Chow et al., 2013; Hillerer et al., 2013; Ma et al., 2012; Roughton et al., 2012). Supporting this distinction, estradiol has recently been found to potentiate fluoxetine-mediated increases in neurogenesis and dendritic complexity in immature neurons, suggesting that serotonergic modulation of hippocampal neurogenesis might underlie sexually dimorphic differences in hippocampal neurogenesis (Vega-Rivera et al., 2015).

I have extensively discussed the functional discrepancies distinguishing the dorsal from the ventral DG. Additional support for the functional distinction of these DG subregions has come from studies showing that dorsal DG neurogenesis correlated with spatial memory task performance, whereas anxiety-related behaviour correlated with ventral DG neurogenesis (Vetreno and Crews, 2015).

We previously described in this Chapter how decreased neurogenesis in some contexts might not affect basal activity, but might exacerbate stress response. A recent advance is the development

of a mouse transgenic line in which neurogenesis is endogenously enhanced; these animals have normal baseline anxiety and antidepressant-like behaviour, but display anxiolytic and antidepressant-like behaviour in a stress-related model of depression (Hill et al., 2015). This supports our assertion that hippocampal neurogenesis can regulate affective behaviour through modulation of stress response, as described earlier in this Chapter.

We also mentioned earlier the multitude of factors that stimulate hippocampal neurogenesis, including through electrical stimulation. A recent publication has found that this is additionally possible through extremely low-frequency electromagnetic fields, which can increase rodent neurogenesis *in vivo* and increase neural stem cell proliferation and neuronal differentiation *in vitro*, with a potentially epigenetic mechanism via histone H3 lysine 9 (H3K9) acetylation and cAMP response element-binding protein (CREB) phosphorylation, contingent upon Ca_v1 channels (Leone et al., 2014). If this can similarly be accomplished in humans, the implications are noteworthy, as this non-invasive treatment could provide therapeutic benefit for a wide variety of psychiatrically related phenotypes.

Finally, the preceding Chapter briefly mentioned the possibility the ventral hippocampal neurogenesis exerts its mood regulating effects via the mPFC. While an intriguing and potentially wide-reaching hypothesis, it had at that point been unsupported. However, it has recently been found that ventromedial PFC stimulation increases hippocampal neurogenesis and dendritogenesis in new neurons, as well as memory task performance, with neurogenesis correlating with performance (Liu et al., 2015). Future studies will hopefully elucidate to what extent the influence of hippocampal neurogenesis on behaviour is mediated by the HPA axis as well as the mPFC.

1.3 SPECIFIC AIMS AND EXPERIMENTS

Having introduced neuregulin-1 and ErbB receptors, the phenomenon and function of hippocampal neurogenesis, and its distinction along the septotemporal axis (the concepts forming the impetus behind the thesis), we move to the specific aims of the thesis and its composite experiments.

AIM 1: Assess whether peripheral NRG1 administration affects adult hippocampal neurogenesis. To accomplish this aim, we subcutaneously administered NRG1 to adult mice and assessed subsequent proliferation and neurogenesis using immunohistochemical techniques, as described in Chapter 2.

AIM 2: Assess whether NRG1-mediated increases in hippocampal neurogenesis are associated with antidepressant-like effects. We assessed animals with NRG1-mediated increases in ventral hippocampal neurogenesis using behavioural tasks, as discussed in Chapter 2.

AIM 3: Characterize the effects of NRG1 on hippocampal neurogenesis, across neurogenic stages and the septotemporal axis. Here we repeated the administration paradigm of experiments in Chapter 2, focusing on immature neurons specifically, as described in Chapter 3, with the addition of neuronal reconstructions from across the septotemporal axis. Cumulative experiments from these chapters revealed that NRG1 has temporally- and subregionally-specific neurogenic effects.

AIM 4: Identify a putative mechanism for the neurogenic and behavioural effects of NRG1. Using BrdU to label cells at various time points, we identified expression patterns of ErbB receptors in DG cells and identified these cells immunohistochemically (Chapters 2 and 4),

revealing that ErbB3 may be the candidate NRG1 receptor mediating the neurogenic and antidepressant-like effects of NRG1 administration. We then used phosphorylation ELISA (Chapter 4) to verify that ventral DG ErbB3 is activated by NRG1 administration.

AIM 5: Determine whether psychopathology is associated with disrupted NRG1-ErbB signaling. As discussed in Chapter 4, we examined whether DG ErbB3 is disrupted in an animal model of depression, then peripherally in depressed patients, and finally in the hippocampus of suicide completers. This was done using genetic and epigenetic techniques, as well as stereological and morphological examination of the DG in suicide completers, and determined that DG ErbB3 is reduced in the DG of mice in a model of depression and in the hippocampus of suicide completers, and that the latter may be due to a substantial deficit in DG granule cell neurons.

CHAPTER 2. Subchronic peripheral neuregulin-1 increases ventral hippocampal neurogenesis and induces antidepressant-like effects.

(Published in PLoS ONE, 2011.)

Ian Mahar[1,3], Stephanie Tan[1], Maria Antonietta Davoli[1], Sergio Dominguez-Lopez[2], Calvin Qiang[1], Adeline Rachalski[1], Gustavo Turecki[1,2,3], Naguib Mechawar[1,2,3]*

[1]McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Verdun, Québec, Canada. [2]Depts. of Psychiatry and [3]Neurology & Neurosurgery, McGill University, Montréal, Québec, Canada.

***Corresponding author**

Key words: Hippocampal neurogenesis, Neuregulin, Antidepressant, Proliferation, ErbB, Ventral hippocampus

Abstract

Background

Adult hippocampal neurogenesis has been implicated in the mechanism of antidepressant action, and neurotrophic factors can mediate the neurogenic changes underlying these effects. The neurotrophic factor neuregulin-1 (NRG1) is involved in many aspects of brain development, from cell fate determination to neuronal maturation. However, nothing is known about the influence of NRG1 on neurodevelopmental processes occurring in the mature hippocampus.

Methods

Adult male mice were given subcutaneous NRG1 or saline to assess DG proliferation and neurogenesis, as well as cell fate determination. Mice also underwent behavioural testing. Expression of ErbB3 and ErbB4 NRG1 receptors in newborn DG cells was assessed at various time points between birth and maturity. The phenotype of ErbB-expressing progenitor cells was also characterized with cell type-specific markers.

Results

The current study shows that subchronic peripheral NRG1β administration selectively increased cell proliferation (by 71%) and neurogenesis (by 50%) in the caudal DG within the ventral hippocampus. This pro-proliferative effect did not alter neuronal fate, and may have been mediated by ErbB3 receptors, which were expressed by newborn DG cells from cell division to maturity and colocalized with SOX2 in the subgranular zone. Furthermore, four weeks after cessation of subchronic treatment, animals displayed robust antidepressant-like behaviour in the absence of changes in locomotor activity, whereas acute treatment did not produce antidepressant effects.

Conclusions

These results show that neuregulin-1β has pro-proliferative, neurogenic and antidepressant properties, further highlight the importance of peripheral neurotrophic factors in neurogenesis and mood, and support the role of hippocampal neurogenesis in mediating antidepressant effects.

2.1 Introduction

Adult hippocampal neurogenesis, the evolutionarily conserved process by which new granule cell neurons are added to the DG of the hippocampus, has been implicated in several brain functions, including affective modulation. According to the neurogenic theory of antidepressant effects, increasing hippocampal neurogenesis can ameliorate depressive symptoms ((Boldrini et al., 2009; Jacobs et al., 2000; Jayatissa et al., 2006; Kempermann, 2002; Kempermann and Kronenberg, 2003; Malberg et al., 2000; Santarelli et al., 2003; Surget et al., 2008); see (Drew and Hen, 2007; Sahay and Hen, 2007; Vaidya et al., 2007) for review), although it is controversial whether deficits in neurogenesis are sufficient to cause a depressive phenotype. In support of the involvement of neurogenesis in antidepressant effects, antidepressant treatments increase hippocampal neurogenesis (Banasr et al., 2006; Boldrini et al., 2009), ablating hippocampal neurogenesis can prevent antidepressant effects (Airan et al., 2007; Jiang et al., 2005; Santarelli et al., 2003; Surget et al., 2008 (although see David et al., 2009; Holick et al., 2008), and the latency of these effects in humans and animals correlates with the time required for newborn DG neurons to integrate into mature circuits (Jacobs et al., 2000).

Neurotrophic factors have been proposed to mediate the neurogenic response to antidepressants, with strong evidence that peripherally circulating neurotrophic factors can modulate both mood and neurogenesis (Aberg et al., 2000; Duman et al., 2009; Scharfman et al.,

2005; Schmidt and Duman, 2010; Sen et al., 2008; Wang et al., 2009). In particular, chronic peripheral administration of brain-derived neurotrophic factor (BDNF) has been reported to increase neurogenesis (Schmidt and Duman, 2010) and to induce antidepressant and anxiolytic effects (Schmidt and Duman, 2010). Interestingly, serum levels of BDNF are also reduced in depressed patients and increased by antidepressant treatment (Diniz et al., 2010; Sen et al., 2008), raising the possibility that peripheral activity of this neurotrophic factor could affect mood by modulating central neurophysiological processes such as adult hippocampal neurogenesis. Further support for a peripheral influence of neurotrophins on mood arises from the demonstration that peripheral administration of the pro-neurogenic hormone insulin-like growth factor-I, which can cross the BBB, produces antidepressant-like behaviour (Duman et al., 2009).

The neuregulin (NRG) family of EGF-related proteins comprises a wide variety of soluble and membrane-bound proteins that mediate their effects through ErbB tyrosine kinase receptors (Olayioye et al., 2000; Talmage and Role, 2004). The diversity of NRG proteins, arising mainly from alternative splicing, is particularly well-documented for the neurotrophic factor NRG1, which includes at least fifteen isoforms. In addition to being expressed in the brain, some NRG1 isoforms are also widely expressed in the periphery (Shibuya et al., 2010; Wen et al., 1994; Zhang et al., 2008). In particular, NRG1β, the most widespread NRG1 isoform in the brain, is found in the circulation, from which it can readily cross the adult BBB via carrier-mediated transport and affect brain activity (Carlsson et al., 2011; Kastin et al., 2004; Xu et al., 2006; Xu et al., 2004). Studies of NRG1 signaling in the brain indicate prominent roles for this protein during development, such as cell fate determination (Sardi et al., 2006), axon guidance (Lopez-Bendito et al., 2006), radial glia elongation (Anton et al., 1997; Gierdalski et al., 2005;

Schmid et al., 2003), neuronal migration (Flames et al., 2004; Yau et al., 2003) and dendritic growth (Rieff and Corfas, 2006).

Despite our rapidly expanding knowledge of NRG1 functions in the developing brain, very little is currently known about the influence of this neurotrophic factor on neurodevelopmental processes occurring at maturity. NRG1 administration in the adult subventricular zone (SVZ) has been shown to affect progenitor organization and migration (Ghashghaei et al., 2006). However, a possible role for NRG1 signaling in modulating hippocampal neurogenesis remains to be examined (Jaaro-Peled et al., 2009). Here, we exploited the fact that NRG1β readily crosses the BBB to study the consequences of subchronic peripheral administration of this neurotrophic factor on hippocampal neurogenesis. Based on previous studies showing that NRG1 is pro-proliferative in vitro (Eto et al., 2010; Liu et al., 2005) and that neurotrophic factors increase proliferation and neurogenesis in the adult hippocampus (Aberg et al., 2000; Scharfman et al., 2005; Schmidt and Duman, 2010), we hypothesized that NRG1β treatment would stimulate DG proliferation, leading to an increase in hippocampal neurogenesis. We further hypothesized that this increase in neurogenesis would lead to antidepressant effects, in line with studies demonstrating an association between these phenomena (Airan et al., 2007; Jiang et al., 2005; Santarelli et al., 2003; Surget et al., 2008), as well as those showing antidepressant effects after neurotrophic factor administration (Duman et al., 2009; Schmidt and Duman, 2010). We show that this treatment strongly stimulates DG cell proliferation exclusively in the ventral hippocampus, leading to an increase in neurogenesis that is accompanied by robust antidepressant-like behaviour.

2.2 Methods and materials

2.2.1 Animals

Adult male C57BL/6 mice were purchased from Charles River Canada. For ErbB3/SOX2/nestin triple labeling, group-housed transgenic mice expressing green fluorescent protein (GFP) at the nestin promoter (nestin-GFP (Mignone et al., 2004)) on a C57BL/6 background were used. All animals were 2 months of age and housed in groups (except animals for neurogenesis and behavioural experiments, which were isolated) on a 12:12 light:dark cycle with *ad libitum* access to food and water. All experiments followed the policies and guidelines of the Canadian Council on Animal Care and were approved by McGill University's Animal Care Committee (approval ID: 5473).

2.2.2 NRG1β and BrdU administration

After anesthesia with isoflurane, mice were implanted with subcutaneous osmotic mini-pumps (Alzet) containing either recombinant NRG1β type-I (EGF domain dissolved in sterile 0.9% saline, administered at a constant rate of 10 μg/d; R&D Systems; accession # NP_039250) or vehicle (randomized groups assignment). Animals were given a subcutaneous saline injection and placed on a heating pad to recover, and were monitored post-operatively for complications. An anti-inflammatory Carprofen tablet was placed in each cage. Depleted mini-pumps were removed from anesthetized animals after the administration period, and pump depletion was verified.

To evaluate proliferation, mice (n=6/group) were sacrificed at the end of a 24 h NRG1β or vehicle administration period, having received two injections of bromodeoxyuridine (BrdU; Sigma; 50 mg/kg, i.p.) dissolved in sterile 0.9% saline with 0.4 M NaOH: one immediately after mini-pump implantation, and the other two hours prior to sacrifice. To evaluate neurogenesis

(encompassing proliferation, differentiation and survival), mice (n=7/group) received BrdU twice daily during the 72 h NRG1β or vehicle administration period and sacrificed 30 d after mini-pump implantation. For immunohistochemistry receptor colocalization experiments, naive animals received BrdU 2 h, 24 h, 7 d, or 28 d prior to sacrifice (n=2/survival period), with the latter two groups receiving two BrdU injections on the day of administration. To determine the acute behavioral effects of NRG1β, mice were given three 0.1 ml i.p. injections of NRG1β (3.33 μg; n=7) or vehicle (n=8), 24 h, 12 h and 1 h prior to testing, and then sacrificed (see **Fig. 5** for experimental timelines).

2.2.3 Tissue processing

Animals were deeply anesthetized with a cocktail of ketamine, xylazine and acepromazine (0.1 ml/100 g), and perfused through the heart with ice-cold phosphate-buffered saline (PBS) followed by 4% formaldehyde in 0.1 M phosphate buffer. Brains were then rapidly removed, postfixed at 4°C for 24 h in fixative, then transferred to a 30% sucrose solution until equilibrium was reached. Brains were cut using a cryostat into serial 40 μm-thick coronal sections, which were placed in a cryoprotectant solution (glycerol:ethylene glycol:PBS, 3:3:4) and stored at -20˚C.

2.2.4 BrdU immunohistochemistry

Unless otherwise specified, all immunohistochemistry incubations were at room temperature. The section-sampling fraction was 1/8. Omitting primary antibodies resulted in an absence of specific staining for all immunohistochemistry protocols. Rinses with PBS preceded all steps except the addition of primary antibodies and addition of H_2O_2. For BrdU light

microscopy immunohistochemistry, sections were pre-treated for 1.5 h in PBS containing 0.2% Triton X-100 (PBS-T; Fisher), 10 min in PBS containing 0.9% H_2O_2, and 30 min at 37˚C in PBS with 2N HCl to denature DNA. Sections were then incubated in PBS-T containing 2% Normal goat serum (NGS) for 30 min, and then overnight at 4˚C in the same solution with monoclonal rat anti-BrdU (1:1000; Serotec). Sections were then incubated for 1 h in biotinylated goat anti-rat antibody (1:200; Vector Laboratories), followed for 30 min by the avidin-biotin complex procedure (ABC Kit, Vectastain Elite, Vector). Labeling was revealed with a diaminobenzidine kit (Vector). Sections were mounted on glass slides, dehydrated, and coverslipped with Permount (Fisher Scientific).

2.2.5 BrdU/NeuN double-labeling

To determine the proportions of newborn cells that became neurons, coronal brain sections from the neurogenesis experiment were incubated overnight at 4˚C with rat anti-BrdU (as above) and mouse anti-NeuN (1:200; Chemicon) antibodies in 2% NGS in PBS-T, after consecutive incubations in PBS-T (2 h), 2N HCl in PBS at 37˚C (30 min) and PBS-T containing 2% NGS (1 h). This was followed by a 1.5 h incubation in fluorescent DyLight 594-labeled goat anti-rat (1:1500; Jackson) and DyLight 488-labeled goat anti-mouse (1:500; Jackson) antibodies in 2% NGS in PBS-T. Sections were mounted on glass slides and coverslipped with ProLong Gold antifade reagent with DAPI (Invitrogen).

2.2.6 BrdU/ErbB double-labeling

Sections from animals injected with BrdU 2 h, 24 h, 7 d, or 28 d prior to sacrifice were incubated for 2 h in PBS-T. Sections were then incubated for 30 min in PBS at 37˚C in 1N HCl

to denature DNA, and incubated in 2% NGS in PBS-T for 2 h before an overnight incubation at 4˚C in rat anti-BrdU (as above) and monoclonal mouse anti-ErbB4 (Thermo Scientific; 1:100) or monoclonal rabbit anti-ErbB3 (accession # Q61526, Santa Cruz; 1:100) in the same solution. Sections were then incubated for 1.5 h in DyLight 594 goat anti-rat (1:1500) and DyLight 488 goat anti-rabbit (1:500) or goat anti-mouse (1:100 or 1:200) antibodies (Jackson) in PBS-T containing 2% NGS. Sections were mounted on slides and coverslipped and coded as above.

The anti-ErbB4 antibody is directed against an extracellular domain of the ErbB4 receptor (Neddens and Buonanno, 2009), and the anti-ErbB3 antibody is directed against the C-terminal domain (Gerecke et al., 2001). The specificity of the latter antibody has been validated previously in ErbB3-knockout tissues (Jackson-Fisher et al., 2008; Lee et al., 2009). In addition, we confirmed the staining pattern using a different anti-ErbB3 antibody raised in mice (Millipore, 1:25, 1:50 and 1:100; accession # P21860; not shown) under light microscopy conditions with DAB. Furthermore, immunofluorescence double-labeling with both anti-ErbB3 antibodies revealed colocalization throughout the DG (wide-field and confocal microscopy).

2.2.7 ErbB3/SOX2/nestin triple-labeling

Nestin-GFP mouse brain sections were incubated for 2 h in PBS-T and 1 h in 2% NGS in PBS-T, then overnight at 4˚C in rabbit anti-ErbB3 (as above) and mouse anti-SOX2 (R&D; 1:200; accession # P48431). The staining pattern of the latter (which has been validated previously in SOX2-knockout tissue (Cavallaro et al., 2008)) was confirmed with an additional anti-SOX2 antibody (Millipore; 1:12 000 and 1:20 000; accession # P48431; not shown) under light microscopy conditions with DAB. Secondary antibodies (DyLight 594 goat anti-rabbit and Dylight 649 goat anti-mouse, 1:500, Jackson) were added for 1.5 h. Sections were mounted on

slides and coverslipped as above. As a negative control, a nestin-GFP-negative sibling did not show GFP staining using wide-field and confocal microscopy.

2.2.8 Cell quantification

All quantifications and analyses were done by an investigator blind to experimental groups. BrdU-IR cells in the DG of the hippocampus were counted using a Leica CME with a 40X 0.65NA E2 achromat objective (proliferation) or a Leica DM 2500 with a 40X 0.75 NA HCX PL Fluotar objective (neurogenesis). Cell counts were expressed as average numbers per DG, with left and right DGs per section averaged, and for subregion analyses DG counts were averaged between DGs in each subregion. Proliferation of cells in the SVZ was determined at 40X (0.75 NA UPlan FL N objective) on an Olympus BX51 microscope equipped with a motorized stage and CX-9000 camera (MBF), using an optical fractionator (OF) probe (Stereo Investigator, MBF). A pilot study conducted to establish sampling parameters revealed a Gunderson coefficient of error (CE) ($m = 1$) < 0.05. The size of the counting frame for cell estimation was 500 μm^2, dissector height was set to 14 μm, and guard zone distance to 1 μm. The average sampling area was 6072.5 μm^2. As the OF procedure provided cell count estimates and not absolute cell numbers, density of BrdU-IR cells was determined as the estimated population using section thickness divided by the Cavalieri volume corrected for overprojection. Gunderson CEs ($m = 1$) for all animals were < 0.05.

2.2.9 Surface area and volumetric analyses

To rule out group differences in surface area or volume (as calculated by planimetry and Cavalieri estimation), the SVZs and DGs of each brain used in cell quantification were traced on

the Olympus BX51 microscope mentioned above with a 10X UPlan FL N 0.3 NA objective using the Stereo Investigator software package (MBF). SVZs and DGs as well as rostral (-1.46 to -2.54 mm from bregma (Franklin and Paxinos, 2007)) and caudal (-2.55 to -3.80 mm from bregma) DG subregions presented no significant difference between groups for any of the measures of this analysis, including total and average surface area and total and average volume, both corrected and uncorrected for overprojection ($ps > 0.05$).

2.2.10 Confocal microscopy

Multiple-labeling analyses were conducted at 40X (1.3 NA Plan-Neofluar 40x Oil DIC objective) on a Zeiss LSM510 Meta confocal microscope equipped with an Axiovert 200M stand and motorized stage (Carl Zeiss Canada), using 405 nm, 488 nm, 543nm, and 633 nm wavelength lasers. Images were obtained using the Zeiss Aim software package (Carl Zeiss Canada), at a pixel size of 0.11 μm for x and y, a scan average of ≥ 4 frames, a pixel dwell time of ≥ 3.20 μs, and optical slice of < 3 μm sampled at an interval of < 1.5 μm. Images were unaltered except for overall brightness and contrast. For quantitative colocalization analyses, 20 random cells from the rostral and caudal DG (10/subregion from multiple DGs) of each animal were analyzed, and data expressed as the percentage of BrdU-IR cells that were also NeuN-IR or ErbB-IR.

2.2.11 Behaviour

Animals in the neurogenesis experiment were assessed in a locomotor task and in the FST 28 d and 29 d, respectively, after the onset of the 72 h NRG1β or saline administration period, or 40 min and 1 h after the final injection in the acute behaviour experiment. The FST

was chosen for its implication as a hippocampal neurogenesis-dependent task (Airan et al., 2007; Jiang et al., 2005 (although see David et al., 2009; Holick et al., 2008)), and the locomotor task served to determine whether putative antidepressant effects were due to changes in general locomotor activity. To study locomotor behaviour, mice were placed individually into acrylic Versamax RS2USB v4.00 Animal Activity Monitor boxes (.2m L x .2m W x .3m H; Accuscan) in a room lit by red light for a period of 90 min (20 min for the acute behaviour experiment). Locomotor behaviour was analyzed automatically by the Versamax VMX 1.4B software system (AccuScan). Three periods of activity were analyzed: no habituation (first 10 min activity), 10 min of habituation (second 10 min activity) and extended habituation for the neurogenesis experiment (30 min habituation, 60 min activity). For the FST, mice were placed in 4 L glass beakers (25 cm depth, 15 cm diameter) 1/3rd filled with water (25˚C) for a period of 10 minutes in a dimly lit room. Behaviour was recorded by video equipment and subsequently analyzed using Videotrack behavioural tracking software (Viewpoint). The first two minutes were treated as habituation, and the following four minutes analyzed for duration of swimming and immobility behaviour.

2.2.12 Statistics

Normality of data was assessed using Shapiro-Wilk tests. Parametric pair-wise comparisons were made using Student's t-tests. Non-parametric pair-wise comparisons were made with Mann-Whitney U-tests. Comparisons within testing period time points across the duration of the FST were made with Bonferroni-corrected t- and U-tests. Values of $p < 0.05$ were considered to be statistically significant.

2.3 Results

2.3.1 Proliferation

Mice were administered NRG1β or saline for 24 h (to evaluate proliferation immediately after) or 72 h (to evaluate neurogenesis and behaviour four weeks later), and received injections of BrdU during administration (see **Fig. 5**). After 24 h of NRG1 administration, cell proliferation in the DG, as assessed by numbers of BrdU-IR cells, increased by 38% compared to controls ($p = 0.013$). Subregional analyses revealed that this increase was highly significant in the caudal DG (71%; $p = 0.0011$), but non-significant in the rostral DG ($p = 0.29$) (**Fig. 6A**). Proliferation in the SVZ did not differ between groups ($p = 0.34$; **Fig. 7**).

2.3.2 Neurogenesis

Overall, BrdU-IR cell numbers in the DG increased four weeks after NRG1β administration (31%; $p = 0.042$). As with proliferation however, this increase was only significant in the caudal DG (50%, $p = 0.013$; rostral: $p = 0.20$) (**Fig. 6B**). The proportion of cells that differentiated into neurons in these animals did not vary with treatment (overall: $p = 0.91$; rostral DG: $p = 1.00$ or caudal DG: $p = 0.90$; **Fig. 8**), indicating that the NRG1β-induced increase in proliferation within the caudal DG led to increased neurogenesis in this region.

2.3.3 BrdU-ErbB colocalization

To examine if these effects may have resulted from direct stimulation of NRG1 receptors expressed by progenitor cells, we determined at different post-BrdU injection time points whether BrdU-IR in the DG colocalized with ErbB3- or ErbB4-IR (see **Fig. 5**), as presence of one of these receptors is required for NRG1-ErbB signaling. IR for both receptors were observed

in the DG in expression patterns similar to previous studies using these and other antibodies (Chaudhury et al., 2003; Gerecke et al., 2001; Neddens and Buonanno, 2009). In naive animals 70 ±8% (rostral: 55 ±16%; caudal: 85 ±3%; mean % ±SEM) of newborn cells were found to express ErbB3 during the proliferative period (2 h and 24 h following BrdU injection; **Fig.9, A1** and **A2**), whereas BrdU-IR and ErbB4-IR were not found to be colocalized in the DG during this period. Interestingly, BrdU/ErbB3 colocalization was also observed 7 d and 28 d post-injection (**Appendix Figures A1** and **A2**), suggesting a life-long influence for NRGs on the activity of adult-born granule cells.

2.3.4 ErbB3/nestin/SOX2 colocalization

To examine which neurogenic cell subtypes in the DG expressed ErbB3, we analyzed the colocalization of ErbB3, nestin and SOX2 in nestin-GFP animals. Colocalization was predominately limited to the subgranular zone. Although both SOX2 and nestin colocalized with ErbB3 in the rostral and caudal DG, nestin and ErbB3 were only co-expressed in cells expressing SOX2 (i.e. triple-labeled cells; **Fig.10A and A3A**) and not in the absence of SOX2, whereas ErbB3 and SOX2 also colocalized in the absence of nestin (**Fig.10B and A3B**), suggesting that the neuronal precursor cells involved in the NRG1-induced proliferative increase express SOX2.

2.3.5 Behaviour

The effects of subchronic (72 h) NRG1β treatment on antidepressant-like behaviour were assessed in the same animals in which neurogenesis was examined. For all analyses of the locomotor task, experimental groups did not differ in either total distance traveled (1^{st} 10 min: $p = 0.30$; 2^{nd} 10 min: $p = 0.28$; 60 min after 30 min habituation: $p = 0.26$) or activity duration (1^{st}

10 min: p = 0.25; 2nd 10 min: p = 0.23; 60 min after 30 min habituation: p = 0.29) (**Fig. 11**). Treatment groups did not differ in locomotor activity after acute treatment (24 h), either initially (distance: p = 0.67; duration: p = 0.96) or after 10 min of habituation (distance: p = 0.60; duration: p = 0.67) (**Fig. 12**).

NRG1β-treated animals showed decreased immobility (44%; p = 0.0062) and increased swimming (194%; p = 0.0062) in the FST four weeks after the administration period, suggesting that NRG1β treatment had antidepressant effects. Bonferroni-corrected temporal analyses revealed that these animals had significantly increased swimming and decreased immobility throughout the testing period ($0.019 \leq ps \leq 0.039$) except the fifth minute (ps = 0.11) (**Fig. 13**). Acute treatment with NRG1β did not produce antidepressant effects in the FST compared to vehicle-treated animals (immobility: p = 0.15; swimming, p = 0.15). During the testing period treatment groups did not differ at any point ($0.37 \leq ps \leq 1.0$) (**Fig. 14**).

2.4 Discussion

This study shows that peripheral NRG1β administration increases cell proliferation in the caudal (but not rostral) DG within 24 h, and that this treatment leads to a similar increase in local neurogenesis. This pro-proliferative effect did not influence cell fate, and was likely mediated by ErbB3 receptors in SOX2-expressing neuronal precursor cells. Given the magnitude of this effect within such a relatively short period, it is likely that the progenitor cells involved in this increase are the rapidly proliferating type-II cells, as opposed to the more quiescent type-I stem cell-like progenitors that have been found to give rise (predominately through symmetric division) to both stem cell-like progenitors and neuronal fate-determined progenitors (Kempermann et al., 2004; Namba et al., 2011). However, the widespread expression of ErbB3 in the DG leaves open the

possibility of mature granule cells contributing to this phenomenon. In addition, the large increase in DG cell numbers was accompanied by antidepressant-like behaviours four weeks after cessation of subchronic treatment, but not acutely. NRG1β administration did not increase proliferation in the SVZ, in agreement with previous findings (Ghashghaei et al., 2006), suggesting that the pro-proliferative influence of NRG1β in neurogenic regions of the adult brain *in vivo* is restricted to the caudal DG. This region is localized within the ventral hippocampus (Banasr et al., 2006; Maren and Holt, 2004; van Strien et al., 2009), which is structurally and functionally distinct from the dorsal hippocampus (Bannerman et al., 2004; Fanselow and Dong, 2010; van Strien et al., 2009). Adult ventral hippocampal lesions are anxiolytic and disrupt fear conditioning and memory (Bannerman et al., 2004; Fanselow and Dong, 2010; Hobin et al., 2006; Kjelstrup et al., 2002; Pentkowski et al., 2006), whereas neonatal ventral hippocampus lesions are a well-characterized model of schizophrenia (Lipska and Weinberger, 2002; Wilson and Alvin V. Terry, 2010). In addition to its role in anxiety, the ventral hippocampus and ventral hippocampal neurogenesis have been implicated in depression, antidepressant-related behaviour and response to stress (Airan et al., 2007; Banasr et al., 2006; Elizalde et al., 2010; Jayatissa et al., 2006; Maggio and Segal, 2007a, b; Nettles et al., 2000; Oomen et al., 2010; Paizanis et al., 2010; Sahay and Hen, 2007). The involvement of the ventral hippocampus in emotional modulation may be linked to its connectivity, which includes projections to the PFC, amygdala, nucleus accumbens, olfactory bulb, and HPA axis-related structures (Bannerman et al., 2004; Fanselow and Dong, 2010; Ishikawa and Nakamura, 2006; Miller et al., 2010; van Groen and Wyss, 1990), and to its increased serotonergic innervation in comparison with the dorsal hippocampus (Bjarkam et al., 2003; Gage and Thompson, 1980; Sahay and Hen, 2007; Wilson

and Molliver, 1991). Interestingly, NRG1 hypomorphic mice display disrupted ventral hippocampal–nucleus accumbens transmission (Nason et al., 2011).

Treatments that induce neurogenic, antidepressant and anxiolytic effects typically do so after chronic (but not subchronic) duration (Banasr et al., 2006; Detke et al., 1997; Dulawa et al., 2004; Jiang et al., 2005; Malberg et al., 2000; Santarelli et al., 2003). The present study demonstrates that NRG1 has neurogenic and antidepressant effects with subchronic (72 h) treatment. These effects were measured 28 d after cessation of treatment (and long after exogenous NRG1β would have degraded), a delay that mirrors the maturation period of neurons born during treatment (Ge et al., 2007). Given that these newborn neurons are then in a state of increased plasticity (Ge et al., 2007), our results suggest that boosting the number of highly plastic neurons in the ventral hippocampus can mediate antidepressant effects. Notably, 24 h of treatment was sufficient to increase ventral hippocampal cell proliferation but not to produce antidepressant effects, supporting the hypothesis that the latter are due to the hyperplastic state of immature neurons and not simply an increased number of DG cells. A recent study has suggested that behaviour in the FST may be neurogenesis-independent (David et al., 2009), in opposition to previous studies (Airan et al., 2007; Jiang et al., 2005). However, our temporal paradigm shows that the antidepressant effects seen here are concomitant with an increase in adult-born granule cells. Although the current data indicate that the antidepressant effects of NRG1 in particular may be neurogenesis-dependent, they also offer support to the hypothesis that established antidepressants such as SSRIs have a delayed onset of clinical effectiveness (and require chronic treatment) due to the delay required for treatment-induced newborn neurons to mature and integrate into DG circuitry.

NRG1 has attracted widespread interest for its involvement in the etiology of psychiatric conditions. Single nucleotide polymorphisms of the NRG1 gene have been associated with schizophrenia and bipolar disorder (Georgieva et al., 2008; Goes et al., 2009; Lu et al., 2010; Naz et al., 2011; Prata et al., 2009; Seshadri et al., 2010; Stefansson et al., 2003; Stefansson et al., 2002; Walker et al., 2010). Mice hypomorphic for NRG1 or ErbB4 show behavioural abnormalities consistent with existing animal models for schizophrenia, including abnormal prepulse inhibition and enhanced response to cannabinoid and dopaminergic agonists (Boucher et al., 2007a; Boucher et al., 2007b; Kato et al., 2011). Studies of clinical populations have revealed decreased peripheral expression of NRG1β in schizophrenic patients that increased with antipsychotic treatment (Shibuya et al., 2010; Zhang et al., 2008), which is particularly interesting as NRG1β can cross the adult BBB and affect brain activity and behaviour (Carlsson et al., 2011; Kastin et al., 2004; Xu et al., 2006; Xu et al., 2004). Schizophrenia has been associated with both abnormal NRG1 type-I signaling in the PFC (Bertram et al., 2007; Mei and Xiong, 2008; Parlapani et al., 2010) and decreased hippocampal neurogenesis (Cui et al., 2009; Reif et al., 2006). In particular, some studies suggest that NRG1 levels are decreased in the brains of depressed and schizophrenic patients (Bertram et al., 2007; Parlapani et al., 2010). Decreased NRG1 activity may negatively affect hippocampal neurogenesis in schizophrenic patients, contributing to the etiology of this disorder. NRG1 administration could both reverse decreases in hippocampal neurogenesis and ameliorate psychiatric symptoms. The potential utility of NRG1β as a therapeutic that could be delivered peripherally is supported by a recent Phase II clinical study examining NRG1β as a treatment for chronic heart conditions, which

revealed that peripheral NRG1β administration does not lead to serious adverse effects in humans (Gao et al., 2010).

In conclusion, this study is the first to suggest that NRG1β, a peripherally and centrally expressed neurotrophic factor that crosses the BBB, increases ventral hippocampal neurogenesis and modulates mood as a putative antidepressant. It is also the first to show that newborn DG cells express ErbB3 from birth to maturity. That the behaviourbehavioural effects were not observed acutely but were present four weeks after subchronic treatment cessation in a novel administration paradigm is consistent with the neurogenic theory of antidepressant effects, as this time frame corresponds to the maturation and functional integration of newborn neurons within ventral hippocampal circuitry following treatment-stimulated proliferation. These results also highlight the potential for modulation of brain plasticity and behaviour by peripheral neurotrophic factors.

Acknowledgements

The authors acknowledge the Cell Imaging and Analysis Network (CIAN) at McGill University, Montreal, for expert advice in confocal microscopy, as well as Amanda Knezevic for technical assistance and Marissa Maheu, Jesse Jackson, Sylvain Williams and Lalit Srivastava for constructive feedback on the manuscript. We thank the laboratory of Dr. Derek van der Kooy for the generous donation of nestin-GFP mice. IM was supported in part by a scholarship from the Réseau Québécois de Recherche sur le Suicide (FRSQ). SDL is a CONACYT scholar. NM is an FRSQ chercheur-boursier.

FIGURES:

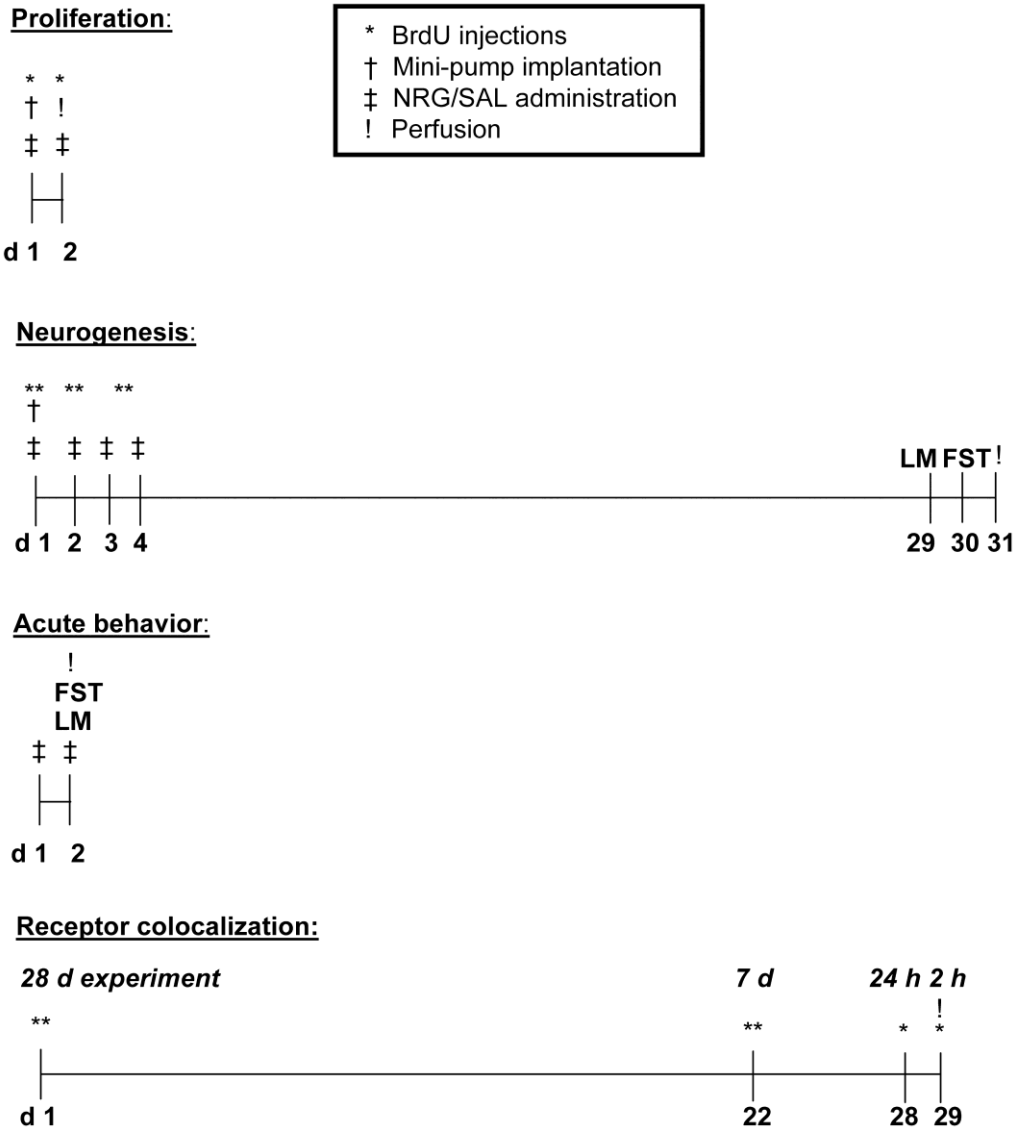


Figure 5. Experimental timelines. FST, forced swim test; LM, locomotor task; NRG, neuregulin; SAL, saline.

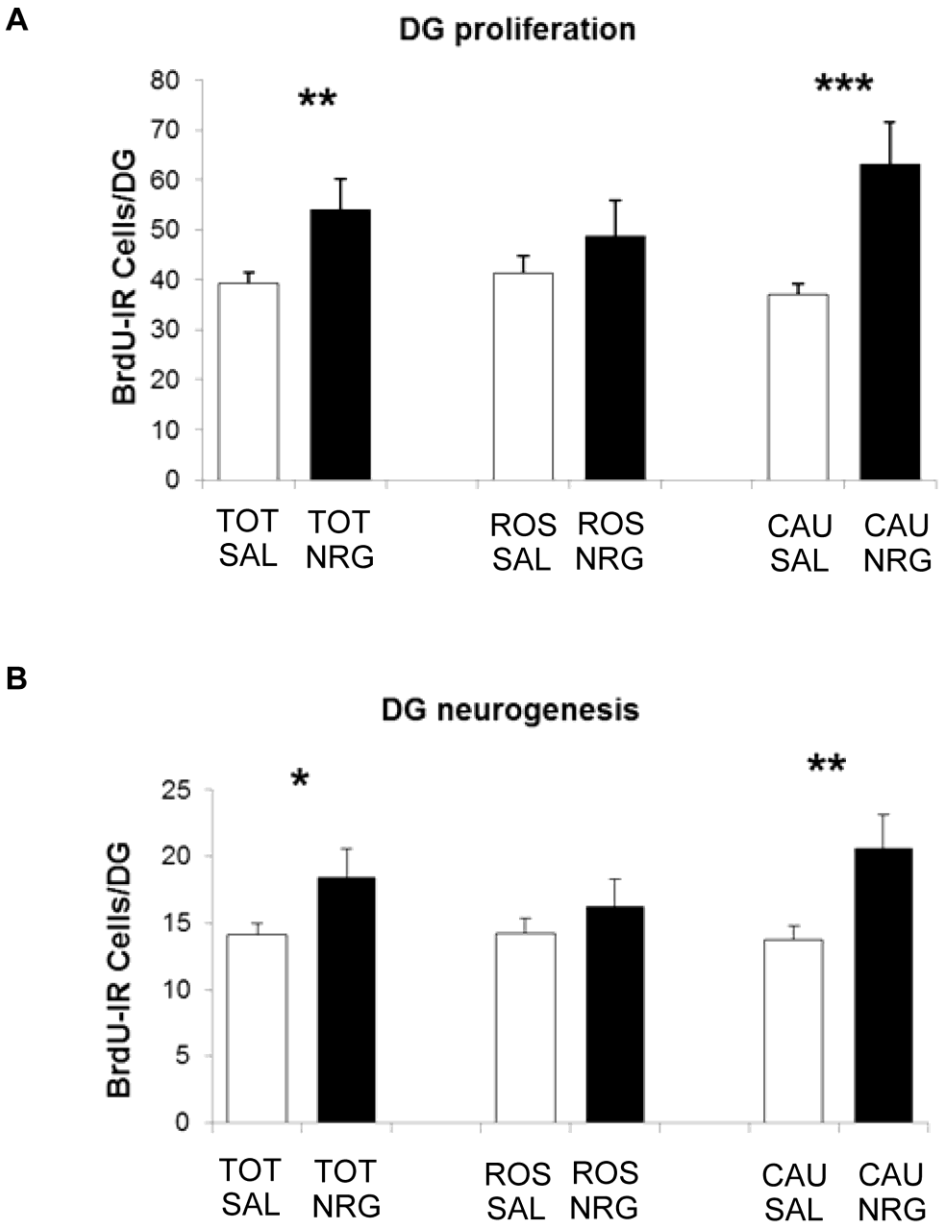


Figure 6. Proliferative and neurogenic effects of peripheral NRG1 administration. (**A**) NRG1 increases dentate gyrus (DG) cell proliferation, in the caudal (71%; $p = 0.0011$), but not rostral ($p = 0.20$), DG. (**B**) NRG1 increased the number of BrdU-IR cells 28 d after administration. As with proliferation, this increase is significant in the caudal (50%; $p = 0.013$), but not rostral ($p = 0.29$), DG. Bars represent mean ± SEM. *$p < 0.05$; **$p < 0.025$; ***$p < 0.005$. CAU, caudal; ROS, rostral; SAL, saline; TOT, total.

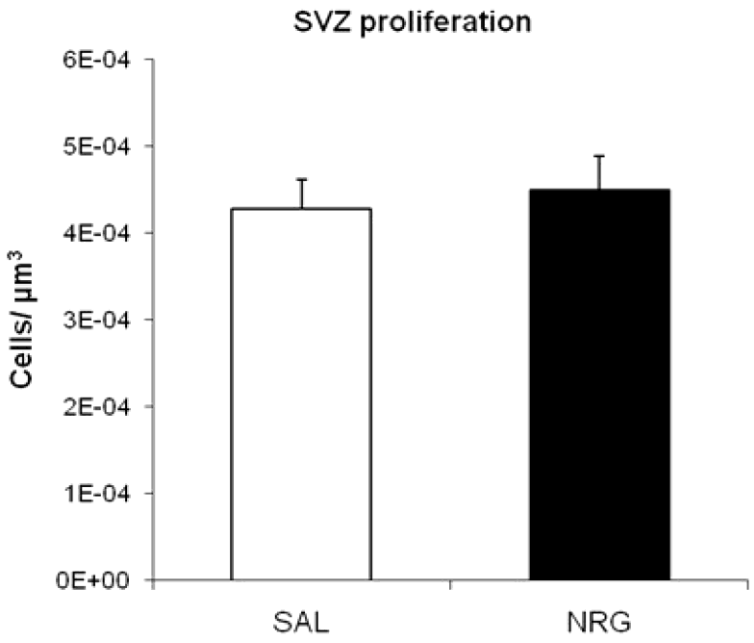


Figure 7. SVZ cell proliferation. NRG1 administration does not affect SVZ proliferation ($p = 0.34$). Bars represent mean ± SEM.

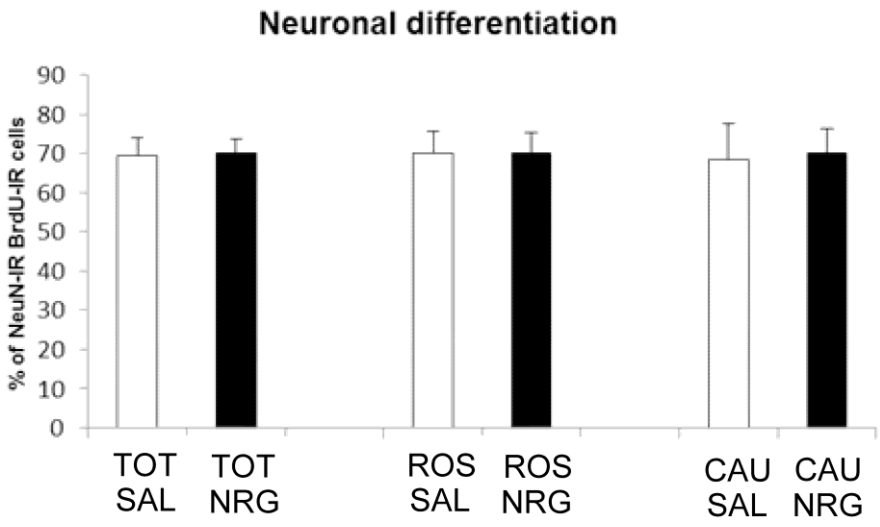


Figure 8. Neuronal differentiation. NRG1 administration does not affect the proportion of BrdU-IR cells that differentiated into NeuN-IR neurons, either overall ($p = 0.91$) or in the rostral ($p = 1.0$) or caudal ($p = 0.90$) subregions. Bars represent mean ± SEM. TOT, total.

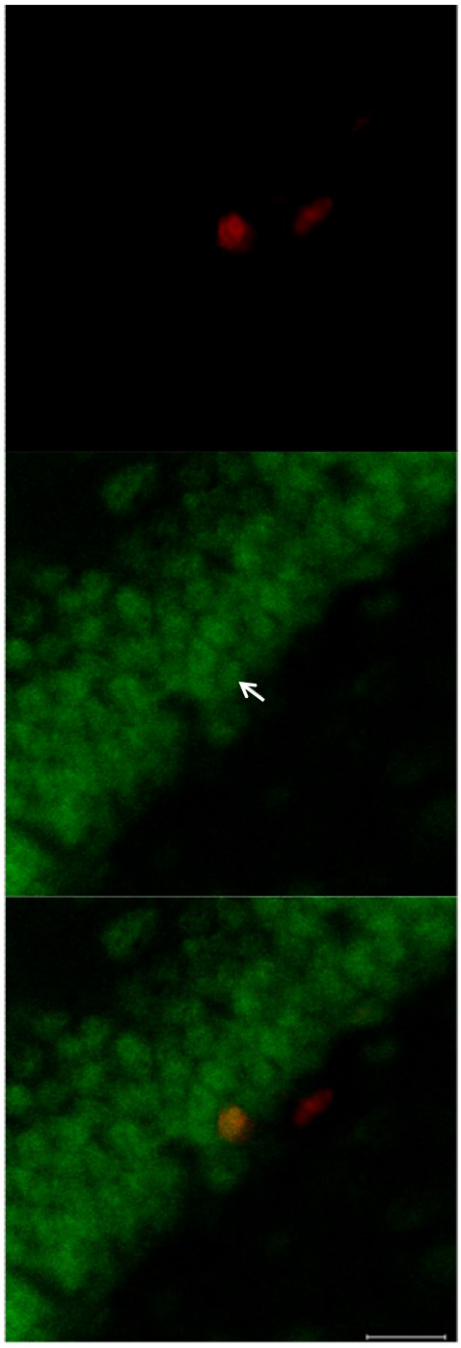


Figure 9. BrdU/ErbB3 colocalization. BrdU-IR cells (**red**) express ErbB3 (**green**), at 2 h (**Fig.A1** and **A2**) and 24 h (**above**), corresponding to the period in which cell proliferation is increased following NRG1 administration, as well as 7 d and 28 d after birth (**Fig. A1 and A2**). Scale bar = 20 μm.

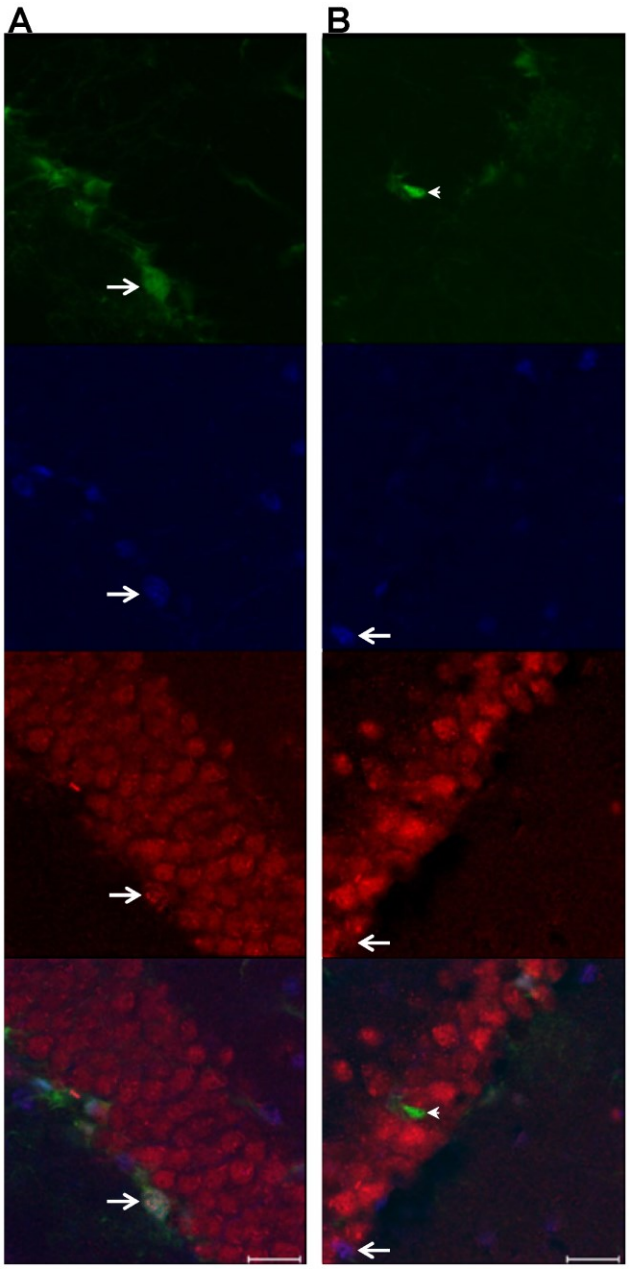


Figure 10. ErbB3/nestin/SOX2 colocalization. ErbB3-IR cells (**red**) in the DG colocalize with nestin (**green**) and SOX2 (**blue**) (arrow in **A**; **Fig. A3A**), or with SOX2 in the absence of nestin (arrow in **B**; **Fig. A3B**), but not nestin in the absence of SOX2. Arrowhead in **B** denotes a nestin-IR cell that is negative for both ErbB3 and SOX2. Scale bar = 20 μm.

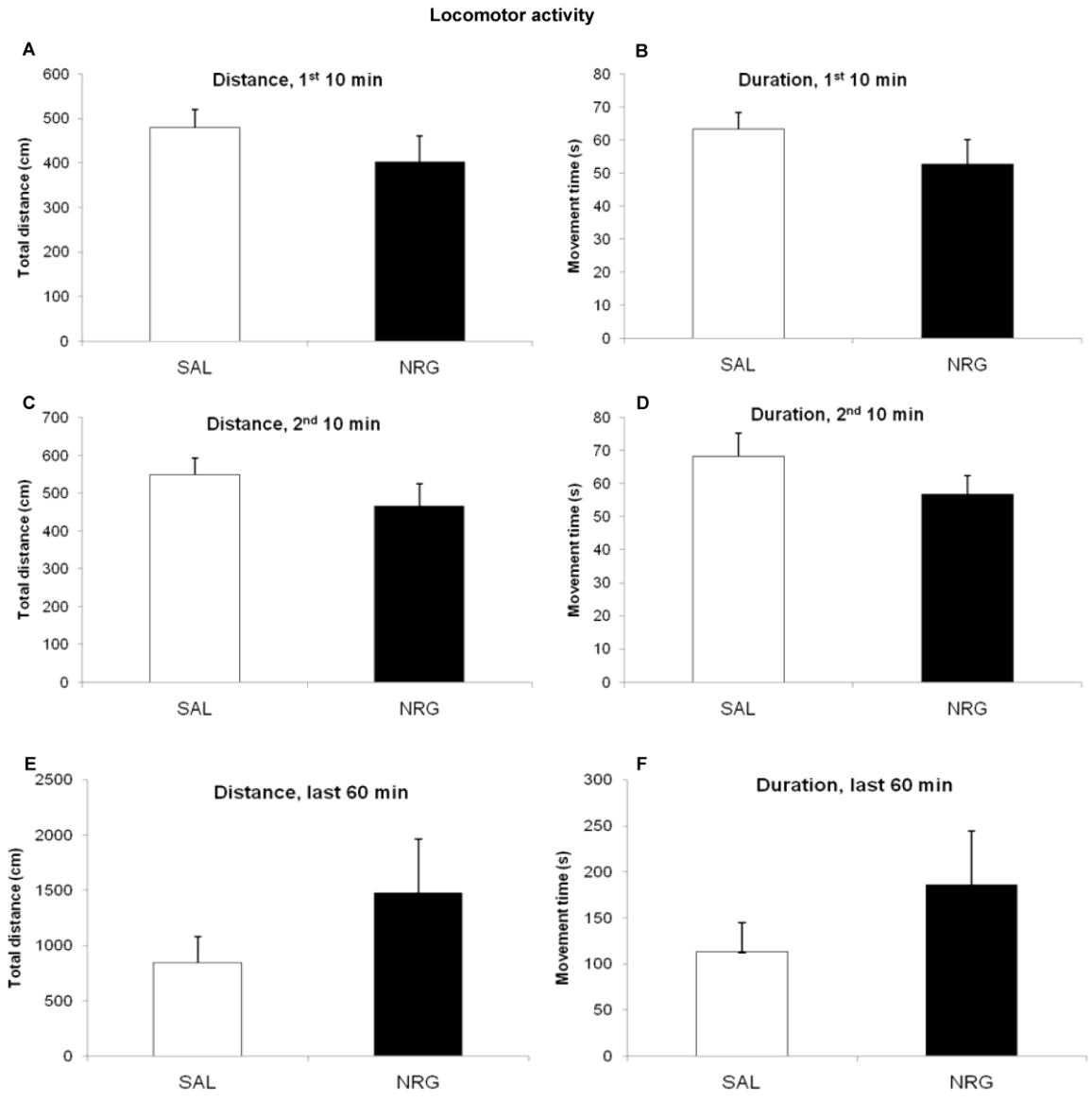


Figure 11. Locomotor activity does not change in response to NRG1 administration. NRG1 administration does not affect locomotor activity 28 d after subchronic administration, either in distance (**A**, **C**, **E**) or duration (**B**, **D**, **F**). **A**, **B**: First 10 min of activity (distance: $p = 0.30$; duration: $p = 0.25$). **C**, **D**: 10 min of activity after 10 min habituation (distance: $p = 0.28$; duration: $p = 0.23$). **E**, **F**: 60 min of activity after 30 min habituation (distance: $p = 0.26$; duration: $p = 0.29$). Bars represent mean ± SEM.

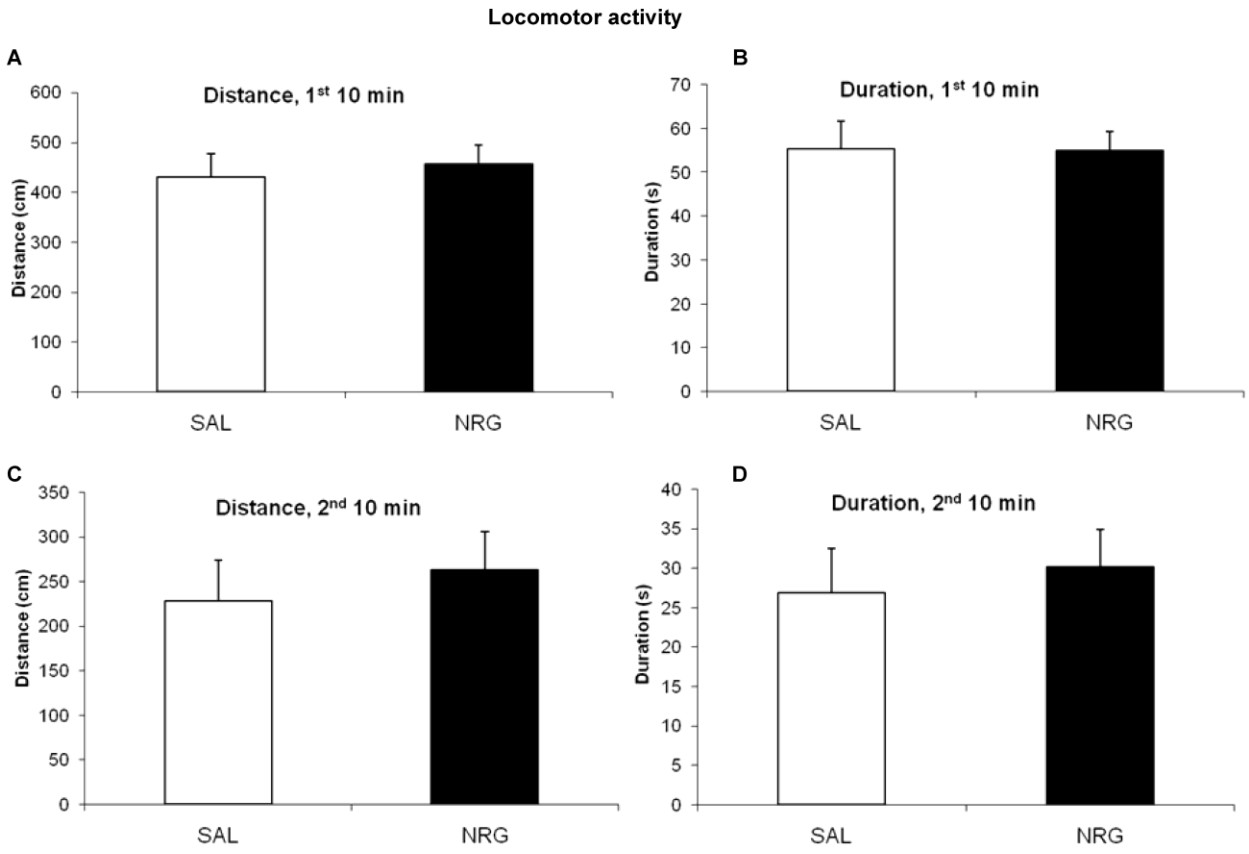


Figure 12. Acute locomotor activity does not change in response to NRG1 administration. NRG1 administration does not affect locomotor activity acutely after administration, either in distance (**A**, **C**) or duration (**B**, **D**). **A**, **B**: First 10 min of activity (distance: $p = 0.67$; duration: $p = 0.96$). **C**, **D**: 10 min of activity after 10 min habituation (distance: $p = 0.60$; duration: $p = 0.67$). Bars represent mean ± SEM.

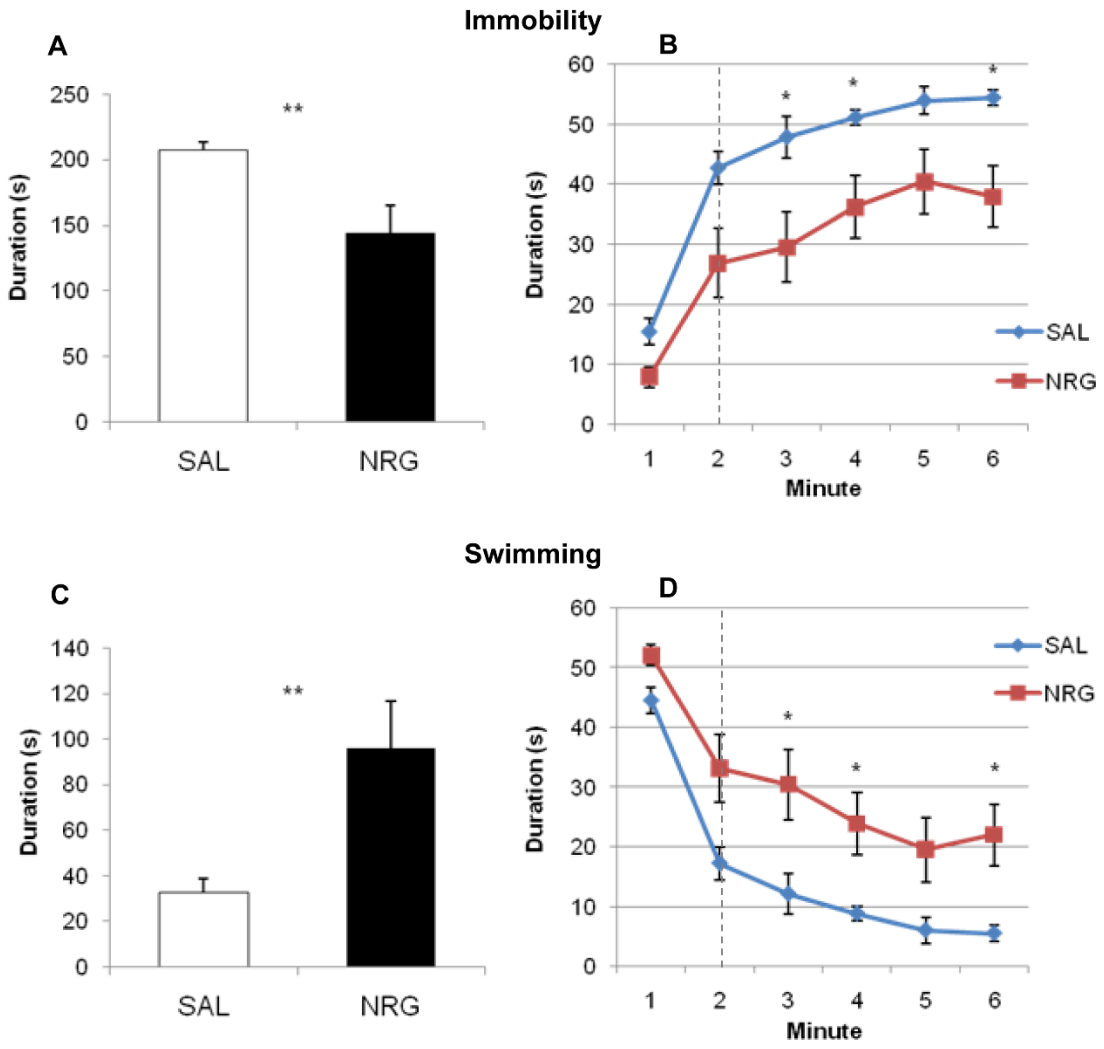


Figure 13. Animals treated with NRG display antidepressant behaviour in a forced swim test 28 d after administration. NRG-treated animals show decreased immobility (**A**, 44%, $p = 0.0062$; **B**) and increased swimming behaviour (**C**, 194%, $p = 0.0062$; **D**), both overall during the testing phase (**A**, **C**) and throughout the task (**B**, **D**). Dashed lines denote beginning of testing phase. Bars and points represent means ± SEM. *$p < 0.05$, **$p < 0.01$.

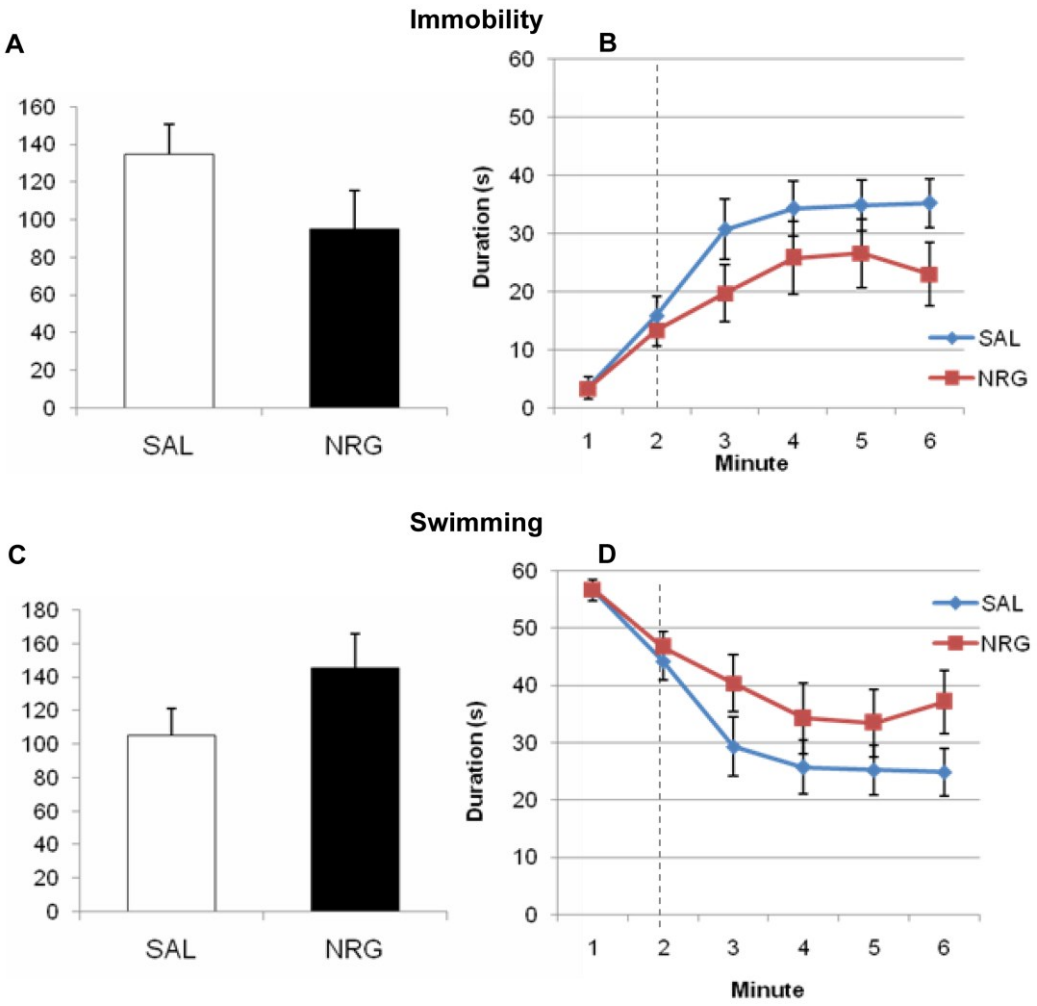


Figure 14. Animals treated with NRG do not display antidepressant behaviour in a forced swim test acutely after administration. Treatment groups did not differ with respect to immobility **(A**, p=0.15; **B**) or swimming behaviour (**C**, p=0.15; **D**), both overall during the testing phase (**A**, **C**) and throughout the task (**B**, **D**). Dashed lines denote beginning of testing phase. Bars and points represent means ± SEM.

Supplementary materials are available in Appendix.

2.5 ADDENDUM

As the first manuscript to result from our experiments, the preceding Chapter was published comparatively early during my doctoral studies (2011). As such, several of its key findings and implications have subsequently been further developed by additional research performed outside of our group. The following is a brief synopsis of these relevant interim developments.

Earlier we discussed the propensity for NRG1 to cross the blood-brain barrier. Subsequent studies have validated this property, administering radiolabeled NRG1β-I and subsequently detecting it in multiple brain regions. Peripherally-administered NRG1 has also been shown to increase phosphorylation of Akt and ErbB receptors in the brain, alter neuronal firing activity, act as a neurotrophic factor, and protect against phencyclinide-induced behavioural and neurotransmission alterations (Abe et al., 2011; Carlsson et al., 2011; Cui et al., 2013; Depboylu et al., 2015; Engel et al., 2014; Rosler et al., 2011).

Following our demonstration of antidepressant effects resulting from NRG1 administration, the majority of subsequent studies examining NRG1/ErbBs and behaviour involved transgenic mice. NRG1 mutants lacking the transmembrane domain were shown to have an exacerbated corticosterone response to acute stress in young mice and a decreased anxiety response to acute stress in older mice, and the normally anxiolytic effects of cannabidiol are ablated in these mice (Chesworth et al., 2012; Long et al., 2012). Conversely, inducible NRG1 overexpression in forebrain regions led to schizophrenia-like alterations in open-field, prepulse inhibition, memory, and social interaction (but not anxiety) behaviours, which were partially reversed by antipsychotic treatment and normalized at adulthood when NRG1 levels were restored (Yin et al., 2013). These results support the hypothesis that imbalances of NRG1 affect behaviour bidirectionally (Deng et al., 2013), with forebrain increases contributing to schizophrenia-like behaviours and decreases affecting anxiety and stress response. With the antidepressant-like

activity and normal locomotor activity in our studies, this potentially suggests that the impetus behind our effects was not increased forebrain NRG1 but is likely more limbic, such as in the hippocampus specifically.

Subsequent to the publication of the preceding Chapter, additional studies supported the role of NRG1 and ErbB3 (as well as related proteins) in cell proliferation, in a variety of cell types. The NRG1β isoform was shown to induce proliferation of cancer cells (Ruan et al., 2012). Intracerebroventricular infusion of betacellulin, an additional EGF family member, was shown to increase neurogenesis in the DG and SVZ, and neurospheres *in vitro*, and betacellulin hypomorphs display diminished recovery of neuroblast populations following ablation of proliferation (Gomez-Gaviro et al., 2012). EGFR is involved in PI3K, ERK/MAPK, and PLCγ pathway activation in neural stem cells, providing a mechanism for its pro-proliferative/survival influence on these cells (Sütterlin et al., 2013). NRG1-ErbB3 signaling drives proliferation in melanoma cells through PI3K/Akt (Belleudi et al., 2012). ErbB3 was also found to positively influence cell proliferation through a Cyclin D1-related mechanism (Andrique et al., 2012). Furthermore, ErbB3's C-terminal interacts with NEDD4's WW domains, and this interaction occurs even in the absence of NRG1. Knocking down NEDD4 increases ErbB3 expression and cancer cell proliferation in vivo and in vitro, offering further support for ErbB3 as pro-proliferative and providing further insight into its regulation (Huang et al., 2015). Conversely, ErbB3 knockdown in colon cancer cells causes cell cycle arrest in G1, with dramatic reductions in cell proliferation within 72 hours (Lee et al., 2014a). Finally, ErbB3 is required for the proliferation of Bergmann glial cells in the cerebellum (Sathyamurthy et al., 2015).

Of particular relevance to the role of ErbB3 in regulating cell proliferation were striking findings that ErbB3, previously thought to have insufficient kinase activity for signaling (Guy et al.,

1994), is in fact capable of signaling (Deng et al., 2013; Shi et al., 2010a; Steinkamp et al., 2014). This not only suggests a functional role of the receptor even in a homodimeric conformation, but supports the plausibility of ErbB3 is the mechanistic receptor from our findings as discussed earlier in this Chapter.

Regarding our methodology in quantifying numbers of BrdU-IR cells in the DG, we considered an absolute quantification of the number of labeled cells to be a more accurate and relevant option than an estimate derived from stereological assessment. A recent publication has supported this position, concluding that absolute quantification may be preferable to stereology for DG cell proliferation, based largely on the heterogenous distribution of proliferating cells (Noori and Fornal, 2011).

Previously in this Chapter we discussed our efforts to characterize ErbB3 and ErbB4 expression in the murine DG, both temporally and along the septotemporal axis. Subsequent studies have thoroughly examined the expression of ErbB4 across brain regions and species, using immunohistochemical and transgenic techniques. In mice, cells expressing ErbB4 are particularly dense in the cortex and amygdala, and sparse in the thalamus, hindbrain, and cerebellum, with intermediate density in the olfactory bulb, basal ganglia, and hypothalamus. Although some ErbB4-expressing cells are GAD67-negative, particularly in subcortical areas, in the cortex the majority of ErbB4-expressing cells are GABAergic interneurons, particularly parvalbuminergic basket/chandelier interneurons. In the hippocampus, ErbB4-expressing cells are found in all areas except for the subiculum, and particularly in the pyramidal and molecular layers of cornu ammonis regions, as well as the granule cell layer and hilus of the DG. Most hippocampal ErbB4+ cells are GAD67+, with 30% also expressing parvalbumin. Interestingly, in the DR, a sizable proportion of serotonergic neurons also express ErbB4 (Bean et al., 2014). In

primates, ErbB4 expression appears to be largely or completely restricted to GABAergic interneurons (Neddens and Buonanno, 2011), as in mice. However, it is important to point out that there is some electrophysiological, morphological, and immunohistochemical murine support for ErbB4 expression in principal hippocampal and cortical excitatory neurons as well (Cooper and Koleske, 2014; Mechawar et al., 2007; Pitcher et al., 2011).

Part of the association between increased hippocampal neurogenesis and antidepressant-like behaviour in the preceding Chapter was contingent upon the previously-discovered temporally-restricted critical period of hyperplasticity of immature adultborn hippocampal neurons (Ge et al., 2007). Subsequent studies have both supported and further elucidated this critical period. This plasticity was validated as being NR2B NMDAR-dependent, is accompanied by a transiently-increased excitation/inhibition balance in immature granule cells relative to mature granule cells, and is potentially related to pattern-separation functions (Kheirbek et al., 2012; Marin-Burgin et al., 2012; Nakashiba et al., 2012). Experience-dependent synaptic input remodeling occurs during this period (Bergami et al., 2015), as does the source of inputs, switching from local circuits to more distal cortical inputs, particularly from the perirhinal and lateral entorhinal cortices (Vivar et al., 2012). The development of GABAergic inputs may also contribute, as these differ between mature and immature neurons, as does their integration into feedback coupling circuits with a temporal association to this critical period, and these GABAergic inputs may help contribute (and temporally regulate) the hyperplasticity observed in these neurons (Li et al., 2012b; Temprana et al., 2015). Neurogenic ablation, by irradiation or transgenic techniques, causes memory impairments specifically 4-6 weeks following the manipulation, as does optogenetic silencing of 4 week-old adultborn granule cells, indicating that newborn neurons functionally contribute specifically during this period of hyperplasticity

(Denny et al., 2012; Gu et al., 2012). The process of continually producing a unique pattern of immature hyperplastic neurons in circuits containing mature granule cells has been hypothesized to serve as a putative 'time-stamp' for memories during consolidation (Rangel et al., 2014), although the relevance of this to the more affective functions of the ventral hippocampus has not yet been shown. In addition, some aspects of the increased plasticity of adultborn neurons may persist into neuronal maturity and have relevance for spatial memory (Lemaire et al., 2012), and potentially other neurogenesis-related functions.

Finally, regarding subsequent relevant human clinical findings, a panel of blood biomarkers of depression, including ErbB3, showed high validity for MDD and response to cognitive-behavioural therapy (Keri et al., 2014). The preceding Chapter also discussed the clinical implications of our findings in the context of human clinical trials, based on a phase-II neuregulin-1 administration heart failure trial (Gao et al., 2010). Additional studies have further supported the safety of therapeutic agents targeting NRG1 and ErbB signaling, indicating that NRG1 administration in the treatment of psychiatric conditions would likely be well-tolerated with a low incidence of side effects (Jabbour et al., 2011; Macbeath et al., 2014; Tjulandin et al., 2014).

CHAPTER 3. Effects of neuregulin-1 administration on neurogenesis in the adult mouse hippocampus and characterization of immature neurons along the septotemporal axis

3.1 PREAMBLE

The previous Chapter established that NRG1 administration has neurogenic effects; specifically, this increases proliferation and overall neurogenesis in the ventral DG, but does not affect neuronal differentiation. However, there are other stages of neurogenesis that were not examined by this manuscript (see **Fig. 3** in Chapter 1). Particularly, it was unclear what the effects of NRG1 administration on intermediate stages of neurogenesis might be, when cells have differentiated into immature developing neurons. To this end, we aimed to characterize the effects of NRG1 on the remaining stages of hippocampal neurogenesis, specifically survival and morphological development (dendritogenesis, synaptogenesis, and cell body morphology). To specifically target this population of cells, we repeated the NRG1 administration paradigm of the previous study, but altered the timing of the BrdU administration, in order to track a population of cells undergoing intermediate neurogenic stages at the time of NRG1 administration.

From these experiments, we determined that NRG1 administration does not affect cell survival or morphology in immature granule cell neurons, and thus that the neurogenic effects of NRG1 in the hippocampus are temporally and subregionally specific to proliferation and overall neurogenesis in the ventral DG.

In addition, we took advantage of the extensive cellular characterization data across the septotemporal axis from these studies to thoroughly investigate whether immature neurons differ between the dorsal and ventral DG. We determined that immature neurons in these two

subregions differ in several key developmental aspects, with potential relevance to their subsequent functional distinctions. We also found that overall cytogenesis is higher in the ventral DG than in the dorsal DG.

Together, the results in this Chapter complete our characterization of the effects of NRG1 on hippocampal neurogenesis, determine features characterizing immature granule cell neurons during their development, and identify distinguishing markers between dorsal and ventral immature neurons and neurogenesis that may have relevance to their particular functions.

3.2 Effects of neuregulin-1 administration on neurogenesis in the adult mouse hippocampus and characterization of immature neurons along the septotemporal axis

Authors: Ian Mahar[1,2], Angus MacIsaac[1], John Junghan Kim[1], Gustavo Turecki[1,2,3], Naguib Mechawar[1,2,3,*]

[1] McGill Group for Suicide Studies, Douglas Mental Health University Institute, 6875 LaSalle Blvd, Verdun, Québec, Canada H4H 1R3

[2] McGill University, Integrated Program in Neuroscience, Montreal Neurological Institute

[3] McGill University, Department of Psychiatry

***Corresponding author**

ABSTRACT

Adult hippocampal neurogenesis has been associated with behavioural regulation, including learning and emotional behaviour. Its diverse functionality is segregated along the septotemporal axis from the dorsal to ventral hippocampus, with the former region associated with memory and the latter with stress and emotional regulation. However, features defining and distinguishing immature neurons in these distinct regions have yet to be characterized. In addition, although we have previously shown that administration of neuregulin-1 (NRG1), a neurotrophic factor implicated in psychopathology, selectively increases proliferation and overall neurogenesis in the mouse ventral DG, effects of administration at intermediate stages of neurogenesis in immature neurons are unknown.

In the current study, we labeled adultborn cells in mice using BrdU, and later administered NRG1 to examine its *in vivo* neurogenic effects on immature neurons with respect to cell survival, morphology, and synaptogenesis. In addition, we characterized features of immature neurons along the septotemporal axis.

We found that the neurogenic effects of NRG1 are temporally and subregionally specific to proliferation in the ventral DG. We also determined particular features that differentiate immature neurons in the dorsal and ventral DG, including cell body size, synaptic density, and dendritic branching. In addition, we found that rates of cytogenesis differed between the dorsal and ventral DG, but were correlated between the two regions, suggesting that a common set of factors regulate cytogenesis in both regions. Finally, we identified a novel heterogeneity in synaptic density in the molecular layer surrounding the granule cell layer, which is maintained along the septotemporal axis.

These results indicate that the neurogenic involvement of NRG1-induced antidepressant-like behaviour is associated with increased ventral DG cell proliferation in particular, and identify novel distinctions between neurogenic development in the dorsal and ventral hippocampus, likely contributing to their functional heterogeneity.

KEYWORDS: Neurogenesis, neuregulins, cell morphology, dendritogenesis, synaptogenesis

HIGHLIGHTS:

- **Hippocampal neurogenesis has been shown to be increased by neuregulin-1 (NRG1) administration.**
- **However, the effects of NRG1 administration on immature neuronal stages of neurogenesis are unknown.**

- **Here we show that the neurogenic effects of NRG1 are restricted to proliferation in the ventral dentate gyrus, without affecting immature neurons.**
- **We also thoroughly characterize differences in immature neuronal features along the septotemporal axis.**

3.2.1 Introduction

Adult hippocampal neurogenesis is the process by which new neurons are added throughout life to the granule cell layer of the DG in the hippocampus (Kempermann and Gage, 2000). This phenomenon has been associated with a number of different functions, most notably learning and memory, affective regulation, and response to stress (Deng et al., 2010; Snyder et al., 2011b). In addition, depression has been associated with reduced number of DG granule cells (Boldrini et al., 2014), whereas antidepressant treatment increases hippocampal neurogenesis in rodents, non-human primates, and humans (Boldrini et al., 2013; Boldrini et al., 2009; Malberg et al., 2000; Perera et al., 2011). The functional role of new granule cell neurons appears to be related to their unique electrophysiological properties during a hyperplastic window extending from approximately four to six weeks of cellular age in rodents (Ge et al., 2007), whereas mature granule cells are comparatively quiescent (Aimone et al., 2010).

Anatomically, the hippocampus follows a septotemporal axis, progressing from the dorsal (or septal) hippocampus rostrally to the ventral (or temporal) hippocampus caudally (Fanselow and Dong, 2010; Mahar et al., 2011; Tanti et al., 2013). Recent studies have established that the dorsal and ventral hippocampus are anatomically and functionally distinct regions, with the dorsal hippocampus (analogous to the posterior hippocampus in humans) being more involved in spatial learning and memory, whereas the ventral hippocampus (anterior hippocampus in humans) is associated more with emotional regulation (Bannerman et al., 2004; Fanselow and

Dong, 2010; van Strien et al., 2009). This functional distinction is maintained at the level of the DG and DG neurogenesis, as studies have shown that dorsal hippocampal neurogenesis is necessary for contextual discrimination, whereas the ventral DG has been associated with affective behaviour, antidepressant response, and response to stress (Banasr et al., 2006; Elizalde et al., 2010; Lehmann et al., 2013; Mahar et al., 2011; Tanti et al., 2013). As a result, it has been proposed that the influence of adult hippocampal neurogenesis on emotion-related behaviour is contingent upon the number of functional immature hyperplastic neurons present in the ventral DG specifically at the time of behavioural assessment, potentially due to connections between the ventral hippocampus and the HPA axis, amygdala, nucleus accumbens, and PFC (Mahar et al., 2014). However, despite the identified importance of these cells in emotion-related behaviour, there is a lack of studies specifically examining developmental, morphological, and synaptic features distinguishing immature ventral and dorsal hippocampal neurons.

In addition to the influence of monoaminergic agents, such as antidepressant medications, on adult hippocampal neurogenesis, neurotrophic factors have also been shown to increase hippocampal neurogenesis (Mahar et al., 2014). These include brain-derived neurotrophic factor (BDNF) (Scharfman et al., 2005; Schmidt and Duman, 2010), VEGF (Jin et al., 2002), IGF (Aberg et al., 2000), and most recently NRG1 (Mahar et al., 2011). NRG1, which had previously been associated with psychopathology (Bertram et al., 2007; Georgieva et al., 2008; Stefansson et al., 2002), was found in this study to rapidly increase hippocampal proliferation and neurogenesis selectively in the caudal DG within the ventral hippocampus when administered peripherally to adult mice (Mahar et al., 2011). This neurogenic effect of subchronic NRG1β administration likely resulted from local binding to NRG1 receptor ErbB3 in neural progenitor cells, and was associated with antidepressant-like behaviour (Mahar et al., 2011). However,

although these results identified a temporal association between increased proliferation/neurogenesis and antidepressant-like behaviour, the effects of NRG1 on intermediate stages of hippocampal neurogenesis, occurring between cell proliferation and functionality several weeks later, remain to be assessed.

In the present study, we examined the consequences of NRG1 administration on immature neurons in the adult hippocampus, to determine if the mood-related behavioural effects of NRG1 administration previously associated with increased hippocampal neurogenesis can be specifically identified as being due to changes to proliferation/neurogenesis, or alternatively whether other morphological and synaptic changes to neurons in intermediate stages of development may be involved in this phenomenon. This was accomplished through assessments of neuronal survival, morphometric analyses of reconstructed immature neurons, and quantifications of synaptic densities. In addition, we applied these data to characterize immature neurons in the dorsal and ventral DG, in order to identify both common and distinguishing features between these cell populations contributing to vastly distinct behaviourally- and psychopathologically-relevant functions.

Our findings reveal that the effects of NRG1 on hippocampal neurogenesis are temporally and subregionally specific to proliferation, and lead to increased neurogenesis in the ventral DG. Furthermore, our results indicate that immature neurons differ between the dorsal and ventral DG with respect to specific developmental features that may contribute to their specific functional contributions.

3.2.2 Methods and materials

3.2.2.1 Animals

Adult male C57Bl/6 mice were obtained from Charles River (Quebec), and were approximately nine weeks of age at the time of experimentation. Mice were housed on a 12:12 light:dark cycle, with *ad libitum* access to food and water. Animals were randomly assigned to groups aside from weight matching at time of arrival, and did not differ by weight at time of experimentation ($p=0.55$). All experiments adhered to the policies and guidelines of the Canadian Council on Animal Care, and were approved by McGill University's Animal Care Committee.

3.2.2.2 NRG1β and BrdU administration

BrdU (50 mg/kg) was injected i.p. three times, spaced ≥3 hours apart within a 24 h period in order to label dividing cells. 15 days after BrdU administration, mice were given either recombinant NRG1β type-I (R&D Systems; EGF domain dissolved in sterile 0.9% saline, administered at a constant rate of 10 μg/d; n=9) or vehicle (n=10) for three days in subcutaneously implanted osmotic mini-pumps (Alzet; model 1003D), as performed and described previously (Mahar et al., 2011). This latency was chosen as it corresponds to a specific critical period during which adultborn neurons undergo a period of apoptosis or survival, extend dendrites into the molecular layer, and form synapses (Grossman et al., 2010; Snyder et al., 2009a; Toni et al., 2007; Zhao et al., 2006) (see **Fig. A4** for experimental timeline). Mice were left to recover post-operatively on a heating pad, and each animal was given an anti-inflammatory Carprofen tablet.

3.2.2.3 Tissue processing

Mice were deeply anesthetized with a ketamine, xylazine and acepromazine cocktail (0.1 ml/100 g), and perfused intracardially with ice-cold PBS, then with 4% formaldehyde in 0.1 M phosphate buffer. Brains were rapidly removed, postfixed at 4˚C for 24 h in the fixative solution, then transferred to 30% sucrose in PBS until equilibrium was reached. Brains were flash frozen to -40ºC in isopentane and cut with a cryostat into serial 40 µm coronal sections that were immersed in a cryoprotectant solution (glycerol:ethylene glycol:PBS, 3:3:4) and stored at −20˚C.

3.2.2.4 Immunohistochemistry

Immunohostochemistry was conducted on free-floating sections. For all protocols, sections were washed with PBS (pH=7.2) between incubations, except between blocking and primary incubation. All steps occurred at room temperature except for incubation in HCl/PBS, which was at 37˚C, and overnight primary incubations, which were at 4˚C. Steps following addition of fluorescent antibodies included protection from light.

For BrdU single-labeling immunohistochemistry to assess cell survival and cytogenesis, sections were incubated for 1.5 h in PBS-T, then 10 min in 0.9% H_2O_2 in PBS, followed by 30 min in 2 N HCl in PBS, 30 min in block solution containing PBS-T with 2% NGS (Vector), and then overnight in block solution containing rat anti-BrdU antibody (1:1000; Serotec). This was followed by incubation in biotinylated goat anti-rat secondary antibody (1:200; Vector) in block solution for 1 h, and the labeling revealed with DAB using VectaStain Elite and DAB kits (both by Vector), and quenching in double-distilled water (ddH2O) for 5 min before transferring sections to PBS. Sections were mounted on SuperFrost Plus slides (Fisher) in PBS-T, left to dry

to affix to slides, then dehydrated using increasing concentrations of ethanol followed by xylene, and coverslipped using Permount (Fisher).

For BrdU/DCX double-labeling immunohistochemistry for neuronal reconstructions, we used a combination of light and fluorescence microscopy to optimize cell identification while avoiding issues of photobleaching during tracing. Sections were incubated in PBS-T for 2 h, then 10 min in 0.9% H_2O_2 in PBS, followed by 1 h in block solution containing PBS-T with 2% normal horse serum (Vector), then 24 h in block solution containing goat anti-DCX primary antibody (1:250; Santa Cruz). Sections were then incubated for 90 min in block solution containing biotinylated horse anti-goat secondary antibody (1:200), then VectaStain Elite and DAB as performed with BrdU immunohistochemistry. We then incubated sections for 30 min in 1 N HCl in PBS, then 1 h in block solution comprised of PBS-T with 2% NGS, then anti-BrdU in block solution overnight. Goat anti-rat DyLight 594 (1:500; Jackson) secondary antibody in block solution was added for 90 min. Sections were mounted in PBS-T on to slides, then rinsed in ddH2O and coverslipped with ProLong Gold antifade reagent with DAPI (Life Technologies) using #1.5 thickness cover slips (Corning).

For BrdU/DCX/PSD-95 triple-labeling to assess synaptic density, sections were treated for 1 h with PBS-T, then 1 h in blocking solution (PBS-T containing 2% normal donkey serum (Jackson)) followed by overnight incubation in blocking solution containing anti-DCX and rabbit anti-PSD-95 (1:250; Invitrogen) antibodies. Sections were then incubated in blocking solution containing donkey anti-goat DyLight 488 and donkey anti-rabbit DyLight 647 secondary antibodies (1:500; Jackson), followed by 1 N HCl in PBS for 30 min, then 1 h block in PBS-T containing 2% NGS, followed by overnight incubation in blocking solution containing anti-BrdU, then 75 min in PBS-T containing goat anti-rat DyLight 594 secondary antibody (1:500;

Jackson). Sections were mounted on slides, rinsed in ddH2O, and coverslipped with ProLong Gold antifade reagent with DAPI using #1.5 thickness cover slips. A similar protocol was used for staining for confocal microscopy in order to verify PSD-95 staining specificity.

3.2.2.5 Cell quantification

BrdU-IR cells were counted across the septotemporal axis on a Leica CME microscope, using a 40X 0.65 NA E2 achromat objective. For this and other experiments discussed herein, experimenters were blind to group identity. Dorsal and ventral DG regions were identified using previously established criteria (Franklin and Paxinos, 2007; Mahar et al., 2011; Sierksma et al., 2013a; Sierksma et al., 2013b); dorsal: −1.46 to −2.54 mm from bregma; ventral: −2.55 to −3.80 mm from bregma). Data are expressed as the average number of BrdU-IR cells per section.

3.2.2.6 Neuronal reconstruction

At least six clearly-labeled BrdU-/DCX-IR immature neurons (**Fig. 16A-B**) per subject across the septotemporal axis from multiple DGs were randomly selected to be reconstructed using Neurolucida software (MBF) (**Fig. 16B**). Neurons were traced using an Olympus BX51 microscope with a motorized stage and CX-9000 camera using a 100X (1.40 NA UPlansApo oil objective), and analyzed using Neurolucida Explorer (MBF Bioscience). The microscope was calibrated for grid tuning and parcentric/parfocal parameters. Labeled cells with primary or secondary dendrites which appeared to be cut off by the upper or lower boundaries of the tissue, or without a visible process extending through the granule cell layer, were excluded from

analysis. The third panel of **Fig. 16A** was produced in Adobe Illustrator by overlaying a fluorescence (50% transparency) image over a brightfield image of the same field.

3.2.2.7 Synaptic density assessment

Synaptic density was assessed in two stages, with the first examining overall synaptic density within the molecular layer surrounding the granule cell layer, and the second examining synapses specifically associated with approximately 18 day-old immature BrdU-IR neurons. These experiments used an Axio Imager.M2 microscope with motorized stage, AxioCam MR camera, and Apotome.2 system (Zeiss), at 63x (1.40 NA plan-apochromat oil objective). The microscope was calibrated for grid tuning and parcentric/parfocal parameters.

To assess overall synaptic density, four zones in the molecular layer were chosen: adjacent to the basal aspect of the suprapyramidal blade, adjacent to the apical suprapyramidal blade, adjacent to the basal infrapyramidal blade, and adjacent to the apical infrapyramidal blade. For each zone, a z-stack of images from the top to the bottom of the tissue was obtained using Neurolucida software. Four DGs (two dorsal, two ventral) were included per subject. Image acquisition parameters were maintained between subjects. For each image stack, the intensity of PSD-95 staining was obtained from luminance information in each slice for which the molecular layer was in focus without tissue aberration, then averaged between slices. Additional analyses using all images in each stack throughout the issue did not change statistical significance status of primary comparisons.

To assess synaptic density specifically for immature neurons, we traced BrdU/DCX-IR cells at 63X using NeuroLucida software, and quantified PSD-95-IR puncta across dendritic branch

orders and on the cell soma (**Fig.17C, E; Fig. A5**) for 122 cells across all subjects and the septotemporal axis.

3.2.2.8 Confocal microscopy

PSD-95 staining specificity and reliability were assessed with a Zeiss LSM510 Meta confocal microscope equipped with an Axiovert 200 M stand and motorized stage, and 488 nm, 543 nm, and 633 nm wavelength lasers (Carl Zeiss Canada). Objectives used were 40X Plan-Neofluar 1.3 NA oil and 100X Plan-Apochromat 1.4 NA oil. Images were obtained using the Zeiss Aim software package (Carl Zeiss Canada), with a pixel dwell time of ≥3.20 µs, optical slice 1-3 µm sampled at 1 µm intervals. PSD-95 was absent in no-primary control tissue. PSD-95 staining was absent in corpus callosum but present in synapse-rich regions such as cortical and hippocampal regions (**Fig. A6**), suggesting specificity in PSD-95 labeling.

3.2.2.9 Statistics

Data normality was assessed using the D'Agostino and Pearson omnibus normality test. Pairwise comparisons were made using unpaired or paired t-tests with or without Welch's correction for parametric data, or Mann-Whitney or Wilcoxon tests for non-parametric data. Region x treatment analyses were conducted using two-way mixed-model ANOVAs, with Holm-Sidak multiple comparisons post-hoc tests; treatment was a between-subjects factor, and DG subregion was a within-subjects factor. Cytogenic relationships were analyzed by Pearson correlations. All statistical tests were two-tailed, and p values ≤0.05 were considered statistically significant. Figures show mean ± SEM.

3.2.3 RESULTS

3.2.3.1 Effects of neuregulin-1 administration on cell survival

Overall DG cell survival, as assessed by average numbers of BrdU-IR cells per section, was unaffected by NRG1 treatment. This was true for the overall number of BrdU-IR cells in the DG (p=0.27; **Fig. 15A**), for the subgranular zone and granule cell layer (SGZ/GCL) alone (p=0.21; **Fig. 15B**), and for the hilus alone (p=0.89; **Fig. 15C**). In examining the effect of NRG1 on hippocampal subregions, there was also no difference specifically in the dorsal DG (total: p=0.19; hilus: p=0.67; SGZ/GCL: p=0.21; **Fig. A7A-C**) or in the ventral DG (total: p=0.44; hilus: p=0.67; SGZ/GCL: p=0.30; **Fig. A7D-F**).

3.2.3.2 Effects of neuregulin-1 administration on morphological development

Cell body perimeter did not differ significantly between NRG1- and saline- treated mice overall (averaged across the septotemporal axis; p=0.36; **Fig. A8A**), or in the dorsal (p=0.07; **Fig. A7B**) or ventral (p=0.54; **Fig. A7C**) subregions. This was also the case for cell body area (overall: p=0.49; dorsal: p=0.13; ventral: p=0.54; **Fig. 16C, A8AB-AC**), feret max (p=0.59; p=0.13; p=0.54; **Fig.A8D-F**), feret min (p=0.60; p=0.39; p=0.83; **Fig.A8G-I**), aspect ratio (p=0.95; p=0.20; p=0.83; **Fig.A8J-L**), compactness (p=0.98; p=0.46; p=0.82; **Fig.A8M-O**), convexity (p=0.098; p=0.16; p=0.61; **Fig.A8P-R**), form factor (p=0.21; p=0.14; p=0.99; **Fig.A8S-U**), roundness (p=0.92; p=0.49; p=0.88; **Fig.A8V-X**), and solidity (p=0.39; p=0.31; p=0.62; **Fig.A8Y-AA**).

Total dendritic length did not differ with treatment overall (p=0.81; **Fig. A9A**), or in the dorsal (p=0.86; **Fig. A9B**) or ventral (p=0.70; **Fig. A9C**) subregions. Examining length by branch

order, there was no effect of treatment overall (p=0.82; **Fig. A10A**), or in the dorsal (p=0.96; **Fig. A9B**) or ventral (p=0.60; **Fig. A10C**) subregions, and this was also the case for Sholl analysis of length (overall: p=0.81; dorsal: p=0.98; ventral: p=0.60; **Fig.16D, A10W-X**). Mean dendritic length (length per branch) by branch order did not differ with NRG1 treatment overall (p=0.91; **Fig. A10D**), or in the dorsal (p=0.74; **Fig. A10E**) or ventral (p=0.38**; Fig. A10F**) subregions. There was a significant effect for branch order overall and in both subregions (ps<0.0001); length was highest in the 2nd and 3rd branch orders versus subsequent branches (ps≤0.0021), and these tended to be higher than the 1st order (ps≤0.089).

Total dendritic volume did not differ with treatment overall (p=0.69; **Fig. A6G**), or in the dorsal (p=0.81; **Fig. A8AM**) or ventral (p=0.65; **Fig. A8AN**) subregions. Examining volume by branch order, there was no effect of treatment overall (p=0.70; **Fig. 16E**), or in the dorsal (p=0.99; **Fig. A10S**) or ventral (p=0.56; **Fig. A9T**) subregions. Mean dendritic volume by branch order did not differ with NRG1 treatment overall (p=0.67; **Fig. A10G**), or in the dorsal (p=0.91; **Fig. A10H**) or ventral (p=0.46; **Fig. A10I**) subregions.

Total dendritic surface area did not differ with treatment overall (p=0.66; **Fig. A6E**), or in the dorsal (p>0.99; **Fig. A8AK**) or ventral (p=0.66; **Fig. A8AL**) subregions. Examining surface area by branch order, there was no effect of treatment overall (p=0.68; **Fig. A10J**), or in the dorsal (p=0.89; **Fig. A10K**) or ventral (p=0.54; **Fig. A10L**) subregions. Mean dendritic surface area by branch order did not differ with NRG1 treatment overall (p=0.64; **Fig. A10M**), or in the dorsal (p=0.98; Fig. A7N) or ventral (p=0.38; **Fig. A10O**) subregions.

Total dendritic nodes did not differ with treatment overall (p=0.84; **Fig. A8D**), or in the dorsal (p=0.37; **Fig. A10AE**) or ventral (p=0.61; **Fig. A7AF**) subregions. Examining nodes by branch order, there was no effect of treatment overall (p=0.84; **Fig. A10P**), or in the dorsal (p=0.59; **Fig.**

A10Q) or ventral (p=0.82; **Fig. A10R**) subregions, and this was also the case for Sholl analysis of nodes (overall: p=0.84; dorsal: p=0.56; ventral: p=0.82; **Fig.A10Y-AA**). There was a significant effect for Sholl distance overall and in both subregions (ps<0.0001). Overall nodes were most prevalent 10-70um from the cell body (ps≤0.0004, except 60-70 versus 70-80), in the dorsal DG nodes were most prevalent 10-60um from cell body (ps≤0.0048, except versus 60-70um and 40-50 versus 70-80), and ventrally nodes were most prevalent 10-60um from cell body (ps≤0.0024, except versus 60-70um).

Quantity by branch order did not differ with NRG1 treatment overall (p=0.79; **Fig. 16F**), or in the dorsal (p=0.55; **Fig. A10U**) or ventral (p=0.88; **Fig. A10V**) subregions.

Total dendritic endings did not differ with treatment overall (p=0.73; **Fig. 16K**), or in the dorsal (p=0.33; **Fig. A8AG**) or ventral (p=0.73; **Fig. A8AH**) subregions, and this was also the case for Sholl analysis of endings (overall: p=0.73; dorsal: p=0.49; ventral: p=0.83; **Fig.A10AB-AD**).

Dendritic intersections by Sholl analysis did not differ with NRG1 treatment overall (p=0.66; **Fig. A10AE**), or in the dorsal (p=0.85; **Fig. A10AF**) or ventral (p=0.56; **Fig. A10AG**) subregions.

Total dendritic tortuosity did not differ between NRG1- and saline- treated mice overall (p=0.68; **Fig. A9P**), or in the dorsal (p=0.70; **Fig. A9Q**) or ventral (p=0.85; **Fig. A9R**) subregions.

3.2.3.3 Effects of neuregulin-1 administration on synapse development

NRG1 treatment did not affect overall molecular layer synaptic density averaged across zones (p=0.68; **Fig. 17A**). This was consistent along the dorsal (p=0.42; **Fig. A11A**) and ventral

(p=0.17; **Fig. A11B**) subregions. When analyzing individual zones, there was no significant effect of treatment overall (p=0.68; **Fig. 17B**) or in the dorsal (p=0.42; **Fig. A11C**) or ventral (p=0.17; **Fig. A11D**) subregions.

Examining synapses specifically for immature neurons, treatment with NRG1 did not affect overall dendritic synapse numbers (p=0.43; **Fig. A11E**) or density (p=0.82; **Fig. A11H**). This was also the case for the dorsal (total synapses: p=0.82, **Fig. A11F**; density: p=0.48; **Fig. A11I**) and ventral (total synapses: p=0.53, **Fig. A11G**; density: p=0.19; **Fig. A11J**) subregions.

For branch order synaptic analyses, we analyzed the first four branch orders, as all subjects had cells with dendrites reaching at least this order (barring one subject with fewer dorsal branch orders). Analyzing by overall branch order, NRG1 treatment did not affect number (p=0.43; **Fig. A11K**) or density (p=0.82; **Fig. 17D**) of dendritic synapses, although there were significant effects of branch order (ps<0.0001), with lower numbers of synapses in the first two branches than subsequent two branches (ps≤0.0020) and lower numbers in 1st versus 2nd order branches (p=0.0019), and highest density in the 1st branch order (ps<0.0001). In the dorsal DG, NRG1 did not affect number (p=0.86; **Fig. A10L**) or density (p=0.75; **Fig. A11N**) of synapses, although there were significant effects of branch order (ps<0.0001), with lower numbers of synapses in the 1st branch than subsequent three branches (ps≤0.01), and highest density in the 1st branch order (ps<0.0001). Ventrally, NRG1 did not affect number (p=0.53; **Fig. A10M**) or density (p=0.19; **Fig. A11O**) of synapses, although there were significant effects of branch order (ps<0.0001), with lower numbers of synapses in the 1st branch order versus 3rd and 4th order branches (ps=0.0003), and highest density in the 1st branch order versus subsequent three branch orders (ps≤0.0003).

Somatic synapses did not differ overall (p=0.63; **Fig. A11P**) or in the dorsal (p=0.89; **Fig. A11Q**) or ventral (p=0.59; **Fig. A11R**) subregions; this was also the case for somatic synapse density (overall: p=0.39; dorsal: p=0.67; ventral: p=0.23; **Fig.17F, A11S-T**).

3.2.3.4 Septotemporal characterization of cytogenesis

With treatments pooled, cytogenesis was higher in the ventral DG overall (p=0.0007; **Fig. 18A**), and in the SGZ/GCL (p=0.028; **Fig. A12A**) and hilus (p<0.0001; **Fig. A12B**) specifically. Overall DG cytogenesis was significantly correlated between the dorsal and ventral DG (r=0.47; p=0.041; **Fig. 18B**), and this tended to be the case specifically in the SGZ/GCL (r=0.39; p=0.099; **Fig. 18C**) but not the hilus (r=0.23; p=0.35; **Fig. 18D**).

3.2.3.5 Septotemporal characterization of immature neuronal morphology

Summarized characteristics of dorsal and ventral DG immature neurons are available in **Table 6**. With treatments pooled, cell body perimeter (p=0.027; **Fig. A12C**) and area (p=0.049; **Fig. 19A**) were smaller for immature neurons in the ventral DG. Feret max (p=0.065; **Fig. A12D**), but not feret min (p=0.13; **Fig. A12E**), tended to be larger in the dorsal DG. Cell body aspect ratio (p=0.31; **Fig. A12F**), compactness (p=0.27; **Fig. A12G**), convexity (p>0.99; **Fig. A12H**), form factor (p=0.30; **Fig. A12I**), roundness (p=0.30; **Fig. A12J**), and solidity (p=0.70; **Fig. A12K**) did not differ between subregions.

Overall, dendritic length (p=0.89; **Fig. A12L**), volume (p=0.77; **Fig. A12M**), surface area (p=0.80; **Fig. A12N**), nodes (p=0.49; **Fig. A12O**), endings (p=0.51; **Fig. A12P**), and tortuosity (p=0.95; **Fig. A12Q**) did not differ between subregions.

Dendritic length did not differ between dorsal and ventral subregions by branch order (p=0.87; **Fig. A12R**) or sholl analysis (p=0.85), although length was highest for 2nd and 3rd order branches

(ps≤0.0034) and between 10-80 μm from the cell body (ps<0.0001; **Fig. 19B**). Mean dendritic length differed by branch order (p<0.0001) and was highest for the first three branch orders versus subsequent orders (ps<0.0001; **Fig. A12S**) but did not differ between subregions (p=0.57).

Dendritic volume did not differ by subregion (p=0.95), although volume differed by branch order (p<0.0001) with volume highest in the first three branch orders (ps≤0.0041; **Fig. 19C**). Dendritic mean volume did not differ by subregion (p=0.93), although mean volume differed by branch order (p<0.0001) and was higher in the first two branch orders than subsequent orders (ps≤0.036; **Fig. A12T**).

Dendritic surface area did not differ by subregion (p=0.73), although area was significantly higher in the first three branch orders (ps<0.0001; **Fig. A12U**). Dendritic mean surface area did not differ by subregion (p=0.64), but was significantly higher in the first three branch orders (ps≤0.0020; **Fig. A12V**).

There was a tendency for number of branches to be highest in the ventral DG (p=0.092), and they were highest in the 2nd and 3rd branch orders (ps≤0.0001; **Fig. A12W**).

Number of nodes tended to be higher ventrally (p=0.084), and were highest in the first three branch orders (ps≤0.0001) and from 10-60 μm from the cell body (ps≤0.013, except 40-50 vs 60-70: p=0.48; **Fig. 19D**).

For dendritic intersections, dorsal and ventral subregions did not differ by Sholl analysis (p=0.90), and intersections were more common 10-80 μm from the cell body than at other distances (ps≤0.0061; **Fig. 19E**).

For dendritic endings, Sholl analysis revealed an interaction between subregion and Sholl distance ($p=0.0013$); specifically, immature neurons in the ventral DG had significantly more endings 60-70 μm from the cell body ($p=0.0002$; **Fig. 19F**).

3.2.3.6 Septotemporal characterization of synaptic distribution

With treatments pooled, overall molecular layer synapses did not differ between the dorsal and ventral DG ($p=0.84$; **Fig. 20A**). However, there was heterogeneity in synaptic density in specific zones around the molecular layer (**Fig. 17B**). Specifically, overall synaptic density varied by zone ($p<0.0001$), with the highest density in the suprapyramidal basal zone compared to the suprapyramidal apical ($p=0.0005$), infrapyramidal apical ($p<0.0001$), and infrapyramidal basal ($p=0.039$) zones. Suprapyramidal apical density was higher than infrapyramidal apical density ($p=0.0004$), and infrapyramidal basal density was higher than that of the infrapyramidal apical zone ($p<0.0001$).

Notably, these distinctions are largely preserved across the septotemporal axis. In the dorsal DG, the highest density was in the suprapyramidal basal zone compared to the suprapyramidal apical ($p=0.0045$), and infrapyramidal apical ($p<0.0001$) zones, but not the infrapyramidal basal zone ($p=0.26$; **Fig. A11C**). Infrapyramidal apical density was also lower than suprapyramidal apical ($p=0.011$) and infrapyramidal basal density ($p<0.0001$). Ventrally, the highest density was in the suprapyramidal basal zone compared to the suprapyramidal apical $p=0.0086$), infrapyramidal apical ($p<0.0001$), and infrapyramidal basal zones ($p=0.037$; **Fig. A11D**). Infrapyramidal apical density was also lower than suprapyramidal apical ($p=0.0032$) and infrapyramidal basal density ($p=0.0004$).

Pooled overall number of dendritic synapses per immature neuron did not differ between the dorsal and ventral DG ($p>0.99$; **Fig. A13A**), and this was maintained when controlling for dendritic length ($p=0.71$; **Fig. A13B**). Analyzing by branch order, there were no overall septotemporal differences for number ($p=0.81$; **Fig. A13C**) or density ($p=0.97$; **Fig. 20D**) of dendritic synapses; however, there were significant main effects for branch order for synapse number and density ($ps<0.0001$). Specifically, first-order synapses were less abundant than second- ($p=0.0065$), third- ($p<0.0001$), and fourth-order synapses ($p<0.0001$), and second-order synapses were less abundant than third- ($p=0.0022$) and fourth-order ($p=0.0068$) synapses. Conversely, when correcting for branch-order length, first-order synaptic density was higher than that of other branch-orders ($ps<0.0001$).

Number of somatic synapses did not differ by subregion ($p=0.22$; **Fig. A13D**), but ventral DG immature neurons showed increased somatic synapse density ($p=0.028$; **Fig. 20C**).

3.2.4 DISCUSSION

Our results suggest that NRG1β does not affect intermediate stages of neurogenesis in immature adultborn DG neurons. This indicates that the influence of NRG1 on hippocampal neurogenesis is temporally- and subregionally-specific, with effects only for proliferation in the ventral DG leading to neurogenic increases in this same subregion (**Table 5**). Furthermore, this NRG1-mediated increase in neurogenesis is accompanied, four weeks after treatment cessation, by antidepressant-like effects (Mahar et al., 2011). The current results suggest that to the extent that changes in ventral DG neurogenesis underlie this change in emotion-related behaviour four weeks after NRG1 administration, it is changes to cell proliferation alone (and subsequent

increases in immature functional neurons four weeks after proliferation), as opposed to additional contributions from other neurogenic stages, that contribute to this phenomenon.

The lack of effect of NRG1β administration on dendritogenesis or synaptogenesis may seem counter-intuitive, as previous *in vitro* studies examining cortical interneurons have reported increased dendritogenesis and synaptogenesis following NRG1β administration (Cahill et al., 2012; Ting et al., 2011). However, this appeared to be contingent upon ErbB4 expression (Cahill et al., 2012; Ting et al., 2011). Newborn granule cells in the adult DG lack ErbB4 but express ErbB3 (Mahar et al., 2011), suggesting that NRG1-ErbB4 signaling can modulate dendritogenesis and synaptogenesis, whereas NRG1-ErbB3 signaling is involved in cell proliferation. Indeed, NRG1-ErbB3 signaling not only seems to stimulate the proliferation of neuronal precursors in the mature hippocampus (Mahar et al., 2011) but also stimulates proliferation in cancer cell lines (Andrique et al., 2012). In support of this, Ting et al. (2011) found that neither size nor number of synapses made by glutamatergic neurons, which have been shown to largely or completely lack ErbB4 (Bean et al., 2014; Chen et al., 2010; Neddens et al., 2011; Vullhorst et al., 2009) were affected by NRG1β administration.

Comparison of the specificity of hippocampal neurogenic effects following NRG1 administration with other neurotrophic factors is partially confounded by incomplete characterization of these factors' effects along the neurogenic timeline from proliferation to functionality. However, it has been shown that BDNF administration increases cell survival but not proliferation (Schmidt and Duman, 2010), IGF administration increases proliferation and neuronal differentiation (Aberg et al., 2000), and VEGF increases proliferation but not survival (Fournier et al., 2012; Jin et al., 2002). These results highlight the potential specificity of neurogenic effects of neurotrophic factors, and underscore the importance of assessing at which particular neurogenic stages pro-

neurogenic neurotrophic factors induce their effects.

To our knowledge, we also provide the first characterization of parameters that define and distinguish immature neurons in the dorsal and ventral DG. These include differences between these subregions in cell body size, somatic synapses, and dendritic branching. Although associating the differences observed between these particular immature neuronal populations with the distinct behavioural functions associated with either the dorsal or ventral hippocampus is beyond the scope of this study, these differences likely contribute to neuronal function. The smaller cell body size in ventral DG immature neurons is plausibly related to the previously-established maturational distinction between dorsal and ventral immature neurons, in which ventral DG immature neurons appear to mature more slowly with a wider window of excitability (Piatti et al., 2011; Snyder et al., 2012; Tanti et al., 2013). The observed increase in somatic synapse density for ventral DG immature neurons is likely due to this reduction in cell body size, as the total number of somatic synapses per neuron did not differ by DG subregion. Interestingly, the increase in terminal dendritic branches in ventral DG immature neurons occurred 60-70μm from the cell body, which roughly corresponds to the point at which developing dendrites emerge from the granule cell layer into the molecular layer, selectively receiving input from mossy cells (Buckmaster et al., 1992; Scheff and Price, 1998). This suggests that immature neurons in the ventral DG receive comparatively more input from neighboring mossy cells.

We find that cytogenesis, as determined by the number of BrdU-IR cells that proliferated prior to treatment and survived until the animal was sacrificed, was consistently higher in the ventral DG. Previous studies have found that, after water maze training, cell proliferation and neurogenesis were higher in the dorsal than ventral DG (Snyder et al., 2009b; Snyder et al., 2011a). However, these studies examined density of neurogenic cells by volume, as opposed to the absolute

number of cells as analyzed here, and thus this discrepancy may be explained by the volumetric difference between the dorsal and ventral DG. Consequently, it may be that the density of new cells is lower in the ventral DG, whereas overall cytogenesis as measured by the total number of adultborn cells is higher in this region, as reported here. This cytogenic heterogeneity along the septotemporal axis suggests that cytogenic assessments in which cell counts are averaged across the septotemporal axis may be less accurate than those assessing septotemporal subregions separately. However, we find that dorsal and ventral DG cytogenesis are significantly correlated, supporting the notion that a common set of factors regulates this dynamic phenomenon in both regions.

In addition, we identified heterogeneity in dendritic synaptic density in the molecular layer that was maintained along the septotemporal axis; specifically, density was generally highest in the suprapyramidal basal zone and lowest in the infrapyramidal apical zone. This result indicates for the first time an unequal distribution in granule cell synapses in the molecular layer, and suggests that the resulting synaptic profile of adultborn neurons may be contingent on their precise position of integration within the granule cell layer.

Together, these results further characterize the effects of exogenous NRG1 administration on hippocampal neurogenesis across neurogenic stages. They indicate that the neurogenic influence of NRG1 is temporally and subregionally restricted to increases in ventral DG cell proliferation and resulting increases in the number of functional immature DG neurons. In addition, we also determined that the morphology and connectivity of immature neurons differs along the septotemporal axis of the DG, and hypothesize that the specificity of these subregional features may contribute to the functional differences that exist between the dorsal and ventral DG.

Acknowledgments

This research was supported by CIHR (MOP-111022) and NSERC Discovery (341479-07) and RTI (345952-07) grants to NM. NM is a FRQS Chercheur-bouriser Junior 2 and CIHR New Investigator, AM was supported by NSERC, and IM was supported by FRQS. We acknowledge the Cell Imaging and Analysis Network at McGill University for use of their confocal microscopy facilities, Chris Salmon for valuable consultation during experimental optimization, and Sarah Bulin and Susana G. Torres-Platas for manuscript figure advice. The authors declare that they have no financial conflict of interest related to the content of this manuscript.

FIGURES:

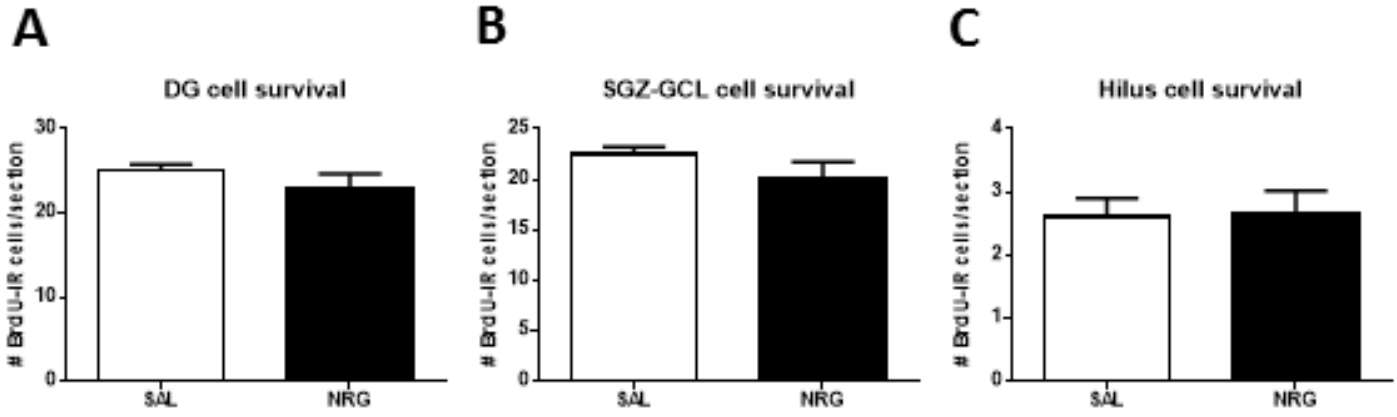


Figure 15. Effects of neuregulin-1 administration on cell survival. Cell survival is not affected by neuregulin (NRG) administration in the overall DG (A), or specifically in the subgranular zone (SGZ) and granule cell layer (GCL) (B) or hilus (C). SAL, saline.

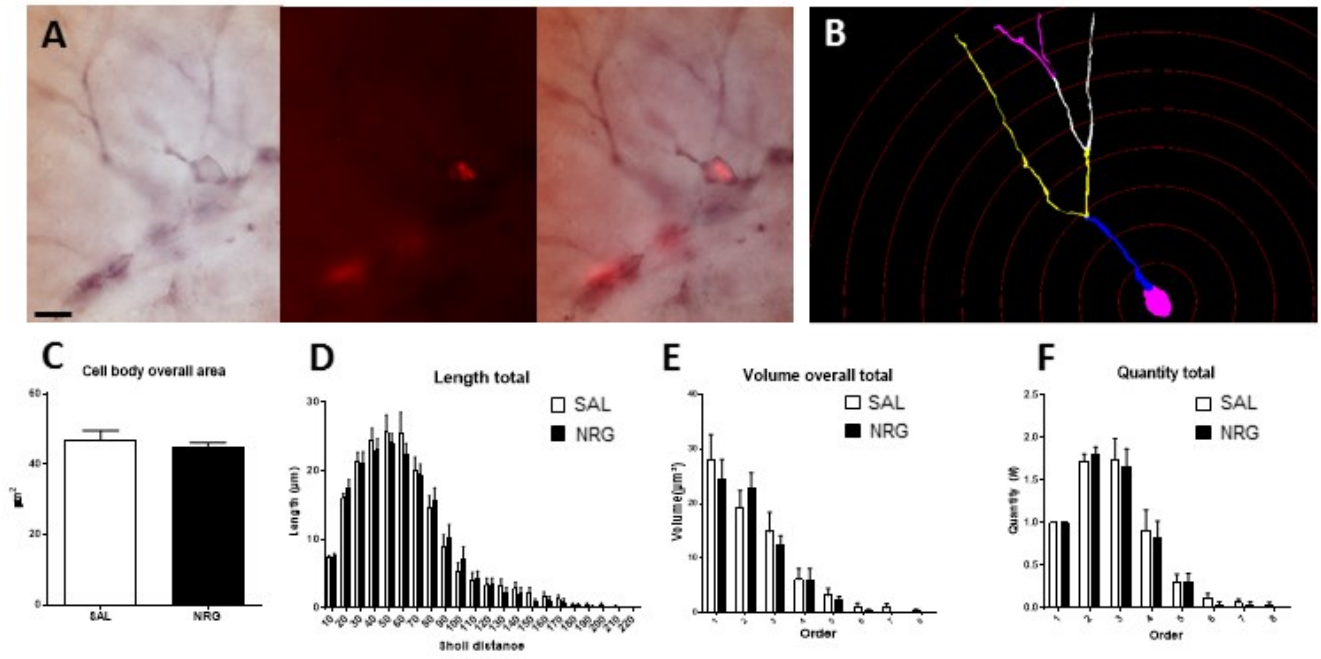


Figure 16. Effects of neuregulin-1 administration on dendritic morphological development. **A**, Immature neuron labeled with DCX (**left**), and BrdU (**middle**) in the same field, with an overlay at **right**. **B**, example reconstructed neuron showing dendritic branch orders and Sholl distances (10 µm). NRG1 administration did not affect immature neuron morphology, including cell body size (C**),** dendritic length (**D**), dendritic volume (**E**), and number of dendritic branches (**F**). Scale bar = 10 µm.

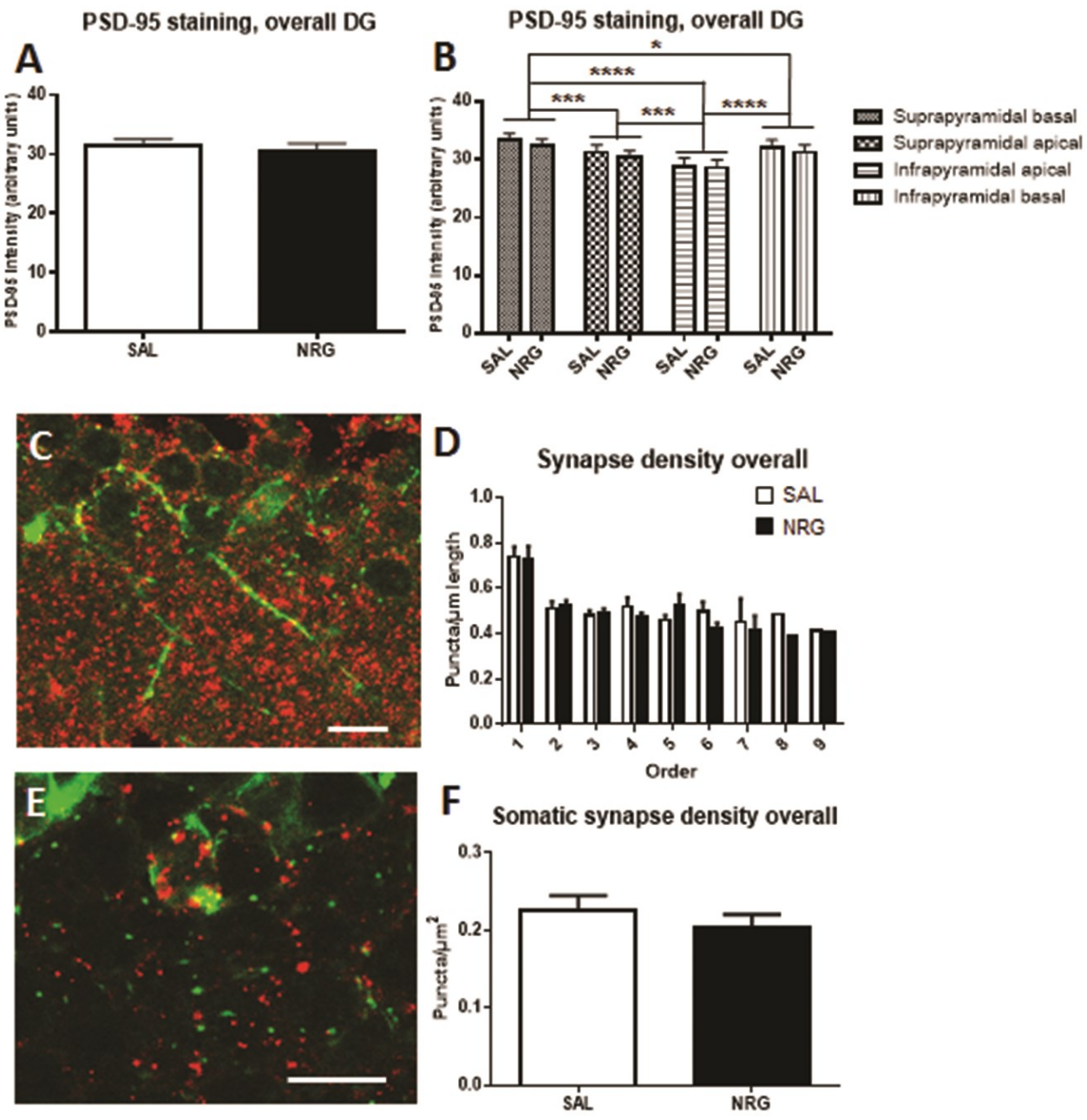


Figure 17. Effects of neuregulin-1 administration on synapse development. Overall synaptic density in the molecular layer surrounding the DG was not affected by NRG1 administration (A), and this was also the case in analyzing particular zones adjacent to various aspects of the DG (B,

although there was heterogeneity in synaptic density between these zones. C, dendritic synaptic labeling by doublecortin (DCX; **green**) and postsynaptic density protein 95 (PSD-95; **red**) staining in BrdU-identified immature neurons. D, Dendritic synaptic density was unaffected by NRG1 administration. E, somatic synaptic labeling by DCX (green) and PSD-95 (red) staining in BrdU-identified immature neurons. Somatic synapse density was unaffected by NRG1 administration (F). Single-channel images of the planes in **C** and **E** are shown in **Fig. A5**. Scale bar = 10 μm. **, $p \leq 0.01$; ****, $p \leq 0.0001$.

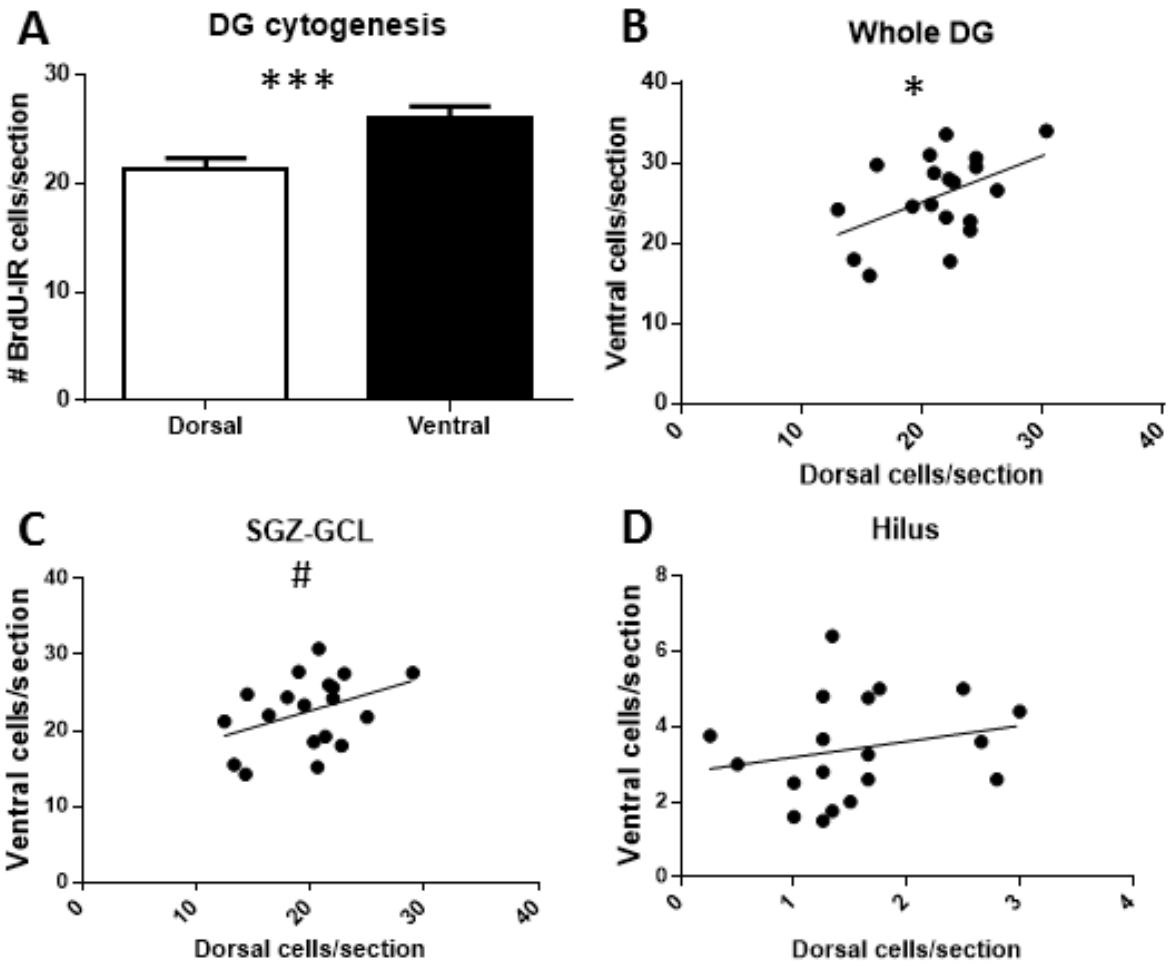


Figure 18. Septotemporal characterization of cytogenesis. A, cytogenesis was higher in the ventral DG than in the dorsal DG. Cytogenesis in the subregions correlated significantly for the overall DG (B), and tended to correlate specifically in the SGZ-GCL (C) but not the hilus (D). #, $p<0.1$; *, $p\leq0.05$; **, $p\leq0.01$; ***, $p\leq0.001$. BrdU, bromodeoxyuridine.

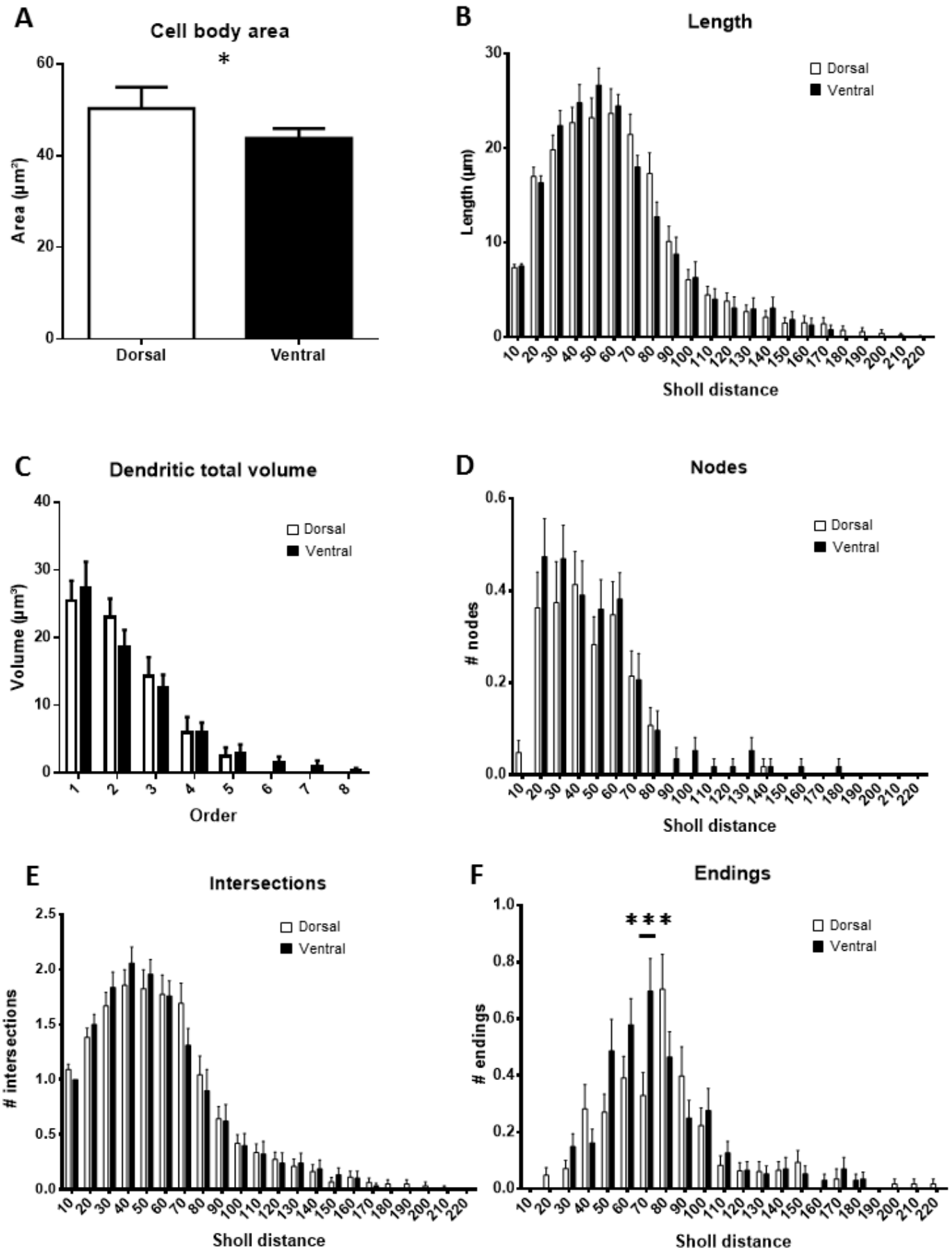

A
Cell body area
*
Area (µm²)
60
40
20
0
Dorsal
Ventral
B
Length
Dorsal
Ventral
Length (µm)
30
20
10
0
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220
Sholl distance
C
Dendritic total volume
Dorsal
Ventral
Volume (µm³)
40
30
20
10
0
1 2 3 4 5 6 7 8
Order
D
Nodes
Dorsal
Ventral
nodes
0.6
0.4
0.2
0.0
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220
Sholl distance
E
Intersections
Dorsal
Ventral
intersections
2.5
2.0
1.5
1.0
0.5
0.0
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220
Sholl distance
F
Endings
***
Dorsal
Ventral
endings
1.0
0.8
0.6
0.4
0.2
0.0
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220
Sholl distance

Figure 19. Septotemporal characterization of immature neuronal morphology. **A**, cell body size was decreased for immature neurons in the ventral DG versus the dorsal DG. Dendritic length (**B**), volume (**C**), nodes (**D**), and intersections (**E**) did not differ for immature neurons between septotemporal subregions, although terminal dendritic endings were increased for ventral DG immature neurons (**F**) specifically 60-70 μm from the cell body.

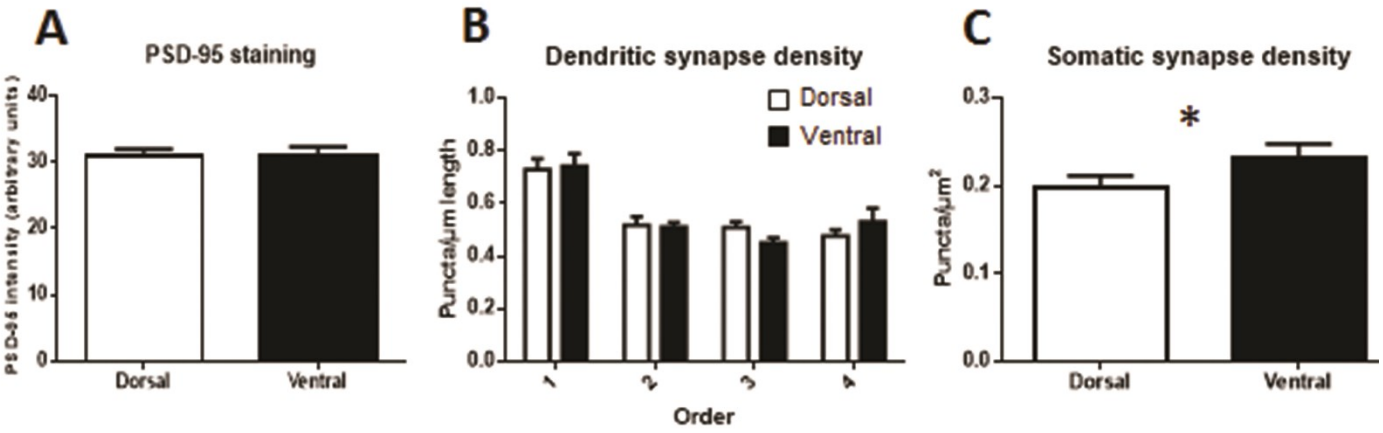


Figure 20. Septotemporal characterization of synaptic distribution. **A**, Synaptic density in the molecular layer surrounding the DG does not differ between DG subregions along the septotemporal axis. **B**, Dendritic synapse density does not differ for immature neurons in the dorsal versus ventral DG; overall, density is highest in the first dendritic branch. **C**, Somatic synapse density is higher for immature neurons in the ventral DG. *, $p \leq 0.05$.

STAGE	OVERALL	DORSAL	VENTRAL
PROLIFERATION	↑	X	↑
DIFFERENTIATION	X	X	X
SURVIVAL	X	X	X
CELL BODY MORPHOLOGY	X	X	X
DENDRITIC MORPHOLOGY	X	X	X
SYNAPSE DEVELOPMENT	X	X	X
OVERALL NEUROGENESIS	↑	X	↑

Table 5. Specificity of effects of neuregulin-1 on adult hippocampal neurogenesis. Neuregulin-1 has temporally and subregionally specific neurogenic effects, wherein cell proliferation and overall neurogenesis are increased selectively in the ventral DG following administration (Mahar et al., 2011), and other neurogenic features (including neuronal differentiation (Mahar et al., 2011), cell survival, cell body and dendritic morphology, and synapse formation) in immature neurons are unaffected.

Immature neuronal parameters		
	Dorsal	**Ventral**
Cell body area	*50.28 ± 4.6 μm*	*43.81 ± 2.1 μm*
Dendritic length	191.5 ± 15.16 μm	182.7 ± 11.31 μm
Dendritic surface area	364.9 ± 35.39 μm^2	338.5 ± 28.34 μm^2
Dendritic volume	73.14 ± 7.89 μm^3	69.27 ± 8.49 μm^3
Dendritic nodes	2.31 ± 0.25	2.49 ± 0.23
Highest length	2^{nd} and 3^{rd} branch orders	2^{nd} and 3^{rd} branch orders
Nodes	Most prevalent 10-60 μm from the cell body	Most prevalent 10-60 μm from the cell body
Dendritic endings	*Fewer 60-70 μm from the cell body*	*More 60-70 μm from the cell body*
Dendritic synapses	Lowest (but most dense) in 1^{st} branch	Lowest (but most dense) in 1^{st} branch
Somatic synapse density	*Decreased versus ventral*	*Increased versus dorsal*
Overall differentiating features	**Larger cell body; decreased somatic synapse density; fewer terminal dendritic endings 60-70 μm from the cell body**	**Smaller cell body; increased somatic synapse density; more terminal dendritic endings 60-70 μm from the cell body**

Table 6. Parameters of immature neurons in the dorsal and ventral DG. Statistically significant differences between immature neurons in the dorsal and ventral DG subregions are shown in italics.

Supplemental materials are available in Appendix.

CHAPTER 4. Disrupted hippocampal neuregulin-1/ErbB3 signaling and dentate gyrus granule cell alterations in suicide

4.1 Preamble

The subsequent Chapter details the majority of the remainder of the experiments conducted during the course of my PhD, and as such is possibly the broadest in terms of scope. Having to this point established that NRG1 administration has temporally and subregionally specific neurogenic effects and concurrent antidepressant-like effects, we aimed to determine if the antithesis of this would be true in psychiatrically relevant populations. More specifically, we hypothesized that depression or suicidality may be associated with disrupted NRG1-ErbB3 signaling and decreased numbers of DG granule cell neurons, as the product of hippocampal neurogenesis. Additionally, we sought to support the role of ErbB3 in the effects of NRG1 in hippocampal function and affective regulation.

The first experiment presented attempted to support ErbB3's mechanistic role in proliferation acutely after NRG1 administration, first hypothesized in our 2011 PLoS ONE publication. We recapitulated the NRG1 administration paradigm from this paper, then dissected out the DG and examined ErbB3 phosphorylation. Strikingly, ErbB3 was phosphorylated in the DG, but only in the ventral DG, mirroring our earlier studies in which this NRG1 administration paradigm only induced cell proliferation in the ventral DG. As ErbB3 is the only receptor to bind NRG1 that is present in these cells (Mahar et al., 2011), this further supports the mechanistic role of ErbB3 in mediating the effects of NRG1 on hippocampal plasticity.

The next experiment presented asks, “If increasing hippocampal NRG1-ErbB3 signaling has antidepressant-like effects, would hippocampal ErbB3 be deleteriously affected in a model of depression?” To this end, we used the social defeat model, a chronic stress paradigm that has been strongly validated as a model of depression (Golden et al., 2011). Following this paradigm, DG tissue was removed by micropunches, RNA was extracted, and we quantified ErbB3 expression. ErbB3 was indeed reduced across the DG; this pattern of ErbB3 reduction, as opposed to DG subregionally-specific alteration, is in line with previous studies showing neurogenic effects of stress that extend across the septotemporal axis (Tanti et al., 2013).

Subsequently, we examined whether NRG1-ErbB3 signaling was disrupted in psychiatrically relevant populations. This was split into two investigations: peripherally, as NRG1 is expressed in blood and can readily cross the blood-brain barrier (Kastin et al., 2004; Rosler et al., 2011), and centrally in the hippocampus, based on the aforementioned animal studies revealing NRG1’s effects on hippocampal plasticity and affect.

The peripheral investigation, in MDD patients and controls before and after antidepressant treatment, revealed that NRG1 levels in blood are not altered in MDD patients with respect to controls, either before or after antidepressant treatment. Additionally, examining by patient response to treatment did reveal differences in NRG1 expression. These experiments suggest that the level of peripheral NRG1 expression is not etiologically related to MDD, nor is it predictive or determinant of response in antidepressant medication in MDD. Thus, to the extent that NRG1-ErbB signaling may be disrupted in psychopathology, this disruption would likely occur centrally as opposed to peripherally.

In the hippocampus, we found that ErbB3, but not other ErbB receptors or NRG1, was sharply reduced for suicide completers in comparison to controls. Although this supported our initial

hypotheses, it also raised the question of the cause of this deficit. We thus examined whether ErbB3 methylation might explain the decrease in hippocampal ErbB3 expression. However, methylation was not altered between suicides and controls, although we did identify a cluster of CpGs in the ErbB3 gene that were hypermethylated with antidepressant treatment.

Finally, as ErbB3 is expressed by neurogenic cells and mature granule cell neurons (Mahar et al., 2011), we examined whether the decreased ErbB3 expression may be due to decreased numbers of granule cells in the DG. Using a stringent stereological protocol including the most detailed human DG tracings performed to date, we determined that in the anterior DG (analogous to the ventral DG in rodents) the number of granule cells was sharply reduced in unmedicated suicides, with a concordant reduction in DG volume, and that granule cell bodies were hypertrophied in the posterior DG. These results not only provide a mechanistic explanation for the decreased ErbB3 in the mRNA studies, but also identify novel suicidal endophenotypes and support hippocampal NRG1-ErbB3 signaling (particularly in the anterior DG) as a candidate pathway in affective regulation and psychopathology.

4.2 Disrupted hippocampal neuregulin-1/ErbB3 signaling and dentate gyrus granule cell alterations in suicide

Ian Mahar[1,2], Benoit Labonte[1,2], Juan Pablo Lopez[1,3], Sandra Yogendran[1,2], Elsa Isingrini[2], Lea Perret[2], Maria Antonietta Davoli[1], Calvin Qiang[1], Adeline Rachalski[1], Bruno Giros[2,4], Gustavo Turecki[1,2,3,4], Naguib Mechawar[1,2,4]*

[1]*McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada.*

[2]*Integrated Program in Neuroscience, McGill University, Montreal, Quebec, Canada.*

[3]*Department of Human Genetics, McGill University, Montreal, Quebec, Canada.*

[4]*Department of Psychiatry, McGill University, Montreal, Quebec, Canada.*

***Corresponding author**

ABSTRACT

Neuregulin-1 (NRG1) and ErbB3 have been associated with psychopathology, and NRG1-ErbB3 signaling has been shown to increase hippocampal neurogenesis and induce antidepressant-like effects. We aimed to determine whether deficits in NRG1 or ErbB3 might be present in an animal model of depression or in human psychopathological samples. We found that DG ErbB3 expression is decreased in an animal model of depression, and that unmedicated suicides show

sharply decreased hippocampal ErbB3 expression and decreased numbers of granule cell neurons in the hippocampal DG (reversed by antidepressant treatment), which express ErbB3. We also found alterations in anterior DG glial cells and cell body morphology in posterior DG granule cells. These results reveal novel suicidal endophenotypes, as well as a putative etiological mechanism underlying suicidality, and suggest that antidepressant or NRG1 treatment may reverse the deficit in DG granule cell neurons observed in suicidal individuals.

4.2.1 Introduction

Suicide has become the leading cause of injury mortality in the United States (Rockett et al., 2012). However, our understanding of the etiology of suicidality remains limited. Although studies have reported genetic and epigenetic associations with suicidality (see (Turecki, 2014) for review), a clear putative etiological candidate mechanism has yet to emerge.

Neuregulin-1 (NRG1) has repeatedly been associated with psychopathology in multiple human populations as well as in animal models. Genetic association studies have linked NRG1 with schizophrenia, MDD, and bipolar disorder (Georgieva et al., 2008; Moon et al., 2011; Rethelyi et al., 2010; Stefansson et al., 2003). Post-mortem investigations have shown altered NRG1 cleavage in the PFC in schizophrenia as well as decreased density of NRG1-expressing neurons in schizophrenia and depression, and reduced hippocampal NRG1 expression in schizophrenia and bipolar disorder (Bertram et al., 2007; Marballi et al., 2012). In animal studies, altering NRG1 activity changes behaviour in tasks modeling schizophrenia, anxiety, and antidepressant response HPA axis response to stress (Bi et al., 2015; Chen et al., 2008; Chohan et al., 2014; Mahar et al., 2011; O'Leary et al., 2014; O'Tuathaigh et al., 2010; Pei et al., 2014). Notably,

NRG1 has been shown to readily cross the blood-brain barrier and affect brain activity (Carlsson et al., 2011; Kastin et al., 2004; Xu et al., 2006; Xu et al., 2004).

The NRG1 receptor ErbB3 has also been associated with multiple psychiatric conditions (Aston et al., 2004, 2005; Le-Niculescu et al., 2009; Li et al., 2009; Milanesi et al., 2012). Although previously considered to be kinase-insufficient (c.f. Cao et al., 2007) based largely on a report of reduced autophosphorylation compared to EGFR in insect cells with induced ErbB3 expression (Guy et al., 1994), recent studies have suggested that ErbB3 has sufficient kinase activity for signaling (Deng et al., 2013; Shi et al., 2010a; Steinkamp et al., 2014), and its downstream signaling primarily involves PI3K/Akt (Cook et al., 2011; Jung et al., 2006; Lee et al., 2014b; Sathyamurthy et al., 2015; Smirnova et al., 2012; Young et al., 2013). ErbB3 has also recently been found to be required for certain types of cell proliferation in the brain, likely due to downstream PI3K signaling (Sathyamurthy et al., 2015).

The process of adult hippocampal neurogenesis, which adds new granule cell neurons to the DG throughout life, has been strongly implicated in the mechanism of antidepressants, response to stress, and affective behaviour (Mahar et al., 2014). In addition, deficits in hippocampal neurogenesis may precipitate vulnerability to stress and psychopathology (Mahar et al., 2014). As a result, the investigation of the human DG and granule cells has become a rapidly developing field, with the few studies to date in psychiatric populations providing interesting psychopathological insight. Recent post-mortem studies from Boldrini and colleagues have found that antidepressants increased numbers of neural progenitor cells in depressed patients, and that granule cells were decreased in untreated MDD patients in the anterior and mid DG (Boldrini et al., 2014; Boldrini et al., 2013; Boldrini et al., 2009). Conversely, a recent report found no difference in the number of granule cells between MDD patients and controls, but

reported an increase in granule cells with age for individuals taking antidepressants, supporting the hypothesis that these therapeutics have a positive effect on granule cell number through increased neurogenesis (Cobb et al., 2013). However, these few studies did not directly compare granule cell numbers between suicide completers and controls.

Previously, we have shown that peripheral NRG1 administration has temporally- and subregionally-specific effects on neurogenesis and affective behaviour. Specifically, NRG1 administration in mice rapidly induces cell proliferation in the ventral DG (but not acute behavioural effects), leading to increased ventral DG neurogenesis (Mahar et al., 2011). This is accompanied by antidepressant-like behaviour at a time at which these cells are functional hyperplastic neurons (Ge et al., 2007; Mahar et al., 2011). These neurogenic effects are restricted to this subregion and also these neurogenic stages, as NRG1 administration does not affect the differentiation or survival of neuronal precursors (Mahar et al., 2011). The neurogenic and affective effects of NRG1 may be mediated through the activation of ErbB3 expressed by neurogenic cells from division to functional maturity. ErbB4, the other primary NRG1 receptor which the ligand binds to directly, was not observed in early neurogenic cells and is potentially limited to GABAergic interneurons in the hippocampus (Bean et al., 2014; Mahar et al., 2011; Neddens and Buonanno, 2009 (although see also Mechawar et al., 2007; Pitcher et al., 2011).

Although increasing NRG1 has neurogenic and antidepressant-like effects, it is unclear whether the converse to these effects may also be true, whereby deficits in NRG1-ErbB3 signaling would be associated with psychopathology. Here, we hypothesized that hippocampal ErbB3 would be disrupted in an animal model of depression, and that NRG1-ErbB3 signaling would be decreased in affective psychopathological populations. We show that peripheral NRG1 activates ErbB3 specifically in the ventral DG in mice, that ErbB3 is ubiquitously expressed in DG granule cells

in humans as in mice, that hippocampal ErbB3 is disrupted in an animal model of depression, and that hippocampal (but not peripheral) NRG1-ErbB3 signaling is disrupted in affective psychopathology. This disruption is commensurate with a deficit in granule cell neurons in the DG that is reversible with antidepressant treatment.

4.2.2 Methods and materials

4.2.2.1 NRG1 administration

Adult (approximately P60) male C57Bl/6 mice (n=8/group; Charles River, QC) were housed in groups until experimentation, with *ad libitum* access to food and water on a 12:12 light:dark cycle. All animal experiments were in accordance with the guidelines and policies of the Canadian Council on Animal Care, and were approved by McGill University's Animal Care Committee. Mice were administered either sterile 0.9% saline or recombinant NRG1β (R&D; EGF domain, 10 μg/d) dissolved in saline for 24 h through subcutaneously implanted osmotic mini-pumps (Alzet), as performed previously (Mahar et al., 2011). Post-operatively, mice were placed on a heating pad to recover and were monitored for complications, and received an anti-inflammatory Carprofen tablet placed in the cage.

4.2.2.2 Mouse dentate gyrus dissection and protein extraction

Following NRG1 or saline administration, mice were deeply anesthetized with isoflurane and decapitated. Brains were rapidly removed and placed in cold PBS under a dissecting microscope. DGs were dissected based on a previously established DG isolation procedure (Hagihara et al.,

2009). Dissected DGs were then separated into dorsal and ventral segments, corresponding to previously established designations (Mahar et al., 2011), and stored at -80°C. Protein was digested in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors, then sonicated and centrifuged at 13200 RPM for 20 min at 4°C. The supernatant was removed, vortexed, aliquoted, and stored at -20°C, and protein verified with a BSA protein assay kit (Pierce) and plate reader at 562 nm.

4.2.2.3 Fluorescence-Assisted Cell Sorting

To validate DG dissections, parallel DG dissections were performed in adult male transgenic mice expressing GFP at the nestin promoter (nestin-GFP (Mahar et al., 2011; Mignone et al., 2004)) on a C57Bl/6 background and non-transgenic littermates, in a manner mimicking the mice used in NRG1/saline administration experiments. DG tissue was digested, triturated, and processed following a previously established cell isolation procedure (Brewer and Torricelli, 2007), and neuronal gradient fraction cells were sorted on a FACSAria machine (BD Biosciences). Fluorescence phenotype of sorted cells was subsequently verified on an Olympus BX51 microscope.

4.2.2.4 pErbB3 quantification

ErbB3 phosphorylation was assessed using a pErbB3 ELISA kit (RayBiotech). Briefly, dorsal and ventral samples from subjects were loaded onto wells of the kit's 96-well plate, with subsequent manufacturers' instructions observed. Samples were read on a SpectraMax plate reader (Molecular Devices) at 450 nm. To verify and correct for protein concentration, samples

were loaded onto 4-12% polyacrylamide gels (Invitrogen), transferred to nitrocellulose membrane (Amersham GE Healthcare), treated with Ponceau (Sigma) and visualized using a charge-coupled device camera and Image Studio Lite software (Licor). Values were normalized to control samples.

4.2.2.5 Chronic social defeat stress

Adult (approximately P60) male C57Bl/6 mice (Charles River, QC) were subjected to social defeat stress following a previously established protocol (Golden et al., 2011). Briefly, animals were pair-housed in identical conditions to the previous animal experiments, and defeated mice (n=13 were exposed to daily 5 min physical interactions with an aggressive male CD1 mouse (approximately 60; Charles River, QC) during 10 consecutive days. CD1 mice were otherwise housed individually and had been previously screened for 3 days to verify aggressive behaviour. Defeated mice remained in the home cage of the aggressor mouse for 24 h post-defeat, separated physically by a perforated plexiglass divider, and were rotated between CD1 aggressors for each session. Control undefeated mice (n=7) were pair-housed, separated by a plexiglass divider. Animals were decapitated, and brains were removed, frozen in -40°C isopentane, and stored at -80°C.

4.2.2.6 DG micropunches

Brains of stressed and control mice were placed in a cryostat at -20°C, and separated into serial 1 mm sections using a mouse brain matrix and razor blades. Micropunches (0.5 mm in diameter)

were obtained from both the dorsal and ventral DG bilaterally, and stored at -80°C prior to RNA extraction.

4.2.2.7 Quantitative RT-PCR for rodent DG

RNA was extracted from micropunches using RNeasy Micro (Qiagen) kits with elution in RNAse-free water. RNA was assessed using a Nanodrop 1000 system (Thermo Scientific). cDNA was synthesized with oligo dT primers. mRNA was measured by quantitative RT-PCR (qRT-PCR) (Life Technologies; 7900HD) using TaqMan probes (Life Technologies; Mm01159999_m1, Mm99999915_g1) for ErbB3 and GAPDH, including water and RNA-free control wells that did not give a positive signal. Expression values as shown are mean relative fold change quantities of sample replicates normalized to GAPDH.

4.2.2.8 ErbB3 immunohistochemistry and *in situ* hybridization in human DG

All steps were at room temperature unless otherwise specified. Washes in wash buffer (Dako) were performed between all steps except between blocking and addition of primary antibody. Based on a previously established ErbB3 immunohistochemistry protocol (Mahar et al, 2011), paraffin-embedded tissue from two control subjects (a 60 year-old male with a post-mortem interval of 7.25 h, and a 74 year-old female with a post-mortem interval of 11 h) was dewaxed and rehydrated in xylene, decreasing concentrations of ethanol, and ddH2O. Tissue was then incubated in a proteinase-K solution (1:1000; Qiagen) for 10 min, PBS-T for 1 h, 3% H_2O_2 for 10 min, blocking solution (2% NGS (Vector) in wash buffer) for 1 h. This was followed by an overnight incubation at 4°C in blocking solution with rabbit anti-ErbB3 antibody (1:25 and

1:100, Santa Cruz), an antibody that has been validated previously (Jackson-Fisher et al., 2008; Lee et al., 2009; Mahar et al., 2011). After primary antibody removal, tissue was incubated in biotinylated goat anti-rabbit secondary antibody (1:500; Vector) in blocking solution for 90 min, and labeling was revealed using the Vectastain Elite and DAB kits (both Vector), quenched in ddH_2O for 5 min, dehydrated in increasing ethanol concentrations followed by xylene, then coverslipped using Permount (Fisher). A no-primary control condition did not produce staining. Sections were visualized on an Olympus BX51 microscope using MBF software. Additional human ErbB3 immunohistochemistry data were obtained from the Human Protein Atlas for a rabbit anti-ErbB3 antibody (HPA045396) with a demonstrated high degree of specificity (Human Protein Atlas, 2015; Uhlen et al., 2015). ErbB3 *in situ* hybridization data were obtained from the Allen Human Brain Atlas (Allen Institute for Brain Science, 2014; Hawrylycz et al., 2012). Specifically, *in situ* hybridization data were obtained from http://human.brain-map.org/ish/search, and microarray data were obtained from http://human.brain-map.org/microarray/search. All images were unaltered except for overall brightness or contrast.

4.2.2.9 MDD blood collection and qRT-PCR

Human blood samples were collected and processed as previously described (Lopez et al., 2014). Briefly, blood samples and Hamilton Depression Scale (HAM-D) scores were obtained from non-depressed control and medication-naive MDD patients at the Douglas Mental Health University Institute in Verdun, Quebec, at two time points; baseline (T0) and 8 weeks later (T8). Patients were referred to the study by a clinician, and received antidepressant treatment with citalopram (10-60 mg/day titrated progressively) between T0 and T8. Blood samples were collected, and RNA extracted, using PAXgene tubes/kits (PreAnalytiX/QIAGEN). Gene

expression was quantified by qRT-PCR using TaqMan probes (Hs00247620_m1, cat. #4310884E). Subject information for this and other human studies is available in **Appendix Table A1**. Controls were classified as not diagnosed primarily with a non-substance psychiatric disorder for all experiments, and did not die by suicide in post-mortem experiments.

4.2.2.10 Post-mortem frozen hippocampus processing for molecular assays

Frozen hippocampal samples from individuals who had undergone toxicological and psychological autopsy assessments (Dumais et al., 2005) were obtained from the Douglas-Bell Canada Brain Bank (Verdun, QC). Medication status was obtained from toxicological assay and medication history. RNA was extracted using RNeasy kits (QIAGEN), and cDNA was produced using oligo dT primers. Amplification and quantification was performed using qRT-PCR and TaqMan probes (Hs00247620_m1, Hs01076078_m1, Hs01001580_m1, Hs00176538_m1, Hs00955525_m1, cat. # 4310884E). Expression values as shown are mean quantities of sample replicates normalized to GAPDH.

For epigenetic analyses, DNA was extracted from frozen human post-mortem hippocampal samples, which was then bisulfite-converted using an EpiTect Bisulfite kit (Qiagen), and samples were processed for epityper (Sequenom) quantification at Genome Quebec (Montreal, Quebec, Canada). To measure ErbB3 methylation, CpGs were analyzed in 3 500 bp regions (spanning from positions 56472794-56474049 on chromosome 12) within the promoter and surrounding the transcription start site of the ErbB3 gene. Data are represented as percentage of methylation, with missing values calculated to allow mixed-model analysis.

4.2.2.11 Post-mortem fixed hippocampus processing for stereological and morphological analyses

Fixed hippocampal samples were also obtained from the Douglas-Bell Canada Brain Bank. Tissues were immersed in a 30% sucrose solution until sinking, flash-frozen in isopentane (-45°C), then sliced coronally at 50 µm on a freezing sliding microtome in PBS, placed on a shaker for 1 hour, then transferred to a cryopreservative solution (glycerol:ethylene glycol:PBS, 3:3:4) for storage at -20°C. Slices were mounted in PBS-T, and after drying were rehydrated in dH2O, stained with a heated cresyl violet solution for three minutes, rinsed in dH2O for 5 minutes, then dehydrated in increasing concentrations of alcohol and xylene, then coverslipped. DGs were carefully traced tightly around the granule cell layer, to assess population and volume as accurately as possible. The hippocampal head was considered as the anterior hippocampus (Duvernoy, 1988). Stereology, cell body measurement, and regional volume assessment were performed using StereoInvestigator software (MBF Bioscience). The parameters for stereology were: counting frame: 30 µm X 30 µm; grid size: 350 µm X 350 µm; dissector height: 10 µm; guard zone height: 3 µm; measuring thickness at each site. Granule cell bodies were assessed with the nucleator probe (eight-ray isotropic uniform random sections). Volume was assessed using the Cavalieri estimator (grid spacing: 20 µm) corrected for overprojection. Population and volumetric data were averaged across sections to account for variation in number of sections. The microscope was parcentric/parfocal and lens grid tune calibrated.

4.2.2.12 Statistics

All statistical tests were two-tailed. Pairwise comparisons were made by Mann-Whitney or t-tests with Welch's correction when required. Social defeat expression data were analyzed by two-way mixed-model ANOVA. Three-group analyses were performed by Kruskal-Wallis or ANOVA with Fisher's post-hoc tests. Time point/CpG x group analyses were performed with two-way mixed-model ANOVAs and Sidak post-hoc tests. Covariate corrections were performed by ANCOVA. Experimental group identity was blinded when applicable. Statistical outliers were identified by Grubbs' tests and removed. P values ≤ 0.05 were considered significant. Data are presented as mean±SEM.

4.2.3 Results

4.2.3.1 Peripheral NRG1 phosphorylates ErbB3 in the ventral DG

To determine whether peripheral NRG1 can activate ErbB3 receptors in the DG of the hippocampus, we administered NRG1 or saline subcutaneously to mice for 24 hours, then dissected the dorsal and ventral DG and assessed ErbB3 phosphorylation by ELISA. ErbB3 phosphorylation was selectively increased in the ventral DG ($t(14)=2.214$; $p=0.044$**; Fig. 21A-B**), in agreement with previous findings showing that this administration paradigm selectively increases cell proliferation in this subregion (Mahar et al, 2011). FACS in transgenic animals revealed a GFP+ population in DG dissections (**Fig. A14**), supporting the anatomical accuracy of DG dissections.

4.2.3.2 Hippocampal ErbB3 is decreased by chronic social defeat stress

As boosting hippocampal NRG1-ErbB3 signaling has been suggested to have antidepressant-like effects, we sought to determine whether hippocampal ErbB3 expression is reduced in an animal

model of depression. Mice subjected to chronic social defeat stress showed reduced expression of overall DG ErbB3 compared to unstressed mice ($F(1,16)=6.16$; $p=0.025$; **Fig. 21C**) without this reduction being specific to either DG subregion.

4.2.3.3 ErbB3 is ubiquitously expressed in human DG granule cells

Although we had previously reported that ErbB3 is ubiquitously expressed in murine DG granule cells, we sought to confirm this expression pattern in the human DG, as had been mentioned in a previous immunohistochemical study (Chaudhury et al., 2003). We find a similar expression pattern in humans, with ubiquitous ErbB3 expression in granule cells throughout the granule cell layer, using both immunohistochemistry (**Fig. 22A**) and *in situ* hybridization (**Fig. 22B-C**) data, with strong expression present in granule cells. These results are supported by data from the Human Protein Atlas (Human Protein Atlas, 2015; Uhlen et al., 2015) (**Fig. A15A**).

4.2.3.4 Peripheral NRG1 expression is not associated with MDD, depressive recovery, or antidepressant response

As NRG1-ErbB3 activity is disrupted in an animal model of depression, and as peripheral NRG1 has been associated with affective behaviour (Mahar et al., 2011), we investigated whether NRG1 is differentially expressed in peripheral blood of depressed individuals before or after 8 weeks of antidepressant (citalopram) treatment. To this end, we found that NRG1 expression did not differ by experimental group or time point (there was a significant interaction ($F(1,43)=7.75$; $p=0.0079$), but post-hoc tests were non-significant ($ps>0.05$); **Fig. 23A**), suggesting that any disruption in NRG1-ErbB3 related to psychopathology would more plausibly be central rather

than peripheral. Dividing MDD subjects by symptomatic remission status did not reveal differences from controls or between time points, suggesting that basal levels of peripheral NRG1 do not predict or mediate response to antidepressants.

4.2.3.5 Hippocampal ErbB3 expression is reduced in suicide completers

Similar to expression of NRG1 in the blood of MDD patients, hippocampal NRG1 expression was unchanged in suicide completers (**Fig. 23B**). However, ErbB3 expression was markedly decreased in suicide completers compared to controls (**Fig. 23E**; $F(2,53)=4.39$, $p=0.017$; unmedicated $t(53)=2.80$, $p=0.0071$; medicated $t(53)=2.37$, $p=0.021$; pooled suicides vs. controls $t=1.78$, $p<0.05$), whereas ErbB1 (EGFR; **Fig. 23C**), ErbB2 (**Fig. 23D**), and ErbB4 (**Fig. 23F**) expression were unchanged between groups.

4.2.3.6 Decreased ErbB3 is not due to epigenetic changes in gene methylation

To attempt to determine a putative explanation for the decreased hippocampal ErbB3 expression observed in suicide completers, we next sought to determine whether epigenetic modifications to ErbB3, in particular differential methylation, could underlie this deficit. We found no overall group differences in hippocampal ErbB3 methylation; however, there was a group x CpG interaction, in that a cluster of CpGs in the third fragment, surrounding the transcription start site, was hypermethylated in suicides who had taken antidepressants ($F(34,626)=2.04$, $p=0.0006$; CpG 22-24 $ps<0.0001$; CpG 25-28 $t(663)=3.41$, $p=0.0021$ vs. unmedicated suicides, $t(663)=2.59$, $p=0.029$ vs. controls; **Fig. 23G**).

4.2.3.7 Assessment of granule cells in suicide completers

As decreased hippocampal ErbB3 in suicides was not attributable to changes in gene methylation, and given the expression pattern of ErbB3 in the human DG, we hypothesized that a loss of granule cells may account for this phenomenon, and tested this hypothesis through detailed tracing and stereology in human DG samples (**Fig. A15B-C**). Stereology, cell body nucleator, and Cavalieri CEs were low (mean optical fractionator CE: 0.044; mean nucleator CE: 0.0064; mean Cavalieri CE: 0.028).

Number of granule cells was decreased selectively in the anterior DG for unmedicated suicides compared to controls (45%; $F(2,10)=5.18$, $p=0.029$; $t(10)=2.78$, $p=0.020$; **Fig.24A, A16A-B**) and medicated suicides (47%; $t(10)=2.85$, $p=0.017$), with the latter not differing from controls ($t(10)=0.23$, $p=0.83$). Similarly, granule cell layer volume was selectively reduced in the anterior DG for unmedicated suicides relative to controls (36%; $F(2,10)=4.50$, $p=0.04$; $t(10)=2.70$, $p=0.022$; **Fig.24B, A16C-D**) and medicated suicides (36%; $t(10)=2.53$, $p=0.030$; controls vs. medicated suicides $t(10)=0.036$, $p=0.97$). Neuronal density was unchanged between groups (**Fig.24C, A16E-F**). Neuronal cell body size was increased in the posterior DG of pooled suicides ($t(9)=2.54$; $p=0.032$; **Fig. 24D**), but not in the anterior or overall (**Fig. A16G-H**) DG. Number of glial cells did not differ between groups overall (**Fig. A16I**) or in the posterior DG (**Fig. A16J**), but were altered in the anterior DG ($F(2,10)=4.32$; $p=0.045$; **Fig. 24E**), with decreased glial cells in unmedicated suicides vs. controls ($t(10)=2.91$, $p=0.016$). Glial density did not differ between groups, either overall or in the anterior or posterior DG (**Fig. A16K-M**). Ratio of neurons to glia did not differ between groups, either overall (**Fig. 24F**) or in the anterior (**Fig. A16N**) or posterior (**Fig. A16O**) DG.

4.2.4 DISCUSSION

Our results are the first to associate the NRG1-ErbB3 signaling pathway with suicidality, by indicating that hippocampal ErbB3 is disrupted in suicide completers, likely owing to deficits in DG granule cells in unmedicated suicides. We also identify hippocampal ErbB3 as being potentially linked to depressive etiology in general, as this receptor was decreased in an animal chronic stress model of depression. Peripheral NRG1 administration (which has been shown to be safe in humans (Gao et al., 2010; Jabbour et al., 2011)) phosphorylates ErbB3 receptors in the DG and increases neurogenesis, producing additional granule cells expressing ErbB3, with concomitant antidepressant-like effects (Mahar et al., 2011). As such, this represents a promising putative therapeutic target for depressed individuals, particularly those with a suicidal phenotype.

The results of the ErbB3 phosphorylation experiment are intriguing, as phosphorylation was increased in the DG *in vivo* following NRG1 administration, yet only in the ventral DG, a subregion repeatedly shown to be involved in the response to stress and antidepressants (Mahar et al., 2014). This aligns with our previous experiments, which showed that the neurogenic effects of NRG1 are also restricted to this subregion (Mahar et al., 2011), supporting our hypothesis that NRG1 has its neurogenic and behavioural effects through ErbB3 phosphorylation in this hippocampal area. The subregional specificity may be related to increased ErbB3 expression in neurogenic cells in the ventral DG (Mahar et al., 2011). These results also add further support to findings indicating that NRG1 readily crosses the blood-brain barrier and affects a variety of brain processes (Carlsson et al., 2011; Kastin et al., 2004; Kato et al., 2015; Kato et al., 2011; Mahar et al., 2011; Xu et al., 2006; Xu et al., 2004). In addition, the finding that DG ErbB3 is decreased in a chronic stress model of depression inversely mirrors the antidepressant-like effects of augmented NRG1-ErbB3 signaling (Mahar et al., 2011), suggesting

that affective regulation by hippocampal NRG1-ErbB3 signaling may be bidirectional in nature. Given the expression of ErbB3 in neurogenic and mature granule cells, this finding is also in accordance with previous studies showing that a rodent chronic stress model of depression reduces DG neurogenesis across the septotemporal axis (Tanti et al., 2013).

Although our epigenetic analysis reveals that decreased hippocampal ErbB3 expression in suicides is likely not related to differential methylation, we found in individuals who had taken antidepressants a cluster of differentially methylated CpGs located around the transcription start site of the ErbB3 gene, in a region enriched in transcription factor binding sites. Though preliminary, this could potentially highlight a mechanism for the positive effects of antidepressants on neurogenesis, especially as increased antidepressant-mediated gene methylation has been shown in previous studies to mediate antidepressant effects (Robison et al., 2014). Future investigation could determine if methylation at these sites affects the response of neural progenitor cells to antidepressant medication.

The decrease in hippocampal ErbB3 can plausibly be attributed to a loss of granule cells in the anterior DG, particularly in unmedicated suicides, as we (and others; see Chaudhury et al., 2003; Gerecke et al., 2001; Hawrylycz et al., 2012; Mahar et al., 2011; Uhlen et al., 2015) show that these cells ubiquitously express ErbB3. These results are also in line with previous studies showing decreased numbers of anterior hippocampal granule cells and neural progenitors in individuals with MDD, as well as increased numbers of mitotic cells in this subregion with antidepressant treatment (Boldrini et al., 2014; Boldrini et al., 2013; Boldrini et al., 2009). The anatomical specificity of the effects is noteworthy, as the anterior hippocampus is more associated with emotional modulation (Bannerman et al., 2004) and, in non-human primates, depression-like behaviours are inversely correlated with anterior DG neurogenesis (Perera et al.,

2011). This subregion is analogous to the ventral hippocampus in rodents, which has been associated with affective regulation (Fanselow and Dong, 2010). More specifically, the ventral DG and DG neurogenesis in particular have been associated with response to stress and antidepressants, as well as affective behaviour (Mahar et al., 2014). It is also the selective subregion in which NRG1 disruption leads to decreased communication efficacy with the nucleus accumbens (Nason et al., 2011) and in which ErbB3 phosphorylation and increased neurogenesis occur following NRG1 administration (Mahar et al., 2011). Thus it seems that chronic stress or other deleterious factors may contribute to suicidality by reducing the number of ErbB3-expressing granule cells in this emotion-related subregion of the hippocampus, whereas this endophenotype is reversed by administration of antidepressants (and potentially NRG1). The mechanism by which reduced numbers of granule cells contribute etiologically to suicidality may also be stress-related, as DG granule cells inhibit HPA axis activity, and in their absence response to stress is augmented, further reducing numbers of granule cells and exacerbating other effects of stress (Mahar et al., 2014). The fact that medicated suicides had significantly more granule cells than unmedicated suicides, but not higher hippocampal ErbB3 expression, was surprising; however, there are at least three potential explanations. First, although adultborn rodent granule cells have shown ErbB3 expression soon after division (Mahar et al., 2011), it is possible that adultborn human granule cell neurons express ErbB3 later in maturation, in accordance to their potentially lengthened maturation profile (Kohler et al., 2011; Perera et al., 2011), with a larger proportion of granule cells being born in response to antidepressants after treatment. In fact, this has been shown in previous studies, with increased ErbB3 expression in DCX+ immature neurons in comparison to SOX2+ early neurogenic cells (Bracko et al., 2012). Alternatively, we show that antidepressants hypermethylate a region of the

ErbB3 gene, which could subsequently suppress ErbB3 expression per cell. Finally, the mRNA expression data were from the hippocampus as a whole, whereas the stereological assessment was specific to a subregion of interest, which may account for the discrepancy.

In contrast to previous studies investigating schizophrenia (Shibuya et al., 2010) and depression (Belzeaux et al., 2010), we did not find that peripheral NRG1 expression was associated with MDD or recovery with antidepressant treatment. This suggests that, to the extent that disrupted NRG1-ErBb3 signaling might underlie depression-related phenotypes, it is likely that this disruption is central (e.g. in the hippocampus), as opposed to peripheral. Although previous post-mortem studies have found alterations in the brain for NRG1 or ErbB4 in the context of psychopathology (Bertram et al., 2007; Hahn et al., 2006; Law et al., 2006; Marballi et al., 2012; Parlapani et al., 2010), we measured no change in NRG1, ErbB1 (EGFR), ErbB2, or ErbB4 expression in the hippocampus of suicides. However, ErbB receptor studies in hippocampal psychopathological samples are sparse, and thus would benefit from analyses in a broader psychopathological perspective, particularly given our finding that hippocampal ErbB3 is disrupted in suicides. Indeed, given the potential relevance of ErbB3 to neurogenesis, affective behaviour, and psychopathology, and its ubiquitous expression profile in the granule cell layer, ErbB3 may represent a promising target of future investigation in the etiology and treatment of psychiatrically relevant phenotypes.

Acknowledgements

Authors declare no competing financial interests to this manuscript. This research was supported by CIHR (MOP-111022) and NSERC Discovery (341479-07) and RTI (345952-07) grants to

NM. IM and EI were supported by FRQS. NM is a FRQS Chercheur-boursier Junior 2 and CIHR New Investigator. FACS data and associated figure were produced with the assistance of the Flow Cytometry Core Facility at the Université de Montréal.

FIGURES:

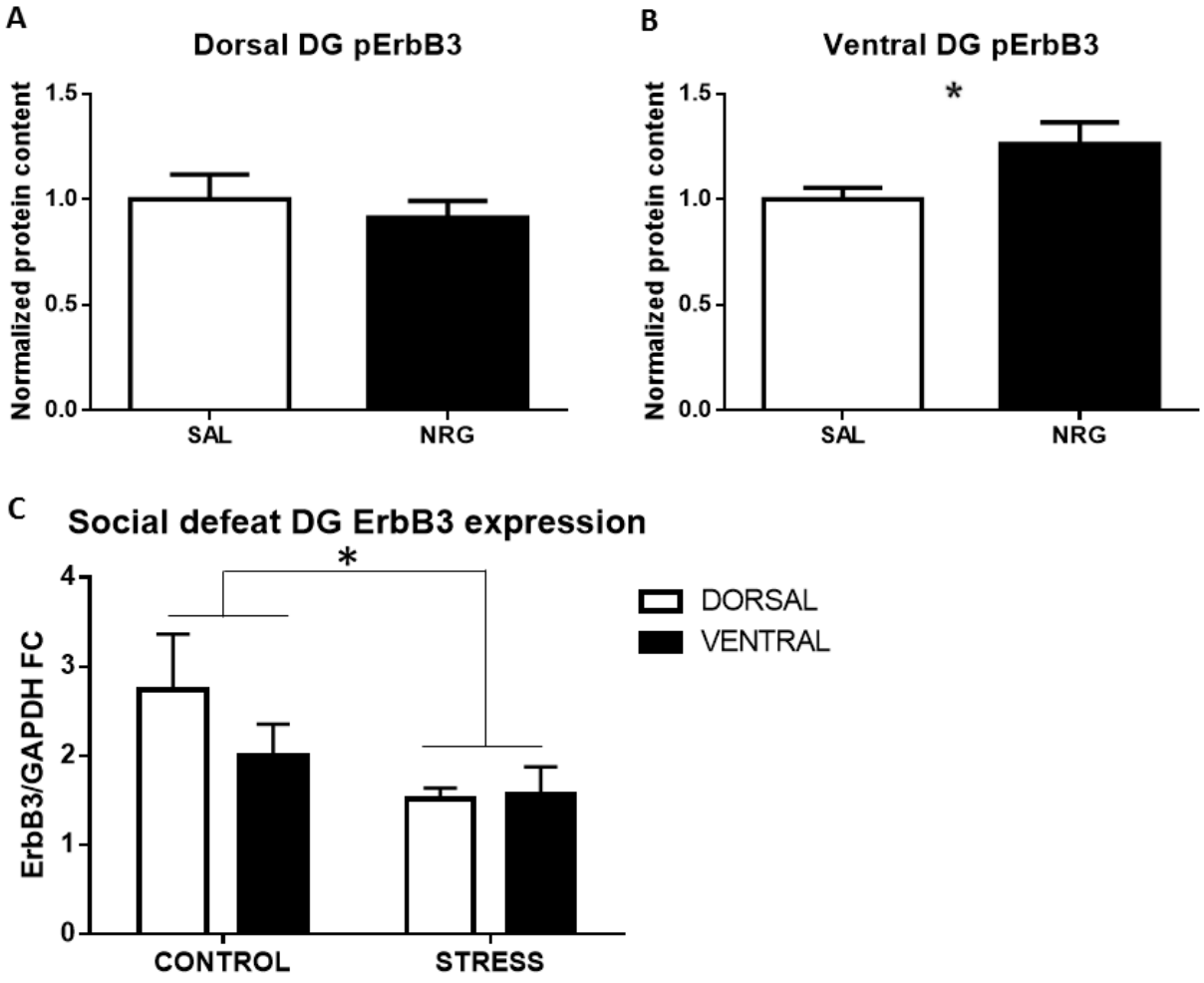


Figure 21. ErbB3 in the DG is activated by peripheral NRG1 administration, and its expression is reduced in a mouse model of depression. A, Ventral DG phosphorylation is increased by 24 h of subcutaneous NRG1. B, chronic social defeat stress reduces ErbB3 expression in the DG. *, $p<0.05$. FC, fold change; NRG, neuregulin; SAL, saline.

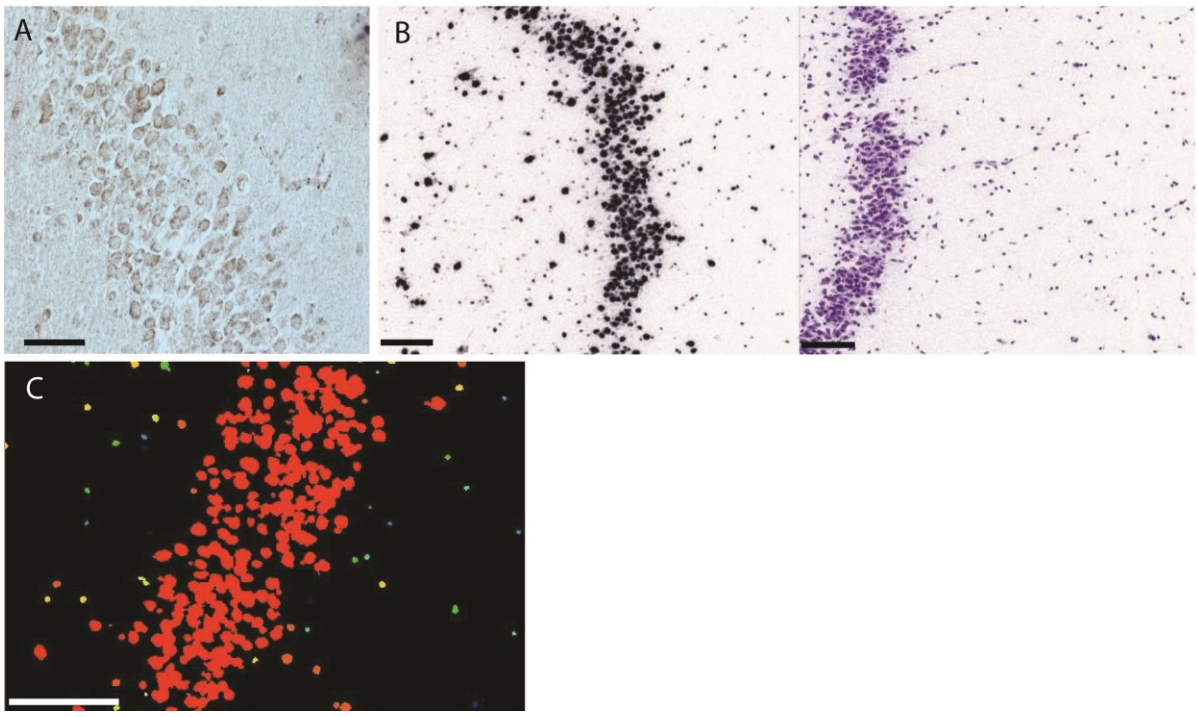
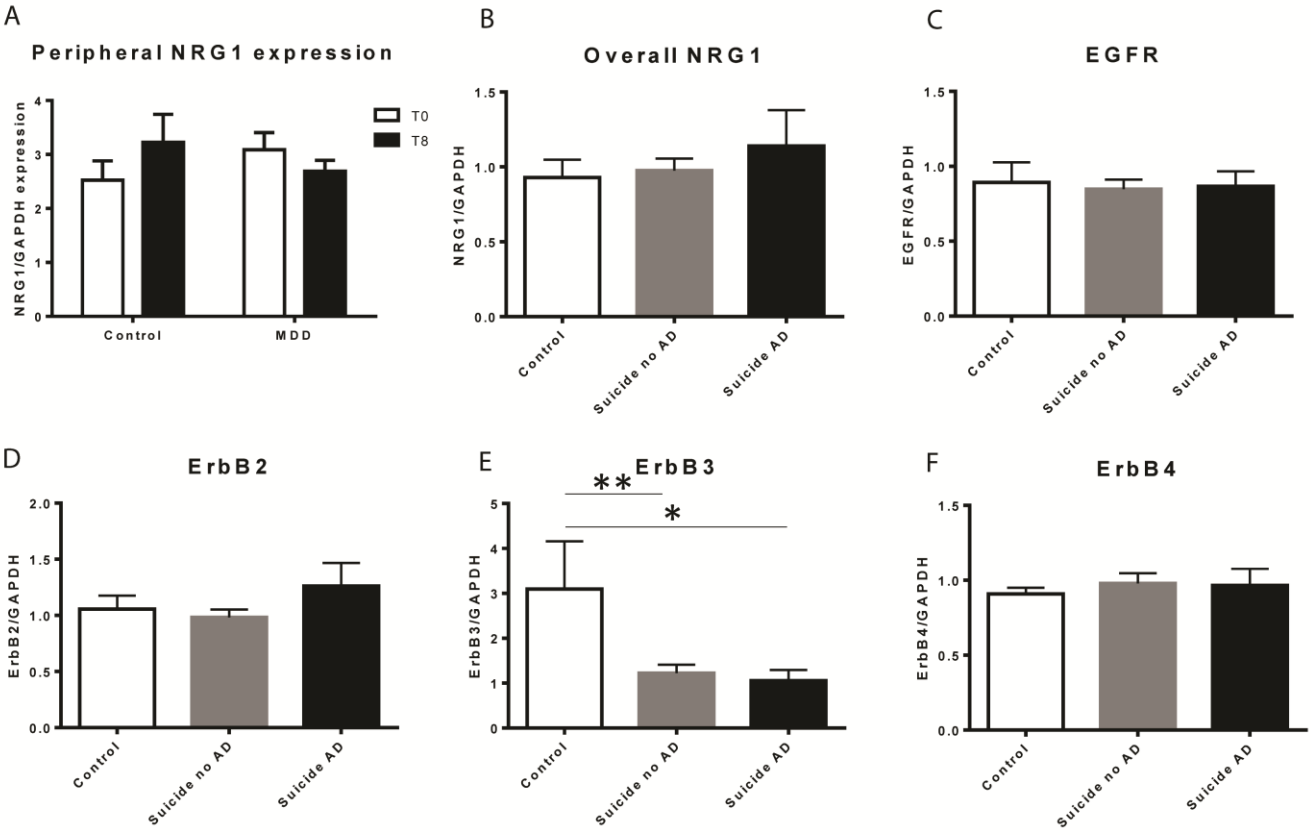


Figure 22. ErbB3 is expressed ubiquitously by granule cell layer neurons in the human DG. A, ErbB3 immunohistochemistry. B, *in situ* hybridization for ErbB3 (left) and corresponding Nissl staining (right). (C) ErbB3 gene expression heatmap. Image credit (B-C): Allen Human Brain Atlas (http://www.brain-map.org/ ; Allen Institute for Brain Science; Hawrylycz et al., 2012. Scale bars: 100 µm.


A
Peripheral NRG1 expression
NRG1/GAPDH expression
T0
T8
Control
MDD
B
Overall NRG1
NRG1/GAPDH
Control
Suicide no AD
Suicide AD
C
EGFR
EGFR/GAPDH
Control
Suicide no AD
Suicide AD
D
ErbB2
ErbB2/GAPDH
Control
Suicide no AD
Suicide AD
E
ErbB3
**
*
ErbB3/GAPDH
Control
Suicide no AD
Suicide AD
F
ErbB4
ErbB4/GAPDH
Control
Suicide no AD
Suicide AD

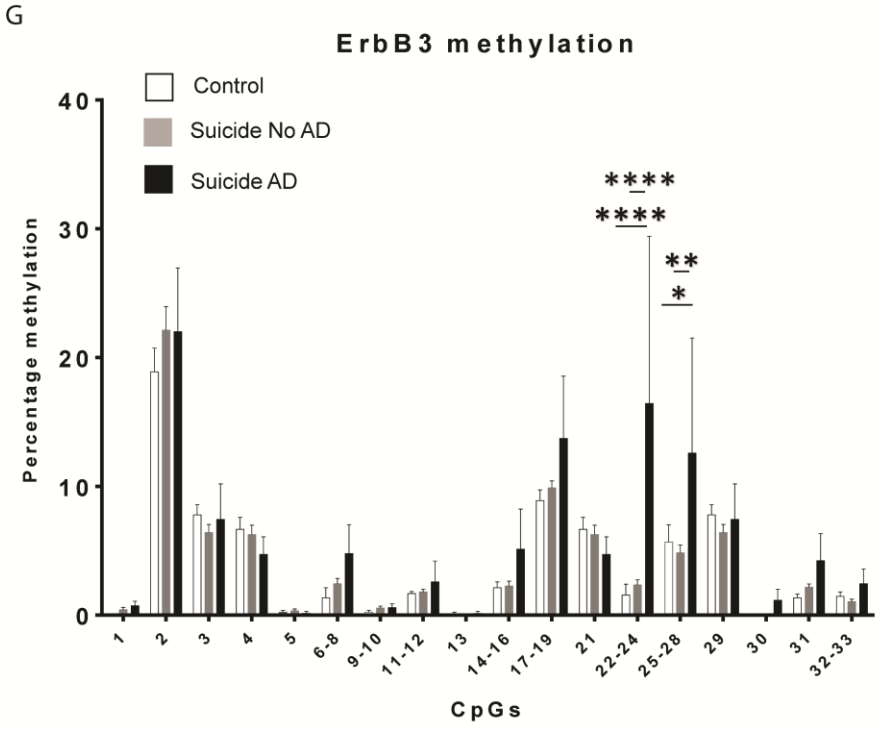

G
ErbB3 methylation
Control
Suicide No AD
Suicide AD
****
****
**
*
Percentage methylation
CpGs
1
2
3
4
5
6-8
9-10
11-12
13
14-16
17-19
21
22-24
25-28
29
30
31
32-33

Figure 23. Peripheral and central NRG1/ErbB gene expression in cases and controls. Peripheral NRG1 expression is not disrupted in major depressive disorder (MDD) patients at baseline (A; T0), is not affected by eight weeks of citalopram treatment (T8), and baseline T0 expression is not predictive of antidepressant response. Hippocampal expression of NRG1 (B), ErbB1 (EGFR; C), ErbB2 (D), and ErbB4 (F) does not differ between controls and suicides regardless of antidepressant treatment; however, ErbB3 expression (E) is reduced. G, reduced hippocampal expression of ErbB3 in suicides is not due to changes in ErbB3 methylation, as unmedicated suicides did not differ from controls in terms of CpG methylation. However, antidepressant usage was associated with increased methylation in a cluster of CpGs is located around the ErbB3 promoter region near the transcription start site, in an area enriched in transcription factor binding sites. *, $p<0.05$; **, $p<0.01$; ****, $p<0.0001$. AD, antidepressant; CTRL, control.

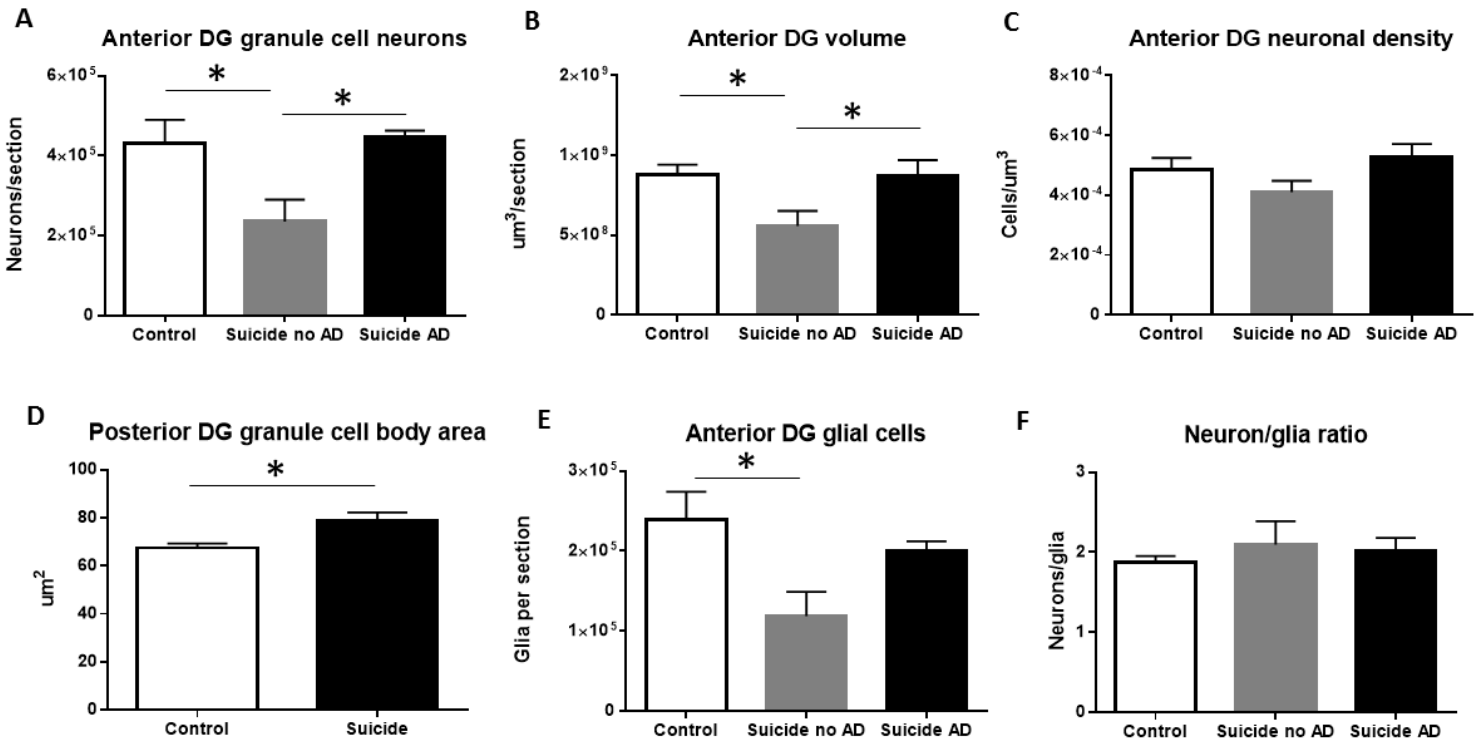


Figure 24. Assessment of granule cells, glia, and granule cell layer in DG of suicides and controls. Unmedicated suicides have decreased numbers of granule cells in the anterior DG (A), as well as decreased volume in the anterior DG (B). Granule cell neuronal density was unchanged in the anterior DG (C). Granule cell body size was increased in suicides in the posterior DG (D). Number of glial cells was decreased for unmedicated suicides compared to controls in the anterior DG (E). Ratio of granule cell neurons to glia in the overall DG did not differ between groups (F). AD, antidepressant.

Supplemental materials are available in Appendix.

CHAPTER 5. DISCUSSION AND CONCLUSIONS

5.1 Discussion of results and implications

As our results are discussed at length in their respective Chapters, I will attempt to avoid a lengthy reiteration of our findings here in the interest of concision and limiting redundancy. As mentioned in Chapter 1, our overall aims were to determine whether NRG1 administration affects hippocampal neurogenesis and affective behaviour, characterize NRG1-induced neurogenic affects across neurogenic stages and the septotemporal axis, identify a putative mechanism for the effects of NRG1 that we identified, and determine whether NRG1-ErbB signaling is etiologically associated with psychiatrically relevant contexts such as depression and suicide. In brief, through our experiments we determined that NRG1 has temporally- and subregionally-specific neurogenic effects that are limited to increasing proliferation and subsequent neurogenesis in the ventral DG. This increased neurogenesis was associated with antidepressant-like behavioural effects at a time when these new neurons were immature hyperplastic neurons, but not earlier. We identified ErbB3 as a candidate mechanistic receptor in these phenomena, and verified that ErbB3 in the ventral DG is phosphorylated by peripheral NRG1 administration. Finally, we associated NRG1-ErbB3 signaling with suicidality, including decreased ErbB3 expression in the hippocampus of suicide completers that was likely due to a deficit in DG granule cell neurons.

These results are the first to identify NRG1 as a neurogenic regulator, the first to identify hippocampal NRG1-ErbB3 as regulators of antidepressant-like behaviour, and the only results to date associating the NRG1-ErbB3 pathway with suicidality. The finding that hippocampal NRG1-ErbB3 signaling regulates neurogenesis and affective behaviour suggests that this pathway may have etiological relevance for psychiatrically relevant contexts, and as such may

lead to treatments targeting this pathway. In addition, one of the specific endophenotypes that we identified in suicide completers (reduced numbers of granule cell neurons) was shown to potentially be reversible through administration of NRG1 from our animal studies, indicating that this may serve as a useful and valuable therapeutic in future practice. Finally, the endophenotypic hallmarks of suicidality that we have observed in the hippocampus shed light on the etiology of this phenomenon, furthering our understanding of how suicidality may originate and be therapeutically addressed. The subsequent section proposes specific measures by which these results may be furthered in these and other areas through future research.

5.2 Future directions

Although the sum total of the research we performed serves as an attempt to address the research questions and hypotheses we presented in Chapter 1, as a natural consequence many additional questions have been raised from our results.

In terms of clinical relevance, our data converge upon the hypothesis that a deficit in ErbB3 (possibly due to decreased numbers of granule cell neurons in the DG) exists in the hippocampus in psychopathological contexts, particularly with respect to suicidality, and that this might be reversed by treatment with NRG1, which has shown to be safe in human trials. Future studies might establish or disprove this hypothesis through the administration of NRG1 to depressed and suicidal patients to determine if depressive or suicidal symptoms are ameliorated. In terms of logistics, clinical trials for the use of NRG1 in heart failure have established dosage ranges that can be administered without causing substantial side effects, and thus would serve as initial heuristics in determining a putatively therapeutic dosage for psychiatrically relevant treatment.

The potential benefits of this treatment are threefold; 1) NRG1 treatment may produce antidepressant effects after only a brief administration paradigm (followed by a delay in therapeutic efficacy), limiting exposure for individuals who might otherwise experience side effects; 2) side effects may be less than those of conventional antidepressants, based on clinical trial data and NRG1's status as an endogenously-found protein; and 3) it could easily be added as an adjunct therapy in combination with traditional antidepressants. Such treatment, based on our findings, could increase hippocampal neurogenesis, reversing deficits in hippocampal granule cells and ErbB3 expression, with commensurate amelioration of psychiatric symptoms.

As an aside, it is important to address one potential risk in NRG1 as a clinical therapeutic or adjuvant. Overexpression of ErbB receptors has been found in a variety of cancer cell types, particularly in breast and ovarian cancer (Jeong et al., 2014; Montero et al., 2008), and stimulation of these cells by NRG1 could induce cell proliferation, worsening oncological prognosis, although there is no indication to date that NRG1 treatment might actually induce cancerous development in the absence of cancerous cells. As this has yet to be assessed in the existing NRG1 clinical trials as a counter-indication for NRG1 treatment, this remains a concern for clinical usage of NRG1 in patients who also have cancer, and possibly also those in cancer remission. Thus, until this concern is allayed by clinical trial data to the contrary, clinical usage of NRG1 should be limited to cancer-free individuals, and might not be ideal for longer-term administration.

The mechanism behind affective regulation by NRG1-ErbB3 signaling is currently speculative, and could be expanded by subsequent research. Our current hypothesis is that NRG1 binds to ErbB3 receptors expressed by neurogenic cells, leading to increases in proliferation through PI3K/Akt signaling, increasing neurogenesis in DG subregions (ventral in rodents, anterior in

humans), which could prevent or reduce depressive or suicidal symptoms, potentially through improving regulation of the HPA axis via the PVN/BNST. This could be tested in several preclinical investigations. First, although mice lacking ErbB3 are embryonically lethal and ErbB3-specific antagonists are not yet commercially available, the latter could be used upon commercial release (ideally through selective administration into the DG) in tandem with NRG1 administration to determine whether ErbB3 expression in the DG is critical for the neurogenic and antidepressant effects of NRG1 administration. In addition, conditional suppression of ErbB3 in granule cells, through RNA interference, lentiviral vectors, or optogenetic techniques, could also determine how (and when, with respect to administration) ErbB3 is required in these phenomena. Neurogenic studies have recently begun to investigate whether HPA regulation is critical to neurogenesis-related antidepressant-like effects following antidepressant administration (Schloesser et al., 2014; Schloesser et al., 2009; Snyder et al., 2011b). These could be extended to examining the effects of NRG1 administration in the absence of changes in HPA function (e.g. in the context of glucocorticoid antagonists or adrenalectomy), to determine if the mechanism by which NRG1 administration and its resulting neurogenic effects affect behaviour is through this system. Finally, it is possible (although unlikely, given the temporal dynamics of our behavioural findings), that NRG1's behavioural effects are due to a neurogenesis-independent mechanism. This could be examined by repeating these earlier studies in mice with ablated neurogenesis. Although the means to do so were not available to us at the time that we performed the initial experiments, several labs are capable of doing so with minimal ancillary or confounding effects, including methods to selectively irradiate the DG and transgenic ablations of neurogenesis (Lagace et al., 2010; Snyder et al., 2011b; Wu and Hen, 2014). These techniques could determine if NRG1 induces antidepressant-like effects through a

neurogenesis-independent mechanism (i.e. if administration induces behavioural changes in the absence of neurogenesis), or conversely whether neurogenesis is required for these behavioural phenotypes to emerge.

Similarly, as ErbB receptors are also expressed in the periphery and in non-hippocampal brain regions, our current experiments cannot preclude the possibility that our peripheral NRG1 administration paradigm induces its neurogenic and behavioural effects through NRG1-ErbB binding outside of the hippocampus or the brain as a whole. To address this, our initial animal experiments could be repeated with direct intrahippocampal administration replacing our subcutaneous administration. If our neurogenic and antidepressant results were replicated, this would support our hypothesis that peripheral administration acts through the hippocampus specifically. Conversely, if our results were not replicated, this would suggest that the mechanism for our findings may be extra-hippocampal, through binding either in other brain regions or in the periphery. In either case, selective administration paradigms could further establish the specific site of NRG1-ErbB binding required to produce these effects, and these could be verified through localized ErbB inactivation.

Based on the model presented in Chapter 1, we suspect that the decrease in ErbB3 observed in socially defeated mice from Chapter 4 may be due to chronic stress-induced increases in glucocorticoids that negatively regulated hippocampal neurogenesis and numbers of granule cells. This could be tested through repetition of this experiment in tandem with adrenalectomization (to reduce HPA activation-induced hypersecretion of glucocorticoids) or glucocorticoid antagonist administration, to determine if this prevents or ameliorates social defeat-induced ErbB3 decreases.

It was particularly interesting to us that our primary findings were restricted to the ventral DG in mice, and the anterior DG in humans, as these represent DG subregions that are particularly associated with affective regulation. However, it is not entirely clear why these effects were so subregionally restricted. The subregional specificity of effects of NRG1 on neurogenesis may be partially explained by the increased proportion of neurogenic cells expressing ErbB3 in the ventral DG in mice, as discussed in Chapter 2, and this could be paralleled in the anterior DG in humans. However, this may not be a completely sufficient explanation, as ErbB3 was not entirely absent in the dorsal DG in mice, yet proliferation was unchanged in these animals following NRG1 administration. Similarly, the mechanism underlying the deficit in anterior (but not posterior) DG granule cells in suicides is not yet clear. These questions will hopefully be addressed by subsequent studies examining distinctions between DG subregions, similar to those we performed in Chapter 3.

Similarly, is this deficit in anterior DG granule cells the cause, or consequence, of suicidality? This is an especially difficult question to answer, as the logistics of human research (particularly post-mortem suicide research) precludes us from manipulating numbers of DG granule cells and observing causality. Further, no valid animal models for suicide exist, limiting the potential to determine causality in animals. It is possible that decreased numbers of DG granule cells predispose to suicide by exacerbating stress responses, or alternatively that the stress of experiencing suicidal ideation (or stressors that cause it) subsequently reduce numbers of DG granule cells. Distinguishing between these possibilities may be difficult for future studies to address, especially as animal studies have shown both increased stress responses resulting from neurogenic ablation (Schloesser et al., 2014; Schloesser et al., 2009; Snyder et al., 2011b), and stress reducing neurogenesis (Dagyte et al., 2011; Elizalde et al., 2010). Ultimately, it is

plausible that both mechanisms co-occur in the suicidal brain, potentially leading to a positive feedback loop that further exacerbates both stress and stress response, as proposed in the context of depression in Chapter 1.

Along the same lines, does the deficit in hippocampal ErbB3 in suicides merely reflect the decreased numbers of granule cells, or is it possible that ErbB3 expression is reduced per cell, subsequently reducing NRG1 binding and resulting neurogenesis? The latter hypothesis could be tested with laser-capture microdissection of the granule cell layer in tandem with qRT-PCR.

Although decreased numbers of granule cells is a plausible and sufficient potential explanation for the decreased ErbB3 expression observed in unmedicated suicides, we cannot rule out alternative possibilities. For example, disrupted regulation of ErbB3 by altered expression or function of ErbB3-targeting miRNAs could plausibly cause the observed ErbB3 deficit, which future studies could examine. This could also be said for long non-coding RNAs, as well. In addition, when we conducted studies to examine epigenetic regulation of ErbB3 in suicides, we examined methylation and determined that it was not supported as a candidate mechanism for altered ErbB3 expression in suicides. However, additional epigenetic processes, such as acetylation and chromatin remodeling, could potentially influence ErbB3 expression.

Relating to these epigenetic studies, we identified a cluster of CpGs in the ErbB3 gene that were hypermethylated with antidepressant treatment in suicides. These CpGs were found in the promoter region and surrounded the transcription start site, in a region enriched in transcription factor binding sites. It is tempting to speculate that this could relate to the mechanism by which antidepressants increase hippocampal neurogenesis, in that antidepressant treatment potentially hypermethylates this region of the ErbB3 gene and affects ErbB3-related cell proliferation and subsequent neurogenesis. This is especially intriguing given the necessity of neurogenesis for the

behavioural effects of antidepressants, and the decreases in granule cells observed in depression (Boldrini et al., 2013; Santarelli et al., 2003). However, at this early point this hypothesis is merely speculative, and it remains for subsequent research to investigate whether this finding has any clinical relevance.

Although we did not detect ErbB4, the other receptor apart from ErbB3 that binds NRG1, in early neurogenic cells, ErbB2 co-expression was not examined in these cells. However, previous studies have shown that even an extremely low ratio of ErbB2 to ErbB3 is sufficient to assist or facilitate signaling of ErbB3 complexes through heterodimeric pairing (Steinkamp et al., 2014). Studies ablating ErbB2 function and assessing the effects on cellular proliferation in a NRG1 administration paradigm would determine whether this coreceptor contributes to ErbB3 signaling and neurogenesis.

Our studies have focused on the adult brain. Although previous studies have examined the effects of early-life NRG1 administration on behaviour (Kato et al., 2015; Kato et al., 2011), it is unclear whether NRG1 administration during development would affect developmental neurogenesis. If this is the case, this could lead to increased numbers of DG granule cells, buffering against stress effects through HPA regulation. Alternatively, this manipulation may lead to DG hypertrophy or aberrant DG formation, which could underlie the behavioural disruptions seen in animals administered NRG1 during development (Kato et al., 2015; Kato et al., 2011).

Finally, it is undeniable that our experiments (indeed, the contents of this document in total) focus on NRG1 as the ligand of interest, particularly NRG1βI. As such, our results do not necessarily generalize to other neuregulins or other EGF family members, such as betacellulin, heparin-binding EGF-like growth factor, amphiregulin, epiregulin, and epigen (Singh and Harris,

2005). The rationale behind our experiments could also be applied to a lesser extent to these proteins; although not expressed in the brain to the extent of NRG1 (or at all, in some cases), as ErbB ligands it is possible that they might also affect neurogenesis, affective behaviour, and psychopathology, which future research might investigate.

5.3 CONCLUDING REMARKS

This document has described the context, hypotheses, methods, findings, conclusions, and implications of the past seven years of my research into neuregulin-1, ErbB receptors, hippocampal neurogenesis, affective regulation, and psychopathology, particularly suicide and depression. In brief, we believe that we have supported our hypotheses that NRG1 has temporally- and subregionally-specific neurogenic effects in an emotion-related subregion of the hippocampal DG, that the mechanism for these effects involves binding to the ErbB3 receptor on neurogenic cells in this subregion, that these neurogenic effects are concurrent with antidepressant-like behavioural alterations, and that conversely models of depression and suicide completers show decreased hippocampal ErbB3. This latter finding is plausibly related to a deficit in DG granule cell neurons that is reversible with antidepressant treatment. These findings have clinical implications regarding the potential for NRG1 as a novel therapeutic or treatment adjuvant, and may shed light into the etiology of suicidality and other psychopathology. The logical follow-up research to these studies should focus on supporting or disproving our proposed models, as well as determining the therapeutic utility of NRG1 in clinical populations. NRG1-ErbB signaling appears to play a fascinating and powerful role in hippocampal plasticity, affective regulation, and psychopathology, and it is my hope that the current document has conveyed its importance in these phenomena.

REFERENCES

Abe, Y., Namba, H., Kato, T., Iwakura, Y., Nawa, H., 2011. Neuregulin-1 signals from the periphery regulate AMPA receptor sensitivity and expression in GABAergic interneurons in developing neocortex. J Neurosci 31, 5699-5709.

Aberg, M.A., Aberg, N.D., Hedbacker, H., Oscarsson, J., Eriksson, P.S., 2000. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. J Neurosci 20, 2896-2903.

Abrous, D.N., Adriani, W., Montaron, M.F., Aurousseau, C., Rougon, G., Le Moal, M., Piazza, P.V., 2002. Nicotine self-administration impairs hippocampal plasticity. J Neurosci 22, 3656-3662.

Adachi, M., Barrot, M., Autry, A.E., Theobald, D., Monteggia, L.M., 2008. Selective loss of brain-derived neurotrophic factor in the dentate gyrus attenuates antidepressant efficacy. Biological psychiatry 63, 642-649.

Ahmad, A., Rasheed, N., Banu, N., Palit, G., 2010. Alterations in monoamine levels and oxidative systems in frontal cortex, striatum, and hippocampus of the rat brain during chronic unpredictable stress. Stress 13, 355-364.

Aimone, J.B., Deng, W., Gage, F.H., 2010. Put them out to pasture? What are old granule cells good for, anyway...? Hippocampus 20, 1124-1125.

Airan, R.D., Meltzer, L.A., Roy, M., Gong, Y., Chen, H., Deisseroth, K., 2007. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science 317, 819-823.

Akiskal, H.S., Hirschfeld, R.M., Yerevanian, B.I., 1983. The relationship of personality to affective disorders. Arch Gen Psychiatry 40, 801-810.

Albert, P.R., Benkelfat, C., Descarries, L., 2012. The neurobiology of depression--revisiting the serotonin hypothesis. I. Cellular and molecular mechanisms. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 367, 2378-2381.

Allen Institute for Brain Science, 2014. Allen Human Brain Atlas.

Allen, N.B., Badcock, P.B., 2006. Darwinian models of depression: a review of evolutionary accounts of mood and mood disorders. Prog Neuropsychopharmacol Biol Psychiatry 30, 815-826.

Alme, C.B., Buzzetti, R.A., Marrone, D.F., Leutgeb, J.K., Chawla, M.K., Schaner, M.J., Bohanick, J.D., Khoboko, T., Leutgeb, S., Moser, E.I., Moser, M.B., McNaughton, B.L., Barnes, C.A., 2010. Hippocampal granule cells opt for early retirement. Hippocampus 20, 1109-1123.

Altar, C.A., Whitehead, R.E., Chen, R., Wortwein, G., Madsen, T.M., 2003. Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. Biological psychiatry 54, 703-709.

Altman, J., Das, G.D., 1965. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 124, 319-335.

Amat, J., Aleksejev, R.M., Paul, E., Watkins, L.R., Maier, S.F., 2010. Behavioral control over shock blocks behavioral and neurochemical effects of later social defeat. Neuroscience 165, 1031-1038.

Amat, J., Baratta, M.V., Paul, E., Bland, S.T., Watkins, L.R., Maier, S.F., 2005. Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. Nature neuroscience 8, 365-371.

Amat, J., Paul, E., Watkins, L.R., Maier, S.F., 2008. Activation of the ventral medial prefrontal cortex during an uncontrollable stressor reproduces both the immediate and long-term protective effects of behavioral control. Neuroscience 154, 1178-1186.

American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (5th ed.). American Psychiatric Publishing, Arlington, VA.

Anacker, C., Cattaneo, A., Luoni, A., Musaelyan, K., Zunszain, P.A., Milanesi, E., Rybka, J., Berry, A., Cirulli, F., Thuret, S., Price, J., Riva, M.A., Gennarelli, M., Pariante, C.M., 2013a. Glucocorticoid-Related Molecular Signaling Pathways Regulating Hippocampal Neurogenesis. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 38, 872-883.

Anacker, C., Cattaneo, A., Musaelyan, K., Zunszain, P.A., Horowitz, M., Molteni, R., Luoni, A., Calabrese, F., Tansey, K., Gennarelli, M., Thuret, S., Price, J., Uher, R., Riva, M.A., Pariante, C.M., 2013b. Role for

the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis. Proceedings of the National Academy of Sciences of the United States of America 110, 8708-8713.

Anacker, C., Zunszain, P.A., Cattaneo, A., Carvalho, L.A., Garabedian, M.J., Thuret, S., Price, J., Pariante, C.M., 2011. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Molecular psychiatry 16, 738-750.

Andersson, R.H., Johnston, A., Herman, P.A., Winzer-Serhan, U.H., Karavanova, I., Vullhorst, D., Fisahn, A., Buonanno, A., 2012. Neuregulin and dopamine modulation of hippocampal gamma oscillations is dependent on dopamine D4 receptors. Proceedings of the National Academy of Sciences of the United States of America 109, 13118-13123.

Andrique, L., Fauvin, D., El Maassarani, M., Colasson, H., Vannier, B., Seite, P., 2012. ErbB3(80 kDa), a nuclear variant of the ErbB3 receptor, binds to the Cyclin D1 promoter to activate cell proliferation but is negatively controlled by p14ARF. Cellular signalling 24, 1074-1085.

Anton, E.S., Marchionni, M.A., Lee, K.F., Rakic, P., 1997. Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. Development 124, 3501-3510.

Araneda, R., Andrade, R., 1991. 5-Hydroxytryptamine2 and 5-hydroxytryptamine 1A receptors mediate opposing responses on membrane excitability in rat association cortex. Neuroscience 40, 399-412.

Arango, V., Underwood, M.D., Boldrini, M., Tamir, H., Kassir, S.A., Hsiung, S., Chen, J.J., Mann, J.J., 2001. Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. Neuropsychopharmacology 25, 892-903.

Arborelius, L., Hawks, B.W., Owens, M.J., Plotsky, P.M., Nemeroff, C.B., 2004. Increased responsiveness of presumed 5-HT cells to citalopram in adult rats subjected to prolonged maternal separation relative to brief separation. Psychopharmacology 176, 248-255.

Ashby, C.R., Jr., Edwards, E., Wang, R.Y., 1994. Electrophysiological evidence for a functional interaction between 5-HT1A and 5-HT2A receptors in the rat medial prefrontal cortex: an iontophoretic study. Synapse 17, 173-181.

Aso, E., Ozaita, A., Valdizan, E.M., Ledent, C., Pazos, A., Maldonado, R., Valverde, O., 2008. BDNF impairment in the hippocampus is related to enhanced despair behavior in CB1 knockout mice. Journal of neurochemistry 105, 565-572.

Aso, E., Renoir, T., Mengod, G., Ledent, C., Hamon, M., Maldonado, R., Lanfumey, L., Valverde, O., 2009. Lack of CB1 receptor activity impairs serotonergic negative feedback. Journal of neurochemistry 109, 935-944.

Aston, C., Jiang, L., Sokolov, B.P., 2004. Microarray analysis of postmortem temporal cortex from patients with schizophrenia. Journal of neuroscience research 77, 858-866.

Aston, C., Jiang, L., Sokolov, B.P., 2005. Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. Mol Psychiatry 10, 309-322.

Avgustinovich, D.F., Kovalenko, I.L., Kudryavtseva, N.N., 2005. A model of anxious depression: persistence of behavioral pathology. Neurosci Behav Physiol 35, 917-924.

Bambico, F.R., Duranti, A., Tontini, A., Tarzia, G., Gobbi, G., 2009a. Endocannabinoids in the treatment of mood disorders: evidence from animal models. Current pharmaceutical design 15, 1623-1646.

Bambico, F.R., Gobbi, G., 2008. The cannabinoid CB1 receptor and the endocannabinoid anandamide: possible antidepressant targets. Expert Opin Ther Targets 12, 1347-1366.

Bambico, F.R., Katz, N., Debonnel, G., Gobbi, G., 2007. Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 27, 11700-11711.

Bambico, F.R., Nguyen, N.T., Gobbi, G., 2009b. Decline in serotonergic firing activity and desensitization of 5-HT1A autoreceptors after chronic unpredictable stress. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 19, 215-228.

Banasr, M., Duman, R.S., 2008. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biological psychiatry 64, 863-870.

Banasr, M., Hery, M., Printemps, R., Daszuta, A., 2004. Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 29, 450-460.

Banasr, M., Soumier, A., Hery, M., Mocaer, E., Daszuta, A., 2006. Agomelatine, a new antidepressant, induces regional changes in hippocampal neurogenesis. Biol Psychiatry 59, 1087-1096.

Bannerman, D.M., Rawlins, J.N., McHugh, S.B., Deacon, R.M., Yee, B.K., Bast, T., Zhang, W.N., Pothuizen, H.H., Feldon, J., 2004. Regional dissociations within the hippocampus--memory and anxiety. Neurosci Biobehav Rev 28, 273-283.

Bao, J., Lin, H., Ouyang, Y., Lei, D., Osman, A., Kim, T.W., Mei, L., Dai, P., Ohlemiller, K.K., Ambron, R.T., 2004. Activity-dependent transcription regulation of PSD-95 by neuregulin-1 and Eos. Nature neuroscience 7, 1250-1258.

Barnes, A., Isohanni, M., Barnett, J.H., Pietilainen, O., Veijola, J., Miettunen, J., Paunio, T., Tanskanen, P., Ridler, K., Suckling, J., Bullmore, E.T., Jones, P.B., Murray, G.K., 2012. Neuregulin-1 genotype is associated with structural differences in the normal human brain. NeuroImage 59, 2057-2061.

Barr, A.M., Markou, A., Phillips, A.G., 2002. A 'crash' course on psychostimulant withdrawal as a model of depression. Trends Pharmacol Sci 23, 475-482.

Bean, J.C., Lin, T.W., Sathyamurthy, A., Liu, F., Yin, D.M., Xiong, W.C., Mei, L., 2014. Genetic labeling reveals novel cellular targets of schizophrenia susceptibility gene: distribution of GABA and non-GABA ErbB4-positive cells in adult mouse brain. J Neurosci 34, 13549-13566.

Beck, K.D., Powell-Braxton, L., Widmer, H.R., Valverde, J., Hefti, F., 1995. Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. Neuron 14, 717-730.

Bekris, S., Antoniou, K., Daskas, S., Papadopoulou-Daifoti, Z., 2005. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. Behavioural brain research 161, 45-59.

Belanoff, J.K., Rothschild, A.J., Cassidy, F., DeBattista, C., Baulieu, E.E., Schold, C., Schatzberg, A.F., 2002. An open label trial of C-1073 (mifepristone) for psychotic major depression. Biological psychiatry 52, 386-392.

Bell, C., Abrams, J., Nutt, D., 2001. Tryptophan depletion and its implications for psychiatry. The British journal of psychiatry : the journal of mental science 178, 399-405.

Belleudi, F., Marra, E., Mazzetta, F., Fattore, L., Giovagnoli, M.R., Mancini, R., Aurisicchio, L., Torrisi, M.R., Ciliberto, G., 2012. Monoclonal antibody-induced ErbB3 receptor internalization and degradation inhibits growth and migration of human melanoma cells. Cell Cycle 11, 1455-1467.

Belmaker, R.H., Agam, G., 2008. Major depressive disorder. The New England journal of medicine 358, 55-68.

Belzeaux, R., Formisano-Treziny, C., Loundou, A., Boyer, L., Gabert, J., Samuelian, J.C., Feron, F., Naudin, J., Ibrahim, E.C., 2010. Clinical variations modulate patterns of gene expression and define blood biomarkers in major depression. Journal of psychiatric research 44, 1205-1213.

Benkelfat, C., Ellenbogen, M.A., Dean, P., Palmour, R.M., Young, S.N., 1994. Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. Archives of general psychiatry 51, 687-697.

Bergami, M., Masserdotti, G., Temprana, S.G., Motori, E., Eriksson, T.M., Gobel, J., Yang, S.M., Conzelmann, K.K., Schinder, A.F., Gotz, M., Berninger, B., 2015. A critical period for experience-dependent remodeling of adult-born neuron connectivity. Neuron 85, 710-717.

Bergstrom, A., Jayatissa, M.N., Mork, A., Wiborg, O., 2008. Stress sensitivity and resilience in the chronic mild stress rat model of depression; an in situ hybridization study. Brain research 1196, 41-52.

Bertram, I., Bernstein, H.G., Lendeckel, U., Bukowska, A., Dobrowolny, H., Keilhoff, G., Kanakis, D., Mawrin, C., Bielau, H., Falkai, P., Bogerts, B., 2007. Immunohistochemical evidence for impaired neuregulin-1 signaling in the prefrontal cortex in schizophrenia and in unipolar depression. Ann N Y Acad Sci 1096, 147-156.

Bhagwagar, Z., Rabiner, E.A., Sargent, P.A., Grasby, P.M., Cowen, P.J., 2004. Persistent reduction in brain serotonin1A receptor binding in recovered depressed men measured by positron emission tomography with [11C]WAY-100635. Mol Psychiatry 9, 386-392.

Bhutani, M.K., Bishnoi, M., Kulkarni, S.K., 2009. Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. Pharmacology, biochemistry, and behavior 92, 39-43.

Bi, L.L., Sun, X.D., Zhang, J., Lu, Y.S., Chen, Y.H., Wang, J., Geng, F., Liu, F., Zhang, M., Liu, J.H., Li, X.W., Mei, L., Gao, T.M., 2015. Amygdala NRG1-ErbB4 is critical for the modulation of anxiety-like behaviors. Neuropsychopharmacology 40, 974-986.

Bijak, M., Tokarski, K., Czyrak, A., Mackowiak, M., Wedzony, K., 1996. Imipramine increases the 5-HT1A receptor-mediated inhibition of hippocampal neurons without changing the 5-HT1A receptor binding. Eur J Pharmacol 305, 79-85.

Billings, A.G., Cronkite, R.C., Moos, R.H., 1983. Social-environmental factors in unipolar depression: comparisons of depressed patients and nondepressed controls. Journal of abnormal psychology 92, 119-133.

Bjarkam, C.R., Sorensen, J.C., Geneser, F.A., 2003. Distribution and morphology of serotonin-immunoreactive axons in the hippocampal region of the New Zealand white rabbit. I. Area dentata and hippocampus. Hippocampus 13, 21-37.

Black, C., Miller, B.J., 2015. Meta-Analysis of Cytokines and Chemokines in Suicidality: Distinguishing Suicidal Versus Nonsuicidal Patients. Biol Psychiatry 78, 28-37.

Blier, P., de Montigny, C., 1999. Serotonin and drug-induced therapeutic responses in major depression, obsessive-compulsive and panic disorders. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 21, 91S-98S.

Blier, P., Pineyro, G., el Mansari, M., Bergeron, R., de Montigny, C., 1998. Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. Ann N Y Acad Sci 861, 204-216.

Boldrini, M., Butt, T.H., Santiago, A.N., Tamir, H., Dwork, A.J., Rosoklija, G.B., Arango, V., Hen, R., Mann, J.J., 2014. Benzodiazepines and the potential trophic effect of antidepressants on dentate gyrus cells in mood disorders. Int J Neuropsychopharmacol 17, 1923-1933.

Boldrini, M., Hen, R., Underwood, M.D., Rosoklija, G.B., Dwork, A.J., Mann, J.J., Arango, V., 2012. Hippocampal Angiogenesis and Progenitor Cell Proliferation Are Increased with Antidepressant Use in Major Depression. Biological psychiatry.

Boldrini, M., Santiago, A.N., Hen, R., Dwork, A.J., Rosoklija, G.B., Tamir, H., Arango, V., John Mann, J., 2013. Hippocampal granule neuron number and dentate gyrus volume in antidepressant-treated and untreated major depression. Neuropsychopharmacology 38, 1068-1077.

Boldrini, M., Underwood, M.D., Hen, R., Rosoklija, G.B., Dwork, A.J., John Mann, J., Arango, V., 2009. Antidepressants increase neural progenitor cells in the human hippocampus. Neuropsychopharmacology 34, 2376-2389.

Booij, L., Szyf, M., Carballedo, A., Frey, E.M., Morris, D., Dymov, S., Vaisheva, F., Ly, V., Fahey, C., Meaney, J., Gill, M., Frodl, T., 2015. DNA methylation of the serotonin transporter gene in peripheral cells and stress-related changes in hippocampal volume: a study in depressed patients and healthy controls. PloS one 10, e0119061.

Borsini, F., Ceci, A., Bietti, G., Donetti, A., 1995. BIMT 17, a 5-HT1A receptor agonist/5-HT2A receptor antagonist, directly activates postsynaptic 5-HT inhibitory responses in the rat cerebral cortex. Naunyn-Schmiedeberg's archives of pharmacology 352, 283-290.

Bosker, F.J., Klompmakers, A., Westenberg, H.G., 1997. Postsynaptic 5-HT1A receptors mediate 5-hydroxytryptamine release in the amygdala through a feedback to the caudal linear raphe. European journal of pharmacology 333, 147-157.

Boucher, A.A., Arnold, J.C., Duffy, L., Schofield, P.R., Micheau, J., Karl, T., 2007a. Heterozygous neuregulin 1 mice are more sensitive to the behavioural effects of Delta9-tetrahydrocannabinol. Psychopharmacology (Berl) 192, 325-336.

Boucher, A.A., Hunt, G.E., Karl, T., Micheau, J., McGregor, I.S., Arnold, J.C., 2007b. Heterozygous neuregulin 1 mice display greater baseline and Delta(9)-tetrahydrocannabinol-induced c-Fos expression. Neuroscience 149, 861-870.

Bracko, O., Singer, T., Aigner, S., Knobloch, M., Winner, B., Ray, J., Clemenson, G.D., Jr., Suh, H., Couillard-Despres, S., Aigner, L., Gage, F.H., Jessberger, S., 2012. Gene expression profiling of neural stem cells and their neuronal progeny reveals IGF2 as a regulator of adult hippocampal neurogenesis. J Neurosci 32, 3376-3387.

Branchi, I., Santarelli, S., Capoccia, S., Poggini, S., D'Andrea, I., Cirulli, F., Alleva, E., 2013. Antidepressant treatment outcome depends on the quality of the living environment: a pre-clinical investigation in mice. PloS one 8, e62226.

Brenes Saenz, J.C., Villagra, O.R., Fornaguera Trias, J., 2006. Factor analysis of Forced Swimming test, Sucrose Preference test and Open Field test on enriched, social and isolated reared rats. Behav Brain Res 169, 57-65.

Brewer, G.J., Torricelli, J.R., 2007. Isolation and culture of adult neurons and neurospheres. Nat Protoc 2, 1490-1498.

Brezun, J.M., Daszuta, A., 2000a. Serotonergic reinnervation reverses lesion-induced decreases in PSA-NCAM labeling and proliferation of hippocampal cells in adult rats. Hippocampus 10, 37-46.

Brezun, J.M., Daszuta, A., 2000b. Serotonin may stimulate granule cell proliferation in the adult hippocampus, as observed in rats grafted with foetal raphe neurons. European Journal of Neuroscience 12, 391-396.

Broadbent, N.J., Gaskin, S., Squire, L.R., Clark, R.E., 2010. Object recognition memory and the rodent hippocampus. Learning & memory 17, 5-11.

Brown, E.S., Varghese, F.P., McEwen, B.S., 2004. Association of depression with medical illness: does cortisol play a role? Biological psychiatry 55, 1-9.

Brummelte, S., Galea, L.A., 2010. Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. Neuroscience 168, 680-690.

Buckmaster, P.S., Strowbridge, B.W., Kunkel, D.D., Schmiege, D.L., Schwartzkroin, P.A., 1992. Mossy cell axonal projections to the dentate gyrus molecular layer in the rat hippocampal slice. Hippocampus 2, 349-362.

Cahill, M.E., Jones, K.A., Rafalovich, I., Xie, Z., Barros, C.S., Muller, U., Penzes, P., 2012. Control of interneuron dendritic growth through NRG1/erbB4-mediated kalirin-7 disinhibition. Mol Psychiatry 17, 1, 99-107.

Cahill, M.E., Remmers, C., Jones, K.A., Xie, Z., Sweet, R.A., Penzes, P., 2013. Neuregulin1 signaling promotes dendritic spine growth through kalirin. Journal of neurochemistry 126, 625-635.

Cai, X., Gu, Z., Zhong, P., Ren, Y., Yan, Z., 2002. Serotonin 5-HT1A receptors regulate AMPA receptor channels through inhibiting Ca2+/calmodulin-dependent kinase II in prefrontal cortical pyramidal neurons. The Journal of biological chemistry 277, 36553-36562.

Camprecios, G., Lorita, J., Pardina, E., Peinado-Onsurbe, J., Soley, M., Ramirez, I., 2011. Expression, localization, and regulation of the neuregulin receptor ErbB3 in mouse heart. J Cell Physiol 226, 450-455.

Cannon, D.M., Walshe, M., Dempster, E., Collier, D.A., Marshall, N., Bramon, E., Murray, R.M., McDonald, C., 2012. The association of white matter volume in psychotic disorders with genotypic variation in NRG1, MOG and CNP: a voxel-based analysis in affected individuals and their unaffected relatives. Transl Psychiatry 2, e167.

Canoll, P.D., Musacchio, J.M., Hardy, R., Reynolds, R., Marchionni, M.A., Salzer, J.L., 1996. GGF/neuregulin is a neuronal signal that promotes the proliferation and survival and inhibits the differentiation of oligodendrocyte progenitors. Neuron 17, 229-243.

Cao, L., Deng, W., Guan, L., Yang, Z., Lin, Y., Ma, X., Li, X., Liu, Y., Ye, B., Lao, G., Chen, Y., Liang, H., Wu, Y., Ou, Y., Huang, W., Liu, W., Wang, Q., Wang, Y., Zhao, L., Li, T., Hu, X., 2014. Association of the 3' region of the neuregulin 1 gene with bipolar I disorder in the Chinese Han population. J Affect Disord 162, 81-88.

Cao, Z., Wu, X., Yen, L., Sweeney, C., Carraway, K.L., 3rd, 2007. Neuregulin-induced ErbB3 downregulation is mediated by a protein stability cascade involving the E3 ubiquitin ligase Nrdp1. Mol Cell Biol 27, 2180-2188.

Carlsson, T., Schindler, F.R., Hollerhage, M., Depboylu, C., Arias-Carrion, O., Schnurrbusch, S., Rosler, T.W., Wozny, W., Schwall, G.P., Groebe, K., Oertel, W.H., Brundin, P., Schrattenholz, A., Hoglinger, G.U., 2011. Systemic administration of neuregulin-1beta1 protects dopaminergic neurons in a mouse model of Parkinson's disease. Journal of neurochemistry 117, 1066-1074.

Caspi, A., Hariri, A.R., Holmes, A., Uher, R., Moffitt, T.E., 2010. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. The American journal of psychiatry 167, 509-527.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386-389.

Cavallaro, M., Mariani, J., Lancini, C., Latorre, E., Caccia, R., Gullo, F., Valotta, M., DeBiasi, S., Spinardi, L., Ronchi, A., Wanke, E., Brunelli, S., Favaro, R., Ottolenghi, S., Nicolis, S.K., 2008. Impaired generation of mature neurons by neural stem cells from hypomorphic Sox2 mutants. Development 135, 541-557.

Celada, P., Puig, M.V., Casanovas, J.M., Guillazo, G., Artigas, F., 2001. Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: Involvement of serotonin-1A, GABA(A), and glutamate receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience 21, 9917-9929.

Chaudhury, A.R., Gerecke, K.M., Wyss, J.M., Morgan, D.G., Gordon, M.N., Carroll, S.L., 2003. Neuregulin-1 and erbB4 immunoreactivity is associated with neuritic plaques in Alzheimer disease brain and in a transgenic model of Alzheimer disease. Journal of neuropathology and experimental neurology 62, 42-54.

Chen, P., Chen, J., Huang, K., Ji, W., Wang, T., Li, T., Wang, Y., Wang, H., He, L., Feng, G., 2012. Analysis of association between common SNPs in ErbB4 and bipolar affective disorder, major depressive

disorder and schizophrenia in the Han Chinese population. Progress in Neuro-Psychopharmacology and Biological Psychiatry 36, 17-21.

Chen, Y.J., Johnson, M.A., Lieberman, M.D., Goodchild, R.E., Schobel, S., Lewandowski, N., Rosoklija, G., Liu, R.C., Gingrich, J.A., Small, S., Moore, H., Dwork, A.J., Talmage, D.A., Role, L.W., 2008. Type III neuregulin-1 is required for normal sensorimotor gating, memory-related behaviors, and corticostriatal circuit components. J Neurosci 28, 6872-6883.

Chen, Y.J., Zhang, M., Yin, D.M., Wen, L., Ting, A., Wang, P., Lu, Y.S., Zhu, X.H., Li, S.J., Wu, C.Y., Wang, X.M., Lai, C., Xiong, W.C., Mei, L., Gao, T.M., 2010. ErbB4 in parvalbumin-positive interneurons is critical for neuregulin 1 regulation of long-term potentiation. Proceedings of the National Academy of Sciences of the United States of America 107, 21818-21823.

Cheng, Y., Rodriguiz, R., Murthy, S., Senatorov, V., Thouennon, E., Cawley, N., Aryal, D., Ahn, S., Lecka-Czernik, B., Wetsel, W., 2014. Neurotrophic factor-α1 prevents stress-induced depression through enhancement of neurogenesis and is activated by rosiglitazone. Mol Psychiatry.

Chesworth, R., Yulyaningsih, E., Cappas, E., Arnold, J., Sainsbury, A., Karl, T., 2012. The response of neuregulin 1 mutant mice to acute restraint stress. Neurosci Lett 515, 82-86.

Chiba, S., Numakawa, T., Ninomiya, M., Richards, M.C., Wakabayashi, C., Kunugi, H., 2012. Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. Prog Neuropsychopharmacol Biol Psychiatry 39, 112-119.

Chohan, T.W., Boucher, A.A., Spencer, J.R., Kassem, M.S., Hamdi, A.A., Karl, T., Fok, S.Y., Bennett, M.R., Arnold, J.C., 2014. Partial genetic deletion of neuregulin 1 modulates the effects of stress on sensorimotor gating, dendritic morphology, and HPA axis activity in adolescent mice. Schizophr Bull 40, 1272-1284.

Chong, V.Z., Thompson, M., Beltaifa, S., Webster, M.J., Law, A.J., Weickert, C.S., 2008. Elevated neuregulin-1 and ErbB4 protein in the prefrontal cortex of schizophrenic patients. Schizophrenia research 100, 270-280.

Chow, C., Epp, J.R., Lieblich, S.E., Barha, C.K., Galea, L.A., 2013. Sex differences in neurogenesis and activation of new neurons in response to spatial learning and memory. Psychoneuroendocrinology 38, 1236-1250.

Christiansen, S., Bouzinova, E.V., Palme, R., Wiborg, O., 2012. Circadian activity of the hypothalamic-pituitary-adrenal axis is differentially affected in the rat chronic mild stress model of depression. Stress 15, 647-657.

Cobb, J.A., Simpson, J., Mahajan, G.J., Overholser, J.C., Jurjus, G.J., Dieter, L., Herbst, N., May, W., Rajkowska, G., Stockmeier, C.A., 2013. Hippocampal volume and total cell numbers in major depressive disorder. Journal of psychiatric research 47, 299-306.

Commons, K.G., 2008. Evidence for topographically organized endogenous 5-HT-1A receptor-dependent feedback inhibition of the ascending serotonin system. The European journal of neuroscience 27, 2611-2618.

Cook, R.S., Garrett, J.T., Sanchez, V., Stanford, J.C., Young, C., Chakrabarty, A., Rinehart, C., Zhang, Y., Wu, Y., Greenberger, L., Horak, I.D., Arteaga, C.L., 2011. ErbB3 ablation impairs PI3K/Akt-dependent mammary tumorigenesis. Cancer research 71, 3941-3951.

Cooper, M.A., Koleske, A.J., 2014. Ablation of ErbB4 from excitatory neurons leads to reduced dendritic spine density in mouse prefrontal cortex. J Comp Neurol 522, 3351-3362.

Cowen, P.J., Power, A.C., Ware, C.J., Anderson, I.M., 1994. 5-HT1A receptor sensitivity in major depression. A neuroendocrine study with buspirone. Br J Psychiatry 164, 372-379.

Crawford, L.K., Craige, C.P., Beck, S.G., 2010. Increased intrinsic excitability of lateral wing serotonin neurons of the dorsal raphe: a mechanism for selective activation in stress circuits. J Neurophysiol 103, 2652-2663.

Crespi, F., 2009. Apamin increases 5-HT cell firing in raphe dorsalis and extracellular 5-HT levels in amygdala: a concomitant in vivo study in anesthetized rats. Brain research 1281, 35-46.

Crespi, F., 2010. SK channel blocker apamin attenuates the effect of SSRI fluoxetine upon cell firing in dorsal raphe nucleus: a concomitant electrophysiological and electrochemical in vivo study reveals implications for modulating extracellular 5-HT. Brain research 1334, 1-11.

Cryan, J.F., Holmes, A., 2005. The ascent of mouse: advances in modelling human depression and anxiety. Nature reviews. Drug discovery 4, 775-790.

Cui, K., Ashdown, H., Luheshi, G.N., Boksa, P., 2009. Effects of prenatal immune activation on hippocampal neurogenesis in the rat. Schizophr Res 113, 288-297.

Cui, W., Tao, J., Wang, Z., Ren, M., Zhang, Y., Sun, Y., Peng, Y., Li, R., 2013. Neuregulin1beta1 antagonizes apoptosis via ErbB4-dependent activation of PI3-kinase/Akt in APP/PS1 transgenic mice. Neurochem Res 38, 2237-2246.

Czubak, A., Nowakowska, E., Kus, K., Burda, K., Metelska, J., Baer-Dubowska, W., Cichocki, M., 2009. Influences of chronic venlafaxine, olanzapine and nicotine on the hippocampal and cortical concentrations of brain-derived neurotrophic factor (BDNF). Pharmacological reports : PR 61, 1017-1023.

Dagyte, G., Crescente, I., Postema, F., Seguin, L., Gabriel, C., Mocaer, E., Boer, J.A., Koolhaas, J.M., 2011. Agomelatine reverses the decrease in hippocampal cell survival induced by chronic mild stress. Behav Brain Res 218, 121-128.

Dagyte, G., Trentani, A., Postema, F., Luiten, P.G., Den Boer, J.A., Gabriel, C., Mocaer, E., Meerlo, P., Van der Zee, E.A., 2010. The novel antidepressant agomelatine normalizes hippocampal neuronal activity and promotes neurogenesis in chronically stressed rats. CNS neuroscience & therapeutics 16, 195-207.

Dahlstroem, A., Fuxe, K., 1964. Evidence for the Existence of Monoamine-Containing Neurons in the Central Nervous System. I. Demonstration of Monoamines in the Cell Bodies of Brain Stem Neurons. Acta physiologica Scandinavica. Supplementum, SUPPL 232:231-255.

Dang, H., Chen, Y., Liu, X., Wang, Q., Wang, L., Jia, W., Wang, Y., 2009a. Antidepressant effects of ginseng total saponins in the forced swimming test and chronic mild stress models of depression. Progress in neuro-psychopharmacology & biological psychiatry 33, 1417-1424.

Dang, H., Sun, L., Liu, X., Peng, B., Wang, Q., Jia, W., Chen, Y., Pan, A., Xiao, P., 2009b. Preventive action of Kai Xin San aqueous extract on depressive-like symptoms and cognition deficit induced by chronic mild stress. Experimental biology and medicine 234, 785-793.

David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., Drew, M., Craig, D.A., Guiard, B.P., Guilloux, J.P., Artymyshyn, R.P., Gardier, A.M., Gerald, C., Antonijevic, I.A., Leonardo, E.D., Hen, R., 2009. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62, 479-493.

Dawood, T., Anderson, J., Barton, D., Lambert, E., Esler, M., Hotchkin, E., Haikerwal, D., Kaye, D., Lambert, G., 2007. Reduced overflow of BDNF from the brain is linked with suicide risk in depressive illness. Mol Psychiatry 12, 981-983.

De Montigny, C., 1981. Enhancement of the 5-HT neurotransmission by antidepressant treatments. Journal de physiologie 77, 455-461.

Deakin, I.H., Nissen, W., Law, A.J., Lane, T., Kanso, R., Schwab, M.H., Nave, K.A., Lamsa, K.P., Paulsen, O., Bannerman, D.M., Harrison, P.J., 2012. Transgenic overexpression of the type I isoform of neuregulin 1 affects working memory and hippocampal oscillations but not long-term potentiation. Cerebral cortex 22, 1520-1529.

Delgado, P.L., Miller, H.L., Salomon, R.M., Licinio, J., Krystal, J.H., Moreno, F.A., Heninger, G.R., Charney, D.S., 1999. Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. Biological psychiatry 46, 212-220.

Deng, C., Pan, B., Engel, M., Huang, X.F., 2013. Neuregulin-1 signalling and antipsychotic treatment: potential therapeutic targets in a schizophrenia candidate signalling pathway. Psychopharmacology (Berl) 226, 201-215.

Deng, W., Aimone, J.B., Gage, F.H., 2010. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nature reviews. Neuroscience 11, 339-350.

Denny, C.A., Burghardt, N.S., Schachter, D.M., Hen, R., Drew, M.R., 2012. 4- to 6-week-old adult-born hippocampal neurons influence novelty-evoked exploration and contextual fear conditioning. Hippocampus 22, 1188-1201.

Depboylu, C., Rösler, T.W., Andrade, A., Oertel, W.H., Höglinger, G.U., 2015. Systemically administered neuregulin‐1β1 rescues nigral dopaminergic neurons via the ErbB4 receptor tyrosine kinase in MPTP mouse models of Parkinson's disease. Journal of neurochemistry 133, 590-597.

Descarries, L., Watkins, K.C., Garcia, S., Beaudet, A., 1982. The serotonin neurons in nucleus raphe dorsalis of adult rat: a light and electron microscope radioautographic study. J Comp Neurol 207, 239-254.

Detke, M.J., Johnson, J., Lucki, I., 1997. Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. Exp Clin Psychopharmacol 5, 107-112.

Diaz, S.L., Doly, S., Narboux-Neme, N., Fernandez, S., Mazot, P., Banas, S.M., Boutourlinsky, K., Moutkine, I., Belmer, A., Roumier, A., Maroteaux, L., 2012. 5-HT2B receptors are required for serotonin-selective antidepressant actions. Molecular psychiatry 17, 154-163.

Diniz, B.S., Teixeira, A.L., Talib, L.L., Mendonca, V.A., Gattaz, W.F., Forlenza, O.V., 2010. Serum brain-derived neurotrophic factor level is reduced in antidepressant-free patients with late-life depression. World J Biol Psychiatry 11, 550-555.

Douet, V., Chang, L., Lee, K., Ernst, T., Pediatric Imaging, N., Genetics, C., 2015. ERBB4 polymorphism and family history of psychiatric disorders on age-related cortical changes in healthy children. Brain Imaging Behav 9, 128-140.

Douet, V., Chang, L., Pritchett, A., Lee, K., Keating, B., Bartsch, H., Jernigan, T.L., Dale, A., Akshoomoff, N., Murray, S., Bloss, C., Kennedy, D.N., Amaral, D., Gruen, J., Kaufmann, W.E., Casey, B.J., Sowell, E., Ernst, T., 2014. Schizophrenia-risk variant rs6994992 in the neuregulin-1 gene on brain developmental trajectories in typically developing children. Transl Psychiatry 4, e392.

Dranovsky, A., Hen, R., 2006. Hippocampal neurogenesis: regulation by stress and antidepressants. Biological psychiatry 59, 1136-1143.

Drevets, W.C., Thase, M.E., Moses-Kolko, E.L., Price, J., Frank, E., Kupfer, D.J., Mathis, C., 2007. Serotonin-1A receptor imaging in recurrent depression: replication and literature review. Nuclear medicine and biology 34, 865-877.

Drew, M.R., Hen, R., 2007. Adult hippocampal neurogenesis as target for the treatment of depression. CNS Neurol Disord Drug Targets 6, 205-218.

Dulawa, S.C., Hen, R., 2005. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. Neurosci Biobehav Rev 29, 771-783.

Dulawa, S.C., Holick, K.A., Gundersen, B., Hen, R., 2004. Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology 29, 1321-1330.

Dumais, A., Lesage, A.D., Lalovic, A., Seguin, M., Tousignant, M., Chawky, N., Turecki, G., 2005. Is violent method of suicide a behavioral marker of lifetime aggression? Am J Psychiatry 162, 1375-1378.

Duman, C.H., Schlesinger, L., Terwilliger, R., Russell, D.S., Newton, S.S., Duman, R.S., 2009. Peripheral insulin-like growth factor-I produces antidepressant-like behavior and contributes to the effect of exercise. Behav Brain Res 198, 366-371.

Duric, V., Banasr, M., Stockmeier, C.A., Simen, A.A., Newton, S.S., Overholser, J.C., Jurjus, G.J., Dieter, L., Duman, R.S., 2013. Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 16, 69-82.

Duvernoy, H.M., 1988. The Human Hippocampus: An Atlas of Applied Anatomy. Springer-Verlag.

Egeland, M., Zhang, X., Millan, M.J., Mocaer, E., Svenningsson, P., 2012. Pharmacological or genetic blockade of the dopamine D3 receptor increases cell proliferation in the hippocampus of adult mice. Journal of neurochemistry 123, 811-823.

Eisch, A.J., Petrik, D., 2012. Depression and hippocampal neurogenesis: a road to remission? Science 338, 72-75.

El Hage, W., Powell, J.F., Surguladze, S.A., 2009. Vulnerability to depression: what is the role of stress genes in gene x environment interaction? Psychological medicine 39, 1407-1411.

Elizalde, N., Garcia-Garcia, A.L., Totterdell, S., Gendive, N., Venzala, E., Ramirez, M.J., Del Rio, J., Tordera, R.M., 2010. Sustained stress-induced changes in mice as a model for chronic depression. Psychopharmacology (Berl) 210, 393-406.

Engel, M., Snikeris, P., Jenner, A., Karl, T., Huang, X.-F., Frank, E., 2014. Neuregulin 1 prevents phencyclidine-induced behavioral impairments and disruptions to GABAergic signaling in mice. International Journal of Neuropsychopharmacology, pyu114.

Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., Gage, F.H., 1998. Neurogenesis in the adult human hippocampus. Nature medicine 4, 1313-1317.

Ernst, C., Deleva, V., Deng, X., Sequeira, A., Pomarenski, A., Klempan, T., Ernst, N., Quirion, R., Gratton, A., Szyf, M., Turecki, G., 2009. Alternative splicing, methylation state, and expression profile of tropomyosin-related kinase B in the frontal cortex of suicide completers. Arch Gen Psychiatry 66, 22-32.

Eto, K., Hommyo, A., Yonemitsu, R., Abe, S.I., 2010. ErbB4 signals Neuregulin1-stimulated cell proliferation and c-fos gene expression through phosphorylation of serum response factor by mitogen-activated protein kinase cascade. Mol Cell Biochem 339, 119-125.

Fabre, V., Beaufour, C., Evrard, A., Rioux, A., Hanoun, N., Lesch, K.P., Murphy, D.L., Lanfumey, L., Hamon, M., Martres, M.P., 2000. Altered expression and functions of serotonin 5-HT1A and 5-HT1B receptors in knock-out mice lacking the 5-HT transporter. The European journal of neuroscience 12, 2299-2310.

Fairbank, J.A., Hansen, D.J., Fitterling, J.M., 1991. Patterns of appraisal and coping across different stressor conditions among former prisoners of war with and without posttraumatic stress disorder. J Consult Clin Psychol 59, 274-281.

Falls, D.L., 2003. Neuregulins: functions, forms, and signaling strategies. Exp Cell Res 284, 14-30.

Fanselow, M.S., Dong, H.W., 2010. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65, 7-19.

Feldmann, R.E., Jr., Sawa, A., Seidler, G.H., 2007. Causality of stem cell based neurogenesis and depression--to be or not to be, is that the question? Journal of psychiatric research 41, 713-723.

Ferland, C.L., Schrader, L.A., 2011. Regulation of histone acetylation in the hippocampus of chronically stressed rats: a potential role of sirtuins. Neuroscience 174, 104-114.

Ferres-Coy, A., Pilar-Cuellar, F., Vidal, R., Paz, V., Masana, M., Cortes, R., Carmona, M.C., Campa, L., Pazos, A., Montefeltro, A., Valdizan, E.M., Artigas, F., Bortolozzi, A., 2013. RNAi-mediated serotonin transporter suppression rapidly increases serotonergic neurotransmission and hippocampal neurogenesis. Translational psychiatry 3, e211.

Firk, C., Markus, C.R., 2007. Review: Serotonin by stress interaction: a susceptibility factor for the development of depression? Journal of psychopharmacology 21, 538-544.

Fischbach, G.D., Rosen, K.M., 1997. ARIA: a neuromuscular junction neuregulin. Annu Rev Neurosci 20, 429-458.

Fitzsimons, C.P., van Hooijdonk, L.W., Morrow, J.A., Peeters, B.W., Hamilton, N., Craighead, M., Vreugdenhil, E., 2009. Antiglucocorticoids, neurogenesis and depression. Mini reviews in medicinal chemistry 9, 249-264.

Flames, N., Long, J.E., Garratt, A.N., Fischer, T.M., Gassmann, M., Birchmeier, C., Lai, C., Rubenstein, J.L., Marin, O., 2004. Short- and long-range attraction of cortical GABAergic interneurons by neuregulin-1. Neuron 44, 251-261.

Fleck, D., van Bebber, F., Colombo, A., Galante, C., Schwenk, B.M., Rabe, L., Hampel, H., Novak, B., Kremmer, E., Tahirovic, S., Edbauer, D., Lichtenthaler, S.F., Schmid, B., Willem, M., Haass, C., 2013. Dual cleavage of neuregulin 1 type III by BACE1 and ADAM17 liberates its EGF-like domain and allows paracrine signaling. J Neurosci 33, 7856-7869.

Flores, A.I., Mallon, B.S., Matsui, T., Ogawa, W., Rosenzweig, A., Okamoto, T., Macklin, W.B., 2000. Akt-mediated survival of oligodendrocytes induced by neuregulins. J Neurosci 20, 7622-7630.

Flores, B.H., Kenna, H., Keller, J., Solvason, H.B., Schatzberg, A.F., 2006. Clinical and biological effects of mifepristone treatment for psychotic depression. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 31, 628-636.

Fournier, N.M., Lee, B., Banasr, M., Elsayed, M., Duman, R.S., 2012. Vascular endothelial growth factor regulates adult hippocampal cell proliferation through MEK/ERK- and PI3K/Akt-dependent signaling. Neuropharmacology 63, 642-652.

Franco-Gonzalez, J.F., Ramos, J., Cruz, V.L., Martinez-Salazar, J., 2013. Simulation of homology models for the extracellular domains (ECD) of ErbB3, ErbB4 and the ErbB2-ErbB3 complex in their active conformations. J Mol Model 19, 931-941.

Franklin, K., Paxinos, G., 2007. The mouse brain in stereotaxic coordinates. Academic Press, San Diego, CA.

Froger, N., Palazzo, E., Boni, C., Hanoun, N., Saurini, F., Joubert, C., Dutriez-Casteloot, I., Enache, M., Maccari, S., Barden, N., Cohen-Salmon, C., Hamon, M., Lanfumey, L., 2004. Neurochemical and behavioral alterations in glucocorticoid receptor-impaired transgenic mice after chronic mild stress. J Neurosci 24, 2787-2796.

Furczyk, K., Schutova, B., Michel, T.M., Thome, J., Buttner, A., 2013. The neurobiology of suicide - A Review of post-mortem studies. J Mol Psychiatry 1, 2.

Gabbott, P.L.A., Warner, T.A., Jays, P.R.L., Salway, P., Busby, S.J., 2005. Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol 492, 145-177.

Gage, F.H., Thompson, R.G., 1980. Differential distribution of norepinephrine and serotonin along the dorsal-ventral axis of the hippocampal formation. Brain research bulletin 5, 771-773.

Gao, R., Zhang, J., Cheng, L., Wu, X., Dong, W., Yang, X., Li, T., Liu, X., Xu, Y., Li, X., Zhou, M., 2010. A Phase II, randomized, double-blind, multicenter, based on standard therapy, placebo-controlled study of the efficacy and safety of recombinant human neuregulin-1 in patients with chronic heart failure. J Am Coll Cardiol 55, 1907-1914.

Garcia, L., Castillo, C., Carballo, J., Rodriguez, Y., Forsyth, P., Medina, R., Martinez, J.C., Longart, M., 2013. ErbB receptors and PKC regulate PC12 neuronal-like differentiation and sodium current elicitation. Neuroscience 236, 88-98.

Gartside, S.E., Hajos-Korcsok, E., Bagdy, E., Harsing, L.G., Jr., Sharp, T., Hajos, M., 2000. Neurochemical and electrophysiological studies on the functional significance of burst firing in serotonergic neurons. Neuroscience 98, 295-300.

Gartside, S.E., Leitch, M.M., Young, A.H., 2003. Altered glucocorticoid rhythm attenuates the ability of a chronic SSRI to elevate forebrain 5-HT: implications for the treatment of depression. Neuropsychopharmacology 28, 1572-1578.

Gasbarri, A., Verney, C., Innocenzi, R., Campana, E., Pacitti, C., 1994. Mesolimbic dopaminergic neurons innervating the hippocampal formation in the rat: a combined retrograde tracing and immunohistochemical study. Brain research 668, 71-79.

Ge, S., Yang, C.H., Hsu, K.S., Ming, G.L., Song, H., 2007. A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. Neuron 54, 559-566.

Georgieva, L., Dimitrova, A., Ivanov, D., Nikolov, I., Williams, N.M., Grozeva, D., Zaharieva, I., Toncheva, D., Owen, M.J., Kirov, G., O'Donovan, M.C., 2008. Support for neuregulin 1 as a susceptibility gene for bipolar disorder and schizophrenia. Biol Psychiatry 64, 419-427.

Gerecke, K.M., Wyss, J.M., Karavanova, I., Buonanno, A., Carroll, S.L., 2001. ErbB transmembrane tyrosine kinase receptors are differentially expressed throughout the adult rat central nervous system. J Comp Neurol 433, 86-100.

Gersner, R., Toth, E., Isserles, M., Zangen, A., 2010. Site-specific antidepressant effects of repeated subconvulsive electrical stimulation: potential role of brain-derived neurotrophic factor. Biological psychiatry 67, 125-132.

Ghashghaei, H.T., Weber, J., Pevny, L., Schmid, R., Schwab, M.H., Lloyd, K.C., Eisenstat, D.D., Lai, C., Anton, E.S., 2006. The role of neuregulin-ErbB4 interactions on the proliferation and organization of cells in the subventricular zone. Proc Natl Acad Sci U S A 103, 1930-1935.

Gierdalski, M., Sardi, S.P., Corfas, G., Juliano, S.L., 2005. Endogenous neuregulin restores radial glia in a (ferret) model of cortical dysplasia. Journal of Neuroscience 25, 8498-8504.

Glasper, E.R., Llorens-Martin, M.V., Leuner, B., Gould, E., Trejo, J.L., 2010. Blockade of insulin-like growth factor-I has complex effects on structural plasticity in the hippocampus. Hippocampus 20, 706-712.

Gobbi, G., Murphy, D.L., Lesch, K., Blier, P., 2001. Modifications of the serotonergic system in mice lacking serotonin transporters: an in vivo electrophysiological study. The Journal of pharmacology and experimental therapeutics 296, 987-995.

Goes, F.S., Willour, V.L., Zandi, P.P., Belmonte, P.L., MacKinnon, D.F., Mondimore, F.M., Schweizer, B., Gershon, E.S., McMahon, F.J., Potash, J.B., 2009. Family-based association study of Neuregulin 1 with psychotic bipolar disorder. Am J Med Genet B Neuropsychiatr Genet 150B, 693-702.

Golden, S.A., Covington, H.E., 3rd, Berton, O., Russo, S.J., 2011. A standardized protocol for repeated social defeat stress in mice. Nat Protoc 6, 1183-1191.

Golding, J.P., Trainor, P., Krumlauf, R., Gassmann, M., 2000. Defects in pathfinding by cranial neural crest cells in mice lacking the neuregulin receptor ErbB4. Nat Cell Biol 2, 103-109.

Goldstein, G., Fava, M., Culler, M., Fisher, A., Rickels, K., Lydiard, R.B., Rosenbaum, J., 2000. Elevated plasma thymopoietin associated with therapeutic nonresponsiveness in major depression. Biological psychiatry 48, 65-69.

Gomez-Gaviro, M.V., Scott, C.E., Sesay, A.K., Matheu, A., Booth, S., Galichet, C., Lovell-Badge, R., 2012. Betacellulin promotes cell proliferation in the neural stem cell niche and stimulates neurogenesis. Proceedings of the National Academy of Sciences of the United States of America 109, 1317-1322.

Grah, M., Mihanovic, M., Ruljancic, N., Restek-Petrovic, B., Molnar, S., Jelavic, S., 2014. Brain-derived neurotrophic factor as a suicide factor in mental disorders. Acta neuropsychiatrica 26, 356-363.

Grahn, R.E., Will, M.J., Hammack, S.E., Maswood, S., McQueen, M.B., Watkins, L.R., Maier, S.F., 1999. Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. Brain research 826, 35-43.

Gray, N.A., Milak, M.S., DeLorenzo, C., Ogden, R.T., Huang, Y.Y., Mann, J.J., Parsey, R.V., 2013. Antidepressant treatment reduces serotonin-1A autoreceptor binding in major depressive disorder. Biol Psychiatry 74, 26-31.

Grippo, A.J., Beltz, T.G., Weiss, R.M., Johnson, A.K., 2006. The effects of chronic fluoxetine treatment on chronic mild stress-induced cardiovascular changes and anhedonia. Biological psychiatry 59, 309-316.

Grippo, A.J., Sullivan, N.R., Damjanoska, K.J., Crane, J.W., Carrasco, G.A., Shi, J., Chen, Z., Garcia, F., Muma, N.A., Van de Kar, L.D., 2005. Chronic mild stress induces behavioral and physiological changes, and may alter serotonin 1A receptor function, in male and cycling female rats. Psychopharmacology 179, 769-780.

Grossman, A.W., Aldridge, G.M., Lee, K.J., Zeman, M.K., Jun, C.S., Azam, H.S., Arii, T., Imoto, K., Greenough, W.T., Rhyu, I.J., 2010. Developmental characteristics of dendritic spines in the dentate gyrus of Fmr1 knockout mice. Brain Res 1355, 221-227.

Gruber, O., Falkai, P., Schneider-Axmann, T., Schwab, S.G., Wagner, M., Maier, W., 2008. Neuregulin-1 haplotype HAP(ICE) is associated with lower hippocampal volumes in schizophrenic patients and in non-affected family members. Journal of psychiatric research 43, 1-6.

Gu, Y., Arruda-Carvalho, M., Wang, J., Janoschka, S.R., Josselyn, S.A., Frankland, P.W., Ge, S., 2012. Optical controlling reveals time-dependent roles for adult-born dentate granule cells. Nature neuroscience 15, 1700-1706.

Guarneri, V., Lenihan, D.J., Valero, V., Durand, J.B., Broglio, K., Hess, K.R., Michaud, L.B., Gonzalez-Angulo, A.M., Hortobagyi, G.N., Esteva, F.J., 2006. Long-term cardiac tolerability of trastuzumab in metastatic breast cancer: the M.D. Anderson Cancer Center experience. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 24, 4107-4115.

Guiard, B.P., El Mansari, M., Blier, P., 2008a. Cross-talk between dopaminergic and noradrenergic systems in the rat ventral tegmental area, locus ceruleus, and dorsal hippocampus. Molecular pharmacology 74, 1463-1475.

Guiard, B.P., El Mansari, M., Merali, Z., Blier, P., 2008b. Functional interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 11, 625-639.

Guirado, R., Sanchez-Matarredona, D., Varea, E., Crespo, C., Blasco-Ibanez, J.M., Nacher, J., 2012. Chronic fluoxetine treatment in middle-aged rats induces changes in the expression of plasticity-related molecules and in neurogenesis. BMC neuroscience 13, 5.

Guy, P.M., Platko, J.V., Cantley, L.C., Cerione, R.A., Carraway, K.L., 3rd, 1994. Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity. Proceedings of the National Academy of Sciences of the United States of America 91, 8132-8136.

Hagihara, H., Toyama, K., Yamasaki, N., Miyakawa, T., 2009. Dissection of hippocampal dentate gyrus from adult mouse. J Vis Exp.

Hahn, C.G., Wang, H.Y., Cho, D.S., Talbot, K., Gur, R.E., Berrettini, W.H., Bakshi, K., Kamins, J., Borgmann-Winter, K.E., Siegel, S.J., Gallop, R.J., Arnold, S.E., 2006. Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. Nature medicine 12, 824-828.

Hajos, M., Hajos-Korcsok, E., Sharp, T., 1999. Role of the medial prefrontal cortex in 5-HT1A receptor-induced inhibition of 5-HT neuronal activity in the rat. British journal of pharmacology 126, 1741-1750.

Hale, M.W., Hay-Schmidt, A., Mikkelsen, J.D., Poulsen, B., Bouwknecht, J.A., Evans, A.K., Stamper, C.E., Shekhar, A., Lowry, C.A., 2008. Exposure to an open-field arena increases c-Fos expression in a subpopulation of neurons in the dorsal raphe nucleus, including neurons projecting to the basolateral amygdaloid complex. Neuroscience 157, 733-748.

Hall, H., Lundkvist, C., Halldin, C., Farde, L., Pike, V.W., McCarron, J.A., Fletcher, A., Cliffe, I.A., Barf, T., Wikstrom, H., Sedvall, G., 1997. Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]way-100635. Brain research 745, 96-108.

Hamani, C., Diwan, M., Isabella, S., Lozano, A.M., Nobrega, J.N., 2010a. Effects of different stimulation parameters on the antidepressant-like response of medial prefrontal cortex deep brain stimulation in rats. Journal of psychiatric research 44, 683-687.

Hamani, C., Diwan, M., Macedo, C.E., Brandao, M.L., Shumake, J., Gonzalez-Lima, F., Raymond, R., Lozano, A.M., Fletcher, P.J., Nobrega, J.N., 2010b. Antidepressant-like effects of medial prefrontal cortex deep brain stimulation in rats. Biological psychiatry 67, 117-124.

Hanson, N.D., Owens, M.J., Nemeroff, C.B., 2011. Depression, antidepressants, and neurogenesis: a critical reappraisal. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 36, 2589-2602.

Harkin, A., Kelly, J.P., Leonard, B.E., 2003. A review of the relevance and validity of olfactory bulbectomy as a model of depression. Clin Neurosci Res 3, 253-262.

Hasler, G., 2010. Pathophysiology of depression: do we have any solid evidence of interest to clinicians? World Psychiatry 9, 155-161.

Hatzimanolis, A., McGrath, J.A., Wang, R., Li, T., Wong, P.C., Nestadt, G., Wolyniec, P.S., Valle, D., Pulver, A.E., Avramopoulos, D., 2013. Multiple variants aggregate in the neuregulin signaling pathway in a subset of schizophrenia patients. Transl Psychiatry 3, e264.

Hawrylycz, M.J., Lein, E.S., Guillozet-Bongaarts, A.L., Shen, E.H., Ng, L., Miller, J.A., van de Lagemaat, L.N., Smith, K.A., Ebbert, A., Riley, Z.L., Abajian, C., Beckmann, C.F., Bernard, A., Bertagnolli, D., Boe, A.F., Cartagena, P.M., Chakravarty, M.M., Chapin, M., Chong, J., Dalley, R.A., Daly, B.D., Dang, C., Datta, S., Dee, N., Dolbeare, T.A., Faber, V., Feng, D., Fowler, D.R., Goldy, J., Gregor, B.W., Haradon, Z., Haynor, D.R., Hohmann, J.G., Horvath, S., Howard, R.E., Jeromin, A., Jochim, J.M., Kinnunen, M., Lau, C., Lazarz, E.T., Lee, C., Lemon, T.A., Li, L., Li, Y., Morris, J.A., Overly, C.C., Parker, P.D., Parry, S.E., Reding, M., Royall, J.J., Schulkin, J., Sequeira, P.A., Slaughterbeck, C.R., Smith, S.C., Sodt, A.J., Sunkin, S.M., Swanson, B.E., Vawter, M.P., Williams, D., Wohnoutka, P., Zielke, H.R., Geschwind, D.H., Hof, P.R., Smith, S.M., Koch, C., Grant, S.G., Jones, A.R., 2012. An anatomically comprehensive atlas of the adult human brain transcriptome. Nature 489, 391-399.

Hayley, S., Du, L., Litteljohn, D., Palkovits, M., Faludi, G., Merali, Z., Poulter, M.O., Anisman, H., 2015. Gender and brain regions specific differences in brain derived neurotrophic factor protein levels of depressed individuals who died through suicide. Neurosci Lett 600, 12-16.

Henningsen, K., Palmfeldt, J., Christiansen, S., Baiges, I., Bak, S., Jensen, O.N., Gregersen, N., Wiborg, O., 2012. Candidate hippocampal biomarkers of susceptibility and resilience to stress in a rat model of depression. Molecular & cellular proteomics : MCP 11, M111 016428.

Hercher, C., Canetti, L., Turecki, G., Mechawar, N., 2010. Anterior cingulate pyramidal neurons display altered dendritic branching in depressed suicides. Journal of psychiatric research 44, 286-293.

Hervas, I., Artigas, F., 1998. Effect of fluoxetine on extracellular 5-hydroxytryptamine in rat brain. Role of 5-HT autoreceptors. Eur J Pharmacol 358, 9-18.

Heym, J., Trulson, M.E., Jacobs, B.L., 1982. Raphe unit activity in freely moving cats: effects of phasic auditory and visual stimuli. Brain research 232, 29-39.

Hill, A.S., Sahay, A., Hen, R., 2015. Increasing Adult Hippocampal Neurogenesis is Sufficient to Reduce Anxiety and Depression-Like Behaviors. Neuropsychopharmacology.

Hill, M.N., Hillard, C.J., Bambico, F.R., Patel, S., Gorzalka, B.B., Gobbi, G., 2009. The therapeutic potential of the endocannabinoid system for the development of a novel class of antidepressants. Trends in pharmacological sciences 30, 484-493.

Hillerer, K.M., Neumann, I.D., Couillard-Despres, S., Aigner, L., Slattery, D.A., 2013. Sex-dependent regulation of hippocampal neurogenesis under basal and chronic stress conditions in rats. Hippocampus 23, 476-487.

Hirvonen, J., Karlsson, H., Kajander, J., Lepola, A., Markkula, J., Rasi-Hakala, H., Nagren, K., Salminen, J.K., Hietala, J., 2008. Decreased brain serotonin 5-HT1A receptor availability in medication-naive patients with major depressive disorder: an in-vivo imaging study using PET and [carbonyl-11C]WAY-100635. Int J Neuropsychopharmacol 11, 465-476.

Hobin, J.A., Ji, J., Maren, S., 2006. Ventral hippocampal muscimol disrupts context-specific fear memory retrieval after extinction in rats. Hippocampus 16, 174-182.

Hoglinger, G.U., Rizk, P., Muriel, M.P., Duyckaerts, C., Oertel, W.H., Caille, I., Hirsch, E.C., 2004. Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nature neuroscience 7, 726-735.

Holderbach, R., Clark, K., Moreau, J.-L., Bischofberger, J., Normann, C., 2007. Enhanced long-term synaptic depression in an animal model of depression. Biological psychiatry 62, 92-9100.

Holick, K.A., Lee, D.C., Hen, R., Dulawa, S.C., 2008. Behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the serotonin 1A receptor. Neuropsychopharmacology 33, 406-417.

Holmes, A., 2008. Genetic variation in cortico-amygdala serotonin function and risk for stress-related disease. Neuroscience and biobehavioral reviews 32, 1293-1314.

Horowitz, M.A., Zunszain, P.A., Anacker, C., Musaelyan, K., Pariante, C.M., 2013. Glucocorticoids and inflammation: a double-headed sword in depression? How do neuroendocrine and inflammatory pathways interact during stress to contribute to the pathogenesis of depression? Mod Trends Pharmacopsychiatri 28, 127-143.

Huang, G.J., Bannerman, D., Flint, J., 2008. Chronic fluoxetine treatment alters behavior, but not adult hippocampal neurogenesis, in BALB/cJ mice. Mol Psychiatry 13, 119-121.

Huang, Z., Choi, B.K., Mujoo, K., Fan, X., Fa, M., Mukherjee, S., Owiti, N., Zhang, N., An, Z., 2015. The E3 ubiquitin ligase NEDD4 negatively regulates HER3/ErbB3 level and signaling. Oncogene 34, 1105-1115.

Human Protein Atlas, 2015. Human Protein Atlas, www.proteinatlas.org.

Iga, J.I., Ueno, S.I., Yamauchi, K., Numata, S., Tayoshi‐Shibuya, S., Kinouchi, S., Nakataki, M., Song, H., Hokoishi, K., Tanabe, H., 2007. The Val66Met polymorphism of the brain‐derived neurotrophic factor gene is associated with psychotic feature and suicidal behavior in Japanese major depressive patients. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 144, 1003-1006.

Ishikawa, A., Nakamura, S., 2006. Ventral hippocampal neurons project axons simultaneously to the medial prefrontal cortex and amygdala in the rat. J Neurophysiol 96, 2134-2138.

Jaaro-Peled, H., Hayashi-Takagi, A., Seshadri, S., Kamiya, A., Brandon, N.J., Sawa, A., 2009. Neurodevelopmental mechanisms of schizophrenia: understanding disturbed postnatal brain maturation through neuregulin-1-ErbB4 and DISC1. Trends Neurosci 32, 485-495.

Jabbour, A., Hayward, C.S., Keogh, A.M., Kotlyar, E., McCrohon, J.A., England, J.F., Amor, R., Liu, X., Li, X.Y., Zhou, M.D., Graham, R.M., Macdonald, P.S., 2011. Parenteral administration of recombinant human neuregulin-1 to patients with stable chronic heart failure produces favourable acute and chronic haemodynamic responses. Eur J Heart Fail 13, 83-92.

Jackson-Fisher, A.J., Bellinger, G., Breindel, J.L., Tavassoli, F.A., Booth, C.J., Duong, J.K., Stern, D.F., 2008. ErbB3 is required for ductal morphogenesis in the mouse mammary gland. Breast Cancer Res 10, R96.

Jacobs, B.L., van Praag, H., Gage, F.H., 2000. Adult brain neurogenesis and psychiatry: a novel theory of depression. Molecular psychiatry 5, 262-269.

Jacobs, N., Kenis, G., Peeters, F., Derom, C., Vlietinck, R., van Os, J., 2006. Stress-related negative affectivity and genetically altered serotonin transporter function: evidence of synergism in shaping risk of depression. Archives of general psychiatry 63, 989-996.

Jans, L.A., Riedel, W.J., Markus, C.R., Blokland, A., 2007. Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. Molecular psychiatry 12, 522-543.

Janssen, M.J., Leiva-Salcedo, E., Buonanno, A., 2012. Neuregulin directly decreases voltage-gated sodium current in hippocampal ErbB4-expressing interneurons. J Neurosci 32, 13889-13895.

Jayatissa, M.N., Bisgaard, C., Tingstrom, A., Papp, M., Wiborg, O., 2006. Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. Neuropsychopharmacology 31, 2395-2404.

Jayatissa, M.N., Bisgaard, C.F., West, M.J., Wiborg, O., 2008. The number of granule cells in rat hippocampus is reduced after chronic mild stress and re-established after chronic escitalopram treatment. Neuropharmacology 54, 530-541.

Jayatissa, M.N., Henningsen, K., West, M.J., Wiborg, O., 2009. Decreased cell proliferation in the dentate gyrus does not associate with development of anhedonic-like symptoms in rats. Brain research 1290, 133-141.

Jeong, H., Kim, J., Lee, Y., Seo, J.H., Hong, S.R., Kim, A., 2014. Neuregulin-1 induces cancer stem cell characteristics in breast cancer cell lines. Oncol Rep 32, 1218-1224.

Jhaveri, D.J., Mackay, E.W., Hamlin, A.S., Marathe, S.V., Nandam, L.S., Vaidya, V.A., Bartlett, P.F., 2010. Norepinephrine directly activates adult hippocampal precursors via beta3-adrenergic receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience 30, 2795-2806.

Jiang, W., Zhang, Y., Xiao, L., Van Cleemput, J., Ji, S.P., Bai, G., Zhang, X., 2005. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. The Journal of clinical investigation 115, 3104-3116.

Jin, K., Zhu, Y., Sun, Y., Mao, X.O., Xie, L., Greenberg, D.A., 2002. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America 99, 11946-11950.

Juckel, G., Mendlin, A., Jacobs, B.L., 1999. Electrical stimulation of rat medial prefrontal cortex enhances forebrain serotonin output: implications for electroconvulsive therapy and transcranial magnetic stimulation in depression. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 21, 391-398.

Judd, L.L., Akiskal, H.S., Maser, J.D., Zeller, P.J., Endicott, J., Coryell, W., Paulus, M.P., Kunovac, J.L., Leon, A.C., Mueller, T.I., Rice, J.A., Keller, M.B., 1998. Major depressive disorder: a prospective study of residual subthreshold depressive symptoms as predictor of rapid relapse. J Affect Disord 50, 97-108.

Jung, J.I., Chung, E., Seon, M.R., Shin, H.K., Kim, E.J., Lim, S.S., Chung, W.Y., Park, K.K., Park, J.H., 2006. Isoliquiritigenin (ISL) inhibits ErbB3 signaling in prostate cancer cells. Biofactors 28, 159-168.

Kang, C., Yang, X., Xu, X., Liu, H., Su, P., Yang, J., 2012. Association study of neuregulin 1 gene polymorphisms with auditory P300 in schizophrenia. American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 159B, 422-428.

Kang, H.J., Kim, J.M., Lee, J.Y., Kim, S.Y., Bae, K.Y., Kim, S.W., Shin, I.S., Kim, H.R., Shin, M.G., Yoon, J.S., 2013. BDNF promoter methylation and suicidal behavior in depressive patients. J Affect Disord 151, 679-685.

Kang, M., Pyun, K.H., Jang, C.G., Kim, H., Bae, H., Shim, I., 2005. Nelumbinis Semen reverses a decrease in hippocampal 5-HT release induced by chronic mild stress in rats. The Journal of pharmacy and pharmacology 57, 651-656.

Karege, F., Vaudan, G., Schwald, M., Perroud, N., La Harpe, R., 2005. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain Res Mol Brain Res 136, 29-37.

Karg, K., Burmeister, M., Shedden, K., Sen, S., 2011. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. Arch Gen Psychiatry 68, 444-454.

Kastin, A.J., Akerstrom, V., Pan, W., 2004. Neuregulin-1-beta1 enters brain and spinal cord by receptor-mediated transport. Journal of neurochemistry 88, 965-970.

Kato, T., Abe, Y., Hirokawa, S., Iwakura, Y., Mizuno, M., Namba, H., Nawa, H., 2015. Neurobehavioral Differences Between Mice Receiving Distinct Neuregulin Variants as Neonates; Impact on Sensitivity to MK-801. Curr Mol Med 15, 222-236.

Kato, T., Abe, Y., Sotoyama, H., Kakita, A., Kominami, R., Hirokawa, S., Ozaki, M., Takahashi, H., Nawa, H., 2011. Transient exposure of neonatal mice to neuregulin-1 results in hyperdopaminergic states in adulthood: implication in neurodevelopmental hypothesis for schizophrenia. Mol Psychiatry 16, 307-320.

Kaufman, J., Yang, B.Z., Douglas-Palumberi, H., Houshyar, S., Lipschitz, D., Krystal, J.H., Gelernter, J., 2004. Social supports and serotonin transporter gene moderate depression in maltreated children. Proceedings of the National Academy of Sciences of the United States of America 101, 17316-17321.

Keller, M.C., Neale, M.C., Kendler, K.S., 2007. Association of different adverse life events with distinct patterns of depressive symptoms. The American journal of psychiatry 164, 1521-1529.

Keller, S., Sarchiapone, M., Zarrilli, F., Videtic, A., Ferraro, A., Carli, V., Sacchetti, S., Lembo, F., Angiolillo, A., Jovanovic, N., Pisanti, F., Tomaiuolo, R., Monticelli, A., Balazic, J., Roy, A., Marusic, A., Cocozza, S., Fusco, A., Bruni, C.B., Castaldo, G., Chiariotti, L., 2010. Increased BDNF promoter methylation in the Wernicke area of suicide subjects. Arch Gen Psychiatry 67, 258-267.

Kempermann, G., 2002. Regulation of adult hippocampal neurogenesis - implications for novel theories of major depression. Bipolar disorders 4, 17-33.

Kempermann, G., 2006. Adult Neurogenesis: Stem Cells and Neuronal Development in the Adult Brain. Oxford University Press, New York, NY.

Kempermann, G., 2011. Adult Neurogenesis 2, 2 ed. Oxford University Press, New York, NY.

Kempermann, G., Gage, F.H., 2000. Neurogenesis in the adult hippocampus. Novartis Foundation symposium 231, 220-235; discussion 235-241, 302-226.

Kempermann, G., Jessberger, S., Steiner, B., Kronenberg, G., 2004. Milestones of neuronal development in the adult hippocampus. Trends Neurosci 27, 447-452.

Kempermann, G., Kronenberg, G., 2003. Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biol Psychiatry 54, 499-503.

Kendler, K.S., Gardner, C.O., Prescott, C.A., 2002. Toward a comprehensive developmental model for major depression in women. The American journal of psychiatry 159, 1133-1145.

Kendler, K.S., Gardner, C.O., Prescott, C.A., 2006. Toward a comprehensive developmental model for major depression in men. The American journal of psychiatry 163, 115-124.

Kendler, K.S., Kessler, R.C., Walters, E.E., MacLean, C., Neale, M.C., Heath, A.C., Eaves, L.J., 1995. Stressful life events, genetic liability, and onset of an episode of major depression in women. The American journal of psychiatry 152, 833-842.

Kendler, K.S., Kuhn, J.W., Prescott, C.A., 2004. Childhood sexual abuse, stressful life events and risk for major depression in women. Psychological medicine 34, 1475-1482.

Kendler, K.S., Kuhn, J.W., Vittum, J., Prescott, C.A., Riley, B., 2005. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. Archives of general psychiatry 62, 529-535.

Keri, S., 2009. Genes for psychosis and creativity: a promoter polymorphism of the neuregulin 1 gene is related to creativity in people with high intellectual achievement. Psychol Sci 20, 1070-1073.

Keri, S., Szabo, C., Kelemen, O., 2014. Blood biomarkers of depression track clinical changes during cognitive-behavioral therapy. J Affect Disord 164, 118-122.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K.R., Rush, A.J., Walters, E.E., Wang, P.S., National Comorbidity Survey, R., 2003. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA : the journal of the American Medical Association 289, 3095-3105.

Kheirbek, M.A., Hen, R., 2011. Dorsal vs ventral hippocampal neurogenesis: implications for cognition and mood. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 36, 373-374.

Kheirbek, M.A., Tannenholz, L., Hen, R., 2012. NR2B-dependent plasticity of adult-born granule cells is necessary for context discrimination. J Neurosci 32, 8696-8702.

Kim, H., Whang, W.W., Kim, H.T., Pyun, K.H., Cho, S.Y., Hahm, D.H., Lee, H.J., Shim, I., 2003a. Expression of neuropeptide Y and cholecystokinin in the rat brain by chronic mild stress. Brain research 983, 201-208.

Kim, J.Y., Sun, Q., Oglesbee, M., Yoon, S.O., 2003b. The role of ErbB2 signaling in the onset of terminal differentiation of oligodendrocytes in vivo. J Neurosci 23, 5561-5571.

Kim, Y.K., Lee, H.P., Won, S.D., Park, E.Y., Lee, H.Y., Lee, B.H., Lee, S.W., Yoon, D., Han, C., Kim, D.J., Choi, S.H., 2007. Low plasma BDNF is associated with suicidal behavior in major depression. Prog Neuropsychopharmacol Biol Psychiatry 31, 78-85.

Kimata, H., 2005. Differential modulation of cerebrospinal fluid neurotrophins in patients with atopic dermatitis who attempted suicide. The Journal of clinical psychiatry 66, 1193-1194.

Kinney, G.G., Vogel, G.W., Feng, P., 1997. Decreased dorsal raphe nucleus neuronal activity in adult chloral hydrate anesthetized rats following neonatal clomipramine treatment: implications for endogenous depression. Brain research 756, 68-75.

Kiss, I., Kelemen, O., Keri, S., 2012. Decreased peripheral expression of neuregulin 1 in high-risk individuals who later converted to psychosis. Schizophrenia research 135, 198-199.

Kiuchi, T., Lee, H., Mikami, T., 2012. Regular exercise cures depression-like behavior via VEGF-Flk-1 signaling in chronically stressed mice. Neuroscience 207, 208-217.

Kiyasova, V., Bonnavion, P., Scotto-Lomassese, S., Fabre, V., Sahly, I., Tronche, F., Deneris, E., Gaspar, P., Fernandez, S.P., 2013. A subpopulation of serotonergic neurons that do not express the 5-HT1A autoreceptor. ACS Chem Neurosci 4, 89-95.

Kjelstrup, K.G., Tuvnes, F.A., Steffenach, H.A., Murison, R., Moser, E.I., Moser, M.B., 2002. Reduced fear expression after lesions of the ventral hippocampus. Proceedings of the National Academy of Sciences of the United States of America 99, 10825-10830.

Klaassen, T., Riedel, W.J., Deutz, N.E., van Someren, A., van Praag, H.M., 1999a. Specificity of the tryptophan depletion method. Psychopharmacology 141, 279-286.

Klaassen, T., Riedel, W.J., van Someren, A., Deutz, N.E., Honig, A., van Praag, H.M., 1999b. Mood effects of 24-hour tryptophan depletion in healthy first-degree relatives of patients with affective disorders. Biological psychiatry 46, 489-497.

Kobayashi, K., Ikeda, Y., Sakai, A., Yamasaki, N., Haneda, E., Miyakawa, T., Suzuki, H., 2010. Reversal of hippocampal neuronal maturation by serotonergic antidepressants. Proceedings of the National Academy of Sciences of the United States of America 107, 8434-8439.

Kohler, S.J., Williams, N.I., Stanton, G.B., Cameron, J.L., Greenough, W.T., 2011. Maturation time of new granule cells in the dentate gyrus of adult macaque monkeys exceeds six months. Proceedings of the National Academy of Sciences of the United States of America 108, 10326-10331.

Kondo, M., Nakamura, Y., Ishida, Y., Shimada, S., 2014. The 5-HT3 receptor is essential for exercise-induced hippocampal neurogenesis and antidepressant effects. Mol Psychiatry.

Kukshal, P., Bhatia, T., Bhagwat, A.M., Gur, R.E., Gur, R.C., Deshpande, S.N., Nimgaonkar, V.L., Thelma, B.K., 2013. Association study of neuregulin-1 gene polymorphisms in a North Indian schizophrenia sample. Schizophrenia research 144, 24-30.

Kulkarni, V.A., Jha, S., Vaidya, V.A., 2002. Depletion of norepinephrine decreases the proliferation, but does not influence the survival and differentiation, of granule cell progenitors in the adult rat hippocampus. European Journal of Neuroscience 16, 2008-2012.

Kwon, O.B., Paredes, D., Gonzalez, C.M., Neddens, J., Hernandez, L., Vullhorst, D., Buonanno, A., 2008. Neuregulin-1 regulates LTP at CA1 hippocampal synapses through activation of dopamine D4 receptors. Proceedings of the National Academy of Sciences 105, 15587-15592.

Laaris, N., Le Poul, E., Hamon, M., Lanfumey, L., 1997. Stress-induced alterations of somatodendritic 5-HT1A autoreceptor sensitivity in the rat dorsal raphe nucleus--in vitro electrophysiological evidence. Fundam Clin Pharmacol 11, 206-214.

Labonte, B., Suderman, M., Maussion, G., Navaro, L., Yerko, V., Mahar, I., Bureau, A., Mechawar, N., Szyf, M., Meaney, M.J., Turecki, G., 2012. Genome-wide epigenetic regulation by early-life trauma. Arch Gen Psychiatry 69, 722-731.

Lagace, D.C., Donovan, M.H., DeCarolis, N.A., Farnbauch, L.A., Malhotra, S., Berton, O., Nestler, E.J., Krishnan, V., Eisch, A.J., 2010. Adult hippocampal neurogenesis is functionally important for stress-induced social avoidance. Proceedings of the National Academy of Sciences 107, 4436-4441.

Lan, M.J., Hesselgrave, N., Ciarleglio, A., Ogden, R.T., Sullivan, G.M., Mann, J.J., Parsey, R.V., 2013. Higher pretreatment 5-HT1A receptor binding potential in bipolar disorder depression is associated with treatment remission: A naturalistic treatment pilot PET study. Synapse 67, 773-778.

Lanfumey, L., Mongeau, R., Cohen-Salmon, C., Hamon, M., 2008. Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. Neuroscience and biobehavioral reviews 32, 1174-1184.

Lanfumey, L., Pardon, M.C., Laaris, N., Joubert, C., Hanoun, N., Hamon, M., Cohen-Salmon, C., 1999. 5-HT1A autoreceptor desensitization by chronic ultramild stress in mice. Neuroreport 10, 3369-3374.

Larsen, M.H., Mikkelsen, J.D., Hay-Schmidt, A., Sandi, C., 2010. Regulation of brain-derived neurotrophic factor (BDNF) in the chronic unpredictable stress rat model and the effects of chronic antidepressant treatment. Journal of psychiatric research 44, 808-816.

Law, A.J., Lipska, B.K., Weickert, C.S., Hyde, T.M., Straub, R.E., Hashimoto, R., Harrison, P.J., Kleinman, J.E., Weinberger, D.R., 2006. Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5' SNPs associated with the disease. Proceedings of the National Academy of Sciences of the United States of America 103, 6747-6752.

Le-Niculescu, H., Kurian, S.M., Yehyawi, N., Dike, C., Patel, S.D., Edenberg, H.J., Tsuang, M.T., Salomon, D.R., Nurnberger, J.I., Jr., Niculescu, A.B., 2009. Identifying blood biomarkers for mood disorders using convergent functional genomics. Mol Psychiatry 14, 156-174.

Le Francois, B., Czesak, M., Steubl, D., Albert, P.R., 2008. Transcriptional regulation at a HTR1A polymorphism associated with mental illness. Neuropharmacology 55, 977-985.

Le Poul, E., Laaris, N., Hamon, M., Lanfumey, L., 1997. Fluoxetine-induced desensitization of somatodendritic 5-HT1A autoreceptors is independent of glucocorticoid(s). Synapse 27, 303-312.

Ledonne, A., Nobili, A., Latagliata, E.C., Cavallucci, V., Guatteo, E., Puglisi-Allegra, S., D'Amelio, M., Mercuri, N.B., 2015. Neuregulin 1 signalling modulates mGluR1 function in mesencephalic dopaminergic neurons. Mol Psychiatry 20, 959-973.

Lee, D., Yu, M., Lee, E., Kim, H., Yang, Y., Kim, K., Pannicia, C., Kurie, J.M., Threadgill, D.W., 2009. Tumor-specific apoptosis caused by deletion of the ERBB3 pseudo-kinase in mouse intestinal epithelium. The Journal of clinical investigation 119, 2702-2713.

Lee, H., Lee, H., Chin, H., Kim, K., Lee, D., 2014a. ERBB3 knockdown induces cell cycle arrest and activation of Bak and Bax-dependent apoptosis in colon cancer cells. Oncotarget 5, 5138-5152.

Lee, H.S., Cho, H.J., Kwon, G.T., Park, J.H., 2014b. Kaempferol Downregulates Insulin-like Growth Factor-I Receptor and ErbB3 Signaling in HT-29 Human Colon Cancer Cells. J Cancer Prev 19, 161-169.

Lee, Y.S., Liu, F., Segil, N., 2006. A morphogenetic wave of p27Kip1 transcription directs cell cycle exit during organ of Corti development. Development 133, 2817-2826.

Lehmann, M.L., Brachman, R.A., Martinowich, K., Schloesser, R.J., Herkenham, M., 2013. Glucocorticoids orchestrate divergent effects on mood through adult neurogenesis. J Neurosci 33, 2961-2972.

Lemaire, V., Tronel, S., Montaron, M.F., Fabre, A., Dugast, E., Abrous, D.N., 2012. Long-lasting plasticity of hippocampal adult-born neurons. J Neurosci 32, 3101-3108.

Leone, L., Fusco, S., Mastrodonato, A., Piacentini, R., Barbati, S.A., Zaffina, S., Pani, G., Podda, M.V., Grassi, C., 2014. Epigenetic modulation of adult hippocampal neurogenesis by extremely low-frequency electromagnetic fields. Mol Neurobiol 49, 1472-1486.

Lesch, K.P., 2004. Gene-environment interaction and the genetics of depression. Journal of psychiatry & neuroscience : JPN 29, 174-184.

Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274, 1527-1531.

Leussis, M.P., Freund, N., Brenhouse, H.C., Thompson, B.S., Andersen, S.L., 2012. Depressive-like behavior in adolescents after maternal separation: sex differences, controllability, and GABA. Dev Neurosci 34, 210-217.

Levinson, D.F., 2006. The genetics of depression: a review. Biological psychiatry 60, 84-92.

Leyton, M., Ghadirian, A.M., Young, S.N., Palmour, R.M., Blier, P., Helmers, K.F., Benkelfat, C., 2000. Depressive relapse following acute tryptophan depletion in patients with major depressive disorder. Journal of psychopharmacology 14, 284-287.

Li, B., Woo, R.S., Mei, L., Malinow, R., 2007. The neuregulin-1 receptor erbB4 controls glutamatergic synapse maturation and plasticity. Neuron 54, 583-597.

Li, D., Feng, G., He, L., 2009. Case-control study of association between the functional candidate gene ERBB3 and schizophrenia in Caucasian population. World J Biol Psychiatry 10, 595-598.

Li, K.X., Lu, Y.M., Xu, Z.H., Zhang, J., Zhu, J.M., Zhang, J.M., Cao, S.X., Chen, X.J., Chen, Z., Luo, J.H., Duan, S., Li, X.M., 2012a. Neuregulin 1 regulates excitability of fast-spiking neurons through Kv1.1 and acts in epilepsy. Nature neuroscience 15, 267-273.

Li, Q., Wichems, C., Heils, A., Lesch, K.P., Murphy, D.L., 2000. Reduction in the density and expression, but not G-protein coupling, of serotonin receptors (5-HT1A) in 5-HT transporter knock-out mice: gender and brain region differences. The Journal of neuroscience : the official journal of the Society for Neuroscience 20, 7888-7895.

Li, Y., Aimone, J.B., Xu, X., Callaway, E.M., Gage, F.H., 2012b. Development of GABAergic inputs controls the contribution of maturing neurons to the adult hippocampal network. Proceedings of the National Academy of Sciences of the United States of America 109, 4290-4295.

Li, Y.F., Zhang, Y.Z., Liu, Y.Q., Wang, H.L., Yuan, L., Luo, Z.P., 2004. Moclobemide up-regulates proliferation of hippocampal progenitor cells in chronically stressed mice. Acta pharmacologica Sinica 25, 1408-1412.

Lipska, B.K., Weinberger, D.R., 2002. A neurodevelopmental model of schizophrenia: neonatal disconnection of the hippocampus. Neurotox Res 4, 469-475.

Lira, A., Zhou, M., Castanon, N., Ansorge, M.S., Gordon, J.A., Francis, J.H., Bradley-Moore, M., Lira, J., Underwood, M.D., Arango, V., Kung, H.F., Hofer, M.A., Hen, R., Gingrich, J.A., 2003. Altered depression-related behaviors and functional changes in the dorsal raphe nucleus of serotonin transporter-deficient mice. Biological psychiatry 54, 960-971.

Liu, A., Jain, N., Vyas, A., Lim, L.W., 2015. Ventromedial prefrontal cortex stimulation enhances memory and hippocampal neurogenesis in the middle-aged rats. Elife 4.

Liu, X., Bates, R., Yin, D.M., Shen, C., Wang, F., Su, N., Kirov, S.A., Luo, Y., Wang, J.Z., Xiong, W.C., Mei, L., 2011. Specific regulation of NRG1 isoform expression by neuronal activity. J Neurosci 31, 8491-8501.

Liu, X., Gu, X., Li, Z., Li, X., Li, H., Chang, J., Chen, P., Jin, J., Xi, B., Chen, D., Lai, D., Graham, R.M., Zhou, M., 2006. Neuregulin-1/erbB-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy. J Am Coll Cardiol 48, 1438-1447.

Liu, Y., Ford, B., Mann, M.A., Fischbach, G.D., 2001. Neuregulins increase alpha7 nicotinic acetylcholine receptors and enhance excitatory synaptic transmission in GABAergic interneurons of the hippocampus. J Neurosci 21, 5660-5669.

Liu, Y., Ford, B.D., Mann, M.A., Fischbach, G.D., 2005. Neuregulin-1 increases the proliferation of neuronal progenitors from embryonic neural stem cells. Dev Biol 283, 437-445.

Long, L.E., Chesworth, R., Huang, X.F., Wong, A., Spiro, A., McGregor, I.S., Arnold, J.C., Karl, T., 2012. Distinct neurobehavioural effects of cannabidiol in transmembrane domain neuregulin 1 mutant mice. PloS one 7, e34129.

Lopez-Bendito, G., Cautinat, A., Sanchez, J.A., Bielle, F., Flames, N., Garratt, A.N., Talmage, D.A., Role, L.W., Charnay, P., Marin, O., Garel, S., 2006. Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. Cell 125, 127-142.

Lopez, J.P., Lim, R., Cruceanu, C., Crapper, L., Fasano, C., Labonte, B., Maussion, G., Yang, J.P., Yerko, V., Vigneault, E., El Mestikawy, S., Mechawar, N., Pavlidis, P., Turecki, G., 2014. miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. Nature medicine 20, 764-768.

Lu, C.L., Wang, Y.C., Chen, J.Y., Lai, I.C., Liou, Y.J., 2010. Support for the involvement of the ERBB4 gene in schizophrenia: a genetic association analysis. Neurosci Lett 481, 120-125.

Lucki, I., 1997. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol 8, 523-532.

Luo, D.D., An, S.C., Zhang, X., 2008. Involvement of hippocampal serotonin and neuropeptide Y in depression induced by chronic unpredicted mild stress. Brain research bulletin 77, 8-12.

Luo, X., Prior, M., He, W., Hu, X., Tang, X., Shen, W., Yadav, S., Kiryu-Seo, S., Miller, R., Trapp, B.D., Yan, R., 2011. Cleavage of neuregulin-1 by BACE1 or ADAM10 protein produces differential effects on myelination. The Journal of biological chemistry 286, 23967-23974.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature reviews. Neuroscience 10, 434-445.

Ma, X., Hamadeh, M.J., Christie, B.R., Foster, J.A., Tarnopolsky, M.A., 2012. Impact of treadmill running and sex on hippocampal neurogenesis in the mouse model of amyotrophic lateral sclerosis. PloS one 7, e36048.

Macbeath, G., Adiwijaya, B., Liu, J., Sequist, L., Pujade-Lauraine, E., Higgins, M., Tabah-Fisch, I., Pearlberg, J., Moyo, V., Kubasek, W., 2014. 246PA META-ANALYSIS OF BIOMARKERS IN THREE RANDOMIZED, PHASE 2 STUDIES OF MM-121, A LIGAND-BLOCKING ANTI-ERBB3 ANTIBODY, IN PATIENTS WITH OVARIAN, LUNG, AND BREAST CANCERS. Annals of Oncology 25, iv82-iv82.

Macdonald-Obermann, J.L., Adak, S., Landgraf, R., Piwnica-Worms, D., Pike, L.J., 2013. Dynamic analysis of the epidermal growth factor (EGF) receptor-ErbB2-ErbB3 protein network by luciferase fragment complementation imaging. Journal of Biological Chemistry 288, 30773-30784.

Maggio, N., Segal, M., 2007a. Striking variations in corticosteroid modulation of long-term potentiation along the septotemporal axis of the hippocampus. J Neurosci 27, 5757-5765.

Maggio, N., Segal, M., 2007b. Unique regulation of long term potentiation in the rat ventral hippocampus. Hippocampus 17, 10-25.

Mahar, I., Bagot, R.C., Davoli, M.A., Miksys, S., Tyndale, R.F., Walker, C.D., Maheu, M., Huang, S.H., Wong, T.P., Mechawar, N., 2012. Developmental hippocampal neuroplasticity in a model of nicotine replacement therapy during pregnancy and breastfeeding. PloS one 7, e37219.

Mahar, I., Bambico, F.R., Mechawar, N., Nobrega, J.N., 2014. Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. Neurosci Biobehav Rev 38, 173-192.

Mahar, I., Tan, S., Davoli, M.A., Dominguez-Lopez, S., Qiang, C., Rachalski, A., Turecki, G., Mechawar, N., 2011. Subchronic peripheral neuregulin-1 increases ventral hippocampal neurogenesis and induces antidepressant-like effects. PloS one 6, e26610.

Maheu, M., Lopez, J.P., Crapper, L., Davoli, M.A., Turecki, G., Mechawar, N., 2015a. MicroRNA regulation of central glial cell line-derived neurotrophic factor (GDNF) signalling in depression. Transl Psychiatry 5, e511.

Maheu, M.E., Davoli, M.A., Turecki, G., Mechawar, N., 2013. Amygdalar expression of proteins associated with neuroplasticity in major depression and suicide. Journal of psychiatric research 47, 384-390.

Maheu, M.E., Devorak, J., Freibauer, A., Davoli, M.A., Turecki, G., Mechawar, N., 2015b. Increased doublecortin (DCX) expression and incidence of DCX-immunoreactive multipolar cells in the subventricular zone-olfactory bulb system of suicides. Front Neuroanat 9, 74.

Maier, S.F., Amat, J., Baratta, M.V., Paul, E., Watkins, L.R., 2006. Behavioral control, the medial prefrontal cortex, and resilience. Dialogues in clinical neuroscience 8, 397-406.

Maier, S.F., Watkins, L.R., 2005. Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neuroscience and biobehavioral reviews 29, 829-841.

Malberg, J.E., Duman, R.S., 2003. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 28, 1562-1571.

Malberg, J.E., Eisch, A.J., Nestler, E.J., Duman, R.S., 2000. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20, 9104-9110.

Mann, J.J., Huang, Y.Y., Underwood, M.D., Kassir, S.A., Oppenheim, S., Kelly, T.M., Dwork, A.J., Arango, V., 2000. A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. Archives of general psychiatry 57, 729-738.

Marballi, K., Cruz, D., Thompson, P., Walss-Bass, C., 2012. Differential neuregulin 1 cleavage in the prefrontal cortex and hippocampus in schizophrenia and bipolar disorder: preliminary findings. PloS one 7, e36431.

Maren, S., Holt, W.G., 2004. Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral, but not dorsal, hippocampus impair the acquisition of conditional freezing to an auditory conditional stimulus. Behav Neurosci 118, 97-110.

Marenco, S., Geramita, M., van der Veen, J.W., Barnett, A.S., Kolachana, B., Shen, J., Weinberger, D.R., Law, A.J., 2011. Genetic association of ErbB4 and human cortical GABA levels in vivo. J Neurosci 31, 11628-11632.

Marin-Burgin, A., Mongiat, L.A., Pardi, M.B., Schinder, A.F., 2012. Unique processing during a period of high excitation/inhibition balance in adult-born neurons. Science 335, 1238-1242.

Martin-Ruiz, R., Puig, M.V., Celada, P., Shapiro, D.A., Roth, B.L., Mengod, G., Artigas, F., 2001. Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. The Journal of neuroscience : the official journal of the Society for Neuroscience 21, 9856-9866.

Martin-Ruiz, R., Ugedo, L., 2001. Electrophysiological evidence for postsynaptic 5-HT(1A) receptor control of dorsal raphe 5-HT neurones. Neuropharmacology 41, 72-78.

Masuda, T., Nakagawa, S., Boku, S., Nishikawa, H., Takamura, N., Kato, A., Inoue, T., Koyama, T., 2012. Noradrenaline increases neural precursor cells derived from adult rat dentate gyrus through beta2 receptor. Progress in neuro-psychopharmacology & biological psychiatry 36, 44-51.

Maudhuit, C., Hamon, M., Adrien, J., 1995. Electrophysiological activity of raphe dorsalis serotoninergic neurones in a possible model of endogenous depression. Neuroreport 6, 681-684.

Maudhuit, C., Hamon, M., Adrien, J., 1996. Effects of chronic treatment with zimelidine and REM sleep deprivation on the regulation of raphe neuronal activity in a rat model of depression. Psychopharmacology 124, 267-274.

Maussion, G., Yang, J., Yerko, V., Barker, P., Mechawar, N., Ernst, C., Turecki, G., 2012. Regulation of a truncated form of tropomyosin-related kinase B (TrkB) by Hsa-miR-185* in frontal cortex of suicide completers. PloS one 7, e39301.

Mayberg, H.S., Lozano, A.M., Voon, V., McNeely, H.E., Seminowicz, D., Hamani, C., Schwalb, J.M., Kennedy, S.H., 2005. Deep brain stimulation for treatment-resistant depression. Neuron 45, 651-660.

McArthur, R., Borsini, F., 2006. Animal models of depression in drug discovery: a historical perspective. Pharmacology, biochemistry, and behavior 84, 436-452.

McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., Labonte, B., Szyf, M., Turecki, G., Meaney, M.J., 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nature neuroscience 12, 342-348.

McGrath, P.J., Khan, A.Y., Trivedi, M.H., Stewart, J.W., Morris, D.W., Wisniewski, S.R., Miyahara, S., Nierenberg, A.A., Fava, M., Rush, A.J., 2008. Response to a selective serotonin reuptake inhibitor (citalopram) in major depressive disorder with melancholic features: a STAR*D report. J Clin Psychiatry 69, 1847-1855.

Mechawar, N., Lacoste, B., Yu, W.F., Srivastava, L.K., Quirion, R., 2007. Developmental profile of neuregulin receptor ErbB4 in postnatal rat cerebral cortex and hippocampus. Neuroscience 148, 126-139.

Mei, L., Nave, K.A., 2014. Neuregulin-ERBB signaling in the nervous system and neuropsychiatric diseases. Neuron 83, 27-49.

Mei, L., Xiong, W.C., 2008. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nature reviews. Neuroscience 9, 437-452.

Meltzer, C.C., Price, J.C., Mathis, C.A., Butters, M.A., Ziolko, S.K., Moses-Kolko, E., Mazumdar, S., Mulsant, B.H., Houck, P.R., Lopresti, B.J., Weissfeld, L.A., Reynolds, C.F., 2004. Serotonin 1A receptor binding and treatment response in late-life depression. Neuropsychopharmacology 29, 2258-2265.

Menke, A., Klengel, T., Binder, E.B., 2012. Epigenetics, depression and antidepressant treatment. Current pharmaceutical design 18, 5879-5889.

Mignone, J.L., Kukekov, V., Chiang, A.S., Steindler, D., Enikolopov, G., 2004. Neural stem and progenitor cells in nestin-GFP transgenic mice. J Comp Neurol 469, 311-324.

Milanesi, E., Minelli, A., Cattane, N., Cattaneo, A., Mora, C., Barbon, A., Mallei, A., Popoli, M., Florio, V., Conca, A., Bignotti, S., Gennarelli, M., 2012. ErbB3 mRNA leukocyte levels as a biomarker for major depressive disorder. BMC Psychiatry 12, 145.

Miller, E.J., Saint Marie, L.R., Breier, M.R., Swerdlow, N.R., 2010. Pathways from the ventral hippocampus and caudal amygdala to forebrain regions that regulate sensorimotor gating in the rat. Neuroscience 165, 601-611.

Miller, J.M., Hesselgrave, N., Ogden, R.T., Zanderigo, F., Oquendo, M.A., Mann, J.J., Parsey, R.V., 2013. Brain Serotonin 1A Receptor Binding as a Predictor of Treatment Outcome in Major Depressive Disorder. Biol Psychiatry.

Mineur, Y.S., Belzung, C., Crusio, W.E., 2007. Functional implications of decreases in neurogenesis following chronic mild stress in mice. Neuroscience 150, 251-259.

Mo, B., Feng, N., Renner, K., Forster, G., 2008. Restraint stress increases serotonin release in the central nucleus of the amygdala via activation of corticotropin-releasing factor receptors. Brain research bulletin 76, 493-498.

Modarresi, F., Faghihi, M.A., Patel, N.S., Sahagan, B.G., Wahlestedt, C., Lopez-Toledano, M.A., 2011. Knockdown of BACE1-AS Nonprotein-Coding Transcript Modulates Beta-Amyloid-Related Hippocampal Neurogenesis. Int J Alzheimers Dis 2011, 929042.

Monroe, S.M., Simons, A.D., 1991. Diathesis-stress theories in the context of life stress research: implications for the depressive disorders. Psychol Bull 110, 406-425.

Montero, J.C., Rodriguez-Barrueco, R., Ocana, A., Diaz-Rodriguez, E., Esparis-Ogando, A., Pandiella, A., 2008. Neuregulins and cancer. Clin Cancer Res 14, 3237-3241.

Moon, E., Rollins, B., Mesen, A., Sequeira, A., Myers, R.M., Akil, H., Watson, S.J., Barchas, J., Jones, E.G., Schatzberg, A., Bunney, W.E., DeLisi, L.E., Byerley, W., Vawter, M.P., 2011. Lack of association

to a NRG1 missense polymorphism in schizophrenia or bipolar disorder in a Costa Rican population. Schizophrenia research 131, 52-57.

Moreau, J.L., Jenck, F., Martin, J.R., Mortas, P., Haefely, W.E., 1992. Antidepressant treatment prevents chronic unpredictable mild stress-induced anhedonia as assessed by ventral tegmentum self-stimulation behavior in rats. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 2, 43-49.

Moreau, J.L., Scherschlicht, R., Jenck, F., Martin, J.R., 1995. Chronic mild stress-induced anhedonia model of depression; sleep abnormalities and curative effects of electroshock treatment. Behavioural pharmacology 6, 682-687.

Mosko, S.S., Jacobs, B.L., 1974. Midbrain raphe neurons: spontaneous activity and response to light. Physiology & behavior 13, 589-593.

Mudo, G., Belluardo, N., Mauro, A., Fuxe, K., 2007. Acute intermittent nicotine treatment induces fibroblast growth factor-2 in the subventricular zone of the adult rat brain and enhances neuronal precursor cell proliferation. Neuroscience 145, 470-483.

Musazzi, L., Cattaneo, A., Tardito, D., Barbon, A., Gennarelli, M., Barlati, S., Racagni, G., Popoli, M., 2009. Early raise of BDNF in hippocampus suggests induction of posttranscriptional mechanisms by antidepressants. BMC neuroscience 10, 48.

Nakashiba, T., Cushman, J.D., Pelkey, K.A., Renaudineau, S., Buhl, D.L., McHugh, T.J., Rodriguez Barrera, V., Chittajallu, R., Iwamoto, K.S., McBain, C.J., Fanselow, M.S., Tonegawa, S., 2012. Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. Cell 149, 188-201.

Namba, T., Mochizuki, H., Suzuki, R., Onodera, M., Yamaguchi, M., Namiki, H., Shioda, S., Seki, T., 2011. Time-lapse imaging reveals symmetric neurogenic cell division of GFAP-expressing progenitors for expansion of postnatal dentate granule neurons. PloS one 6, e25303.

Nason, M.W., Jr., Adhikari, A., Bozinoski, M., Gordon, J.A., Role, L.W., 2011. Disrupted activity in the hippocampal-accumbens circuit of type III neuregulin 1 mutant mice. Neuropsychopharmacology 36, 488-496.

Navailles, S., Hof, P.R., Schmauss, C., 2008. Antidepressant drug-induced stimulation of mouse hippocampal neurogenesis is age-dependent and altered by early life stress. J Comp Neurol 509, 372-381.

Naz, M., Riaz, M., Saleem, M., 2011. Potential role of Neuregulin 1 and TNF-alpha (-308) polymorphism in schizophrenia patients visiting hospitals in Lahore, Pakistan. Mol Biol Rep 38, 4709-4714.

Neddens, J., Buonanno, A., 2009. Selective populations of hippocampal interneurons express ErbB4 and their number and distribution is altered in ErbB4 knockout mice. Hippocampus 20, 724-744.

Neddens, J., Buonanno, A., 2011. Expression of the neuregulin receptor ErbB4 in the brain of the rhesus monkey (Macaca mulatta). PloS one 6, e27337.

Neddens, J., Fish, K.N., Tricoire, L., Vullhorst, D., Shamir, A., Chung, W., Lewis, D.A., McBain, C.J., Buonanno, A., 2011. Conserved interneuron-specific ErbB4 expression in frontal cortex of rodents, monkeys, and humans: implications for schizophrenia. Biol Psychiatry 70, 636-645.

Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J., Monteggia, L.M., 2002. Neurobiology of depression. Neuron 34, 13-25.

Nettles, K.W., Pesold, C., Goldman, M.B., 2000. Influence of the ventral hippocampal formation on plasma vasopressin, hypothalamic-pituitary-adrenal axis, and behavioral responses to novel acoustic stress. Brain Res 858, 181-190.

Neumaier, J.F., Edwards, E., Plotsky, P.M., 2002. 5-HT(1B) mrna regulation in two animal models of altered stress reactivity. Biological psychiatry 51, 902-908.

Neumeister, A., Bain, E., Nugent, A.C., Carson, R.E., Bonne, O., Luckenbaugh, D.A., Eckelman, W., Herscovitch, P., Charney, D.S., Drevets, W.C., 2004. Reduced serotonin type 1A receptor binding in panic disorder. J Neurosci 24, 589-591.

Neumeister, A., Konstantinidis, A., Stastny, J., Schwarz, M.J., Vitouch, O., Willeit, M., Praschak-Rieder, N., Zach, J., de Zwaan, M., Bondy, B., Ackenheil, M., Kasper, S., 2002. Association between serotonin

transporter gene promoter polymorphism (5HTTLPR) and behavioral responses to tryptophan depletion in healthy women with and without family history of depression. Archives of general psychiatry 59, 613-620.

Nibuya, M., Morinobu, S., Duman, R.S., 1995. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. The Journal of neuroscience : the official journal of the Society for Neuroscience 15, 7539-7547.

Nickl-Jockschat, T., Stocker, T., Krug, A., Markov, V., Huang, R., Schneider, F., Habel, U., Eickhoff, S.B., Zerres, K., Nothen, M.M., Treutlein, J., Rietschel, M., Shah, N.J., Kircher, T., 2014. A Neuregulin-1 schizophrenia susceptibility variant causes perihippocampal fiber tract anomalies in healthy young subjects. Brain and behavior 4, 215-226.

Noori, H.R., Fornal, C.A., 2011. The appropriateness of unbiased optical fractionators to assess cell proliferation in the adult hippocampus. Frontiers in neuroscience 5, 140.

O'Leary, C., Desbonnet, L., Clarke, N., Petit, E., Tighe, O., Lai, D., Harvey, R., Waddington, J.L., O'Tuathaigh, C., 2014. Phenotypic effects of maternal immune activation and early postnatal milieu in mice mutant for the schizophrenia risk gene neuregulin-1. Neuroscience 277, 294-305.

O'Tuathaigh, C.M., Harte, M., O'Leary, C., O'Sullivan, G.J., Blau, C., Lai, D., Harvey, R.P., Tighe, O., Fagan, A.J., Kerskens, C., Reynolds, G.P., Waddington, J.L., 2010. Schizophrenia-related endophenotypes in heterozygous neuregulin-1 'knockout' mice. The European journal of neuroscience 31, 349-358.

Olayioye, M.A., Neve, R.M., Lane, H.A., Hynes, N.E., 2000. The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 19, 3159-3167.

Olivier, J.D., Van Der Hart, M.G., Van Swelm, R.P., Dederen, P.J., Homberg, J.R., Cremers, T., Deen, P.M., Cuppen, E., Cools, A.R., Ellenbroek, B.A., 2008. A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders. Neuroscience 152, 573-584.

Oomen, C.A., Soeters, H., Audureau, N., Vermunt, L., van Hasselt, F.N., Manders, E.M., Joels, M., Lucassen, P.J., Krugers, H., 2010. Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood. J Neurosci 30, 6635-6645.

Ortega, M.C., Bribian, A., Peregrin, S., Gil, M.T., Marin, O., de Castro, F., 2012. Neuregulin-1/ErbB4 signaling controls the migration of oligodendrocyte precursor cells during development. Experimental neurology 235, 610-620.

Overstreet, D.H., 1993. The Flinders sensitive line rats: a genetic animal model of depression. Neurosci Biobehav Rev 17, 51-68.

Paizanis, E., Renoir, T., Lelievre, V., Saurini, F., Melfort, M., Gabriel, C., Barden, N., Mocaer, E., Hamon, M., Lanfumey, L., 2010. Behavioural and neuroplastic effects of the new-generation antidepressant agomelatine compared to fluoxetine in glucocorticoid receptor-impaired mice. Int J Neuropsychopharmacol 13, 759-774.

Pandya, C.D., Pillai, A., 2014. TrkB interacts with ErbB4 and regulates NRG1-induced NR2B phosphorylation in cortical neurons before synaptogenesis. Cell Communication and Signaling 12.

Parihar, V.K., Hattiangady, B., Kuruba, R., Shuai, B., Shetty, A.K., 2011. Predictable chronic mild stress improves mood, hippocampal neurogenesis and memory. Molecular psychiatry 16, 171-183.

Park, J.H., Enikolopov, G., 2010. Transient elevation of adult hippocampal neurogenesis after dopamine depletion. Experimental neurology 222, 267-276.

Parlapani, E., Schmitt, A., Wirths, O., Bauer, M., Sommer, C., Rueb, U., Skowronek, M.H., Treutlein, J., Petroianu, G.A., Rietschel, M., Falkai, P., 2010. Gene expression of neuregulin-1 isoforms in different brain regions of elderly schizophrenia patients. World J Biol Psychiatry 11, 243-250.

Pazos, A., Probst, A., Palacios, J.M., 1987. Serotonin receptors in the human brain--IV. Autoradiographic mapping of serotonin-2 receptors. Neuroscience 21, 123-139.

Pechnick, R.N., Zonis, S., Wawrowsky, K., Cosgayon, R., Farrokhi, C., Lacayo, L., Chesnokova, V., 2011. Antidepressants stimulate hippocampal neurogenesis by inhibiting p21 expression in the subgranular zone of the hipppocampus. PloS one 6, e27290.

Pei, J.C., Liu, C.M., Lai, W.S., 2014. Distinct phenotypes of new transmembrane-domain neuregulin 1 mutant mice and the rescue effects of valproate on the observed schizophrenia-related cognitive deficits. Front Behav Neurosci 8, 126.

Pentkowski, N.S., Blanchard, D.C., Lever, C., Litvin, Y., Blanchard, R.J., 2006. Effects of lesions to the dorsal and ventral hippocampus on defensive behaviors in rats. The European journal of neuroscience 23, 2185-2196.

Perera, T.D., Coplan, J.D., Lisanby, S.H., Lipira, C.M., Arif, M., Carpio, C., Spitzer, G., Santarelli, L., Scharf, B., Hen, R., Rosoklija, G., Sackeim, H.A., Dwork, A.J., 2007. Antidepressant-induced neurogenesis in the hippocampus of adult nonhuman primates. The Journal of neuroscience : the official journal of the Society for Neuroscience 27, 4894-4901.

Perera, T.D., Dwork, A.J., Keegan, K.A., Thirumangalakudi, L., Lipira, C.M., Joyce, N., Lange, C., Higley, J.D., Rosoklija, G., Hen, R., Sackeim, H.A., Coplan, J.D., 2011. Necessity of hippocampal neurogenesis for the therapeutic action of antidepressants in adult nonhuman primates. PloS one 6, e17600.

Petrik, D., Lagace, D.C., Eisch, A.J., 2012. The neurogenesis hypothesis of affective and anxiety disorders: are we mistaking the scaffolding for the building? Neuropharmacology 62, 21-34.

Peyron, C., Petit, J.M., Rampon, C., Jouvet, M., Luppi, P.H., 1998. Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. Neuroscience 82, 443-468.

Piatti, V.C., Davies-Sala, M.G., Esposito, M.S., Mongiat, L.A., Trinchero, M.F., Schinder, A.F., 2011. The timing for neuronal maturation in the adult hippocampus is modulated by local network activity. J Neurosci 31, 7715-7728.

Pinheiro, R.T., Pinheiro, K.A., da Cunha Coelho, F.M., de Avila Quevedo, L., Gazal, M., da Silva, R.A., Giovenardi, M., Lucion, A.B., de Souza, D.O., Portela, L.V., Oses, J.P., 2012. Brain-derived neurotrophic factor levels in women with postpartum affective disorder and suicidality. Neurochem Res 37, 2229-2234.

Pitcher, G.M., Kalia, L.V., Ng, D., Goodfellow, N.M., Yee, K.T., Lambe, E.K., Salter, M.W., 2011. Schizophrenia susceptibility pathway neuregulin 1-ErbB4 suppresses Src upregulation of NMDA receptors. Nature medicine 17, 470-478.

Pitchot, W., Hansenne, M., Pinto, E., Reggers, J., Fuchs, S., Ansseau, M., 2005. 5-Hydroxytryptamine 1A receptors, major depression, and suicidal behavior. Biol Psychiatry 58, 854-858.

Pitychoutis, P.M., Griva, E., Ioannou, K., Tsitsilonis, O.E., Papadopoulou-Daifoti, Z., 2009. Chronic antidepressant treatment exerts sexually dimorphic immunomodulatory effects in an experimental model of major depression: do females lack an advantage? The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 12, 1157-1163.

Pollak, D.D., Monje, F.J., Zuckerman, L., Denny, C.A., Drew, M.R., Kandel, E.R., 2008. An animal model of a behavioral intervention for depression. Neuron 60, 149-161.

Possel, P., 2011. Can Beck's Theory of Depression and the Response Style Theory Be Integrated? J Couns Psychol 58, 618-629.

Prata, D.P., Breen, G., Osborne, S., Munro, J., St Clair, D., Collier, D.A., 2009. An association study of the neuregulin 1 gene, bipolar affective disorder and psychosis. Psychiatr Genet 19, 113-116.

Pregelj, P., Nedic, G., Paska, A.V., Zupanc, T., Nikolac, M., Balazic, J., Tomori, M., Komel, R., Seler, D.M., Pivac, N., 2011. The association between brain-derived neurotrophic factor polymorphism (BDNF Val66Met) and suicide. J Affect Disord 128, 287-290.

Price, J.L., Drevets, W.C., 2010. Neurocircuitry of mood disorders. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 35, 192-216.

Pruessner, M., Hellhammer, D.H., Pruessner, J.C., Lupien, S.J., 2003. Self-reported depressive symptoms and stress levels in healthy young men: associations with the cortisol response to awakening. Psychosomatic medicine 65, 92-99.

Pucilowski, O., Overstreet, D.H., Rezvani, A.H., Janowsky, D.S., 1993. Chronic mild stress-induced anhedonia: greater effect in a genetic rat model of depression. Physiology & behavior 54, 1215-1220.

Qi, X., Lin, W., Li, J., Pan, Y., Wang, W., 2006. The depressive-like behaviors are correlated with decreased phosphorylation of mitogen-activated protein kinases in rat brain following chronic forced swim stress. Behavioural brain research 175, 233-240.

Rabiner, E.A., Bhagwagar, Z., Gunn, R.N., Cowen, P.J., Grasby, P.M., 2004. Preferential 5-HT1A autoreceptor occupancy by pindolol is attenuated in depressed patients: effect of treatment or an

endophenotype of depression? Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 29, 1688-1698.

Rainer, Q., Xia, L., Guilloux, J.P., Gabriel, C., Mocaer, E., Hen, R., Enhamre, E., Gardier, A.M., David, D.J., 2011. Beneficial behavioural and neurogenic effects of agomelatine in a model of depression/anxiety. Int J Neuropsychopharmacol, 1-15.

Raison, C.L., Miller, A.H., 2003. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. The American journal of psychiatry 160, 1554-1565.

Rangel, L.M., Alexander, A.S., Aimone, J.B., Wiles, J., Gage, F.H., Chiba, A.A., Quinn, L.K., 2014. Temporally selective contextual encoding in the dentate gyrus of the hippocampus. Nat Commun 5, 3181.

Rasheed, N., Tyagi, E., Ahmad, A., Siripurapu, K.B., Lahiri, S., Shukla, R., Palit, G., 2008. Involvement of monoamines and proinflammatory cytokines in mediating the anti-stress effects of Panax quinquefolium. Journal of ethnopharmacology 117, 257-262.

Ratta-Apha, W., Hishimoto, A., Yoshida, M., Ueno, Y., Asano, M., Shirakawa, O., Sora, I., 2013. Association study of BDNF with completed suicide in the Japanese population. Psychiatry research 209, 734-736.

Reif, A., Fritzen, S., Finger, M., Strobel, A., Lauer, M., Schmitt, A., Lesch, K.P., 2006. Neural stem cell proliferation is decreased in schizophrenia, but not in depression. Mol Psychiatry 11, 514-522.

Renthal, W., Maze, I., Krishnan, V., Covington, H.E., Xiao, G., Kumar, A., Russo, S.J., Graham, A., Tsankova, N., Kippin, T.E., Kerstetter, K.A., Neve, R.L., Haggarty, S.J., McKinsey, T.A., Bassel-Duby, R., Olson, E.N., Nestler, E.J., 2007. Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. Neuron 56, 517-529.

Ressler, K.J., Nemeroff, C.B., 2000. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depress Anxiety 12 Suppl 1, 2-19.

Rethelyi, J.M., Bakker, S.C., Polgar, P., Czobor, P., Strengman, E., Pasztor, P.I., Kahn, R.S., Bitter, I., 2010. Association study of NRG1, DTNBP1, RGS4, G72/G30, and PIP5K2A with schizophrenia and

symptom severity in a Hungarian sample. American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 153B, 792-801.

Riad, M., Rbah, L., Verdurand, M., Aznavour, N., Zimmer, L., Descarries, L., 2008. Unchanged density of 5-HT(1A) autoreceptors on the plasma membrane of nucleus raphe dorsalis neurons in rats chronically treated with fluoxetine. Neuroscience 151, 692-700.

Riad, M., Zimmer, L., Rbah, L., Watkins, K.C., Hamon, M., Descarries, L., 2004. Acute treatment with the antidepressant fluoxetine internalizes 5-HT1A autoreceptors and reduces the in vivo binding of the PET radioligand [18F]MPPF in the nucleus raphe dorsalis of rat. The Journal of neuroscience : the official journal of the Society for Neuroscience 24, 5420-5426.

Richardson-Jones, J.W., Craige, C.P., Guiard, B.P., Stephen, A., Metzger, K.L., Kung, H.F., Gardier, A.M., Dranovsky, A., David, D.J., Beck, S.G., Hen, R., Leonardo, E.D., 2010. 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. Neuron 65, 40-52.

Richell, R.A., Deakin, J.F., Anderson, I.M., 2005. Effect of acute tryptophan depletion on the response to controllable and uncontrollable noise stress. Biological psychiatry 57, 295-300.

Rieff, H.I., Corfas, G., 2006. ErbB receptor signalling regulates dendrite formation in mouse cerebellar granule cells in vivo. Eur J Neurosci 23, 2225-2229.

Rieff, H.I., Raetzman, L.T., Sapp, D.W., Yeh, H.H., Siegel, R.E., Corfas, G., 1999. Neuregulin induces GABA(A) receptor subunit expression and neurite outgrowth in cerebellar granule cells. J Neurosci 19, 10757-10766.

Rio, C., Rieff, H.I., Qi, P., Khurana, T.S., Corfas, G., 1997. Neuregulin and erbB receptors play a critical role in neuronal migration. Neuron 19, 39-50.

Rizk, P., Salazar, J., Raisman-Vozari, R., Marien, M., Ruberg, M., Colpaert, F., Debeir, T., 2006. The alpha2-adrenoceptor antagonist dexefaroxan enhances hippocampal neurogenesis by increasing the survival and differentiation of new granule cells. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 31, 1146-1157.

Robison, A.J., Vialou, V., Sun, H.S., Labonte, B., Golden, S.A., Dias, C., Turecki, G., Tamminga, C., Russo, S., Mazei-Robison, M., Nestler, E.J., 2014. Fluoxetine epigenetically alters the CaMKIIalpha promoter in nucleus accumbens to regulate DeltaFosB binding and antidepressant effects. Neuropsychopharmacology 39, 1178-1186.

Rockett, I.R., Regier, M.D., Kapusta, N.D., Coben, J.H., Miller, T.R., Hanzlick, R.L., Todd, K.H., Sattin, R.W., Kennedy, L.W., Kleinig, J., Smith, G.S., 2012. Leading causes of unintentional and intentional injury mortality: United States, 2000-2009. Am J Public Health 102, e84-92.

Roman, O., Seres, J., Pometlova, M., Jurcovicova, J., 2004. Neuroendocrine or behavioral effects of acute or chronic emotional stress in Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Endocr Regul 38, 151-155.

Ropret, S., Zupanc, T., Komel, R., Videtic Paska, A., 2015. Single nucleotide polymorphisms in the BDNF gene and suicide in the Slovenian sample. Neurosci Lett 602, 12-16.

Rosa-Neto, P., Diksic, M., Okazawa, H., Leyton, M., Ghadirian, N., Mzengeza, S., Nakai, A., Debonnel, G., Blier, P., Benkelfat, C., 2004. Measurement of brain regional alpha-[11C]methyl-L-tryptophan trapping as a measure of serotonin synthesis in medication-free patients with major depression. Archives of general psychiatry 61, 556-563.

Rosler, T.W., Depboylu, C., Arias-Carrion, O., Wozny, W., Carlsson, T., Hollerhage, M., Oertel, W.H., Schrattenholz, A., Hoglinger, G.U., 2011. Biodistribution and brain permeability of the extracellular domain of neuregulin-1-beta1. Neuropharmacology 61, 1413-1418.

Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., Fabbri, M.E., Tessarollo, L., Maffei, L., Berardi, N., Caleo, M., 2006. Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. European Journal of Neuroscience 24, 1850-1856.

Rouchet, N., Waroux, O., Lamy, C., Massotte, L., Scuvee-Moreau, J., Liegeois, J.-F., Seutin, V., 2008. SK channel blockade promotes burst firing in dorsal raphe serotonergic neurons. The European journal of neuroscience 28, 1108-1115.

Roughton, K., Kalm, M., Blomgren, K., 2012. Sex-dependent differences in behavior and hippocampal neurogenesis after irradiation to the young mouse brain. The European journal of neuroscience 36, 2763-2772.

Roy, K., Murtie, J.C., El-Khodor, B.F., Edgar, N., Sardi, S.P., Hooks, B.M., Benoit-Marand, M., Chen, C., Moore, H., O'Donnell, P., Brunner, D., Corfas, G., 2007. Loss of erbB signaling in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. Proceedings of the National Academy of Sciences of the United States of America 104, 8131-8136.

Ruan, S.Q., Wang, S.W., Wang, Z.H., Zhang, S.Z., 2012. Regulation of HRG-beta1-induced proliferation, migration and invasion of MCF-7 cells by upregulation of GPR30 expression. Mol Med Rep 6, 131-138.

Russo, S.J., Murrough, J.W., Han, M.-H., Charney, D.S., Nestler, E.J., 2012. Neurobiology of resilience. Nature neuroscience 15, 1475-1484.

Sahay, A., Hen, R., 2007. Adult hippocampal neurogenesis in depression. Nat Neurosci 10, 1110-1115.

Sahay, A., Scobie, K.N., Hill, A.S., O'Carroll, C.M., Kheirbek, M.A., Burghardt, N.S., Fenton, A.A., Dranovsky, A., Hen, R., 2011. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 472, 466-470.

Sairanen, M., Lucas, G., Ernfors, P., Castren, M., Castren, E., 2005. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. The Journal of neuroscience : the official journal of the Society for Neuroscience 25, 1089-1094.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., Hen, R., 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805-809.

Sapolsky, R., 2003. Taming stress. Scientific American 289, 86-95.

Sarchiapone, M., Carli, V., Roy, A., Iacoviello, L., Cuomo, C., Latella, M.C., di Giannantonio, M., Janiri, L., de Gaetano, M., Janal, M.N., 2008. Association of polymorphism (Val66Met) of brain-derived neurotrophic factor with suicide attempts in depressed patients. Neuropsychobiology 57, 139-145.

Sardi, S.P., Murtie, J., Koirala, S., Patten, B.A., Corfas, G., 2006. Presenilin-dependent ErbB4 nuclear signaling regulates the timing of astrogenesis in the developing brain. Cell 127, 185-197.

Sargent, P.A., Kjaer, K.H., Bench, C.J., Rabiner, E.A., Messa, C., Meyer, J., Gunn, R.N., Grasby, P.M., Cowen, P.J., 2000. Brain serotonin1A receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. Arch Gen Psychiatry 57, 174-180.

Sathyamurthy, A., Yin, D.M., Barik, A., Shen, C., Bean, J.C., Figueiredo, D., She, J.X., Xiong, W.C., Mei, L., 2015. ERBB3-mediated regulation of Bergmann glia proliferation in cerebellar lamination. Development 142, 522-532.

Scharfman, H., Goodman, J., Macleod, A., Phani, S., Antonelli, C., Croll, S., 2005. Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. Experimental neurology 192, 348-356.

Scheff, S.W., Price, D.A., 1998. Synaptic density in the inner molecular layer of the hippocampal dentate gyrus in Alzheimer disease. Journal of neuropathology and experimental neurology 57, 1146-1153.

Schloesser, R.J., Jimenez, D.V., Hardy, N.F., Paredes, D., Catlow, B.J., Manji, H.K., McKay, R.D., Martinowich, K., 2014. Atrophy of pyramidal neurons and increased stress-induced glutamate levels in CA3 following chronic suppression of adult neurogenesis. Brain structure & function 219, 1139-1148.

Schloesser, R.J., Manji, H.K., Martinowich, K., 2009. Suppression of adult neurogenesis leads to an increased hypothalamo-pituitary-adrenal axis response. Neuroreport 20, 553-557.

Schmid, R.S., McGrath, B., Berechid, B.E., Boyles, B., Marchionni, M., Sestan, N., Anton, E.S., 2003. Neuregulin 1-erbB2 signaling is required for the establishment of radial glia and their transformation into astrocytes in cerebral cortex. Proc Natl Acad Sci U S A 100, 4251-4256.

Schmidt, H.D., Duman, R.S., 2010. Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. Neuropsychopharmacology 35, 2378-2391.

Schultz, W., 2007. Multiple dopamine functions at different time courses. Annual review of neuroscience 30, 259-288.

Schweimer, J.V., Ungless, M.A., 2010. Phasic responses in dorsal raphe serotonin neurons to noxious stimuli. Neuroscience 171, 1209-1215.

Sen, S., Duman, R., Sanacora, G., 2008. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry 64, 527-532.

Seshadri, S., Kamiya, A., Yokota, Y., Prikulis, I., Kano, S., Hayashi-Takagi, A., Stanco, A., Eom, T.Y., Rao, S., Ishizuka, K., Wong, P., Korth, C., Anton, E.S., Sawa, A., 2010. Disrupted-in-Schizophrenia-1 expression is regulated by beta-site amyloid precursor protein cleaving enzyme-1-neuregulin cascade. Proc Natl Acad Sci U S A 107, 5622-5627.

Shamir, A., Kwon, O.B., Karavanova, I., Vullhorst, D., Leiva-Salcedo, E., Janssen, M.J., Buonanno, A., 2012. The importance of the NRG-1/ErbB4 pathway for synaptic plasticity and behaviors associated with psychiatric disorders. J Neurosci 32, 2988-2997.

Sharp, T., Boothman, L., Raley, J., Queree, P., 2007. Important messages in the 'post': recent discoveries in 5-HT neurone feedback control. Trends in pharmacological sciences 28, 629-636.

Sheikh, N., Ahmad, A., Siripurapu, K.B., Kuchibhotla, V.K., Singh, S., Palit, G., 2007. Effect of Bacopa monniera on stress induced changes in plasma corticosterone and brain monoamines in rats. Journal of ethnopharmacology 111, 671-676.

Shi, F., Telesco, S.E., Liu, Y., Radhakrishnan, R., Lemmon, M.A., 2010a. ErbB3/HER3 intracellular domain is competent to bind ATP and catalyze autophosphorylation. Proceedings of the National Academy of Sciences of the United States of America 107, 7692-7697.

Shi, S.S., Shao, S.H., Yuan, B.P., Pan, F., Li, Z.L., 2010b. Acute stress and chronic stress change brain-derived neurotrophic factor (BDNF) and tyrosine kinase-coupled receptor (TrkB) expression in both young and aged rat hippocampus. Yonsei medical journal 51, 661-671.

Shibuya, M., Komi, E., Wang, R., Kato, T., Watanabe, Y., Sakai, M., Ozaki, M., Someya, T., Nawa, H., 2010. Measurement and comparison of serum neuregulin 1 immunoreactivity in control subjects and patients with schizophrenia: an influence of its genetic polymorphism. J Neural Transm 117, 887-895.

Shima, K., Nakahama, H., Yamamoto, M., 1986. Firing properties of two types of nucleus raphe dorsalis neurons during the sleep-waking cycle and their responses to sensory stimuli. Brain research 399, 317-326.

Shin, M.H., Mavila, N., Wang, W.H., Vega Alvarez, S., Hall, M.C., Andrisani, O.M., 2009. Time-dependent activation of Phox2a by the cyclic AMP pathway modulates onset and duration of p27Kip1 transcription. Molecular and cellular biology 29, 4878-4890.

Shirayama, Y., Chen, A.C., Nakagawa, S., Russell, D.S., Duman, R.S., 2002. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. The Journal of neuroscience : the official journal of the Society for Neuroscience 22, 3251-3261.

Sierksma, A.S., Prickaerts, J., Chouliaras, L., Rostamian, S., Delbroek, L., Rutten, B.P., Steinbusch, H.W., van den Hove, D.L., 2013a. Behavioral and neurobiological effects of prenatal stress exposure in male and female APPswe/PS1dE9 mice. Neurobiology of aging 34, 319-337.

Sierksma, A.S., Rutten, K., Sydlik, S., Rostamian, S., Steinbusch, H.W., van den Hove, D.L., Prickaerts, J., 2013b. Chronic phosphodiesterase type 2 inhibition improves memory in the APPswe/PS1dE9 mouse model of Alzheimer's disease. Neuropharmacology 64, 124-136.

Silberg, J., Pickles, A., Rutter, M., Hewitt, J., Simonoff, E., Maes, H., Carbonneau, R., Murrelle, L., Foley, D., Eaves, L., 1999. The influence of genetic factors and life stress on depression among adolescent girls. Archives of general psychiatry 56, 225-232.

Silverman, L.H., 1976. Psychoanalytic theory "the reports of my death are greatly exaggerated". The American psychologist 31, 621-637.

Singh, A.B., Harris, R.C., 2005. Autocrine, paracrine and juxtacrine signaling by EGFR ligands. Cellular signalling 17, 1183-1193.

Smirnova, T., Zhou, Z.N., Flinn, R.J., Wyckoff, J., Boimel, P.J., Pozzuto, M., Coniglio, S.J., Backer, J.M., Bresnick, A.R., Condeelis, J.S., Hynes, N.E., Segall, J.E., 2012. Phosphoinositide 3-kinase signaling is critical for ErbB3-driven breast cancer cell motility and metastasis. Oncogene 31, 706-715.

Smith, M.A., Makino, S., Kim, S.Y., Kvetnansky, R., 1995a. Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary. Endocrinology 136, 3743-3750.

Smith, M.A., Makino, S., Kvetnansky, R., Post, R.M., 1995b. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 15, 1768-1777.

Snyder, J.S., Choe, J.S., Clifford, M.A., Jeurling, S.I., Hurley, P., Brown, A., Kamhi, J.F., Cameron, H.A., 2009a. Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice. J Neurosci 29, 14484-14495.

Snyder, J.S., Ferrante, S.C., Cameron, H.A., 2012. Late maturation of adult-born neurons in the temporal dentate gyrus. PloS one 7, e48757.

Snyder, J.S., Glover, L.R., Sanzone, K.M., Kamhi, J.F., Cameron, H.A., 2009b. The effects of exercise and stress on the survival and maturation of adult-generated granule cells. Hippocampus 19, 898-906.

Snyder, J.S., Ramchand, P., Rabbett, S., Radik, R., Wojtowicz, J.M., Cameron, H.A., 2011a. Septo-temporal gradients of neurogenesis and activity in 13-month-old rats. Neurobiology of aging 32, 1149-1156.

Snyder, J.S., Soumier, A., Brewer, M., Pickel, J., Cameron, H.A., 2011b. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 476, 458-461.

Song, C., Leonard, B.E., 2005. The olfactory bulbectomised rat as a model of depression. Neurosci Biobehav Rev 29, 627-647.

Spalding, K.L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H.B., Bostrom, E., Westerlund, I., Vial, C., Buchholz, B.A., Possnert, G., Mash, D.C., Druid, H., Frisen, J., 2013. Dynamics of hippocampal neurogenesis in adult humans. Cell 153, 1219-1227.

Steciuk, M., Kram, M., Kramer, G.L., Petty, F., 2000. Acute stress does not alter 5-HT1A receptor density. Prog Neuropsychopharmacol Biol Psychiatry 24, 155-161.

Stefansson, H., Sarginson, J., Kong, A., Yates, P., Steinthorsdottir, V., Gudfinnsson, E., Gunnarsdottir, S., Walker, N., Petursson, H., Crombie, C., Ingason, A., Gulcher, J.R., Stefansson, K., St Clair, D., 2003. Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. American journal of human genetics 72, 83-87.

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., Brynjolfsson, J., Gunnarsdottir, S., Ivarsson, O., Chou, T.T., Hjaltason, O., Birgisdottir, B., Jonsson, H., Gudnadottir, V.G., Gudmundsdottir, E., Bjornsson, A., Ingvarsson, B., Ingason, A., Sigfusson, S., Hardardottir, H., Harvey, R.P., Lai, D., Zhou, M., Brunner, D., Mutel, V., Gonzalo, A., Lemke, G., Sainz, J., Johannesson, G., Andresson, T., Gudbjartsson, D., Manolescu, A., Frigge, M.L., Gurney, M.E., Kong, A., Gulcher, J.R., Petursson, H., Stefansson, K., 2002. Neuregulin 1 and susceptibility to schizophrenia. American journal of human genetics 71, 877-892.

Steinbusch, H.W., 1981. Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience 6, 557-618.

Steiner, J., Bielau, H., Brisch, R., Danos, P., Ullrich, O., Mawrin, C., Bernstein, H.G., Bogerts, B., 2008a. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. Journal of psychiatric research 42, 151-157.

Steiner, M.A., Wanisch, K., Monory, K., Marsicano, G., Borroni, E., Bachli, H., Holsboer, F., Lutz, B., Wotjak, C.T., 2008b. Impaired cannabinoid receptor type 1 signaling interferes with stress-coping behavior in mice. Pharmacogenomics J 8, 196-208.

Steinkamp, M.P., Low-Nam, S.T., Yang, S., Lidke, K.A., Lidke, D.S., Wilson, B.S., 2014. erbB3 is an active tyrosine kinase capable of homo- and heterointeractions. Mol Cell Biol 34, 965-977.

Stockmeier, C.A., Shapiro, L.A., Dilley, G.E., Kolli, T.N., Friedman, L., Rajkowska, G., 1998. Increase in serotonin-1A autoreceptors in the midbrain of suicide victims with major depression-postmortem evidence for decreased serotonin activity. J Neurosci 18, 7394-7401.

Sun, Y., Jin, K., Childs, J.T., Xie, L., Mao, X.O., Greenberg, D.A., 2006. Vascular endothelial growth factor-B (VEGFB) stimulates neurogenesis: evidence from knockout mice and growth factor administration. Developmental biology 289, 329-335.

Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., Hen, R., Belzung, C., 2008. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biological psychiatry 64, 293-301.

Surget, A., Tanti, A., Leonardo, E.D., Laugeray, A., Rainer, Q., Touma, C., Palme, R., Griebel, G., Ibarguen-Vargas, Y., Hen, R., Belzung, C., 2011. Antidepressants recruit new neurons to improve stress response regulation. Mol Psychiatry 16, 1177-1188.

Sütterlin, P., Williams, E.J., Chambers, D., Saraf, K., von Schack, D., Reisenberg, M., Doherty, P., Williams, G., 2013. The molecular basis of the cooperation between EGF, FGF and eCB receptors in the regulation of neural stem cell function. Molecular and Cellular Neuroscience 52, 20-30.

Swiergiel, A.H., Leskov, I.L., Dunn, A.J., 2008. Effects of chronic and acute stressors and CRF on depression-like behavior in mice. Behav Brain Res 186, 32-40.

Szamosi, A., Kelemen, O., Keri, S., 2012. Hippocampal volume and the AKT signaling system in first-episode schizophrenia. Journal of psychiatric research 46, 279-284.

Takase, L.F., Nogueira, M.I., Baratta, M., Bland, S.T., Watkins, L.R., Maier, S.F., Fornal, C.A., Jacobs, B.L., 2004. Inescapable shock activates serotonergic neurons in all raphe nuclei of rat. Behavioural brain research 153, 233-239.

Taliaz, D., Stall, N., Dar, D.E., Zangen, A., 2010. Knockdown of brain-derived neurotrophic factor in specific brain sites precipitates behaviors associated with depression and reduces neurogenesis. Molecular psychiatry 15, 80-92.

Talmage, D.A., Role, L.W., 2004. Multiple personalities of neuregulin gene family members. J Comp Neurol 472, 134-139.

Tanti, A., Westphal, W.P., Girault, V., Brizard, B., Devers, S., Leguisquet, A.M., Surget, A., Belzung, C., 2013. Region-dependent and stage-specific effects of stress, environmental enrichment, and antidepressant treatment on hippocampal neurogenesis. Hippocampus 23, 797-811.

Temprana, S.G., Mongiat, L.A., Yang, S.M., Trinchero, M.F., Alvarez, D.D., Kropff, E., Giacomini, D., Beltramone, N., Lanuza, G.M., Schinder, A.F., 2015. Delayed coupling to feedback inhibition during a critical period for the integration of adult-born granule cells. Neuron 85, 116-130.

Ting, A.K., Chen, Y., Wen, L., Yin, D.M., Shen, C., Tao, Y., Liu, X., Xiong, W.C., Mei, L., 2011. Neuregulin 1 promotes excitatory synapse development and function in GABAergic interneurons. J Neurosci 31, 15-25.

Tjulandin, S., Moiseyenko, V., Semiglazov, V., Manikhas, G., Learoyd, M., Saunders, A., Stuart, M., Keilholz, U., 2014. Phase I, dose-finding study of AZD8931, an inhibitor of EGFR (erbB1), HER2 (erbB2) and HER3 (erbB3) signaling, in patients with advanced solid tumors. Invest New Drugs 32, 145-153.

Toni, N., Teng, E.M., Bushong, E.A., Aimone, J.B., Zhao, C., Consiglio, A., van Praag, H., Martone, M.E., Ellisman, M.H., Gage, F.H., 2007. Synapse formation on neurons born in the adult hippocampus. Nature neuroscience 10, 727-734.

Torres-Platas, S.G., Cruceanu, C., Chen, G.G., Turecki, G., Mechawar, N., 2014. Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. Brain, behavior, and immunity 42, 50-59.

Torres-Platas, S.G., Hercher, C., Davoli, M.A., Maussion, G., Labonte, B., Turecki, G., Mechawar, N., 2011. Astrocytic hypertrophy in anterior cingulate white matter of depressed suicides. Neuropsychopharmacology 36, 2650-2658.

Torres-Platas, S.G., Nagy, C., Wakid, M., Turecki, G., Mechawar, N., 2015. Glial fibrillary acidic protein is differentially expressed across cortical and subcortical regions in healthy brains and downregulated in the thalamus and caudate nucleus of depressed suicides. Mol Psychiatry.

Tosato, S., Bellani, M., Bonetto, C., Ruggeri, M., Perlini, C., Lasalvia, A., Marinelli, V., Rambaldelli, G., Cristofalo, D., Bertani, M., Zanoni, M., Lazzarotto, L., Cerini, R., Pozzi Mucelli, R., Tansella, M., Dazzan, P., Di Forti, M., Murray, R.M., Collier, D.A., Brambilla, P., 2012. Is neuregulin 1 involved in determining cerebral volumes in schizophrenia? Preliminary results showing a decrease in superior temporal gyrus volume. Neuropsychobiology 65, 119-125.

Trulson, M.E., Jacobs, B.L., 1979. Raphe unit activity in freely moving cats: correlation with level of behavioral arousal. Brain research 163, 135-150.

Trulson, M.E., Preussler, D.W., 1984. Dopamine-containing ventral tegmental area neurons in freely moving cats: activity during the sleep-waking cycle and effects of stress. Experimental neurology 83, 367-377.

Tsankova, N., Renthal, W., Kumar, A., Nestler, E.J., 2007. Epigenetic regulation in psychiatric disorders. Nature reviews. Neuroscience 8, 355-367.

Turecki, G., 2014. The molecular bases of the suicidal brain. Nature reviews. Neuroscience 15, 802-816.

Ueyama, E., Ukai, S., Ogawa, A., Yamamoto, M., Kawaguchi, S., Ishii, R., Shinosaki, K., 2011. Chronic repetitive transcranial magnetic stimulation increases hippocampal neurogenesis in rats. Psychiatry and clinical neurosciences 65, 77-81.

Uhlen, M., Fagerberg, L., Hallstrom, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, A., Kampf, C., Sjostedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C.A., Odeberg, J., Djureinovic, D., Takanen, J.O., Hober, S., Alm, T., Edqvist, P.H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J.M., Hamsten, M., von Feilitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von Heijne, G., Nielsen, J., Ponten, F., 2015. Proteomics. Tissue-based map of the human proteome. Science 347, 1260419.

Vaidya, V.A., Fernandes, K., Jha, S., 2007. Regulation of adult hippocampal neurogenesis: relevance to depression. Expert Rev Neurother 7, 853-864.

Valente, M.M., Bortolotto, V., Cuccurazzu, B., Ubezio, F., Meneghini, V., Francese, M.T., Canonico, P.L., Grilli, M., 2012. alpha2delta Ligands Act as Positive Modulators of Adult Hippocampal Neurogenesis and Prevent Depression-Like Behavior Induced by Chronic Restraint Stress. Molecular pharmacology 82, 271-280.

Valverde, O., Torrens, M., 2012. CB1 receptor-deficient mice as a model for depression. Neuroscience 204, 193-206.

van Groen, T., Wyss, J.M., 1990. Extrinsic projections from area CA1 of the rat hippocampus: olfactory, cortical, subcortical, and bilateral hippocampal formation projections. J Comp Neurol 302, 515-528.

van Praag, H., 1996. Serotonin-related, anxiety/aggression-driven, stressor-precipitated depression. A psycho-biological hypothesis. European psychiatry : the journal of the Association of European Psychiatrists 11, 57-67.

van Praag, H.M., 2004. Can stress cause depression? Progress in neuro-psychopharmacology & biological psychiatry 28, 891-907.

van Strien, N.M., Cappaert, N.L., Witter, M.P., 2009. The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. Nature reviews. Neuroscience 10, 272-282.

Vartanian, T., Corfas, G., Li, Y., Fischbach, G.D., Stefansson, K., 1994. A role for the acetylcholine receptor-inducing protein ARIA in oligodendrocyte development. Proceedings of the National Academy of Sciences of the United States of America 91, 11626-11630.

Vazquez-Palacios, G., Bonilla-Jaime, H., Velazquez-Moctezuma, J., 2005. Antidepressant effects of nicotine and fluoxetine in an animal model of depression induced by neonatal treatment with clomipramine. Prog Neuropsychopharmacol Biol Psychiatry 29, 39-46.

Veena, J., Rao, B.S., Srikumar, B.N., 2011. Regulation of adult neurogenesis in the hippocampus by stress, acetylcholine and dopamine. Journal of natural science, biology, and medicine 2, 26-37.

Veena, J., Srikumar, B.N., Mahati, K., Bhagya, V., Raju, T.R., Shankaranarayana Rao, B.S., 2009. Enriched environment restores hippocampal cell proliferation and ameliorates cognitive deficits in chronically stressed rats. Journal of neuroscience research 87, 831-843.

Vega-Rivera, N.M., Fernandez-Guasti, A., Ramirez-Rodriguez, G., Estrada-Camarena, E., 2015. Effect of sub-optimal doses of fluoxetine plus estradiol on antidepressant-like behavior and hippocampal neurogenesis in ovariectomized rats. Psychoneuroendocrinology 57, 113-124.

Veldhuis, H.D., De Korte, C.C., De Kloet, E.R., 1985. Glucocorticoids facilitate the retention of acquired immobility during forced swimming. European journal of pharmacology 115, 211-217.

Vetreno, R.P., Crews, F.T., 2015. Binge ethanol exposure during adolescence leads to a persistent loss of neurogenesis in the dorsal and ventral hippocampus that is associated with impaired adult cognitive functioning. Frontiers in neuroscience 9.

Vicentic, A., Francis, D., Moffett, M., Lakatos, A., Rogge, G., Hubert, G.W., Harley, J., Kuhar, M.J., 2006. Maternal separation alters serotonergic transporter densities and serotonergic 1A receptors in rat brain. Neuroscience 140, 355-365.

Vivar, C., Potter, M.C., Choi, J., Lee, J.Y., Stringer, T.P., Callaway, E.M., Gage, F.H., Suh, H., van Praag, H., 2012. Monosynaptic inputs to new neurons in the dentate gyrus. Nat Commun 3, 1107.

Vogel, G., Neill, D., Hagler, M., Kors, D., 1990. A new animal model of endogenous depression: a summary of present findings. Neurosci Biobehav Rev 14, 85-91.

Vollmayr, B., Simonis, C., Weber, S., Gass, P., Henn, F., 2003. Reduced cell proliferation in the dentate gyrus is not correlated with the development of learned helplessness. Biological psychiatry 54, 1035-1040.

Vullhorst, D., Neddens, J., Karavanova, I., Tricoire, L., Petralia, R.S., McBain, C.J., Buonanno, A., 2009. Selective expression of ErbB4 in interneurons, but not pyramidal cells, of the rodent hippocampus. J Neurosci 29, 12255-12264.

Walker, R.M., Christoforou, A., Thomson, P.A., McGhee, K.A., Maclean, A., Muhleisen, T.W., Strohmaier, J., Nieratschker, V., Nothen, M.M., Rietschel, M., Cichon, S., Morris, S.W., Jilani, O., Stclair, D., Blackwood, D.H., Muir, W.J., Porteous, D.J., Evans, K.L., 2010. Association analysis of Neuregulin 1 candidate regions in schizophrenia and bipolar disorder. Neurosci Lett 478, 9-13.

Walletschek, H., Raab, A., 1982. Spontaneous activity of dorsal raphe neurons during defensive and offensive encounters in the tree-shrew. Physiology & behavior 28, 697-705.

Wang, J.-W., Dranovsky, A., Hen, R., 2009. The When and Where of BDNF and the Antidepressant Response. Biological Psychiatry 63, 640-641.

Wang, J.W., David, D.J., Monckton, J.E., Battaglia, F., Hen, R., 2008. Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. The Journal of neuroscience : the official journal of the Society for Neuroscience 28, 1374-1384.

Warner-Schmidt, J.L., Duman, R.S., 2007. VEGF is an essential mediator of the neurogenic and behavioral actions of antidepressants. Proceedings of the National Academy of Sciences of the United States of America 104, 4647-4652.

Waterhouse, E.G., An, J.J., Orefice, L.L., Baydyuk, M., Liao, G.Y., Zheng, K., Lu, B., Xu, B., 2012. BDNF promotes differentiation and maturation of adult-born neurons through GABAergic transmission. The Journal of neuroscience : the official journal of the Society for Neuroscience 32, 14318-14330.

Weiss, J.M., Cierpial, M.A., West, C.H., 1998. Selective breeding of rats for high and low motor activity in a swim test: toward a new animal model of depression. Pharmacol Biochem Behav 61, 49-66.

Wen, D., Suggs, S.V., Karunagaran, D., Liu, N., Cupples, R.L., Luo, Y., Janssen, A.M., Ben-Baruch, N., Trollinger, D.B., Jacobsen, V.L., et al., 1994. Structural and functional aspects of the multiplicity of Neu differentiation factors. Mol Cell Biol 14, 1909-1919.

Wilber, A.A., Walker, A.G., Southwood, C.J., Farrell, M.R., Lin, G.L., Rebec, G.V., Wellman, C.L., 2011. Chronic stress alters neural activity in medial prefrontal cortex during retrieval of extinction. Neuroscience 174, 115-131.

Wilkinson, L.O., Jacobs, B.L., 1988. Lack of response of serotonergic neurons in the dorsal raphe nucleus of freely moving cats to stressful stimuli. Experimental neurology 101, 445-457.

Willner, P., 1990. Animal models of depression: an overview. Pharmacology & therapeutics 45, 425-455.

Willner, P., 1997. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology 134, 319-329.

Willner, P., 2005. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52, 90-110.

Willner, P., Muscat, R., Papp, M., 1992. Chronic mild stress-induced anhedonia: a realistic animal model of depression. Neurosci Biobehav Rev 16, 525-534.

Willner, P., Towell, A., Sampson, D., Sophokleous, S., Muscat, R., 1987. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology 93, 358-364.

Wilson, C., Alvin V. Terry, J., 2010. Neurodevelopmental Animal Models of Schizophrenia: Role in Novel Drug Discovery and Development Clinical Schizophrenia & Related Psychoses 4, 124-137.

Wilson, M.A., Molliver, M.E., 1991. The organization of serotonergic projections to cerebral cortex in primates: retrograde transport studies. Neuroscience 44, 555-570.

Wong, E.Y., Herbert, J., 2004. The corticoid environment: a determining factor for neural progenitors' survival in the adult hippocampus. The European journal of neuroscience 20, 2491-2498.

Wong, E.Y., Herbert, J., 2006. Raised circulating corticosterone inhibits neuronal differentiation of progenitor cells in the adult hippocampus. Neuroscience 137, 83-92.

Wu, L., Walas, S., Leung, W., Sykes, D.B., Wu, J., Lo, E.H., Lok, J., 2015. Neuregulin1-beta decreases IL-1beta-induced neutrophil adhesion to human brain microvascular endothelial cells. Transl Stroke Res 6, 116-124.

Wu, M.V., Hen, R., 2014. Functional dissociation of adult-born neurons along the dorsoventral axis of the dentate gyrus. Hippocampus 24, 751-761.

Xu, Z., Croslan, D.R., Harris, A.E., Ford, G.D., Ford, B.D., 2006. Extended therapeutic window and functional recovery after intraarterial administration of neuregulin-1 after focal ischemic stroke. J Cereb Blood Flow Metab 26, 527-535.

Xu, Z., Jiang, J., Ford, G., Ford, B.D., 2004. Neuregulin-1 is neuroprotective and attenuates inflammatory responses induced by ischemic stroke. Biochem Biophys Res Commun 322, 440-446.

Yang, P., Arnold, S.A., Habas, A., Hetman, M., Hagg, T., 2008. Ciliary neurotrophic factor mediates dopamine D2 receptor-induced CNS neurogenesis in adult mice. The Journal of neuroscience : the official journal of the Society for Neuroscience 28, 2231-2241.

Yang, S.A., 2012. Association between a Missense Polymorphism (rs3924999, Arg253Gln) of Neuregulin 1 and Schizophrenia in Korean Population. Exp Neurobiol 21, 158-163.

Yang, X., Kuo, Y., Devay, P., Yu, C., Role, L., 1998. A cysteine-rich isoform of neuregulin controls the level of expression of neuronal nicotinic receptor channels during synaptogenesis. Neuron 20, 255-270.

Yao, J.-J., Sun, J., Zhao, Q.-R., Wang, C.-Y., Mei, Y.-A., 2013. Neuregulin-1/ErbB4 signaling regulates Kv4. 2-mediated transient outward K+ current through the Akt/mTOR pathway. American Journal of Physiology-Cell Physiology 305, C197-C206.

Yau, H.J., Wang, H.F., Lai, C., Liu, F.C., 2003. Neural development of the neuregulin receptor ErbB4 in the cerebral cortex and the hippocampus: preferential expression by interneurons tangentially migrating from the ganglionic eminences. Cereb Cortex 13, 252-264.

Ye, W., Mairet-Coello, G., Pasoreck, E., Dicicco-Bloom, E., 2009. Patterns of p57Kip2 expression in embryonic rat brain suggest roles in progenitor cell cycle exit and neuronal differentiation. Developmental neurobiology 69, 1-21.

Yi, L.T., Li, J.M., Li, Y.C., Pan, Y., Xu, Q., Kong, L.D., 2008. Antidepressant-like behavioral and neurochemical effects of the citrus-associated chemical apigenin. Life sciences 82, 741-751.

Yin, D.M., Chen, Y.J., Lu, Y.S., Bean, J.C., Sathyamurthy, A., Shen, C., Liu, X., Lin, T.W., Smith, C.A., Xiong, W.C., Mei, L., 2013. Reversal of behavioral deficits and synaptic dysfunction in mice overexpressing neuregulin 1. Neuron 78, 644-657.

Yin, H.-K., Li, X.-Y., Jiang, Z.-G., Zhou, M.-D., 2015. Progress in neuregulin/ErbB signaling and chronic heart failure. World J Hypertens 5, 63-73.

Yoo, H.J., Woo, R.S., Cho, S.C., Kim, B.N., Kim, J.W., Shin, M.S., Park, T.W., Son, J.W., Chung, U.S., Park, S., Park, M., Kim, S.A., 2015. Genetic association analyses of neuregulin 1 gene polymorphism with endopheontype for sociality of Korean autism spectrum disorders family. Psychiatry research 227, 366-368.

Young, C.D., Pfefferle, A.D., Owens, P., Kuba, M.G., Rexer, B.N., Balko, J.M., Sanchez, V., Cheng, H., Perou, C.M., Zhao, J.J., Cook, R.S., Arteaga, C.L., 2013. Conditional loss of ErbB3 delays mammary gland hyperplasia induced by mutant PIK3CA without affecting mammary tumor latency, gene expression, or signaling. Cancer research 73, 4075-4085.

Zai, C.C., Manchia, M., De Luca, V., Tiwari, A.K., Chowdhury, N.I., Zai, G.C., Tong, R.P., Yilmaz, Z., Shaikh, S.A., Strauss, J., Kennedy, J.L., 2012. The brain-derived neurotrophic factor gene in suicidal behaviour: a meta-analysis. Int J Neuropsychopharmacol 15, 1037-1042.

Zalsman, G., Huang, Y.Y., Oquendo, M.A., Burke, A.K., Hu, X.Z., Brent, D.A., Ellis, S.P., Goldman, D., Mann, J.J., 2006. Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR)

polymorphism with stressful life events and severity of depression. The American journal of psychiatry 163, 1588-1593.

Zhang, H.X., Zhao, J.P., Lv, L.X., Li, W.Q., Xu, L., Ouyang, X., Yuan, Z.Q., Huang, J.S., 2008. Explorative study on the expression of neuregulin-1 gene in peripheral blood of schizophrenia. Neurosci Lett 438, 1-5.

Zhang, T.Y., Meaney, M.J., 2010. Epigenetics and the environmental regulation of the genome and its function. Annual review of psychology 61, 439-466, C431-433.

Zhao, C., Teng, E.M., Summers, R.G., Jr., Ming, G.L., Gage, F.H., 2006. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. J Neurosci 26, 3-11.

Zhao, Y.Y., Sawyer, D.R., Baliga, R.R., Opel, D.J., Han, X., Marchionni, M.A., Kelly, R.A., 1998. Neuregulins promote survival and growth of cardiac myocytes. Persistence of ErbB2 and ErbB4 expression in neonatal and adult ventricular myocytes. The Journal of biological chemistry 273, 10261-10269.

Ziabreva, I., Poeggel, G., Schnabel, R., Braun, K., 2003. Separation-induced receptor changes in the hippocampus and amygdala of Octodon degus: influence of maternal vocalizations. The Journal of neuroscience : the official journal of the Society for Neuroscience 23, 5329-5336.

Zunszain, P.A., Hepgul, N., Pariante, C.M., 2013. Inflammation and depression. Current topics in behavioral neurosciences 14, 135-151.

APPENDIX

Supplemental materials from Chapter 2:

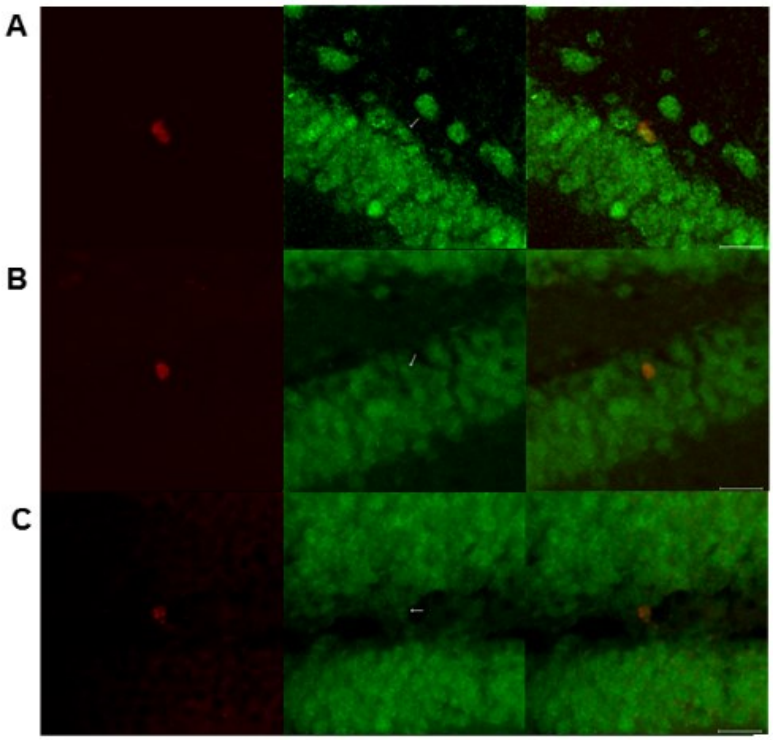


Figure A1. Bromodeoxyuridine (BrdU)/ErbB3 colocalization is present for at least 28 d after cell birth. BrdU-immunoreactive (-IR) cells (**red**) express ErbB3 (**green**), 2 h (**A**), 24 h (**Fig. 9**), 7 d (**B**), and 28 d (**C**) after birth. Scale bars = 20 μm.

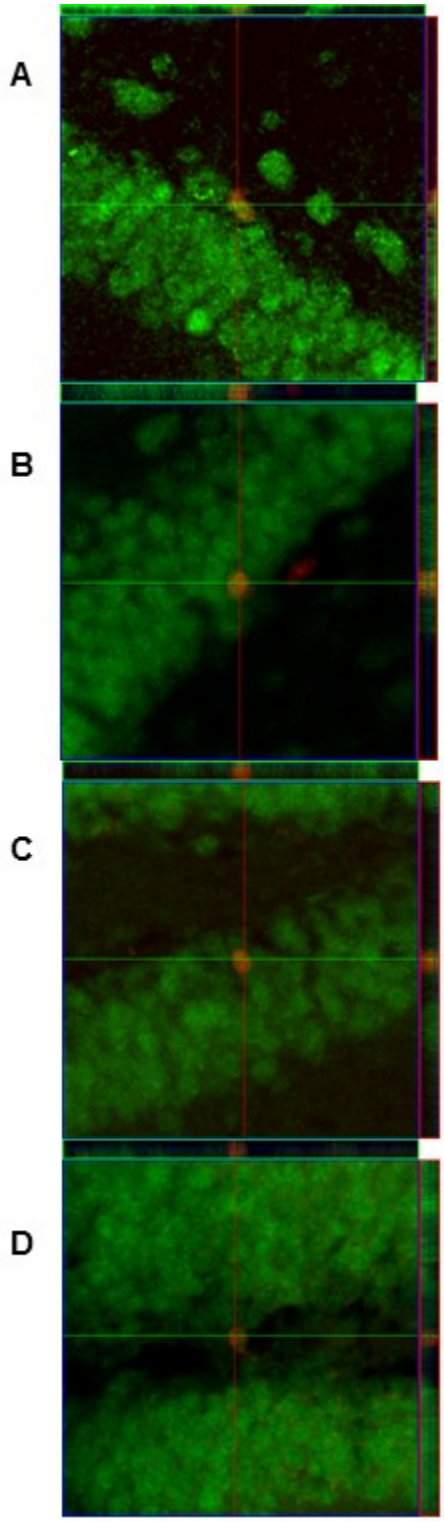


Figure A2. Orthogonal views of BrdU/ErbB3 colocalization. Orthogonal view of BrdU (**red**) / ErbB3 (**green**) colocalized cells from **Fig. 9** and **Fig. A1**. **A**, 2 h; **B**, 24 h; **C**, 7 d; **D**, 28 d.

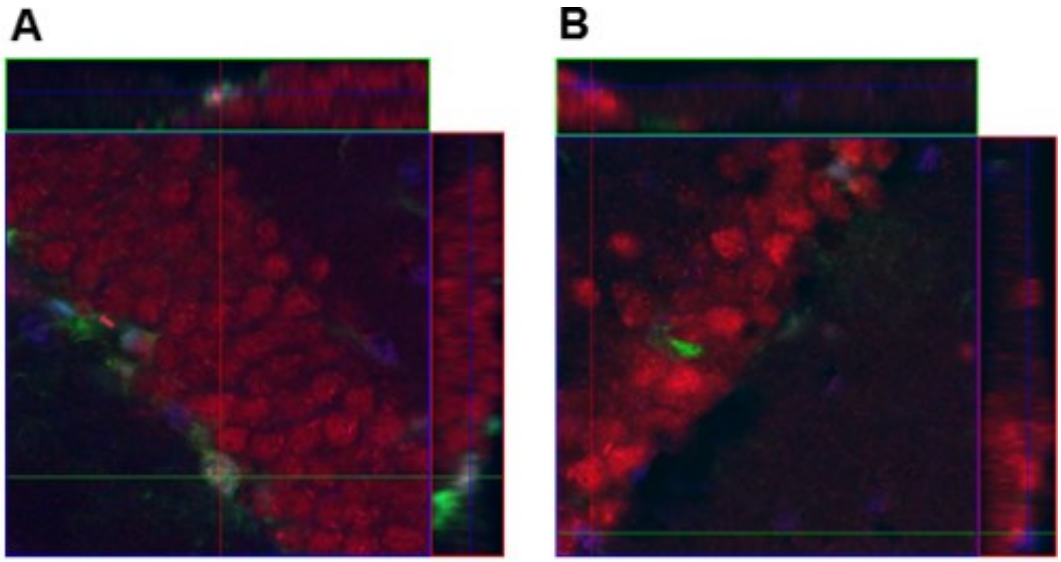


Figure A3. Orthogonal views of cell type colocalization. A, orthogonal view of triple-labeled cell from **Fig. 6A**. **B**, orthogonal view of ErbB3-IR/SOX2-IR/nestin-negative cell from **Fig. 6B**. **Red**, ErbB3; **blue**, SOX2; **green**, nestin.

Supplemental materials from Chapter 3:

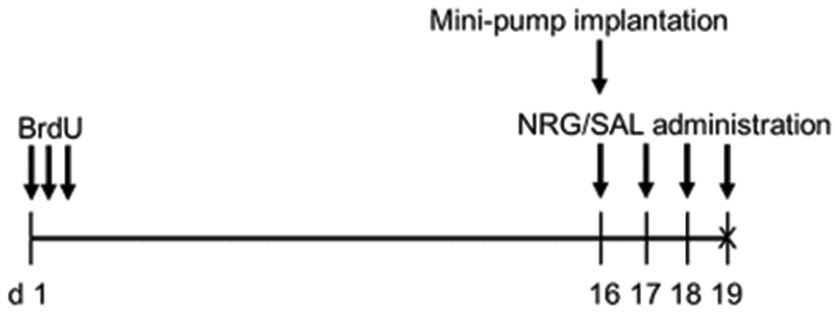


Figure A4. Experimental timeline. Mice were given three injections of BrdU on the first day of experimentation to label newborn cells. At day 16-19, corresponding to a period at which adultborn neurons undergo either apoptosis or morphological and synaptic development, neuregulin-1 (NRG1) or saline (SAL) was administered through subcutaneously implanted osmotic mini-pumps, and animals were sacrificed (X) following administration.

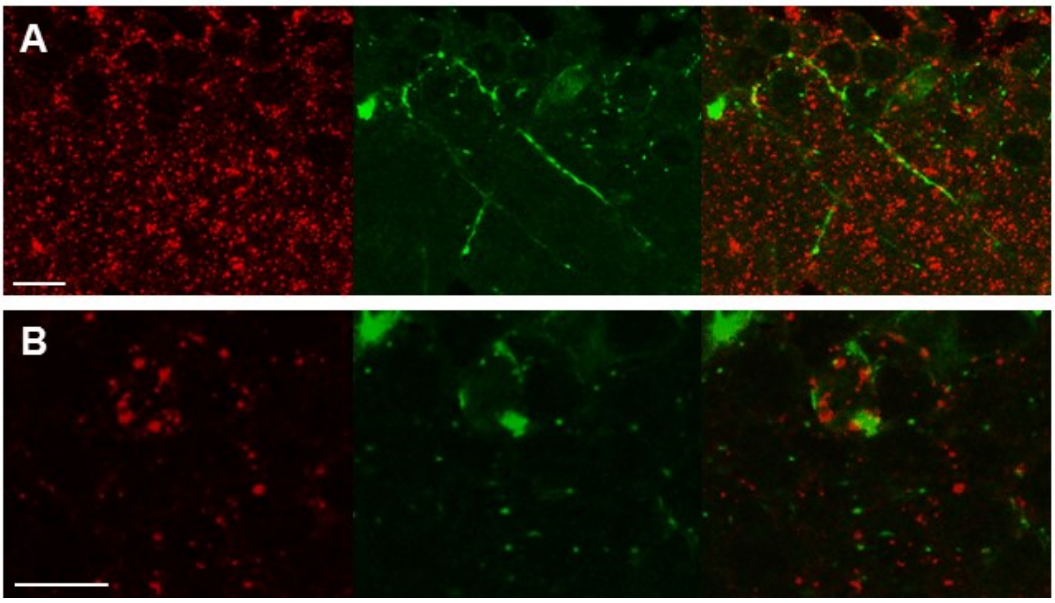


Figure A5. A, B, single-channel images of the planes shown in **Fig. 17C** and **Fig. 17E**, respectively. Red, doublecortin (DCX); green: postsynaptic density protein 95 (PSD-95). Scale bars: 10 μm.

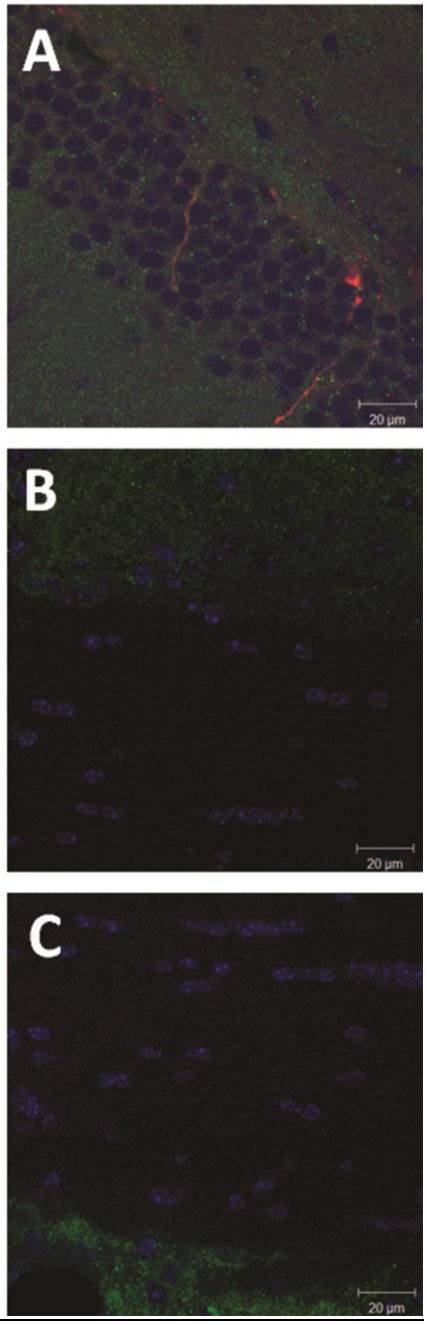


Figure A6. Comparison of PSD-95 staining in corpus callosum and hippocampal molecular layer. PSD-95 staining in the current protocol is absent from the corpus callosum but present in synaptically enriched regions; **A**, Dentate gyrus (DG); **B**, top: cortex; bottom: corpus callosum; **C**, top: corpus callosum; bottom: dorsal boundary of hippocampal molecular layer. Red (**A**), DCX; blue, DAPI; green, PSD-95.

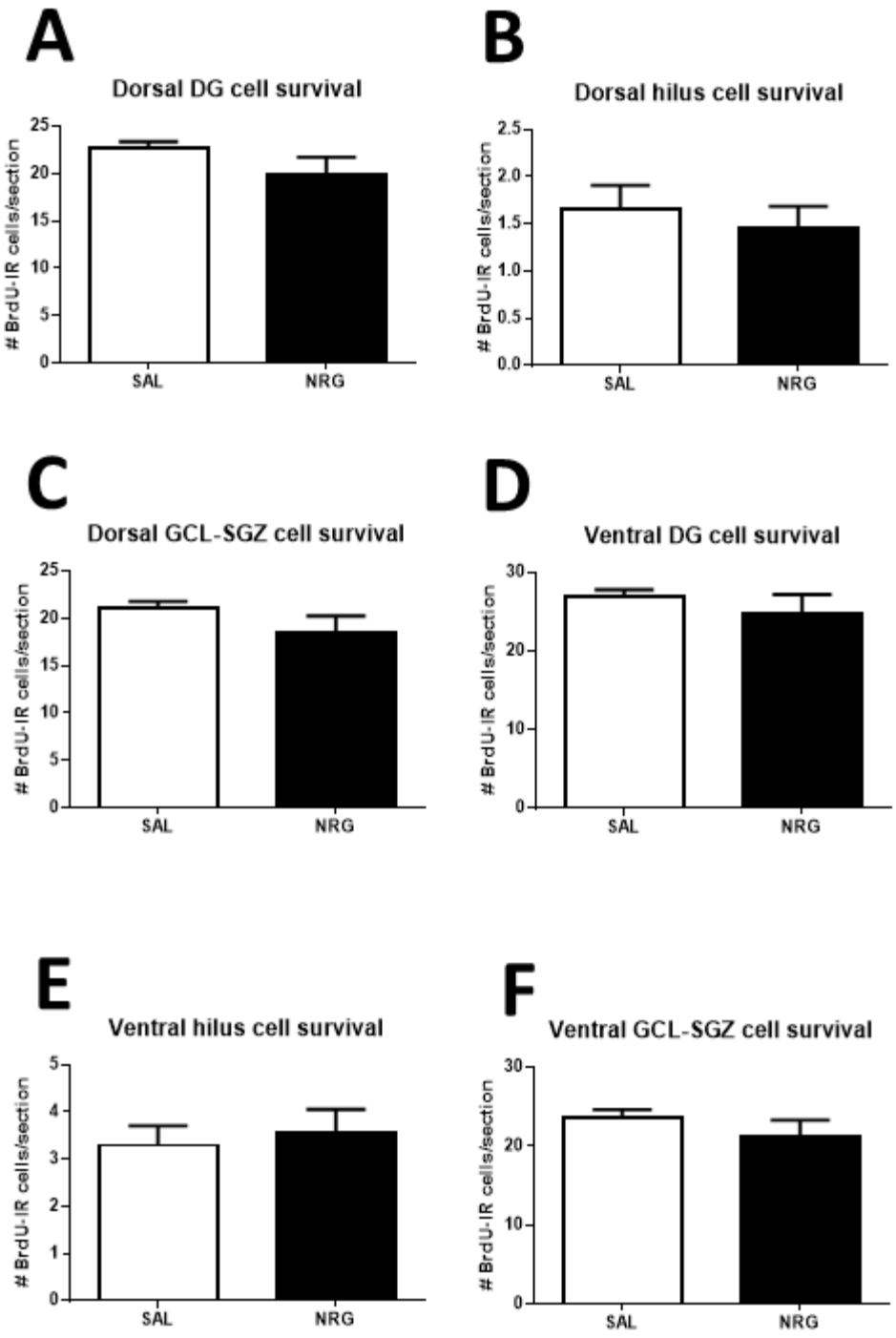
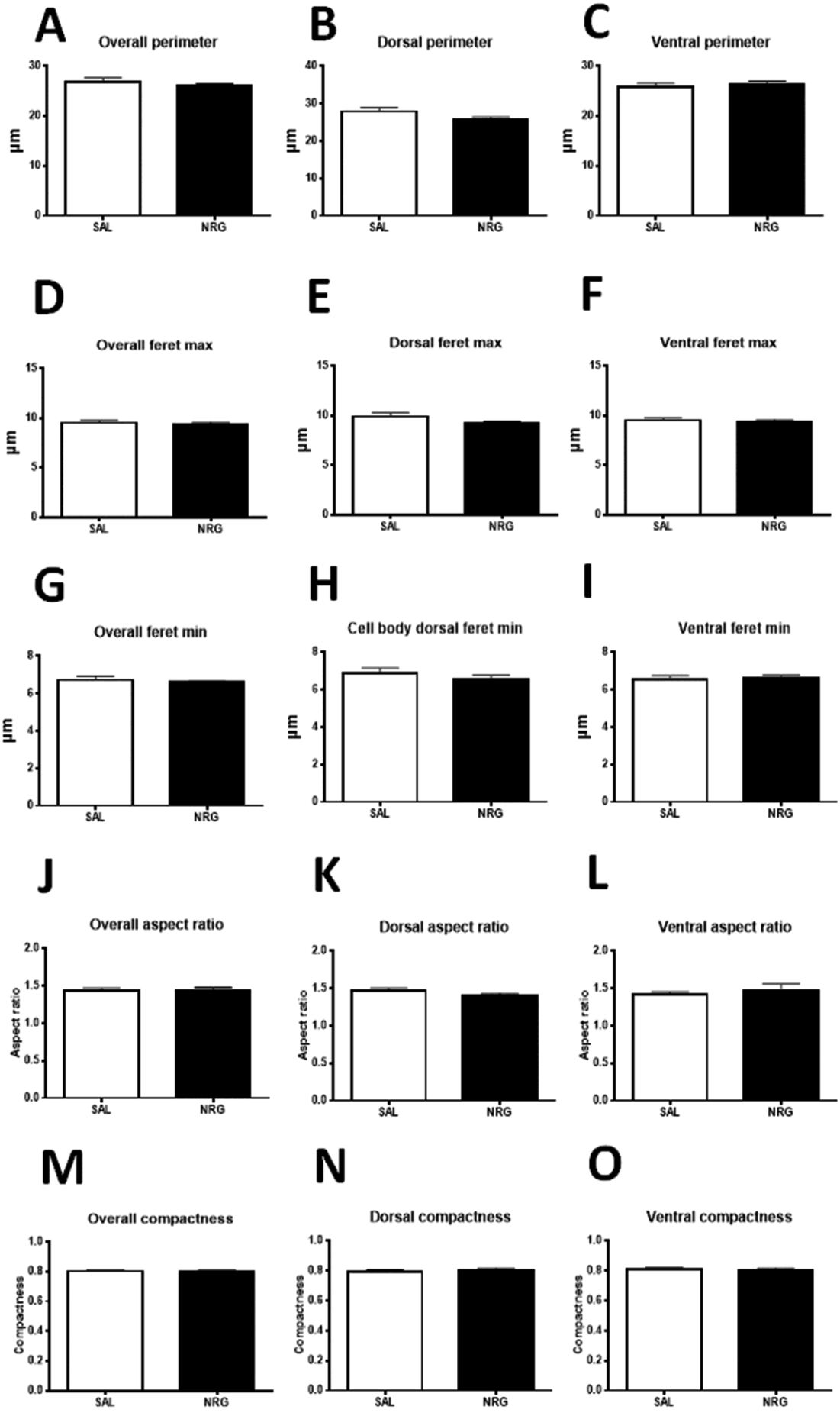


Figure A7. Additional findings on effects of neuregulin-1 administration on cell survival. NRG1 administration did not affect cell survival in the dorsal (**A**) or ventral (**B**) overall DG, or specifically in the subgranular zone and granular cell layer dorsally (**C**) or ventrally (**D**) or in the hilus dorsally (**E**) or ventrally (**F**).


A
Overall perimeter
µm
30
20
10
0
SAL
NRG
B
Dorsal perimeter
µm
40
30
20
10
0
SAL
NRG
C
Ventral perimeter
µm
30
20
10
0
SAL
NRG
D
Overall feret max
µm
15
10
5
0
SAL
NRG
E
Dorsal feret max
µm
15
10
5
0
SAL
NRG
F
Ventral feret max
µm
15
10
5
0
SAL
NRG
G
Overall feret min
µm
8
6
4
2
0
SAL
NRG
H
Cell body dorsal feret min
µm
8
6
4
2
0
SAL
NRG
I
Ventral feret min
µm
8
6
4
2
0
SAL
NRG
J
Overall aspect ratio
Aspect ratio
2.0
1.5
1.0
0.5
0.0
SAL
NRG
K
Dorsal aspect ratio
Aspect ratio
2.0
1.5
1.0
0.5
0.0
SAL
NRG
L
Ventral aspect ratio
Aspect ratio
2.0
1.5
1.0
0.5
0.0
SAL
NRG
M
Overall compactness
Compactness
1.0
0.8
0.6
0.4
0.2
0.0
SAL
NRG
N
Dorsal compactness
Compactness
1.0
0.8
0.6
0.4
0.2
0.0
SAL
NRG
O
Ventral compactness
Compactness
1.0
0.8
0.6
0.4
0.2
0.0
SAL
NRG

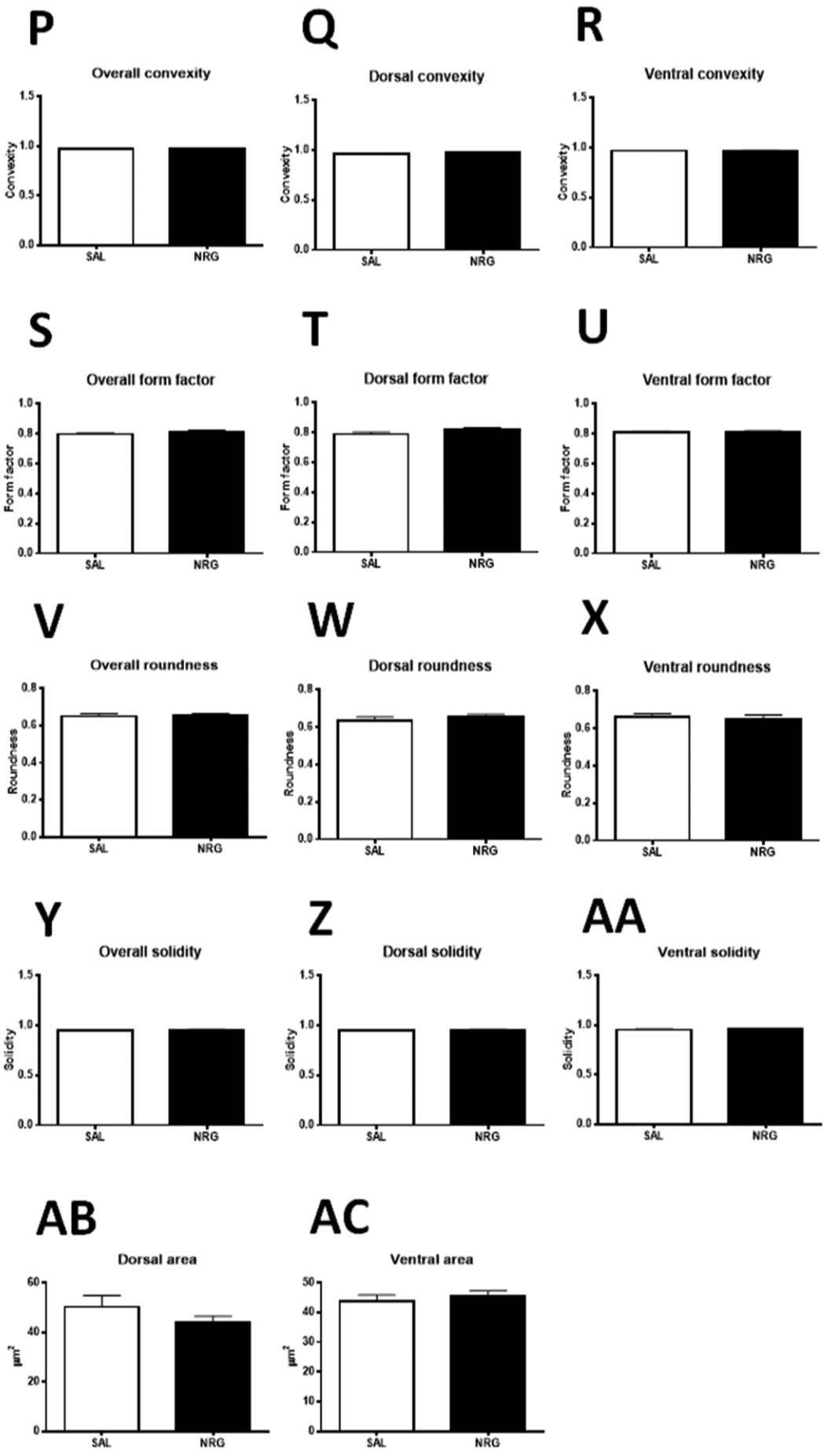
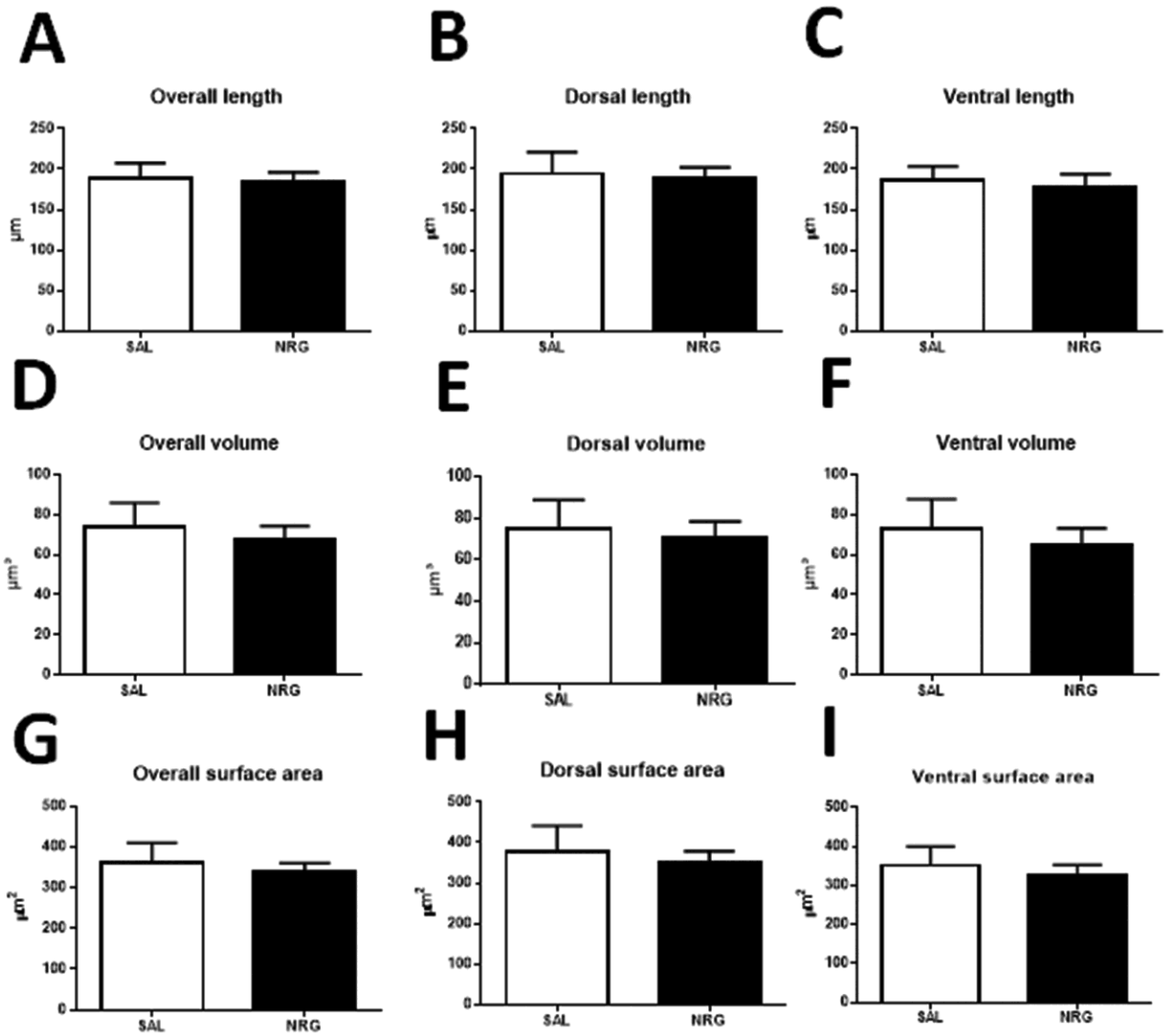

P
Overall convexity
Convexity
1.5
1.0
0.5
0.0
SAL
NRG
Q
Dorsal convexity
Convexity
1.5
1.0
0.5
0.0
SAL
NRG
R
Ventral convexity
Convexity
1.5
1.0
0.5
0.0
SAL
NRG
S
Overall form factor
Form factor
1.0
0.8
0.6
0.4
0.2
0.0
SAL
NRG
T
Dorsal form factor
Form factor
1.0
0.8
0.6
0.4
0.2
0.0
SAL
NRG
U
Ventral form factor
Form factor
1.0
0.8
0.6
0.4
0.2
0.0
SAL
NRG
V
Overall roundness
Roundness
0.8
0.6
0.4
0.2
0.0
SAL
NRG
W
Dorsal roundness
Roundness
0.8
0.6
0.4
0.2
0.0
SAL
NRG
X
Ventral roundness
Roundness
0.8
0.6
0.4
0.2
0.0
SAL
NRG
Y
Overall solidity
Solidity
1.5
1.0
0.5
0.0
SAL
NRG
Z
Dorsal solidity
Solidity
1.5
1.0
0.5
0.0
SAL
NRG
AA
Ventral solidity
Solidity
1.5
1.0
0.5
0.0
SAL
NRG
AB
Dorsal area
μm²
60
40
20
0
SAL
NRG
AC
Ventral area
μm²
50
40
30
20
10
0
SAL
NRG

Figure A8. Additional findings on effects of neuregulin-1 administration on cell body morphological development. NRG1 administration did affect overall, dorsal, or ventral immature neuronal cell body perimeter (**A-C**), feret max (**D-F**), feret min (**G-I**), aspect ratio (**J-L**), compactness (**M-O**), convexity (**P-R**), form factor (**S-U**), roundness (**V-X**), solidity (**Y-AA**), or area (**Fig. 16C, AB-AC**).



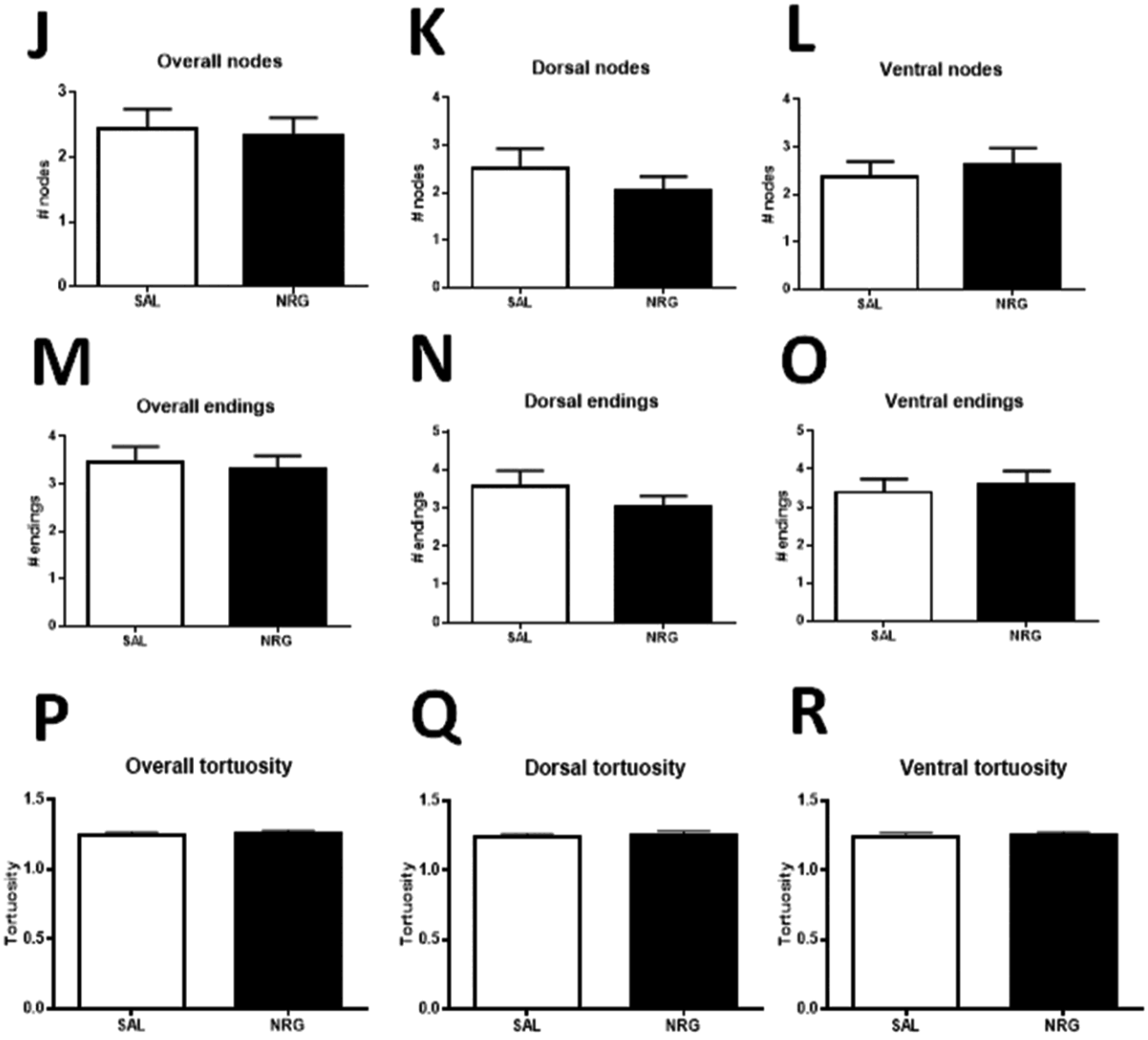
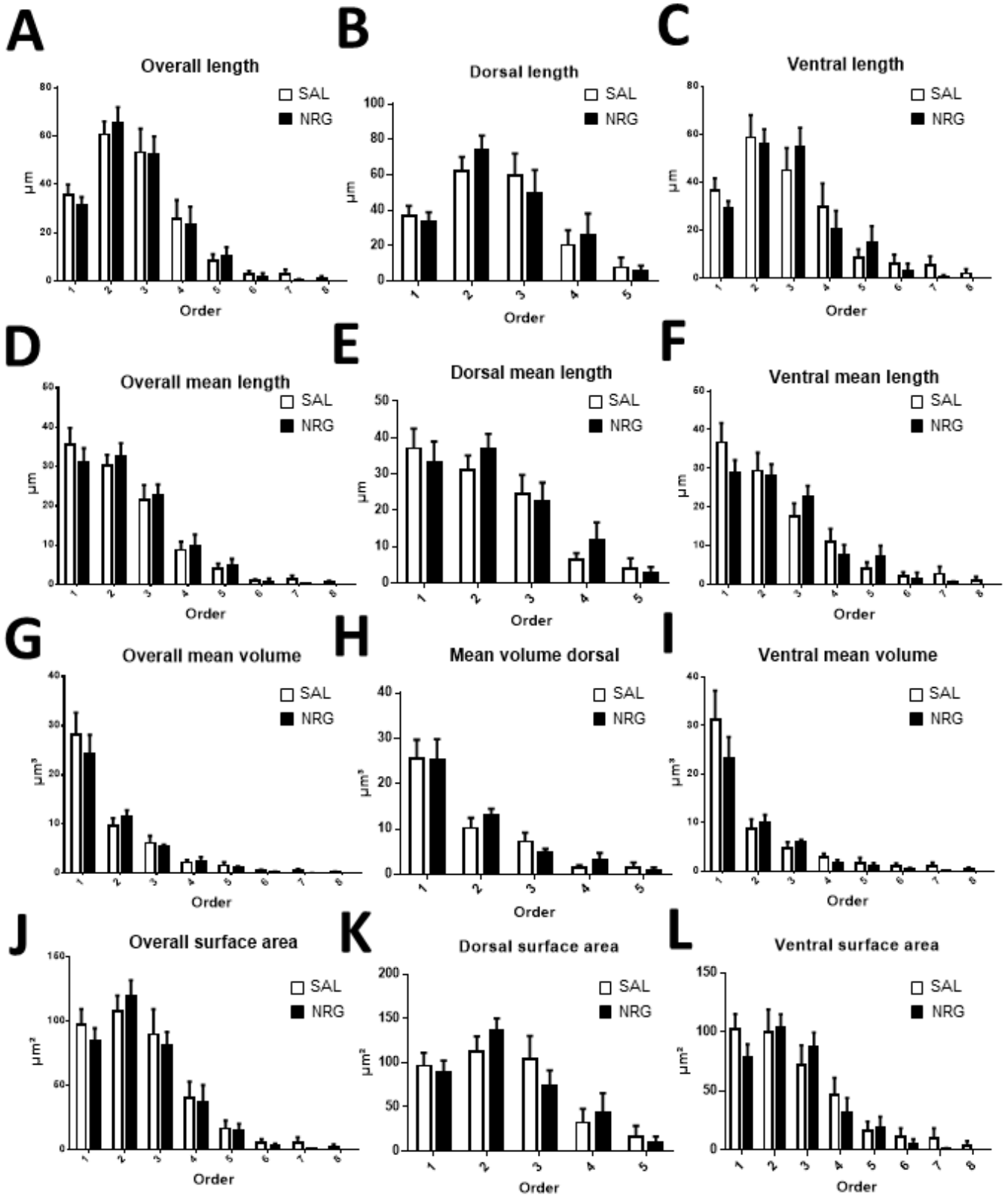
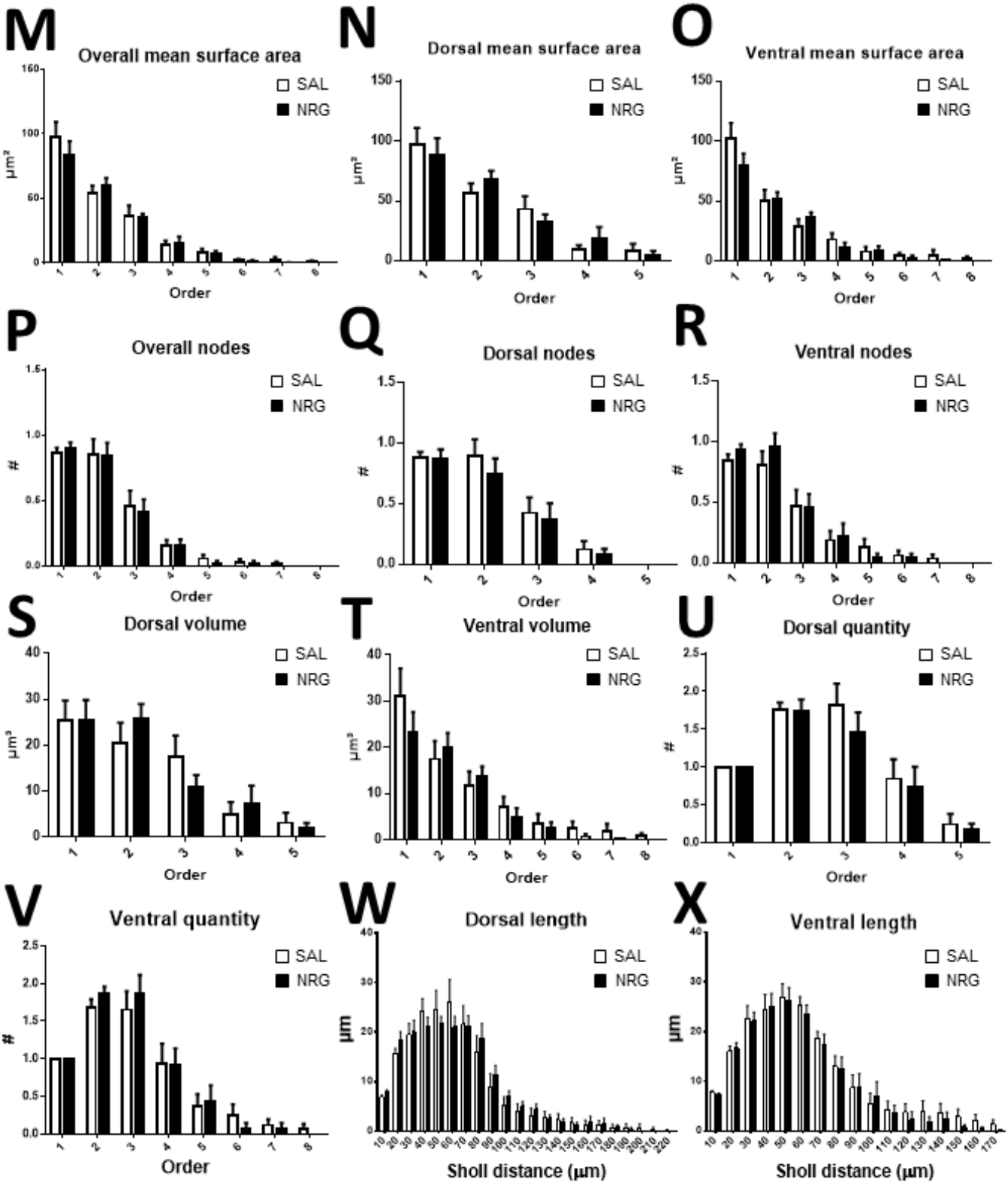


Figure A9. Additional findings on effects of neuregulin-1 administration on total dendritic morphological development. NRG1 administration did affect overall, dorsal, or ventral immature neuronal dendritic length (**A-C**), volume (**D-F**), surface area (**G-I**), nodes (**J-L**), ends (**M-O**), or tortuosity (**P-R**).


A
Overall length
SAL
NRG
µm
Order
B
Dorsal length
SAL
NRG
µm
Order
C
Ventral length
SAL
NRG
µm
Order
D
Overall mean length
SAL
NRG
µm
Order
E
Dorsal mean length
SAL
NRG
µm
Order
F
Ventral mean length
SAL
NRG
µm
Order
G
Overall mean volume
SAL
NRG
µm³
Order
H
Mean volume dorsal
SAL
NRG
µm³
Order
I
Ventral mean volume
SAL
NRG
µm³
Order
J
Overall surface area
SAL
NRG
µm²
Order
K
Dorsal surface area
SAL
NRG
µm²
Order
L
Ventral surface area
SAL
NRG
µm²
Order


M
Overall mean surface area
SAL
NRG
µm²
Order
N
Dorsal mean surface area
SAL
NRG
µm²
Order
O
Ventral mean surface area
SAL
NRG
µm²
Order
P
Overall nodes
SAL
NRG
#
Order
Q
Dorsal nodes
SAL
NRG
#
Order
R
Ventral nodes
SAL
NRG
#
Order
S
Dorsal volume
SAL
NRG
µm³
Order
T
Ventral volume
SAL
NRG
µm³
Order
U
Dorsal quantity
SAL
NRG
#
Order
V
Ventral quantity
SAL
NRG
#
Order
W
Dorsal length
SAL
NRG
µm
Sholl distance (µm)
X
Ventral length
SAL
NRG
µm
Sholl distance (µm)

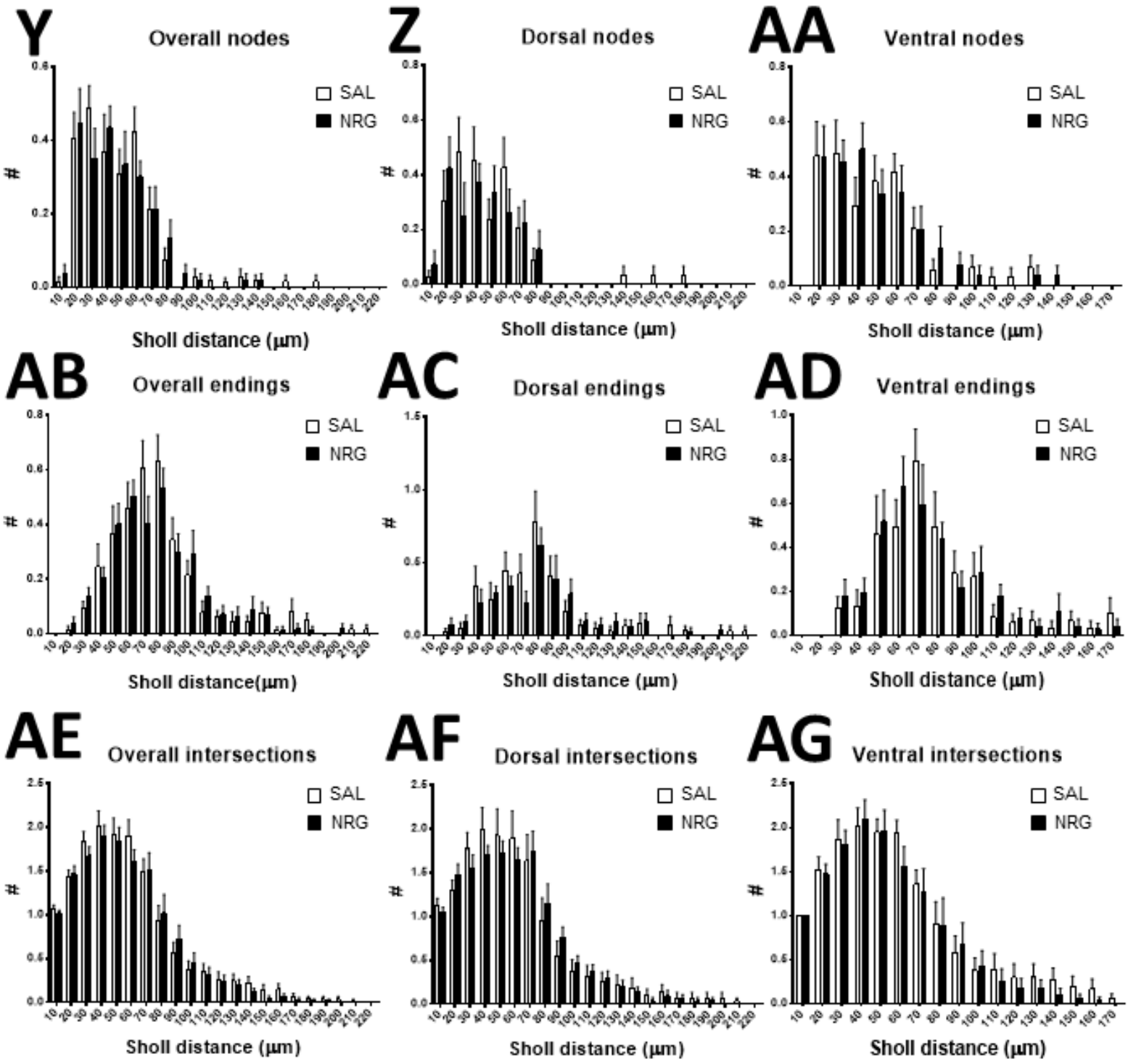
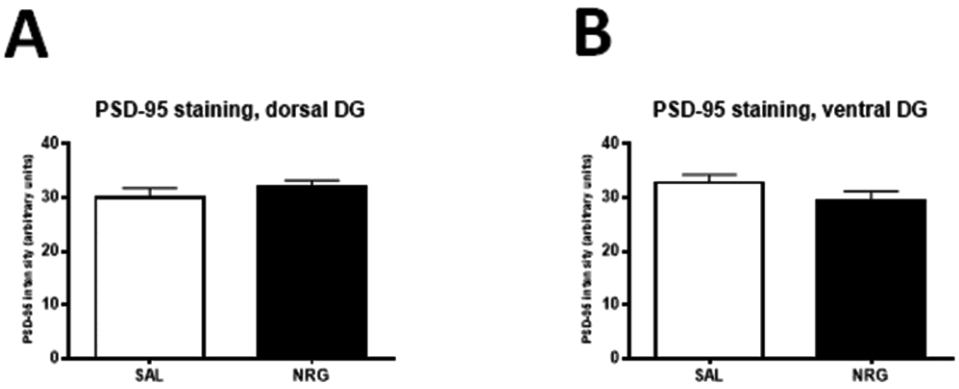
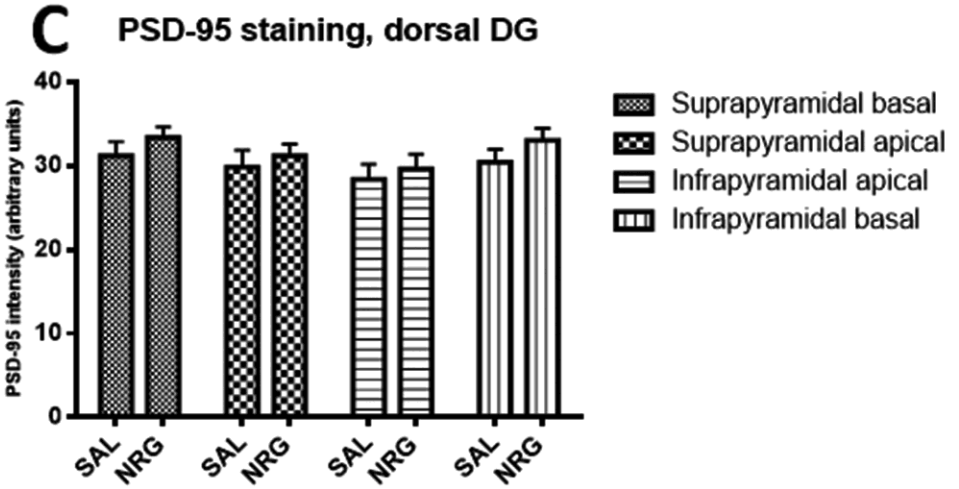
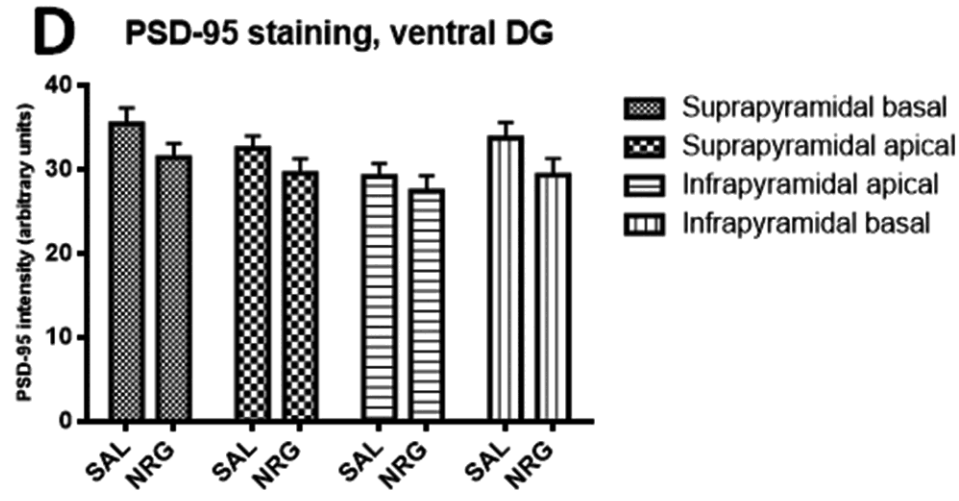
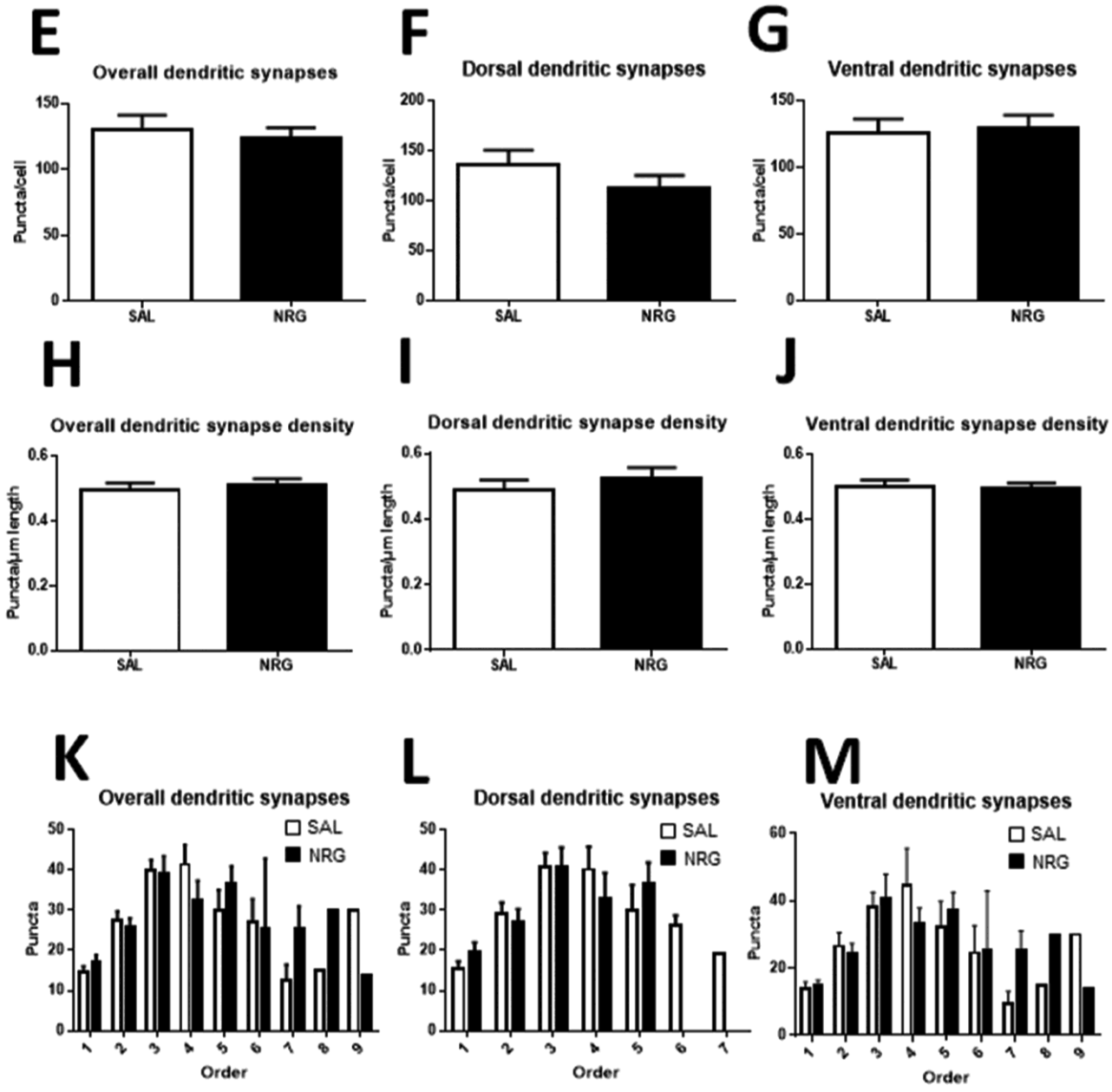

Y
Overall nodes
SAL
NRG
#
Sholl distance (μm)
Z
Dorsal nodes
SAL
NRG
#
Sholl distance (μm)
AA
Ventral nodes
SAL
NRG
#
Sholl distance (μm)
AB
Overall endings
SAL
NRG
#
Sholl distance(μm)
AC
Dorsal endings
SAL
NRG
#
Sholl distance (μm)
AD
Ventral endings
SAL
NRG
#
Sholl distance (μm)
AE
Overall intersections
SAL
NRG
#
Sholl distance (μm)
AF
Dorsal intersections
SAL
NRG
#
Sholl distance (μm)
AG
Ventral intersections
SAL
NRG
#
Sholl distance (μm)

Figure A10. Additional findings on effects of neuregulin-1 administration on dendritic morphological development by branch order and Sholl analyses. By branch order, NRG1 administration did affect overall, dorsal, or ventral immature neuronal dendritic length (A-C), mean length (**D-F**), mean volume (**G-I**), surface area (**J-L**), mean surface area (**M-O**), nodes (**P-R**), ends (**M-O**), volume (**S-T; Fig. 16E**), or quantity (U-W). By Sholl analysis, NRG1 administration did not affect dendritic length (**W-X; Fig. 16D**), nodes (**Y-AA**), endings (**AB-AD**), or intersections (**AE-AG**).


A
PSD-95 staining, dorsal DG
PSD-95 intensity (arbitrary units)
40
30
20
10
0
SAL
NRG
B
PSD-95 staining, ventral DG
PSD-95 intensity (arbitrary units)
40
30
20
10
0
SAL
NRG


C
PSD-95 staining, dorsal DG
PSD-95 intensity (arbitrary units)
40
30
20
10
0
SAL
NRG
SAL
NRG
SAL
NRG
SAL
NRG
Suprapyramidal basal
Suprapyramidal apical
Infrapyramidal apical
Infrapyramidal basal


D
PSD-95 staining, ventral DG
PSD-95 intensity (arbitrary units)
40
30
20
10
0
SAL
NRG
SAL
NRG
SAL
NRG
SAL
NRG
Suprapyramidal basal
Suprapyramidal apical
Infrapyramidal apical
Infrapyramidal basal


E
Overall dendritic synapses
Puncta/cell
150
100
50
0
SAL
NRG
F
Dorsal dendritic synapses
Puncta/cell
200
150
100
50
0
SAL
NRG
G
Ventral dendritic synapses
Puncta/cell
150
100
50
0
SAL
NRG
H
Overall dendritic synapse density
Puncta/µm length
0.6
0.4
0.2
0.0
SAL
NRG
I
Dorsal dendritic synapse density
Puncta/µm length
0.6
0.4
0.2
0.0
SAL
NRG
J
Ventral dendritic synapse density
Puncta/µm length
0.6
0.4
0.2
0.0
SAL
NRG
K
Overall dendritic synapses
SAL
NRG
Puncta
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9
Order
L
Dorsal dendritic synapses
SAL
NRG
Puncta
50
40
30
20
10
0
1 2 3 4 5 6 7
Order
M
Ventral dendritic synapses
SAL
NRG
Puncta
60
40
20
0
1 2 3 4 5 6 7 8 9
Order

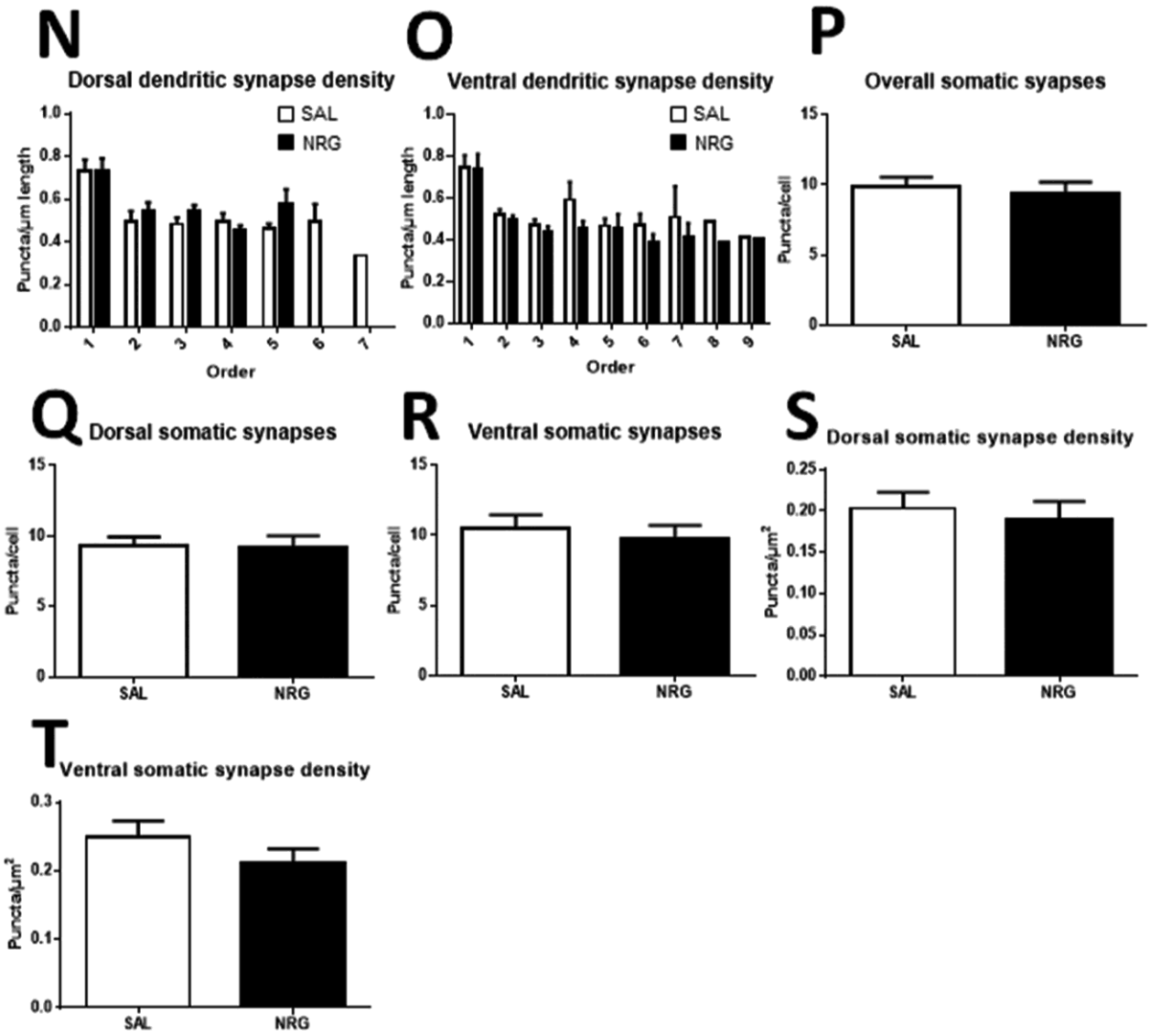
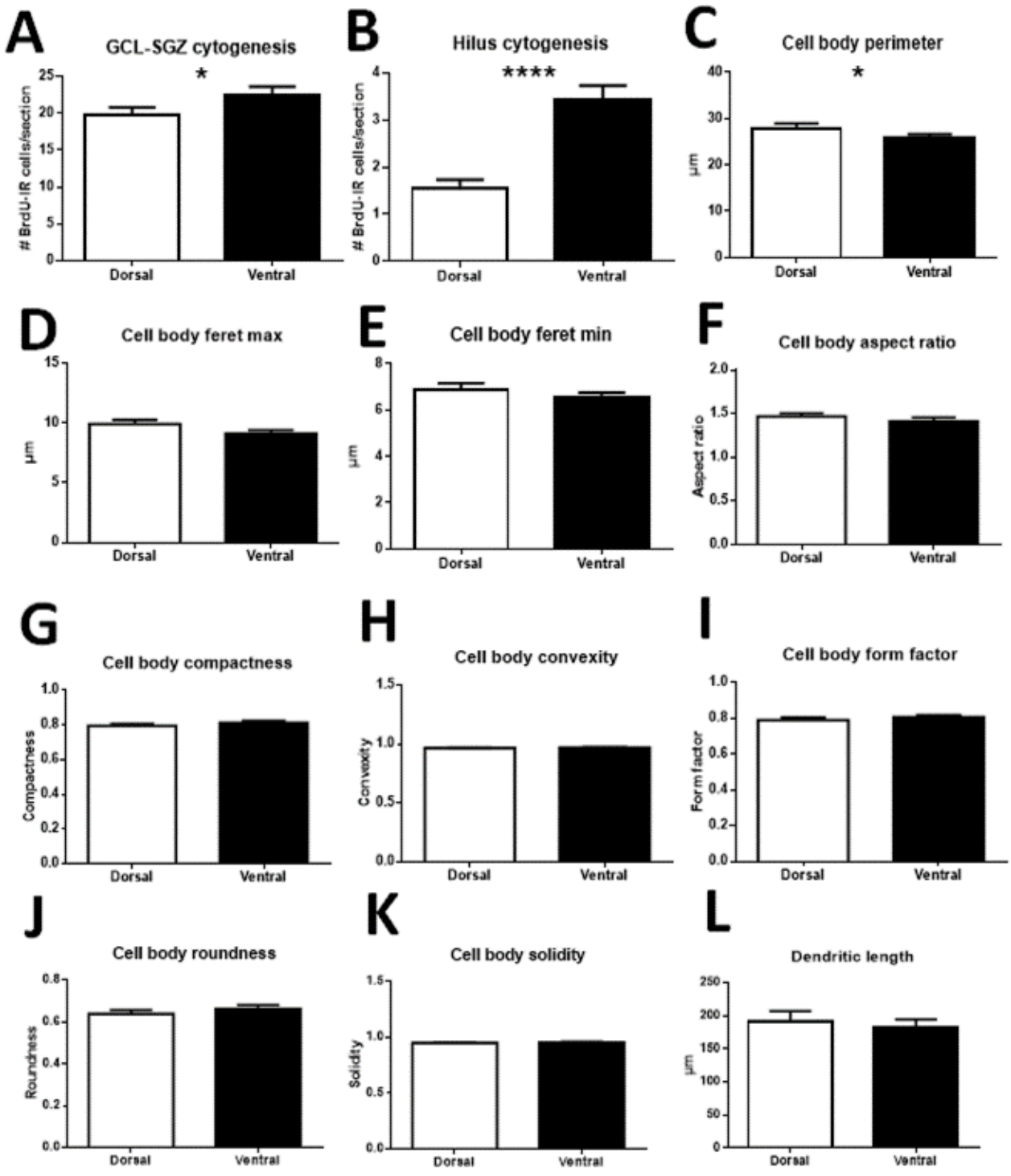


Figure A11. Additional findings on effects of neuregulin-1 administration on synapse development. NRG1 did not affect total molecular layer synaptic density in the dorsal or ventral subregions, overall (**A-B**) or analyzed by molecular layer zone (**C-D**). Examining immature neurons specifically, NRG1 did not affect total dendritic synapse numbers (**E-G**) or density (**H-J**), and this was maintained when examining by dendritic branch order (**K-O; Fig. 17D**). Number (**P-R**) and density (**S-T, Fig. 17F**) of somatic synapses was unaffected by treatment.


A
GCL-SGZ cytogenesis
*
BrdU-IR cells/section
25
20
15
10
5
0
Dorsal
Ventral
B
Hilus cytogenesis
****
BrdU-IR cells/section
4
3
2
1
0
Dorsal
Ventral
C
Cell body perimeter
*
µm
40
30
20
10
0
Dorsal
Ventral
D
Cell body feret max
µm
15
10
5
0
Dorsal
Ventral
E
Cell body feret min
µm
8
6
4
2
0
Dorsal
Ventral
F
Cell body aspect ratio
Aspect ratio
2.0
1.5
1.0
0.5
0.0
Dorsal
Ventral
G
Cell body compactness
Compactness
1.0
0.8
0.6
0.4
0.2
0.0
Dorsal
Ventral
H
Cell body convexity
Convexity
1.5
1.0
0.5
0.0
Dorsal
Ventral
I
Cell body form factor
Form factor
1.0
0.8
0.6
0.4
0.2
0.0
Dorsal
Ventral
J
Cell body roundness
Roundness
0.8
0.6
0.4
0.2
0.0
Dorsal
Ventral
K
Cell body solidity
Solidity
1.5
1.0
0.5
0.0
Dorsal
Ventral
L
Dendritic length
µm
250
200
150
100
50
0
Dorsal
Ventral

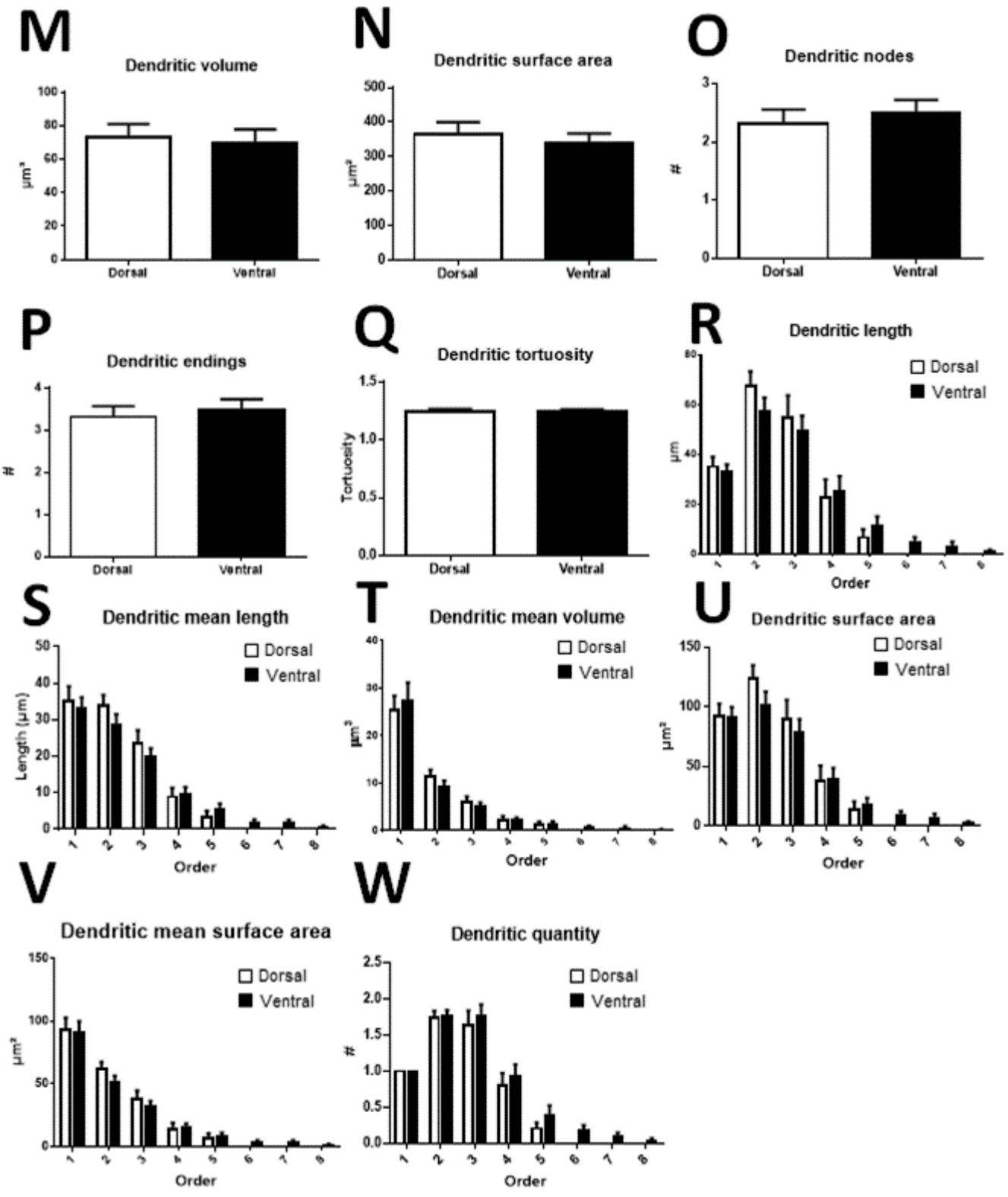

M
Dendritic volume
µm³
Dorsal
Ventral
N
Dendritic surface area
µm²
Dorsal
Ventral
O
Dendritic nodes
#
Dorsal
Ventral
P
Dendritic endings
#
Dorsal
Ventral
Q
Dendritic tortuosity
Tortuosity
Dorsal
Ventral
R
Dendritic length
µm
Dorsal
Ventral
Order
S
Dendritic mean length
Length (µm)
Dorsal
Ventral
Order
T
Dendritic mean volume
µm³
Dorsal
Ventral
Order
U
Dendritic surface area
µm²
Dorsal
Ventral
Order
V
Dendritic mean surface area
µm²
Dorsal
Ventral
Order
W
Dendritic quantity
#
Dorsal
Ventral
Order

Figure A12. Additional findings on septotemporal characterization of cytogenesis and immature neuronal morphology. Cytogenesis was higher in the ventral granule cell layer and subgranular zone (**A**) and hilus (**B**) compared to dorsally. **C**, cell body perimeter was lower in immature neurons in the ventral versus dorsal DG. Dorsal and ventral immature neuronal cell body feret max (**D**), feret min (**E**), aspect ratio (**F**), compactness (**G**), convexity (**H**), form factor (**I**), roundness (**J**), or solidity (**K**) did not differ between groups. Immature neurons did not differ by subregion for total dendritic length (**L**), volume (**M**), surface area (**N**), nodes (**O**), ends (**P**), or tortuosity (**Q**). Analyzing by branch order, there were no septotemporal differences in dendritic length (**R**), mean dendritic length (**S**), mean volume (**T**), dendritic surface area (**U**) or mean surface area (**V**), or dendritic quantity (**W**). *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.

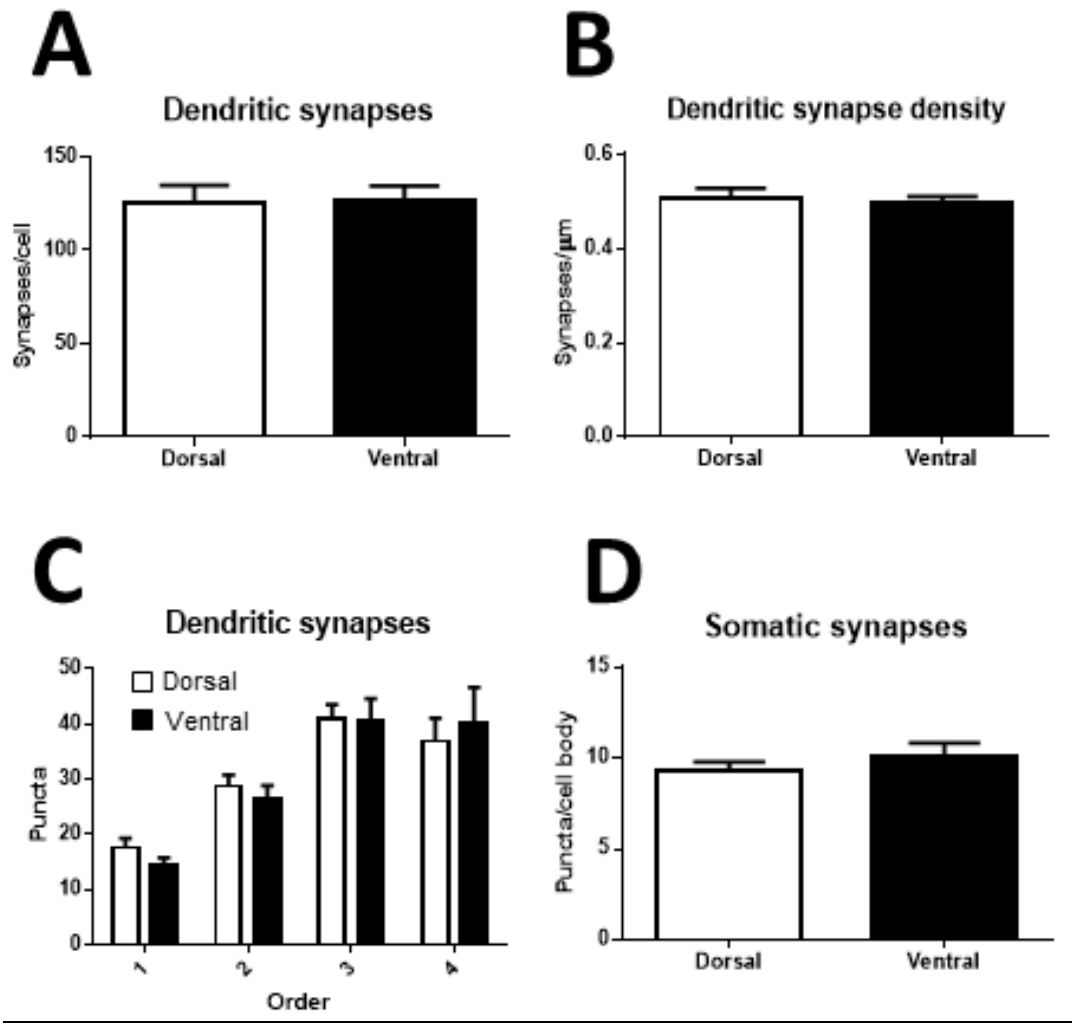


Figure A13. Additional findings on septotemporal characterization of synaptic development. Number (**A**) and density (**B**) of total dendritic synapses did not differ between the dorsal and ventral DG. **C**, Number of synapses did not differ between subregions by branch analysis. **D**, total number of somatic synapses did not differ between subregions.

Supplemental materials from Chapter 4:

Peripheral NRG1 experiment			
	Control	MDD	p value
n	17	28	N/A
Sex (M/F)	9/8	9/19	N/A
Age	38.11 ±2.30	43.68 ±2.33	0.12
Hippocampal mRNA expression experiment			
	Control	Suicide	p value
n	14	49	N/A
Sex (M/F)	14/0	49/0	N/A
Age	38.79 ±3.18	38.80 ±1.61	>0.99
PMI	28.79 ±3.11	31.00 ±1.97	0.73
pH	6.43 ±0.074	6.53± 0.046	0.15
Diagnosis	0	20 MDD, 6 BD, 2 SZ, 6 other	N/A
Medication	1	11	N/A
Cause of death	7 cardiac, 5 MVA, 1 work accident, 1 intoxication	31 hanging, 13 intoxication, 2 jumping, 2 shooting, 1 drowning	N/A
ErbB3 methylation experiment			
	Control	Suicide	p value
n	9	31	N/A
Sex (M/F)	9/0	31/0	N/A
Age	40.22 ±4.65	40.23 ±1.74	>0.99
PMI	23.56 ±2.26	26.86 ±2.00	0.41
pH	6.47 ±0.093	6.57 ±0.053	0.38
Diagnosis	0	10 MDD, 2 BD, 2 SZ, 6 other	N/A
Medication	0	7	N/A
Cause of death	5 cardiac, 3 MVA, 1	19 hanging, 5 intoxication,	N/A

	work accident	2 shooting, 1 jumping, 1 drowning, 1 cutting, 1 asphyxiation, 1 carbon monoxide	
Stereological, volumetric, and morphological experiment			
	Control	Suicide	p value
n	9	14	N/A
Sex (M/F)	7/2	13/1	N/A
Age	44.78 ±6.93	49.43 ±4.98	0.58
PMI	11.39 ±2.00	17.04 ±4.62	0.84
pH	6.52 ±0.088	6.69 ±0.098	0.22
Diagnosis	0	5 MDD, 4 BD, 1 other	N/A
Medication	0	7	N/A
Cause of death	4 cardiac, 3 MVA, 1 pulmonary embolism, 1 unknown	12 hanging, 1 jumping, 1 intoxication	N/A

Table A1. Subject information from human experiments. Diagnosis refers to primary Diagnostic and Statistical Manual of Mental Disorders Axis I diagnosis. Medication refers to antidepressant medication. BD, bipolar disorder; MDD, major depressive disorder; MVA, motor vehicle accident; PMI, post-mortem interval; SZ, schizophrenia.

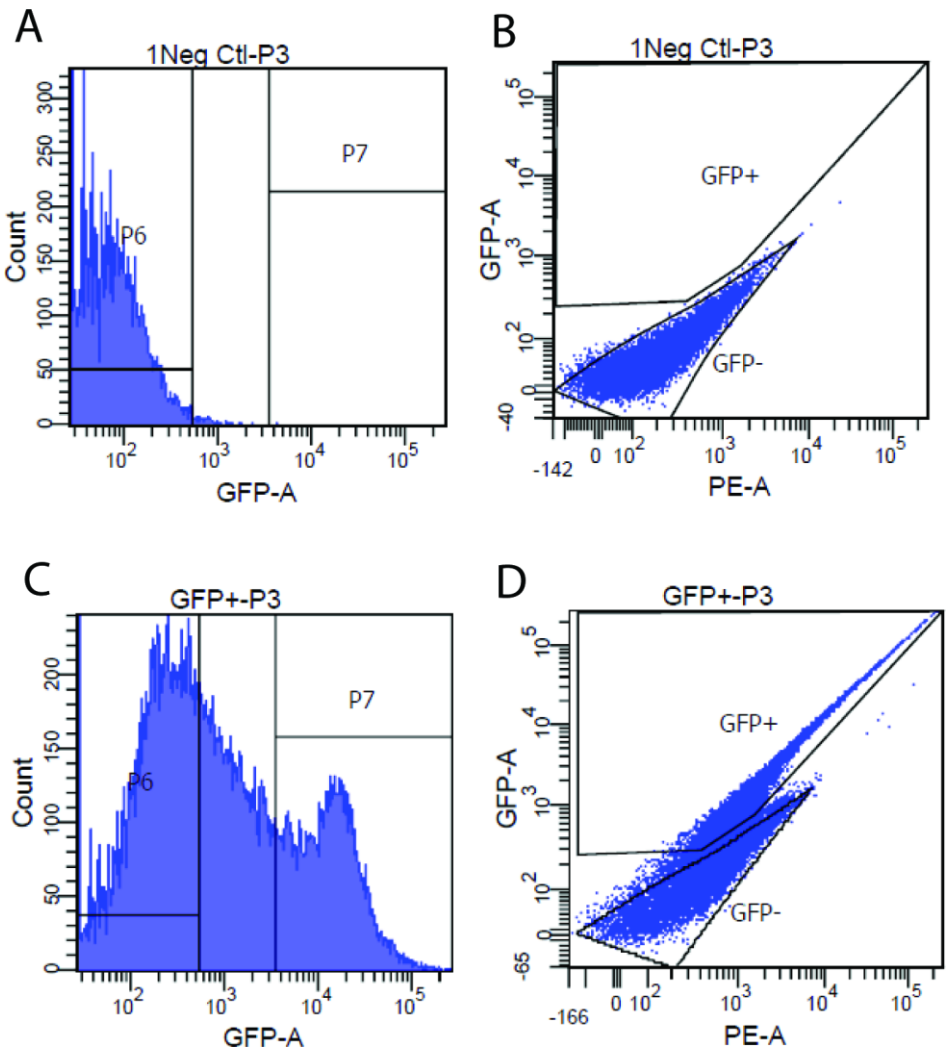


Figure A14. Fluorescence-assisted cell sorting confirmation of nestin-green fluorescent protein (GFP) cells in dissected DG, in order to verify dissection anatomical accuracy. **A**, sample histogram of sorted cell population in a dissection from a nestin-GFP-negative (non-transgenic) mouse, revealing a homogenous low-fluorescence cellular population. **B**, GFP signal intensity dot plot relative to phycoerythrin (PE) autofluorescence from a dissection from a non-transgenic mouse, revealing an absence of GFP+ fluorescence. **C**, sample histogram of a sorted cell population in a dissection from a nestin-GFP+ mouse, revealing a high GFP fluorescence signal and two distinct cellular populations. **D**, GFP signal intensity dot plot of a sorted cell population in a dissection from a nestin-GFP+ mouse, revealing a distinct GFP+ cell population that clusters at higher fluorescent signal intensity.

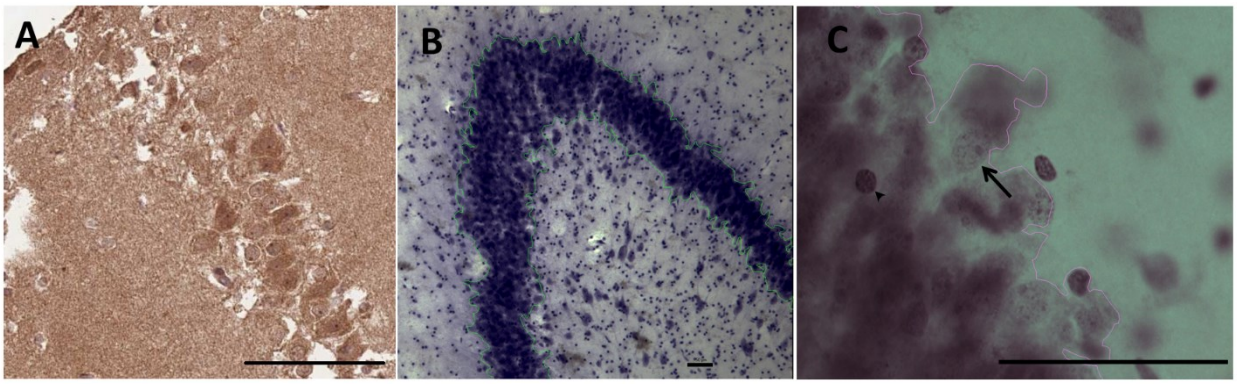


Figure A15. **A**, additional ErbB3 staining in human DG granule cells. **B**, detailed tracing (light green) of human DG for stereological and morphological analyses. **C**, DG at 100X. Arrow: granule cell; arrowhead: glial cell. Scale bar = 100 µm (**A**), 50 µm (**B, C**). Image credit (**A**): Human Protein Atlas (http://www.proteinatlas.org/ ; Uhlen et al., 2015).

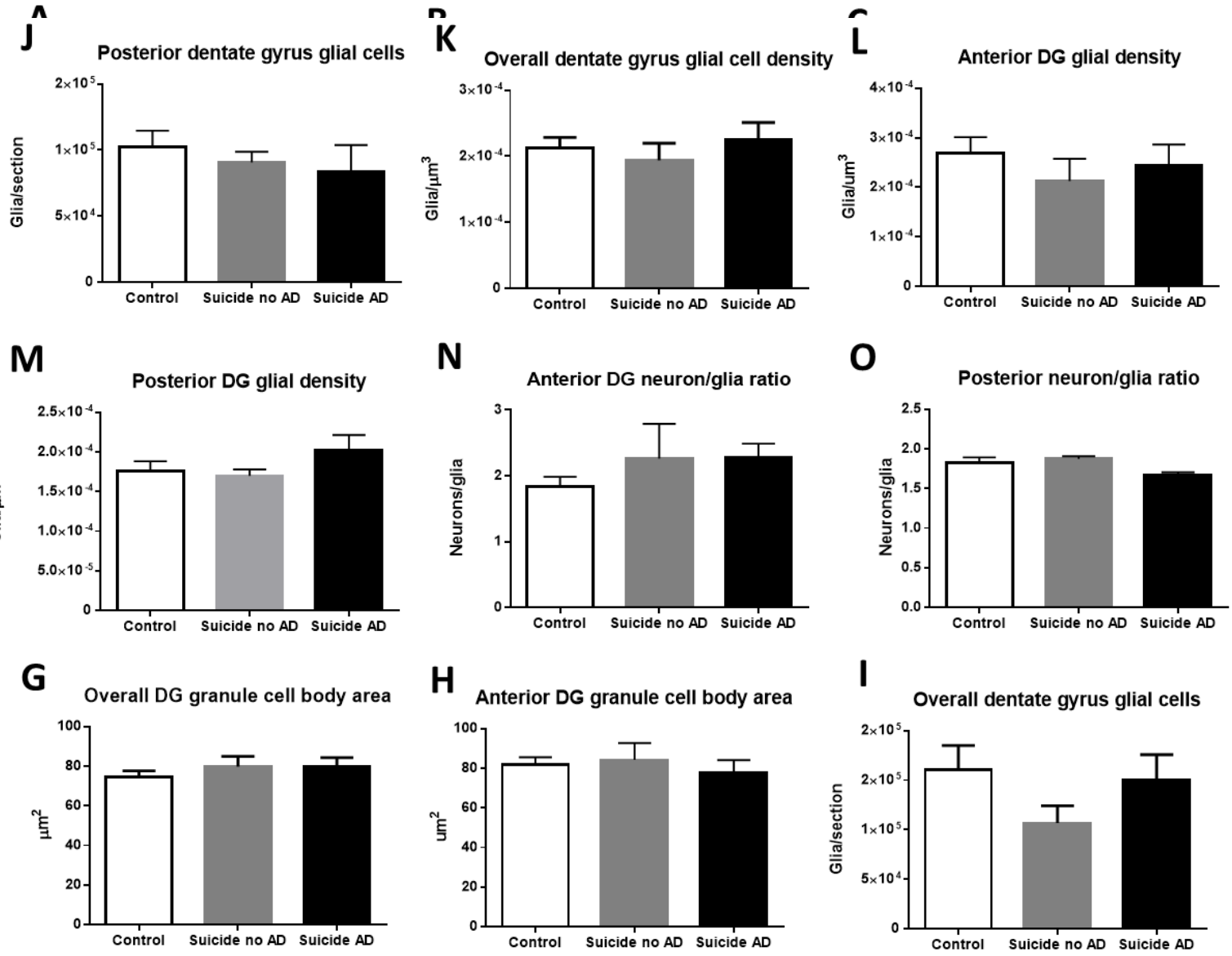

J
Posterior dentate gyrus glial cells
Glia/section
2×10^5
1×10^5
5×10^4
0
Control
Suicide no AD
Suicide AD
K
Overall dentate gyrus glial cell density
Glia/µm^3
3×10^-4
2×10^-4
1×10^-4
0
Control
Suicide no AD
Suicide AD
L
Anterior DG glial density
Glia/um^3
4×10^-4
3×10^-4
2×10^-4
1×10^-4
0
Control
Suicide no AD
Suicide AD
M
Posterior DG glial density
Glia/µm^3
2.5×10^-4
2.0×10^-4
1.5×10^-4
1.0×10^-4
5.0×10^-5
0
Control
Suicide no AD
Suicide AD
N
Anterior DG neuron/glia ratio
Neurons/glia
3
2
1
0
Control
Suicide no AD
Suicide AD
O
Posterior neuron/glia ratio
Neurons/glia
2.5
2.0
1.5
1.0
0.5
0.0
Control
Suicide no AD
Suicide AD
G
Overall DG granule cell body area
µm^2
100
80
60
40
20
0
Control
Suicide no AD
Suicide AD
H
Anterior DG granule cell body area
um^2
100
80
60
40
20
0
Control
Suicide no AD
Suicide AD
I
Overall dentate gyrus glial cells
Glia/section
2×10^5
2×10^5
1×10^5
5×10^4
0
Control
Suicide no AD
Suicide AD

Figure A16. Additional stereological and morphological data from the DGs of controls and suicides with or without antidepressant (AD) treatment. In contrast to the anterior DG, number of granule cells did not differ between groups overall (**A**) or in the posterior DG (**B**), and DG volume did not differ overall (**C**) or in the posterior DG (**D**). Granule cell neuronal density did not differ overall (**E**) or in the posterior DG (**F**). DG granule cell body size did not differ overall (**G**) or in the anterior DG (**H**). Number of glial cells in the DG did not differ between groups overall (**I**) or in the posterior DG (**J**). Glial density did not differ overall (**K**) or in the anterior (**L**) or posterior (**M**) DG. The ratio of neurons to glia in the DG did not differ in the anterior (**N**) or posterior (**O**) DG.