Contribution of NPY system to dysfunctional emotional conditions

Julio César Morales Medina

Department of Neurology and neurosurgery

- McGill University, Montreal

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Abstract

Anxiety and depression are two highly debilitating disorders with high prevalence and co-morbidity with other mental and physical disorders. Current available treatments for these diseases present major caveats, thus the search for novel targets of treatment are the foremost challenges in health research. In this regard, the neuropeptide Y (NPY) system has emerged as a neuromodulator of emotional processing. This peptide mediates its effects by acting on its Y_1, Y_2 and Y_5 receptor subtypes in the brain. However the contribution of each receptor subtype in emotional processes is still not clear, given their differential distribution in the brain as well as in the neuronal synapse. Therefore, the aim of the present study was to dissect the role of each of these receptor subtypes in animal models of emotional dysfunctional conditions. Of particular interest were, the Y_1 agonist, Y_2 antagonist as well as Y_5 agonist as the mediators of emotional dysfunctional conditions. To investigate this, first we fully characterized an animal model of depression and anxiety, the olfactory bulbectomized (OBX) model. Following this characterization we observed that the administration of Y Y_1-agonist reversed hyperlocomotion in open field test (OFT), reduced immobility time in the forced swim test (FST) and increased contacts in the social interaction test (SIT) in OBX rats, however, no effect was observed in corticosterone (CORT)-induced anxiety model. In addition, the Y_2 antagonist decreased immobility in the FST in the OBX rat while increased social contacts in sham animals. Interestingly, this compound also induced an anxiolytic-related effect in CORT-treated rats. Meanwhile, the Y_5 agonist decreased locomotion in OF and increased contacts in the SI test in the OBX rat, induced an anxiolityc-related effect in CORT-treated animals and increased body weight in control animals. Taken together, these

results indicate that the treatment with molecules targeting different NPY receptor subtypes modulate different traits of anxiety and depression. In addition, antagonism of Y_2 receptors elicits a potent effect regardless of the mental state of the animal while the treatment with Y_1 or Y_5 agonists induces differential effects depending on the situation. Thus, targeting these NPY receptors may be of pharmacotherapeutic relevance in the treatment of some forms of anxiety and depression in humans.

Résumé

L'anxiété et la dépression sont deux désordres émotionnels ayant un impact socio-économique majeur. Ils présentent une haute prévalence et co-morbidité avec d'autres désordres mentaux et physiques. Actuellement, les traitements disponibles contre ces maladies possèdent de nombreuses contre-indications. La découverte de nouvelles cibles de traitements est donc un défi crucial dans la recherche en santé mentale. Le neuropeptide Y (NPY) est un neuromodulateur des processus émotionnels. Ce peptide exerce son action dans le cerveau via divers récepteurs incluant les sous-types Y_1, Y_2 et Y_5. La contribution de chacun de ces récepteurs n'est cependant pas claire, et leur distribution varie aussi bien quant à leur localisation pré- ou post-synaptique que régionale. Le but de cette thèse est donc de préciser le rôle de chacun des récepteurs du NPY dans des modèles animaux de dysfonctions émotionnelles. Un intérêt particulier est porté sur les agonistes et antagonistes des récepteurs Y_1, Y_2 et Y_5. Nous avons, dans un premier temps, caractérisé en détail un modèle animal de dépression et d'anxiété: le modèle de la lésion du bulbe olfactif (OBX). Par la suite, nous avons observé que l'administration d'un agoniste Y_1 renverse l'hyperlocomotion lors du test '″open field″ (OF), réduit le temps d'immobilité dans le test de nage forcée (″forced swim test″, FST) et enfin, augmente les interactions entre les animaux lors du test d'interactions sociales (″social interaction″, SI) et ce, spécifiquement chez les rats OBX. Cependant, aucun n'effet n'a été observé dans un autre modèle ou l'anxiété est induite suite à l'injection de la corticostérone. Un antagoniste Y_2, quant à lui, diminue l'immobilité lors du test FST chez les rats OBX alors qu'il augmente les interactions sociales seulement chez les animaux contrôles. Fait intéressant, ce composé induit aussi un effet anxiolytique chez les

rats traités à la corticostérone. Enfin, un antagoniste Y_5 diminue l'activité locomotrice dans l'OF, augmente les interactions lors du test SI chez les rats OBX, induit la sédation chez les animaux traités à la corticostérone et augmente le poids des animaux contrôles. Ces résultats indiquent que le traitement avec des molécules ciblant différentes classes de récepteurs du NPY pourraient circonscrire spécifiquement certains symptômes de l'anxiété et de la dépression. De plus, un antagoniste du récepteur Y_2 semble capable de moduler les comportements anxieux et dépressifs alors que les agonistes Y_1 et Y_5 induisent des effets différentiels dépendamment du contexte. Ainsi, les récepteurs du NPY pourraient s'avérer être des cibles pharmaceutiques pertinentes pour le traitement de certaines formes d'anxiété et de dépression.

Contribution of Authors

Chapter III

Design of the study: Julio Cesar Morales-Medina, Naguib Mechawar, Gonzalo Flores and Remi Quirion[c]

Execution and analysis of the study: Julio César Morales-Medina, Ismael Juarez, Erika Venancio-García, Sandra Nayeli Cabrera, Caroline Menard and Wenfeng Yu

Writing of the manuscript: Julio Cesar Morales-Medina

Supervision: Remi Quirion

Chapter IV

Design of the study: Julio César Morales-Medina

Execution and analysis of the study: Julio César Morales-Medina, Yvan Dumont Charles-Etienne Benoit, Stéphane Bastianetto and Gonzalo Flores

Unique agents providing: Alain Fournier

Writing of the manuscript: Julio César Morales-Medina

Supervision: Remi Quirion

Chapter V

Design of the study: Julio César Morales-Medina Gabriella Gobbi and Remi Quirion

Execution and analysis of the study: Julio César Morales-Medina and Sergio Dominguez-Lopez,

Unique agents providing: Annette Beck-Sickinger[d]

Writing of the manuscript; Julio César Morales-Medina

Supervision: Remi Quirion

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5-HT	Serotonin
ACTH	Adrenocorticotropic hormone
BLA	Basolateral amygdala
BDNF	Brain derived nerve growth factor
BrdU	Bromodeoxyuridine
CeA	Central amygdala
CNS	Central nervous system
CSF	Cerebrospinal fluid
CREB	cAMP response element binding protein
CNVs	Copy number of variations
CORT	Corticosterone
CRF	Corticotrophin releasing factor
DG	Dentate gyrus
ECT	Electroconvulsive therapy
EPM	Elevated plus maze
FRL	Flinders resistant line
FSL	Flinders sensitive line
FST	Forced swim test
KO	Knockout
KRP	Krebs Ringer Phosphate
INA	Information not available
ILC	Infralimbic cortex
ICV	Intracerebroventricular
NPY	Neuropeptide Y

NC	No change
PP	Pancreatic polypeptides
PYY	Peptide YY
mPFC	medial prefrontal cortex
MWM	Morris Water Maze
nmol	Nanomolar
NHS	Normal horse serum
OLC	Orbito-lateral cortex
OBX	Olfactory bulbectomized rat
OFT	Open field test
PirC	Piriform cortex
PFC	Prefrontal cortex
SSRI	Selective serotonin reuptake inhibitor
SNPs	Single nucleotide polymorphisms
SIT	Social interaction test
SGZ	Subgranular zone
SVZ	Subventricular zone
TST	Tail suspension test
TDL	Total dendritic length

Chapter I.

Introduction

A converging body of evidence suggests that NPY and its receptors possess antidepressant- and anxiolytic- like effects after acute administration in naïve animals and knockout rodents. However, the dissociation between both affective disorders remains to be dissected. Moreover, the role of NPY and its various receptor subtypes under challenged or stress conditions remains to be established. In order to investigate the potential role of various NPY receptor subtypes in anxiety- and depression-like behaviors, we propose to use two well-known animal models of depression- and anxiety-like behaviors. The olfactory bulbectomized (OBX) rat model of depression-like behavior induces a wide array of behavioral and neurochemical disturbances which resemble several characteristics of depression and anxiety as observed in humans. In addition, stress or disturbances in Corticosterone (CORT) levels have been associated as a risk factor of emotional disorders. Therefore, we used a CORT regimen that elicits anxiety-like behaviors in the rat.

With this in mind, in Chapter III aimed to characterize the main animal model (OBX rat) to be used in the present thesis. The behavioral task used in this study aimed to dissect the depression- and anxiety- related effect of novel compound of action as well as to suggest potential mechanisms of action how the ablation of olfactory bulbs produce various emotional disturbances.

Having determined, the behavioral paradigms that measure anxiety- or depression-related behavior as well as disturbances in adult hippocampal neurogenesis in the OBX rat (Chapter III), the studies in the following chapters were designed to investigate the effect of exogenous administration of agonists and antagonist of the Y_1 and Y_2 receptors (Chapter IV) and Y_5 agonist (Chapter V) in the OBX rat as well as CORT-treated

animals, two well documented animal models of emotional dysfunctional conditions. Most interestingly, in the supplemental data of Chapter IV, we observed that the novel brain penetrant Y_2 antagonist mimicked the effects observed with the Y_2 antagonist BIIE0246 in the OBX rat. At the same time, we quantified changes in adult hippocampal neurogenesis as a possible mechanism of action of the NPY system.

Throughout all these studies, we sought to validate a well-known animal model of depression- and anxiety- related behavior and to establish the contribution of three NPY receptors in emotional processing

Chapter II: Concise review of the current literature

Major depression is a severe and debilitating emotional disorder that is predicted to become the second leading cause of disability worldwide (Murray and Lopez, 1996). Anxiety comprises multiple disorders with an excessive social burden and shows a very high prevalence (Kertz and Woodruff-Borden, 2011).In addition, Kessler et al. (2003) observed up to 60% co-occurrence of major depression with anxiety in the US National Co-morbidity Survey. These complex emotional dysfunctions are often characterized by anhedonia, hopelessness, exacerbated guiltiness and memory deficits (Castaneda et al., 2008). Commonly prescribed antidepressants are not curative and a considerable subpopulation of depressed subjects is resistant to those treatments (Berlim et al., 2008) while current treatments for anxiety-related disorders include various benzodiazepines but their long term administration induces several side effects including cognitive deficits as well as the development of tolerance (Rudolph and Knoflach, 2011). Thus, the search for novel approaches for the treatment of anxiety-related disorders is one of the foremost challenges in mental health research.

NPY is a 36 amino acid polypeptide first isolated from porcine brain by Tatemoto et al. (1982). This peptide, along with pancreatic polypeptides (PP) and peptide YY (PYY), shares differential affinities for at least four G protein-coupled receptors known as the Y_1, Y_2, Y_4 and Y_5 receptors (Michel et al., 1998). The possible relevance of this peptide in various physiological processes is suggested by the finding that NPY is conserved in evolution and across different species (Larhammar and Salaneck, 2004). In addition, NPY is widely distributed in the central nervous system, particularly in stress and emotional processing regions (Dumont et al., 2000, Dumont and Quirion, 2006b).

In this section, we only briefly summarize the current literature on the role of NPY in depression and anxiety on the basis of results obtained in animal as well as human studies. For more exhaustive reviews, we invite the reader to consult two of our recent reviews (Morales-Medina et al., 2010b, Morales-Medina and Quirion, 2011).

2.0 Animal studies

Multiple approaches have evaluated the role of NPY and its receptors in depression and other emotional processes. Direct and indirect evidence on the role of this peptide have been obtained using genetic manipulations and pharmacological treatments in control animals as well as in few models of depression-related behaviours.

2.1 Genetic manipulations

A summary of data on the role of NPY and its receptors in genetically modified animals is presented in table 1 with details discussed below.

2.1.1 Loss of function studies

Numerous studies have investigated the role of NPY and Y_1, Y_2 and Y_4 receptor subtypes using germinal knockout (KO) animals. Bannon et al. (2000) observed a decrease in center distance in the open field, suggestive of an anxiogenic-like phenotype in the NPY KO mice. Data on Y_1 KO mice generated behavioral results which are rather controversial. Painsipp et al. (2010) observed that female Y_1 KO mice show reduced immobility in the tail suspension test (TST) but not in the forced swim test (FST). Reduced immobility is interpreted as an antidepressant phenotype in both tests (Cryan et al., 2005a, 2005b). In contrast, Karlsson et al. (2008) found increased immobility time in the FST in Y_1 KO mice. However, both male and female mice were used in the latter

study. Interestingly, Karl et al. (2006) found that Y_1 KO mice displayed normal behaviour in the elevated plus maze (EPM), whereas stress produced an anxiolytic-like effect in these animals. Additionally, Painsipp et al. (2010) observed an antidepressant effect after stress in Y_1 KO mice in various depression-related paradigms. Early on, Thorsell et al. (2000) had suggested that the NPYergic system, especially the Y_1 receptor subtype, is activated to counterbalance the effects of stress.

In contrast to the mild antidepressant effects observed in Y_1 KO mice, accumulated evidence has revealed the strong antidepressant- and anxiolytic-related phenotype of germinal Y_2 KO mice independent of sex, age or behavioural paradigm (Carvajal et al., 2006b, Painsipp et al., 2008a, Redrobe et al., 2003b, Tschenett et al., 2003). Most interestingly, Tasan et al. (2010) just reported increases in time spent in open arms in the EPM in animals having a targeted deletion of Y_2 receptors in the basolateral (BLA) and central (CeA) amygdala. Additionally, a decrease in immobility time in the TST was specifically observed after the deletion of the Y_2 receptor subtype in the CeA. Taken together, these series of studies strongly support the role of the Y_2 receptor subtype in depression-related behaviours.

Genetically modified animal	Type of deletion	Species	Sex	Strain	Paradigm	Effect	Time of start of the test	Reference
NPY KO	germinal	mice	male	129/SvJ-c57BL/6	OF	↓distance center of arena	INA	Bannon et al., 2000
NPY transgenic	germinal	rat	male	Sprague–Dawley	EPM	No change	INA	Carvajal et al., 2004
					EPM/stress	No change	INA	
					OF	↑entries center of arena/↑center time	INA	
					EPM	NC	INA	Thorsell et al., 2000
					EPM/stress	NC	INA	
Y1 KO	germinal	mice	female	129/SvJ-c57BL/6	TST	↓ immobility	INA	Painsipp et al. 2010
					FST	NC	INA	
			male and female		OF	NC	INA	Karlsson et al. 2008
					EPM	NC	INA	
			INA		OF	↑distance center of arena	14:00	Karl et al. 2006
Y2 KO	germinal	mice	female	129/SvJ-c57BL/6	TST	↓ immobility	10:00	Painsipp et al., 2008b
					EPM	↑ open arm entries/↑ open arm time	10:00	
					OF	↑center time	10:00	
			INA		FST	↓ immobility	INA	Carvajal et al., 2006
					EPM	↑ open arm entries	INA	
					OF	↑distance center of arena	INA	
			female		TST	↓ immobility	10:30	Painsipp et al., 2008a
					OF	↑entries center of arena	10:30	
					EPM	↑ open arm entries	10:30	
			male		EPM	↑ open arm entries/↑ open arm time	INA	Redrobe et al., 2003b
					OF	↑entries center of arena/↑time center of arena	INA	
			male		FST	↓ immobility	9:00	Tschenett et al., 2003
					OF	↑entries center of arena	9:00	
					EPM	↑ open arm entries	9:00	
			male and female	129/SvJ-c57BL/6 backcrossed 8 generations onto a c57BL/6	FST	NC	9:00	Zambello et al. 2010b
					EPM	NC	9:00	
	conditional-CeA		male	129/SvJ-c57BL/6	TST	↓ immobility	8:00	Tasan et al. 2010
					EPM	↑ open arm entries/↑ open arm time	8:00	
	conditional-BLA				TST	NC	8:00	
					EPM	↑ open arm time	8:00	
Y4 KO	germinal	mice	female	129/SvJ-c57BL/6	TST	↓ immobility	10:00	Painsipp et al., 2008b
					EPM	↑ open arm entries/↑ open arm time	10:00	
					OF	↑entries center of arena/↑center time	10:00	
			male		FST	↓ immobility	8:00	Tasan et al. 2009
					TST	↓ immobility	8:00	
					EPM	NC	8:00	
					OF	↑entries center of arena/↑center time	8:00	
PP transgenic	germinal	mice	INA	C57BL/6J	EPM	↓↓ open arm entries/↓ open arm time	INA	Ueno et al., 2007
Y2/Y4 KO	germinal	mice	male	129/SvJ-c57BL/6	FST	↓ immobility	8:00	Tasan et al. 2009
					TST	↓ immobility	8:00	
					EPM	↑ open arm entries/↑ open arm time	8:00	
					OF	↑entries center of arena/↑center time	8:00	

Table II. 1. Summary of data on the role of NPY and its receptors in genetically modified animals. FST: forced swim test; OF: open field; EPM: elevated plus maze; TST: tail suspension test; INA: Information not available; NC: No change

Recently, it has been shown that the Y_4 KO mice display reduced immobility in the FST and TST in male (Tasan et al., 2009) and female (Painsipp et al., 2008a, Painsipp et al., 2008b) mice. Rather interestingly, Tasan et al., (2009) observed that a double deletion of the Y_2 and Y_4 receptors further increased the movement component of the FST in these animals compared to Y_2 KO mice. This group also suggested that the antidepressant effect of the deletion of the Y_4 receptor was induced peripherally rather than centrally.

Finally, although the role of germinal Y_5 KO mice has been exhaustively evaluated in feeding behaviour (Higuchi et al., 2008, Marsh et al., 1998, Raposinho et al., 2004) and seizures (Marsh et al., 1999), data on its role in emotion-related processes are still awaited.

2.1.2 Gain of function studies

The technique of overexpression of certain genes for the NPY peptide family and its receptors has not been widely used so far. Transgenic rats that overexpress NPY present normal behaviour in the EPM, when tested for the first time. However, after repeated stress, the time spent in the open arm of the EPM was increased independently of the age of the animals (Carvajal et al., 2004, Thorsell et al., 2000). Additionally, PP overexpressing transgenic mice, showed decreased number of entries in the open arm of the EPM, a behaviour associated with increased anxiety (Ueno et al., 2007). As PP-

related peptides have particularly high affinity for the Y4 receptor, it may suggest a role for this subtype in the noted behaviour.

In summary, the germinal genetic manipulation of NPY, PP and receptors suggest that the Y_1 receptor subtype induces an antidepressant-like effect following stressful stimuli while Y_2 KO mice display marked antidepressant and anxiolytic phenotypes. Complementary studies of the Y_4 KO and PP transgenic mice also revealed antidepressant-like phenotypes. Since it is now possible to produce inducible knockout or transgenic animals, further studies using such approaches to investigate further the role of NPY and related peptides in emotional processes are certainly warranted.

2.2 Pharmacological treatment in normal naive animals

Different groups have administered various agonists and antagonists of Y_1, Y_2 and Y_5 receptor subtypes to investigate their potential antidepressant related effects in control animals. A summary of these data is shown in table 2. Acute intracerebroventricular (ICV) administration of NPY produced an antidepressant-like effect in the FST in naive rats (Redrobe et al., 2005, Stogner and Holmes, 2000). Similarly, the Y_1-like agonist, [$Leu^{31}Pro^{34}$]PYY, when administered acutely reduced the immobility time in this paradigm in mice (Redrobe et al., 2002). Additionally, Redrobe et al. (2002) showed that the co-administration of the Y_1 antagonist, BIBO3304, abolished the behavioural effects induced by [$Leu^{31}Pro^{34}$]PYY. Acute ICV administration of the Y_2 antagonist, BIIE0246, also decreased the immobility time in the FST in mice (Redrobe et al., 2002).

While Y_4 KO mice induced an antidepressant-like phenotype, the overexpression of Y_4 receptors, PPs, induce behavioral despairs . The intraperitonial administration of PP increased anxiety-related behaviour in mice (Asakawa et al., 2003) whereas the

intracerebroventricular (ICV) administration of this peptide had limited effect on emotion-related behaviours (Asakawa et al., 1999). In this regard, Kasting and Pan (2010) recently highlighted the relevance of the role of peripheral peptides on the brain.

The Y_5 receptor subtype was initially regarded as the "feeding" receptor (Marsh et al., 1998), but has gone through dramatic changes over the years. More recently, various groups have suggested an important role for this receptor in emotional processes, with particular emphasis on anxiety-related behaviours. Acute administration of the Y_5 agonist, [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP, either in the lateral ventricle (Sorensen et al., 2004) or BLA (Sajdyk et al., 2002a) produced an anxiolytic-like effect in control animals.

Additionally, Sajdyk et al. (2002) observed that Novartis 1, a Y_5 antagonist, blocked the anxiolytic-related effects of NPY3-36 in the basolateral amygdala (BLA). However, the administration of this Y_5 antagonist by itself had no effect on anxiety-related behaviours (Sajdyk et al., 2002a). Interestingly, an acute administration of another Y_5 antagonist, CGP71683A, induced an anxiolytic-related effect which was task specific (Kask et al., 2001). Recently, Walker et al. (2009) observed that the novel Y_5 antagonist, Lu AA33810, reduced [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP-induced increase in plasma levels of corticosterone (CORT) and adrenocorticotropic hormone (ACTH) in normal, naive rats. In addition, either acute or repeated administration of Lu AA33810 resulted in an anxiolytic-like effect in the social interaction test (SIT) in control rats.

Ligand	Target	Specie	Strain	Douse	Route	Duration	Test	Effect	Reference
NPY	NPY system	rat	Sprague–Dawley	0.5-10 ng	ICV	twice/24 hours	FST	↓ immobility	Stogner and Holmes, 2000
NPY	NPY system	mice	CD-one	3.0 nmol	ICV	acute	FST	↓ immobility	Redrobe et al., 2005
NPY	NPY system	mice	CD-one	0.1-3.0 nmol	ICV	acute	FST	↓ immobility	Redrobe et al., 2002
[D-His26]NPY	Y1 agonist	rat	Wistar	0.8-3.0 nmol	ICV	acute	EPM	↑ open arm entries	Sorensen et al., 2004
[Leu^{31}Pro^{34}]PYY	Y1/Y5 agonist	mice	CD-one	3.0 nmol	ICV	acute	FST	↓ immobility	Redrobe et al., 2002
BIBP3226	Y1 antagonist	mice	CD-one	3.0 nmol	ICV	acute	FST	NC	Redrobe et al., 2002
BIBO3304	Y1 antagonist	mice	CD-one	0.3 nmol	ICV	acute	FST	NC	Redrobe et al., 2002
C2-NPY	Y2 agonist	rat	Wistar	0.2-3.0 nmol	ICV	acute	EPM	NC	Sorensen et al., 2004
NPY13-36	Y2 agonist	mice	CD-one	0.3-3.0 nmol	ICV	acute	FST	NC	Redrobe et al., 2002
BIIE0246	Y2 antagonist	mice	CD-one	3.0 nmol	ICV	acute	FST	↓ immobility	Redrobe et al., 2002
PP	Y4 agonist	mice	ddy	3.0 nmol	IP	twice a day/14 days	EPM	open arm time	Asakawa et al., 2003
PP	Y4 agonist	mice	ddy	0.003-3.0 nmol	ICV	acute	EPM	NC	Asakawa et al., 1999
[cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP	Y5 agonist	rat	Wistar	0.8-3.0 nmol	ICV	acute	EPM	↑ open arm entries	Sorensen et al., 2004
CGP71683A	Y5 antagonist	rat	Wistar	10 mg/Kg	IP	acute	OF	↓horizontal activity-↓vertical activity	Kask et al., 2001
CGP71683A	Y5 antagonist	rat	Wistar	10 mg/Kg	IP	acute	EPM	NC	Kask et al., 2001
CGP71683A	Y5 antagonist	rat	Wistar	10 mg/Kg	IP	acute	SI	NC	Kask et al., 2001
Lu AA33810	Y5 antagonist	rat	Sprague-Dawley	3-10 mg/Kg	Oral	acute	SI	↑ active social interaction	Walker et al. 2009
Lu AA33810	Y5 antagonist	rat	Sprague-Dawley	10 mg/Kg	Oral	14 days	SI	↑ active social interaction	Walker et al. 2009

Table II. 2. Effects of NPY related ligands in control naive animals. FST: forced swim test; OF: open field; EPM: elevated plus maze; SI: social interaction; INA: Information not available; NC: No change; IP: intraperitonial; ICV: intracerebroventricular

This first generation of pharmacological approaches in normal animals added further evidence on the role of the NPYergic system in emotional processes to those obtained using germline KO and transgenic animals. In contrast to Y_1 KO mice, these studies showed that the acute activation of the Y_1 receptor subtype can induce an antidepressant effect. These pharmacological approaches also suggested that the antidepressant effects of NPY are mostly mediated through the Y_1 receptor subtype. In agreement with data obtained in Y_2 KO mice, the pharmacological blockade of Y_2 receptors induced an antidepressant phenotype. In addition, these pharmacological data suggested that the action of the Y_4 receptor subtype could be peripherally-mediated. As to the Y_5 receptor, this subtype is apparently mostly associated with anxiety but not depression-like behaviors.

2.3 Animal model studies

Animal models are powerful tools to help understand different pathologies including emotional conditions as well as to test potential therapeutic agents (Cryan and Mombereau, 2004). In that regard, data from various animal models have suggested both direct and indirect roles for NPY in depression and anxiety as observed on table 3. For example, the NPYergic system is disturbed in various animal models of depression and the administration of different NPYergic compounds improve behavioral despairs in these models.

Ligand	Target	Model	Specie	Strain	Douse	Route	Duration	Test	Effect	Reference
NPY	NPY system	LH	rat	Sprague-Dawley	5 ng	CA3	acute	LH	↓ scape failure	Ishida et al., 2007
					500 ng	ICV	acute	LH	↓ scape failure	Ishida et al., 2007
					0.5-500 ng	DG	acute	LH	NC	Ishida et al., 2007
					5 ng	CA3	acute	OF	NC	Ishida et al., 2007
					5 ng	DG	acute	OF	NC	Ishida et al., 2007
		OBX			5.0-10.0 nmol	ICV	14 days	OF	↓ hyperlocomotion	Goyal et al., 2009
[Leu^{31}Pro^{34}]PYY	Y1/Y5 agonist	LH			1 ng	CA3	acute	LH	↓ scape failure	Ishida et al., 2007
		OBX			0.3-1.0 nmol	ICV	14 days	FST	↓ immobility	Morales-Medina et al., 2009
					0.3-1.0 nmol	ICV	14 days	OF	↓ hyperlocomotion	Morales-Medina et al., 2009
					0.1-1.0 nmol	ICV	14 days	SI	↑ active social interaction	Morales-Medina et al., 2009
[Leu^{31}Pro^{34}]NPY	Y1/Y5 agonist	OBX			0.5-1.0 nmol	ICV	14 days	OF	↓ hyperlocomotion	Goyal et al., 2009
NPY13-36	Y2 agonist	LH			5-50 ng	CA3	acute	LH	NC	Ishida et al., 2007
PYY3-36		OBX			1.0 nmol	ICV	14 days	FST	↑immobility	Morales-Medina et al., 2009
BIIE0246	Y2 antagonist	LH			10 ng	CA3	acute	LH	↓ scape failure	Ishida et al., 2007
		LH			10 ng	DG	acute	LH	NC	Ishida et al., 2007
		OBX			1.0-10.0 nmol	ICV	14 days	FST	↓ immobility	Morales-Medina et al., 2009
		OBX			1.0-10.0 nmol	ICV	14 days	OF	NC	Morales-Medina et al., 2009
[cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP	Y5 agonist	OBX			1.0-3.o nmol	ICV	14 days	OF	↓ hyperlocomotion	Morales-Medina et al., 2009
		OBX			3.o nmol	ICV	14 days	SI	↑ active social interaction	Morales-Medina et al., 2009
Lu AA33810	Y5 antagonist	FSL		FSL/FRL	10 mg/Kg	IP	14 days	FST	↓ immobility	Walker et al. 2009
		FSL			10 mg/Kg	IP	14 days	SI	↑ active social interaction	Walker et al. 2009
		CMS		Wistar	3-10 mg/Kg	IP	35 days/twice a day	SPT	↑in sucrose consumption	Walker et al. 2009

Table II.3 Effects of NPY related ligands in various animal models of depression related behavior.

2.3.1 Antidepressant treatments

Several studies have investigated the effects of various antidepressants in animal models of depression-like behavior as well as changes in NPY and its receptors following such treatment. Repeated administration of the selective serotonin reuptake inhibitor (SSRI), fluoxetine, increased NPY-like immunoreactivity in Flinders sensitive line (FSL**)** rats, a genetic model of depression related behavior, while NPY levels were decreased in the hippocampus in the Flinders resistant line (FRL) rats (Caberlotto et al., 1998). A repeated treatment with fluoxetine also increased NPY mRNA levels in the dentate gyrus following psychosocial stress in the rat (Zambello *et al.*, 2010). Additionally, repeated electroconvulsive therapy (ECT) increased NPY levels in the hippocampus (Stenfors et al., 1989). Recently, running as well as the combined treatment of running and the SSRI, escitalopram, increased both NPY mRNA and Y_1 receptor mRNA in hippocampus, particularly in the dentate gyrus in the FSL rat (Bjornebekk et al., 2010). Interestingly, running did not increase the hippocampal levels of NPY in the FRL rats (Bjornebekk et al., 2006). Furthermore, these treatments induced antidepressant-like effects in the FST, and those actions were associated with increased levels of NPY and Y_1 receptors in these animals. Recently, Christiansen et al. (2011) observed that repeated fluoxetine treatment decreased immobility time in the FST in a model of stress-induced depression. This treatment increased the mRNA expression of NPY in dentate gyrus and amygdala. However, NPY mRNA levels in these regions were not altered in control animals, and in animals exposed to repeated stress (Christiansen et al., 2011).

2.3.2 Altered levels of NPY and NPY receptors in animal models of depression-related behaviors

Studies in animal models have suggested that the synthesis of NPY as well as the expression of various subclasses of NPY receptors are altered in key brain regions involved in emotional processing including the hippocampus, amygdala and cortex. For example, low levels of NPY have been observed in the dorsal hippocampus of maternally separated adult rat, a model of depression-related behavior (Jimenez-Vasquez et al., 2001). Similarly, NPY-like immunoreactivity is decreased in the dorsal hippocampus of FSL rats (Caberlotto et al., 1999). Likely as a compensatory mechanism, the level of the Y_1 receptor subtype was increased in the hippocampal region in this genetic model of depression (Caberlotto et al., 1999). Another study by Caberlotto et al. (1998) proposed that Y_1-mRNA expression was downregulated in various cortical regions as well as in the hippocampus of FSL rats. This group also suggested that the expression of the Y_2 receptor subtype was not affected in the hippocampus in this model (Caberlotto et al., 1998). Additionally, repeated stress induced increases in NPY levels (protein and mRNA) in the amygdala (de Lange et al., 2008, 1999, Thorsell et al., 1998). Recently, Christiansen et al., (2011) replicated these findings in a model of stress-induced depression. This group also observed that the mRNA levels of NPY in the hippocampus was unchanged in their model. Interestingly, following psychosocial stress, rats displayed decreased levels of NPY mRNA in various hippocampal subfields (Zambello *et al.*, 2010). In theOBX rat, accumulated evidence suggested disturbances in the NPYergic system. The prepro-NPY mRNA levels were found to be increased in the hippocampus and piriform cortex of the OBX rat (Holmes et al., 1998). Additionally, NPY levels were

increased in the amygdala (Rutkoski et al., 2002) in this animal model. In the cortex of OBX animals, Urigen et al. (2008) observed increased gene expression of NPY while Widerlov et al. (1988a) reported decreased NPY-like immunoreactivity.

These studies consistently reported decreased levels of NPY in the hippocampus but its overexpression in the amygdala. However, the expression of the various NPY receptor subtypes was selectively changed depending on the model used. Future studies should aim to evaluate the antidepressant effects of NPY related molecules and to demonstrate if alterations in NPY levels and receptor expression are correlated with behaviors.

2.3.3 Exogenous administration of NPY related molecules in animal models

A variety of well-known animal models of depression-related behaviors has thus far been used to provide additional evidence on the role of NPY and its receptors in emotional processes. In the helplessness model of depression-like behaviours, the acute administration of NPY, [Leu^{31}, Pro^{34}]PYY, as well as the Y_2 antagonist, BIIE0246, in the CA3 subfield of the hippocampus decreased the escape failure in the active avoidance paradigm, inferred as antidepressant-like behaviour (Ishida et al., 2007). Interestingly, the Y_1 antagonist, BIBO3304 blocked the antidepressant-related effect of NPY in this model (Ishida et al., 2007). Additionally, Goyal et al., (2009) showed that the Y_1-like agonist [Leu^{31}Pro^{34}]NPY decreased the hyperlocomotion in the open field (OF)**,** a disturbed behavior only reversed by administration of antidepressants (Song and Leonard, 2005) in the OBX rat.

Recently, the novel Y_5 antagonist, Lu AA33810, was shown to have anxiolytic- and antidepressant-like effects in the FSL rat (Walker et al., 2009). Lu AA33810

increased sucrose consumption compared to vehicle-treated animals, interpreted as an antidepressant-like effect, in the chronic mild stress model (Walker et al., 2009).

Taken together, these studies confirmed the role of NPY in depression-related behaviors in the challenged brain. The activation of the Y_1 receptor subtype as well as the blockade of the Y_2 receptor consistently induced antidepressant effects in various animal models. However, the role of Y_5 receptor subtype is still controversial. Since both a Y_5 agonist as well as an antagonist improved emotional despairs, their co-treatment will be required to clarify the role of the Y_5 receptor in depression- and anxiety- related behaviors.

2.4 Memory processes

Much evidence has shown that in addition to the emotional despairs observed in human subjects who suffer from depression, memory and attention deficits are common traits (Castaneda et al., 2008). However, still relatively little is known on the role of NPY and its receptors in memory processes. Acute ICV administration of NPY improved learning in two well-known models of amnesia, the anisomycin and scopolamine treatments in rats (Flood et al., 1987). In contrast, memory deficits were observed in young (Thorsell et al., 2000) but not in old (Carvajal et al., 2004) NPY-overexpressing rats. NPY KO mice showed no changes in memory-related paradigms (Bannon et al., 2000). In the object recognition test, Y_1 KO mice displayed some cognitive impairments (Costoli et al., 2005). Similarly, Y_2 KO mice showed learning deficits in a variety of memory tasks (Greco and Carli, 2006, Redrobe et al., 2003a). In contrast, Y_4 KO mice have normal learning abilities compared to wild type mice (Painsipp et al., 2008b).

Thus, further research is certainly warranted on the role of NPY and its receptors in learning and memory deficits known to occur in depressive states.

2.5 Human Studies

Cumulative evidence suggested the existence of a negative correlation between NPY levels and depression. This hypothesis is based on the measurement of peripheral levels of this peptide under stressful conditions and in depressed subjects. Similarly, alterations in NPY levels in suicide attempters and completers who suffered from depression-related disorders, increased NPY levels following chronic antidepressant treatments as well as in genetic association studies in humans suggest a role for NPY in depressive disorders.

2.5.1 NPY levels under stressful conditions in humans

Growing evidence shows that stress is a strong risk factor for depression (Lupien et al., 2009). In humans, NPY administration resulted in reduced cortisol levels in the dark phase in healthy subjects (Antonijevic et al., 2000). Subsequently, Morgan et al. (2002, 2001, 2000) observed that psychological stress increased both cortisol and NPY levels in volunteer subjects. Interestingly, the highest levels of NPY were observed in participants whom were apparently less affected by stress (Morgan et al., 2002). Taken together, these findings suggest that stressful conditions can increase NPY levels in humans as observed in animal studies. The subgroup that is most able to manage stressful experiences also expressed the highest level of the peptide, possibly due to genetic predisposition.

2.5.2 Altered levels of NPY in depressed subjects

The measurement of plasma or cerebrospinal fluid (CSF) levels of monoamines and neuropeptides has been widely used as potential state and/or trait dependant marker of various neuropsychiatric conditions including depression (Raedler and Wiedemann, 2006). Numerous studies have suggested that CSF levels of NPY are decreased in depressed subjects as observed in table 4 (Heilig et al., 2004, Hou et al., 2006, Olsson et al., 2004, Widerlov et al., 1988a, Widerlov et al., 1988b). Additionally, Hashimoto et al.(1996) observed that plasma NPY levels were decreased in major depression while Kuromitsu et al. (2001) showed that NPY mRNA expression was downregulated the frontal cortex in brains of bipolar subjects. In complementary studies, Widdowson et al. (1992) reported that NPY-like immunoreactivity was decreased in the frontal cortex of suicide completers. Interestingly, this alteration in NPY-levels was exacerbated in a subgroup of depressed suicide victims. Moreover, NPY Y_2 receptor mRNA level was reported to be increased in the prefrontal cortex of suicide victims (Caberlotto and Hurd, 2001).

Additionally, it would be interesting to dissect the effect of NPY in the CSF compared to plama levels. Indeed, Kastin and Pan (2010) have suggested a key role fo the peripheral effects of NPY, it thus would be interesting to confirm these findings regarding NPY levels in humans.Taken together, these results provide strong evidence that NPY levels are negatively correlated with depression, although few studies have also shown poor association (Gjerris et al., 1992, Nikisch et al., 2005, Ordway et al., 1995, Roy, 1993).

Model	Effect of NPY levels	Condition	Reference
Cerebrospinal fluid	↓	Major depression	*(Widerlov et al., 1988a; Widerlov et al., 1988b; Heilig et al., 2004; Hou et al., 2006)*
	=	Major depression	*(Gjerris et al., 1992; Roy, 1993; Ordway et al., 1995; Nikisch and Mathe, 2008)*
Brain tissue	↓	Suicide attempters	*(Westrin et al., 1999; Olsson et al., 2004)*
	↓	Postmortem suicide brains	*(Widdowson et al., 1992)*
Plasma levels	↑	Major depression	*(Irwin et al., 1991)*
	↓	Major depression	*(Hashimoto et al., 1996)*
	=	Major depression	*(Czermak et al., 2008)*
	Positive correlation between NPY levels and psychiastenia and irritability	Suicide attempters	*(Westrin et al., 1999a, b)*
Plasma levels	↓	Suicide attempters	*(Westrin et al., 1998)*
Platelets	↑	Major depression	*(Nilsson et al., 1996)*
Platelet-poor plasma	↓	Major depression	*(Nilsson et al., 1996)*
Brain tissue	↓	Depression and epilepsy	*(Frisch et al., 2009)*
Antidepressant treatment	↑	Major depression	*(Widerlov et al., 1988a)*
	=	Major depression	*(Olsson et al., 2004)*
mRNA level	↓	Bipolar disorder	*(Kuromitsu et al., 2001)*
	=	Suicide victim	*(Caberlotto and Hurd, 1999)*
	unchanged Y_1 receptor	Major depression and bipolar disorder	*(Caberlotto and Hurd, 2001)*
	↑ Y_2 receptor	Suicide victim	*(Caberlotto and Hurd, 2001)*

Table II.4. Summary of clinical studies, suggesting a role of neuropeptide Y (NPY) in depression

2.5.3 Antidepressant treatment and NPY in human subjects

In animal models of depression-related behaviors, clear correlation between antidepressant treatment and increased levels of NPY have been reported. In contrast, human studies have provided conflicting data. For example, a long term treatment with the SSRI, citalopram, increased CSF NPY levels (Nikisch et al., 2005); a similar effect being observed following ECT (Nikisch and Mathe, 2008). Conversely, a treatment with the selective monoamine oxidase A inhibitor, amiflamine failed to alter CSF NPY levels (Widerlov et al, 1988b). Olsson et al., (2004) also monitored the levels of NPY in depressed subjects but they failed to observe any correlation between antidepressant treatment and NPY levels. In this study, patients received various antidepressants and at different dose regimens. Accordingly, additional studies are required in order to obtain more definitive conclusions on the relevance of concomitant increases in NPY levels and the alleviation of depressive symptoms observed during antidepressant treatment. It is interesting to note here that in contrast to animal studies, a considerable number of depressed subjects are refractory to any antidepressant treatments (Berlim et al., 2008). Thus, conflicting results may be attributable to the lack of efficacy of antidepressant therapies in a subgroup of patients.

2.5.4 Genetic component of NPY in depression

Depression is a multifactorial disorder with a role for a large array of susceptibility genes, epigenetic and environmental factors (Bale et al., 2010, Brown and Harris, 2008, Lupien et al., 2009). Genetic association studies have attempted to link NPY to emotional disorders, particularly depression. Sebat et al., (2009) hypothesized that single nucleotide polymorphisms (SNPs) are most likely linked with limited increase

in the probability of developing a given disease. In contrast, rare copy number of variations (CNVs) is likely more strongly associated to the likelyhood of developing a certain psychiatric disorder. The SNP rs16147, Leu7Pro7 (T/C), a polymorphism observed in the prepro-NPY gene was first examined in control subjects under an exercise paradigm (Kallio et al., 2001). This study suggested that Leu7Pro7 carriers produced higher levels of plasma NPY compared to Leu7Leu7 and in a much shorter time. Further studies found that alterations in this promoter resulted in higher NPY levels (Buckland et al., 2004, Itokawa et al., 2003, Shah et al., 2009) as observed in figure 1.

Since previous findings have suggested that NPY levels are decreased in depression in humans, a working hypothesis was that carriers of this polymorphism had decreased risks to develop depression. Heilig et al., (2004) tested this assumption and observed a negative correlation between depression and this polymorphism in Swedish population. This finding was subsequently replicated in another study (Sjoholm et al., 2009). In contrast, Linderg et al. (2006) reported a lack of correlation between this SNP and depression in a Danish population. This polymorphism was further evaluated in regard to a stress response in a Finnish population (Zhou et al., 2008). Recently, Sommer et al. (2010) and Domschke et al., (2010) suggested that variations in the polymorphism contribute to emotional responses in humans. However, Cotton et al. (2009) failed to observe an association between this polymorphism and emotional arousal in a very large population.

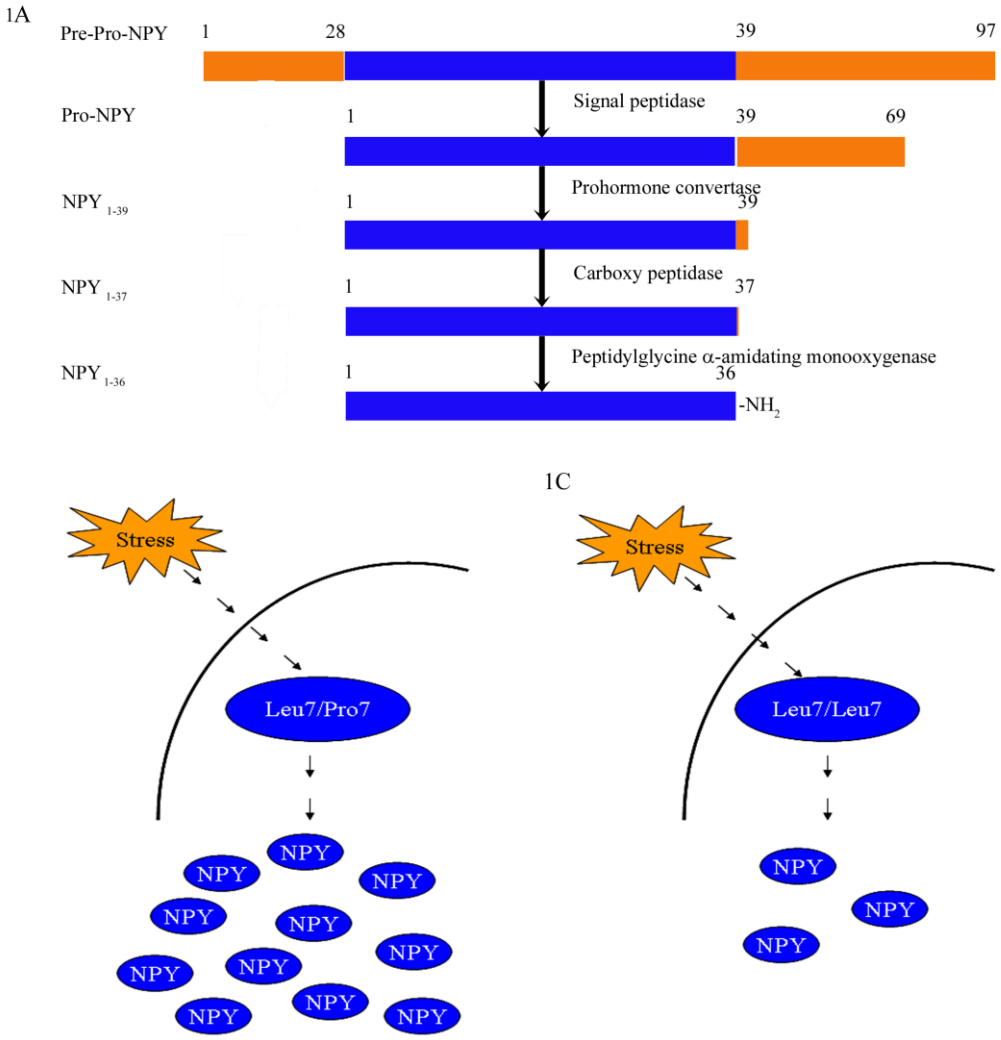

Figure II.1. Synthesis and processing of NPY under normal conditions and after stress. A. This scheme shows the full synthesis of NPY from the 97 aminoacid (a.a.) precursor to its final 36 a.a. form. B. Role of different polymorphisms after the stress cascade is activated. Leu7/Leu7 may result on low levels of NPY, meanwhile, Leu7/Pro7 may produce high levels of NPY. This figure was taken from Morales-Medina JC et al., 2010. Brain Res 1314:194-205.

In summary, these studies globally suggest that this particular SNP plays a key role in the processing of NPY. However, the association between this polymorphism and emotional processes is still controversial. Further studies will thus be necessary to establish the role of this SNP and its impact on NPYergic systems in various subgroups of patients.

2.6 Mechanism of action

Accordingly, we propose adult hippocampal neurogenesis as a possible cellular mechanism of NPY action since neurogenesis or the formation of new neuronal cells occurs during the entire lifespan of an individual (Altman and Das, 1965, Eriksson et al., 1998). Continuous production of neurons has been observed in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) in mammals including rodents and humans (Kuhn et al., 1996). Antidepressants have been shown to enhance this neurogenic process (Santarelli et al., 2003) while various animal models of depression-like behavior display faulty hippocampal neurogenesis including the OBX model (Jaako-Movits and Zharkovsky, 2005). Moreover, *in vitro* studies have suggested that NPY has proliferative action in hippocampal homogenates (Hansel et al., 2001) and may mediate neurogenesis in hippocampus *in vivo* (Howell et al., 2003, Howell et al., 2007). Recent evidence has also shown that NPY modulates neurogenesis in the SVZ (Agasse et al., 2008). Yet the *in vivo* potential role of NPY and its receptors in adult hippocampal neurogenesis in animal models of depression-related behavior, remains to be established.

2.7 Rationale

A converging body of evidence suggests that NPY and its receptors possess antidepressant- and anxiolytic- like effects after acute administration in naïve animals and KO models. However, the dissociation between both affective disorders remains to be dissected. Moreover, the role of NPY and its various receptor subtypes under challenged or stress conditions remains to be established. In order to investigate the potential role of various NPY receptor subtypes in anxiety- and depression-like behavior, we propose to use various well known animal models of depression- and anxiety-like behaviors. The OBX rat model of depression-like behavior induces a wide array of behavioral and neurochemical disturbances which resemble several characteristics of depression observed in humans. In addition, stress or disturbances in Corticosterone (CORT) levels have been associated as a risk factor of emotional disorders. Therefore, acute CORT administration elicits anxiety-like behaviors in the rat.

The behavioral and neuronal consequences of OBX lesions will be characterized with special emphasis on emotional-related processes as well. We will then continuously infuse agonists and antagonist of different NPY receptor subtypes to OBX and CORT treated animals to try to reverse some of the behavioral despairs observed here. At the same time, we will quantify possible alterations in neurogenesis in the DG as possible cellular mechanism (s) of action of the NPY system.

2.8 Hypothesis

The stimulation of Y_1 and Y_5 receptors as well as the blockade of Y_2 receptors will differentially reverse emotional-like deficits observed in animal models of depression- and anxiety-like behaviors (OBX lesions and CORT treatment).

In order to validate this hypothesis, we will first characterize the behavioral changes observed in these models and subsequently evaluate the respective role of the Y_1, Y_2 and Y_5 receptor subtypes in depression and anxiety related behaviors in these same animal models.

2.9 Specific aims:

1. To characterize the OBX model of depression- and anxiety- related behaviors in the rat.
    a. To evaluate the effect of OBX in emotional-related behavioral tests.
    b. To investigate the contribution of this lesion in spatial navigation memory.
    c. To assess the consequences of OBX in the morphology of several brain regions.
    d. To explore the contribution of this lesion in adult cell proliferation and survival in the dentate gyrus of the hippocampal formation.
2. To study the specific contribution of the Y_1 receptor subtype in emotional processes in our chosen animal models.
    a. To evaluate the role of the Y_1 receptor in anxiety- and depression like behavior in the OBX rat model.

b. To assess the role of the Y_1 receptor subtype in anxiety-like behaviors after an acute CORT treatment in rats.

c. To assess the proliferative cellular actions of an NPY analog acting via Y_1 receptors in the dentate gyrus of OBX rodents.

3. To investigate the role of the Y_2 receptor subtype in depression- and anxiety- like behavior in our chosen animal models.

    a. To explore the possible anxiolytic- and antidepressant- like properties of Y_2 receptors in the OBX rat model.

    b. To investigate the effects of a Y_2 agonist and a Y_2 antagonist on anxiety-like behaviors after acute CORT treatment in rats.

4. To explore the role of the Y_5 receptor subtype in affective processes in our chosen animal models.

    a. To assess the role of a Y_5 agonist on anxiety- and depression like behavior in the OBX rat model.

    b. To investigate the possible anxiolytic-like effect of an NPY analog acting via the Y_5 receptor in acutely-treated CORT rats.

Chapter 3: Impaired structural hippocampal plasticity induces emotional and memory deficits in the olfactory bulbectomized rat

3.1 Preface

Significant information regarding the role of the NPY system in emotional processes has been obtained using KO animal models as well as acute administration of this peptide and its analogs. However, a more relevant strategy would be based on the use of well-known animal models of emotional disorders to further characterize the possible anxiolytic- and antidepressive-like effects of NPY and related molecules. In this regard, the OBX rat is one of the most studied and validated animal model of depression-like behavior, since this lesion induces a variety of behavioral, neurological and immunological alterations similar to those observed in human depression (Kelly et al., 1997, Song and Leonard, 2005). In addition, the hyperlocomotion observed in the open field is only reversed after repeated administration of antidepressants, a clinically-relevant timeframe with these drugs (Mar et al., 2000, 2002). Recently, Wang et al., (2007) also suggested the existence of anxiogenic-like behaviors in these animals. However, the mechanism(s) leading to these various behaviors is not fully understood. Therefore, in the present chapter, we propose the use of a battery of behavioral paradigms to underscore possible functional changes in neuronal circuitry as well as adult hippocampal neurogenesis as mechanism(s) of disturbances induced by OBX.

3.2 Manuscript

Impaired structural hippocampal plasticity induces emotional and memory deficits in the olfactory bulbectomized rat

Julio César Morales-Medina[a,c], Ismael Juarez[d], Erika Venancio-García[d], Sandra Nayeli Cabrera[d], Caroline Menard[c], Wenfeng Yu[c], Naguib Mechawar[c], Gonzalo Flores[d] and Rémi Quirion[b,c]

[a]*Dept. of Neurology & Neurosurgery,* [b]*Dept. of Psychiatry,* [c]*Douglas Mental Health University Institute, McGill University, Montréal, QC, Canada, H4G*

[d]*Laboratorio de Neuropsiquiatría, Instituto de Fisiología, Universidad Autónoma de Puebla. 14 Sur 6301, Puebla, México, 72570*

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Abstract

Disturbances in olfactory circuitry have been associated with depression in humans. The removal of olfactory bulbs (OBX lesion) has been largely used as a model of depression-like behavior in the rat. However, cellular disturbances in key brain regions in this animal model have not been evaluated yet. Accordingly, we investigated changes in the hippocampal plasticity as well as behavioral deficits in this animal model. OBX-induced behavioral deficits were studied in a battery of tests, namely the open field test (OFT), forced swim test (FST), and spatial memory disturbances in the Morris Water Maze (MWM). To characterize the cellular disturbances, neuroanatomical rearrangements were investigated in brain regions associated with emotional or olfactory processes including CA1 hippocampus and piriform, medial prefrontal, orbito-lateral, and infralimbic cortices. Cell proliferation and survival of newborn cells in the adult dentate gyrus (DG) were also determined. OBX induced hyperlocomotion and enhanced rearing and grooming in the OFT, increased immobility in the FST as well as required a longer time to find the hidden platform in the MWM. OBX also induced dendritic atrophy in the hippocampus and piriform cortex. In addition, cell proliferation was decreased while the survival remained unchanged in the DG of these animals. These various features are also observed in depressed subjects, adding further support to the validity and usefulness of this model to evaluate potential novel antidepressants.

1. Introduction

Major depression has been associated with disrupted neuronal plasticity (Manji et al., 2001) and deregulated hippocampal neurogenesis (Boldrini et al., 2009). In depressed subjects, neuronal rearrangement has been observed in the hippocampus, prefrontal cortex (PFC), and amygdala (Cotter et al., 2005; Hercher et al., 2009; Rajkowska et al., 1999; Stockmeier et al., 2004), and various animal models of depression-like behavior mimic those neuronal rearrangements. For instance, stress-induced depression-like behaviors are accompanied by hippocampal neuronal hypotrophy (Conrad et al., 2007; Magarinos et al., 1998; Morales-Medina et al., 2009; Watanabe et al., 1992). Hippocampal hypotrophy is also observed in the learned helplessness rat (Hajszan et al., 2009) as well as adult offspring having received low maternal care early in their development (Bagot et al., 2009; Champagne et al., 2008). In animals as well as humans, antidepressants treatment increase adult hippocampal neurogenesis (Boldrini et al., 2009) which is compromised in various animal models of depression (David et al., 2009; Jaako-Movits and Zharkovsky, 2005). Thus, previous findings have consistently suggested that alterations in hippocampal plasticity are key contributors to the disease.

The olfactory bulbectomized (OBX) model of depression produces a wide spectrum of behavioral, neurochemical, endocrine, and immunological changes similar to those observed in humans (Kelly et al., 1997; Song and Leonard, 2005). Interestingly, OBX animals show sensitivity to antidepressant treatments only after repeated administration, similar to the human condition, a feature that increases the validity of this animal model (Mar et al., 2002; Wang et al., 2007). The OBX surgical procedure consistently induces depression-like behaviors in diverse behavioral tests (Han et al.,

2009; Song et al., 1996; Tasset et al., 2008; Wang et al., 2007). However, a comparative analysis of various behavioral tests that measure emotionality in the same cohort of OBX animals remains to be conducted. In addition, attention deficits and memory loss have been reported in depressed subjects (Castaneda et al., 2008). OBX animals show deficits in passive-avoidance behavior (Kelly et al., 1997; Nakagawasai et al., 2003; Sieck, 1972), a condition associated with learning deficits. However, the role of OBX in the Morris Water Maze (MWM), a well-known paradigm used to evaluate hippocampus-dependent spatial memory in rodents (Doggui et al., 2010; Morris, 1984) is rather controversial (Nesterova et al., 2008; Redmond et al., 1994).

Despite the relevant role of compromised neuronal plasticity in depressed subjects and animal models of depression-like behavior, data are scarce with respect to neuronal morphology in OBX animals. In this regard, Nesterova et al. (2008) found abnormalities (pyknosis, karyolysis, and vacuolysis) in the hippocampus and temporal cortex neurons (Bobkova et al., 2004) as well as cell death in the primary olfactory cortex (Heimer and Kalil, 1978) of OBX rodents. These neurodegenerative changes and most of the behavioral deficits are observed only after at least two weeks post lesion (Yamamoto et al., 1997). Therefore, we could hypothesize that OBX induces changes in hippocampal plasticity.

In the present study, we assessed a battery of behavioral tests including the open field test (OFT) and forced swim test (FST) in the same cohort of animals, and MWM in a second cohort of animals using the OBX model. Additionally, dendritic arborization and spine density in five regions involved in emotional or olfactory processes, as well as adult hippocampal neurogenesis were quantitatively evaluated.

2. Materials and Methods

A series of behavioral, neuroanatomical, and immunohistochemical studies were carried out to evaluate the role of OBX in the rat. After OBX surgery, animals were allowed to recover for two weeks, and since generally antidepressant drugs are administered for two weeks, behavioral, neuroanatomical, and immunohistochemical studies were performed four weeks after the removal of olfactory bulbs. Four animal cohorts were studied: the first was used for the OF and FST, the second for the MWM, the third for neuroanatomical analyses, and the fourth for immunohistologial studies of DG proliferation and survival of newborn cells.

2.1 Animals

Male Sprague Dawley rats (Charles River Canada, Montréal, QC, Canada) weighing 150-170g at the beginning of the treatment were housed two per cage and maintained on a 12 h light/dark cycle with *ad libitum* access to food (Purina Lab Chow) and water. All procedures were approved by the McGill Animal Care Committee and according to the guidelines of the Canadian Council on Animal Care.

2.2 OBX surgery

Bilateral olfactory ablation was performed similar to the procedure described in earlier studies (Hasegawa et al., 2005; Watanabe et al., 2003). Briefly, 5% isoflurane was used to induce anesthesia, and subsequently maintained at 2.5% during the surgical procedure. A cranial window, 5.2 mm anterior to the bregma was created in the frontal bone. The olfactory bulbs were cut and aspirated out. Sham operations were performed in the same manner, but the bulbs were left intact. The prevention of blood loss from the cranial window was achieved by filling the open space with a haemostatic sponge.

Following surgery, rats were administered with carprofen and a saline solution (0.9% NaCl) and left in pairs in their cages to recover for two weeks. Only animals with their olfactory bulbs completely removed, and with no damage to their frontal cortex (as determined by an examination following brain removal) were included in data analysis.

2.3 Behavioral tests

Tests were performed on two consecutive days, as reported in another model of depression-related behavior (Kalynchuk et al., 2004). The OF test was carried out on day 27 post-surgery, while the FST was performed on day 28. The behavioral tests were carried out during the light phase of the light-dark cycle (9:00-13:00). Rats were maintained in similar housing conditions throughout all the tests. Ten to sixteen animals per group were assessed.

2.3.1 Open field test

This test was performed under bright light in an OF apparatus (100x100x40 cm) made of a black wooden box with a grey floor and no top, with a field divided into 64 equal-size squares. The dimensions of this arena were similar as used previously (Keilhoff et al., 2006). The locomotor activity was observed and recorded for a total of 10 minutes (min), including locomotion (horizontal behavior) and the frequency of rearing and grooming (vertical behaviors), by an observer blind to the treatment. After each trial, the testing apparatus was cleaned with Peroxigard solution (Bayer Healthcare, Toronto, ON).

2.3.2 Forced swim test

FST is a well-documented tool to screen potential antidepressants (Lucki, 1997; Porsolt et al., 1977). For this behavioral test, rats were placed for a 10 min period in a

white cylindrical tank (29 cm wide x 43 cm high) with no top, filled with water (25°C ± 2°C). A camera was mounted 1 m above the tank, and an observer blind to the experimental conditions evaluated three behaviors; swimming, struggling, and immobility. Following the tests, the rats were removed from the cylinder, cleaned with a towel, and placed under a red lamp until their fur dried.

Swimming time was regarded as the time when an animal performed an active swimming activity beyond the necessary movements to keep itself floating. Time spent struggling was recorded when the front paws of a rat broke through the surface of the water, and it tried to get out of the tank by making quick strong movements. Finally, immobility time was noted when an animal made the minimum necessary movement to keep its body floating. Depression-related behavior was inferred from an increase in the time the rat spent immobile, which is thought to represent a lack of motivation to escape from the water.

2.3.3 Morris Water Maze

The MWM protocol used was as has been described previously (Benoit et al., 2010; Doggui et al., 2010; Morris, 1984). We evaluated hippocampal-dependent spatial memory four weeks after the removal of olfactory bulbs or sham surgery. The animals had to find a hidden platform (14 cm in diameter) located 2 cm below the surface of white-colored water at 24°C in a pool (diameter 1.4 m). For each trial, animals were pseudo-randomly started from a different position. Rats used spatial cues to find the platform that remained in the center of the same quadrant throughout all the training days (Morris 1984). The rats were given three trials per day (90 seconds [s] each) over five consecutive days. Animals were guided to the platform if platform was not located within

90 s. Also, the rats were allowed to rest on the platform and situate themselves for 15 s after each trial.

On day five, the platform was removed and a probe test of 60 s was conducted to evaluate the number of times the animal crosses the platform location. Motivation and visual impairments were controlled with a cued task (platform visible) after the probe test. Animal lacking motivation to escape water or presenting visual impairments were removed from the study. After each trial, animals were dried with a towel and placed under heat lamps to prevent hypothermia. The experimenter was blind to the groups evaluated in the MWM. The results were recorded using a computer-tracking system.

2.4 Golgi-Cox stain method

Four weeks after the removal of the olfactory bulbs or sham surgery, the rats were anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneal injection, ip) and perfused intracardially with saline solution. Brains were removed and stained by using the modified Golgi-Cox method (Alquicer et al., 2008; Flores et al., 2005; Gibb and Kolb, 1998; Juarez et al., 2008) and were left for 14 days in Golgi-Cox solution, followed by 3 days in 30% sucrose solution. Coronal sections of 200 µm thickness of the regions to be studied were obtained by using a vibrotome (Camden Instrument, MA752, Leicester, UK). Sections were treated with ammonium hydroxide for 30 min, followed by 30 min in a Kodak Film Fixer and subsequently rinsed with distilled water. Finally, the sections were dehydrated and mounted on glass slides.

According to the atlas of Paxinos and Watson (1986), the following neurons were studied: Golgi-impregnated neurons from the pyramidal neurons of the CA1 subfield of the hippocampus (Plates 27-36), medial prefrontal cortex (mPFC) layer III (Plates 7-11),

orbito-lateral cortex (OLC) (Plates 5-9), infralimbic cortex (ILC) (Plates 8-10), and piriform cortex (PirC) layer III (Plates 18-32). The criteria used to select neurons for reconstruction was as described earlier by our group (Flores et al., 2005; Juarez et al., 2008). An observer blind to the analysis identified the three-dimensional dendritic tree of five neurons in each hemisphere (10 neurons per animal) in 10 animals per group. Each neuron was reconstructed at a magnification of 250x in a two dimensional plane using a camera Lucida (DM 2000 Microscope, Leica Microsystems, Wetzlar, Germany). Dendritic tracing was quantified by Sholl analysis (Flores et al., 2005; Sholl, 1953). A transparent grid with concentric rings spaced 10 μm apart was placed over the dendritic drawing, and the number of ring intersections was estimated**.** In addition, the total dendritic length (TDL) was calculated by multiplying the total number of intersections of each ring per 10 μm. Another estimate of dendritic arborization is the total number of dendritic branches (branching indicated by bifurcation), which were counted at each order away from the cell body or dendritic shaft. Finally, the density of dendritic spines was estimated by drawing at least 10 μm long segments from the terminal tips at a 1,000x resolution.

2.5 Immunohistochemistry

Two weeks after the removal of the olfactory bulbs, dividing cells in the DG were labeled with the DNA synthesis marker bromodeoxyuridine (BrdU) (50 mg/kg, i.p. dissolved in 0.9% NaCl with 0.4 M NaOH to evaluate neurogenesis (survival). Animals were sacrificed 14 days after receiving three BrdU injections (i.p. at 2h intervals, as previously described (Mechawar et al., 2004). Rats were deeply anesthetized with a ketamine cocktail (5 ml ketamine/2.5 ml xylazine/1 ml acepromazine/1.5 ml sterile

saline, injected 0.10 ml per 100 g, i.p. and their brains were fixed by cardiac perfusion with ice-cold saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) (pH 7.4). The brains were then post-fixed overnight at 4°C in a fixative and stored at -80 °C. Coronal sections (40-μm-thick) of the brains were cut in four series with a cryomicrotome (Microm HM 500M) and processed for immunohistochemistry.

2.5.1 Cell survival

The sections were thoroughly rinsed with PBS solution, pretreated in PBS containing 0.2% Triton X-100 for 2 h at room temperature, and subsequently in 2 M HCl in PBS for 30 min at 37°C to denature the DNA. Sections were then incubated for 24 h at 4°C with monoclonal rat anti-BrdU antibody (1:1000) in 2% normal goat serum (NGS) in PBS+0.2% Triton X-100. After three rinses in PBS, the sections were incubated for 1 h at room temperature with biotinylated goat anti-rat secondary antibody, followed by 1 h of incubation using the avidin–biotin complex procedure. Labeling was developed in DAB-no NiCl, and the reaction was stopped by dipping the sections in water for 5 min., then dehydrated and mounted on glass slides.

2.5.2 Cell proliferation

The sections were rinsed thoroughly with PBS solution, mounted on glass slides using PBS+0.2% Triton X-100, and then dried and incubated overnight in an oven at 70ºC. Antigen retrieval was performed by using a 10mM sodium citrate buffer (pH=6.0) and a steamer for 15 min. The sections were then incubated in 3% H_2O_2/PBS for 10 min. After three washes, the sections were incubated in mouse anti-Ki67 (1:2000) in 2% normal horse serum (NHS) in PBS+0.2% Triton X-100 overnight at 4ºC. Following incubation, the sections were biotinylated with horse anti-mouse, rat-adsorbed secondary

antibody, followed by 1 h with the avidin-biotin complex. The sections were developed on DAB- no NiCl, counterstained 1 min with 0.1% cresyl violet, dehydrated, and coverslipped with permount.

2.7. Antibodies

The clone BU1/75(ICR1) antiBrdU rat monoclonal antibody (Ab Serotec, MCA2060, lot #240408) was raised against synthetic BrdU. The specificity of immunostaining was verified on the control sections derived from the animals not injected with BrdU as previously suggested (Bastien-Dionne et al., 2010; Liu and Martin, 2006). The clone B56 anti-Ki67 mouse monoclonal antibody (BD Pharmingen, #550609, lot #17090) was raised against human Ki67 protein. The specificity has been well characterized previously (Kao et al., 2008; Key et al., 1993). In preliminary experiments, we performed immunostaining experiments by omitting the primary or seconday antibody as well in control animals not injected with BrdU. In all cases,we observed no immunostaining.

2.7. Quantification of immunolabeling

BrdU and Ki67 cells in the DG of the hippocampus were counted using a Nikon eclipse E800 at 40X magnification. The results are expressed as mean of BrdU and Ki67 positive cells per DG. Plates 28, 32, 37 and 42 were evaluated according to Paxinos and Watson (1986). The DG surface area and volume were also calculated with the Cavalieri method, using an Olympus BX51 microscope, CX-9000 camera at 10X magnification and the Stereo Investigator software package, to rule out differences in area or volume in OBX compared to sham animals. Similar area and volume of DG was observed in all the four plates ($p>0.05$).

2.8. Reagents

Kodak Film Fixer was purchased from Kodak (Rochester, USA) and BrdU from Sigma (St. Louis, MO, USA). Triton-X-100 was obtained from Amersham (Piscataway, NJ, USA). Monoclonal rat anti-BrdU antibody was purchased from Ab Serotec (Raleigh, NC, USA). Biotinylated goat anti-rat secondary antibody, biotinylated horse anti-mouse rat absorbed secondary antibody ABC Kit (Vectastain elite), and DAB were acquired from Vector (Burlingane, CA, USA). Mouse anti-Ki67 was obtained from BD Biosciences Pharmingen (Mississauga, ON, Canada). All other chemicals used in this study were of analytical reagent grade, and purchased from local commercial sources.

2.6 Statistical analysis

Data from MWM, dendritic arborization, total dendritic length, and length per branch order were analyzed by two-way ANOVA, followed by a Bonferroni test for post-hoc comparisons with OBX lesion, using time, regions or orders as independent factors. Spine densities, neurogenesis-related studies, FST, and OF were analyzed by an unpaired t-test. $p < 0.05$ was considered significant.

3. Results

OBX lesion induces hyperactivity in the open field and immobility in the forced swim test.

OBX lesion significantly increased the locomotion in the OF arena when compared to sham animals as shown in Fig 1A ($p<0.001$; unpaired t-test). In the same paradigm, OBX lesion augments rearing (Fig 1B, $p<0.01$; unpaired t-test) and grooming (Fig 1C, $p<0.001$; unpaired t-test) events in the rat. The following day after the OF test, animals were placed in a cylinder containing water and three behaviors were evaluated: immobility, struggling, and swimming. OBX animals spent more time immobile (Fig 1D, $p<0.001$; unpaired t-test) by decreasing swimming time but not struggling (Fig 1D, $p<0.001$; unpaired t-test).

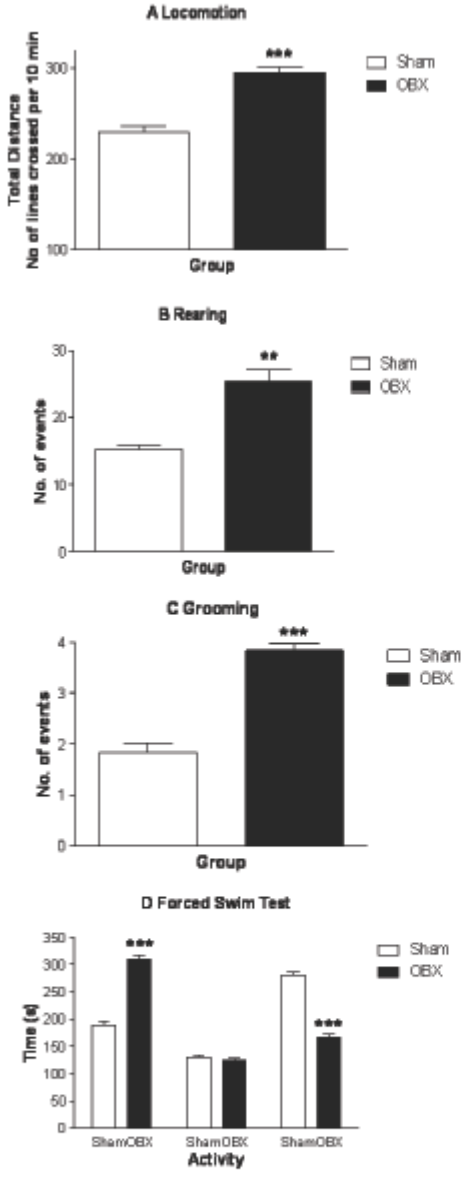

Figure 1

*Figure III.1. The removal of olfactory bulbs (OBX) induced emotion-related deficits in the open field (OF) test and forced swim test (FST) in the rat. OBX increases locomotion (A), number of rearing (B) and grooming (C) in the OF. OBX augments the time spent immobile while decreases swimming time in the FST (D). The results are expressed as mean ± S.E.M. with n=12 animals per group, ** p<0.01 and *** P<0.001*

OBX animals were slower to find the platform in the Morris water maze but presented no difference in the probe test.

Animals were evaluated for the MWM test 23-28 days after the removal of olfactory bulbs or sham surgery (Fig. 2A). This learning curve indicates that OBX rats required more time to find the hidden platform during the second and third day of training. However, both groups of animals crossed the platform position for an equal number of times in the probe test (Fig. 2B). No differences were observed in the visually-cued task after the probe test (data not shown).

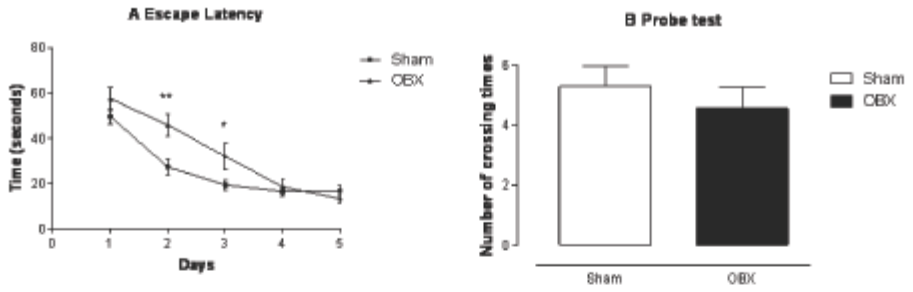

Figure 2

*Figure III. 2. The olfactory bulbectomy (OBX) increases the escape latency in the Morris water maze (MWM). OBX rats show longer escape latencies before finding the platform during days 2 and 3 of training (A). Sham and OBX rats crossed the quadrant with the platform in equal number of times on day five of training and seven days later as well (B). However, time had an effect on the number of crossings. The results are expressed as mean ± S.E.M. with n=16 animals per group, *p<0.05.*

OBX lesion decreases cell proliferation but has no significant effect on newborn cell survival in the DG.

OBX lesion significantly decreased the number of $Ki67^+$ cells in the rat DG ($p<0.05$; unpaired t-test) 28 days after the olfactory bulbs removal or sham surgery (Fig. 3A), indicating a reduction in cell proliferation. In contrast, the number of $BrdU^+$ cells remained similar between groups (Fig. 3B).

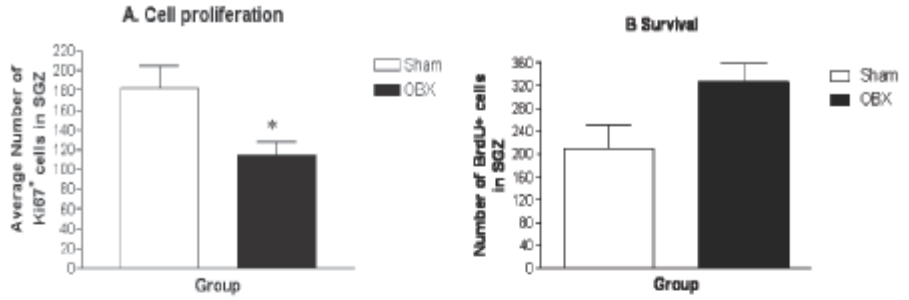

Figure 3

*Figure III.3. Decreased cell proliferation in adult dentate gyrus (DG) in olfactory bulbectomized rat (OBX). OBX rats presented decreased number of Ki67+ cells in the subgranular zone of the DG (A). The number of BrdU+ cells evaluated in the subgranular zone of the DG show no apparent differences between OBX and sham animals (B). The results are expressed as mean ± S.E.M. with n= 5 brains per group in triplicates, *p<0.05.*

OBX lesion rearranges dendritic distribution in the piriform cortex and CA1 hippocampal neurons.

In the OBX rats, we evaluated the dendritic arborization and spine density of PirC, CA1 hippocampus, mPFC, OLC, and ILC. Representative control neurons from each region are presented in Fig 4A (PirC), 4B (CA1 hippocampus), 4C (mPFC), 4D (OLC), and 4E (ILC). Dendritic distal sections of control neurons of these regions are shown in

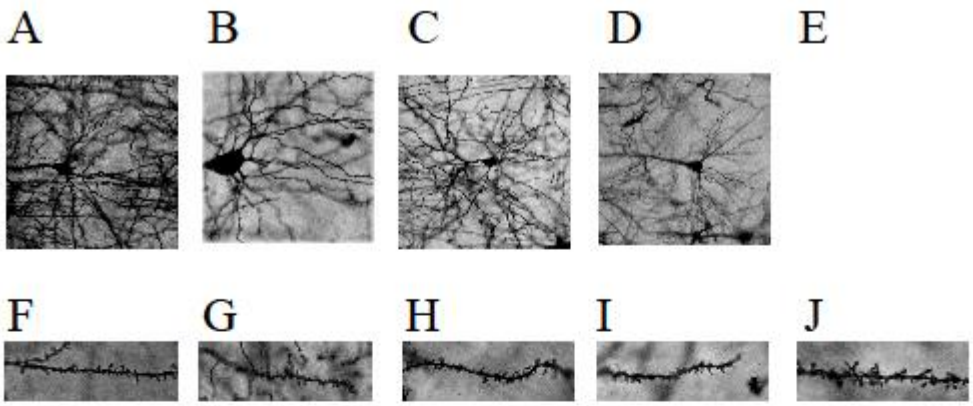

Fig 4F-J.

Figure III.4. Representative photographs of Golgi-stained neurons from the piriform cortex (A), CA1 hippocampus (B), medial prefrontal cortex (C), orbito-lateral cortex, (D) and infralimbic cortex (E) and distal dendritic segments as illustrated in figures F-J in sham animals. The neurons are shown at magnification 250X while the segments of spines at 1000X. Bars (A - E) = 40 µm and (F - J) = 10 µm

Our results indicate that compared to controls, OBX lesion induces reorganization of dendritic arborization (Fig 5A), decreases dendritic length in the third order (Fig 5B, $p<0.05$) and reduces total dendritic length (Fig 5C, $p<0.05$) for the PirC neurons. Neither basal (Fig. 5D) nor distal (Fig 5E) spine density was affected by OBX lesion in the PirC neurons.

Four weeks post OBX lesion, the impact on the neuroplasticity of the CA1 hippocampal pyramidal neurons led to a decrease in dendritic arborization (Fig 6A), particularly at the third order level (Fig 6B, $p<0.05$) with no change in the total dendritic length (Fig 6C). Furthermore, the hippocampal CA1 pyramidal neurons of OBX animals showed no change in either basal (Fig 6D) or apical (Fig 6E) spine density when compared to control rats.

OBX lesion has no effect on the neuronal plasticity of the three cortical regions, cMPF, OLC, and ILC evaluated in the present study. In addition, dendritic arborization (Fig 7-9A), branch order (Fig 7-9B), total dendritic length (Fig 7-9C), and spine density (Fig 7-9D) were not affected by OBX lesion in the cMPF, ILC, and OLC neurons of the rat.

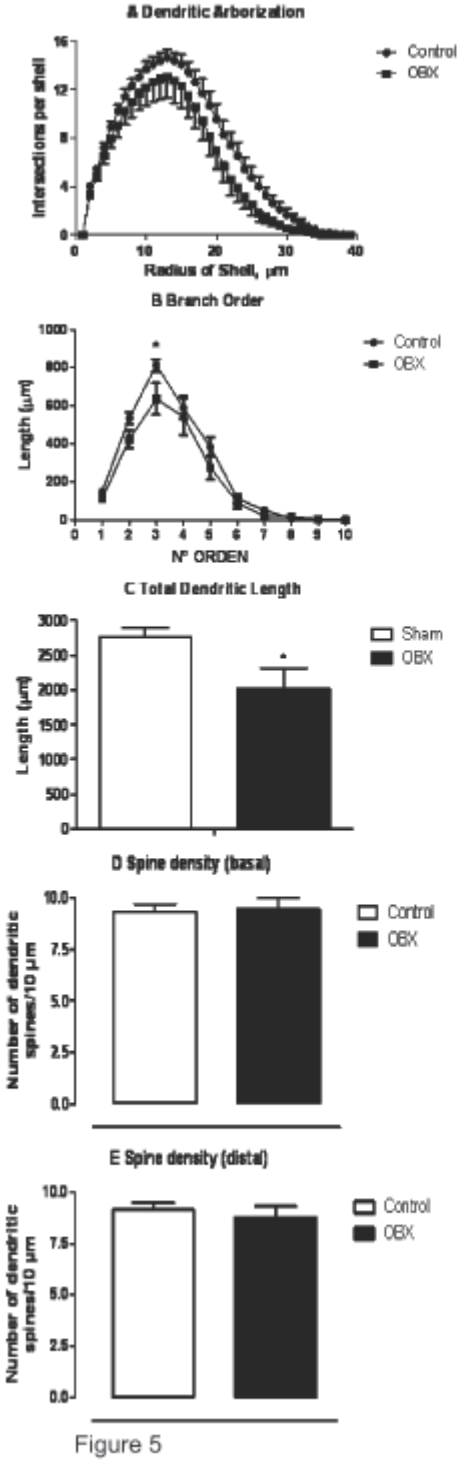

Figure 5

*Figure III.5. Olfactory bulbectomy (OBX) induces hypotrophy in piriform cortex (PirC) pyramidal neurons in OBX rats (A). The branch order analysis indicates a specific reduction in number of neurons in third order in OBX versus sham animals (B). The total dendritic length was decreased in neurons of OBX compared to sham animals (D). No apparent differences were observed in either basal (F) or distal (G) dendritic segments in OBX compared to sham animals. Data expressed as mean ± SEM with n=10 rats per group, 8-10 neurons per dendritic segment and 8-10 sections of basal and distal dendrites were drawn for each animal. *p<0.05.*

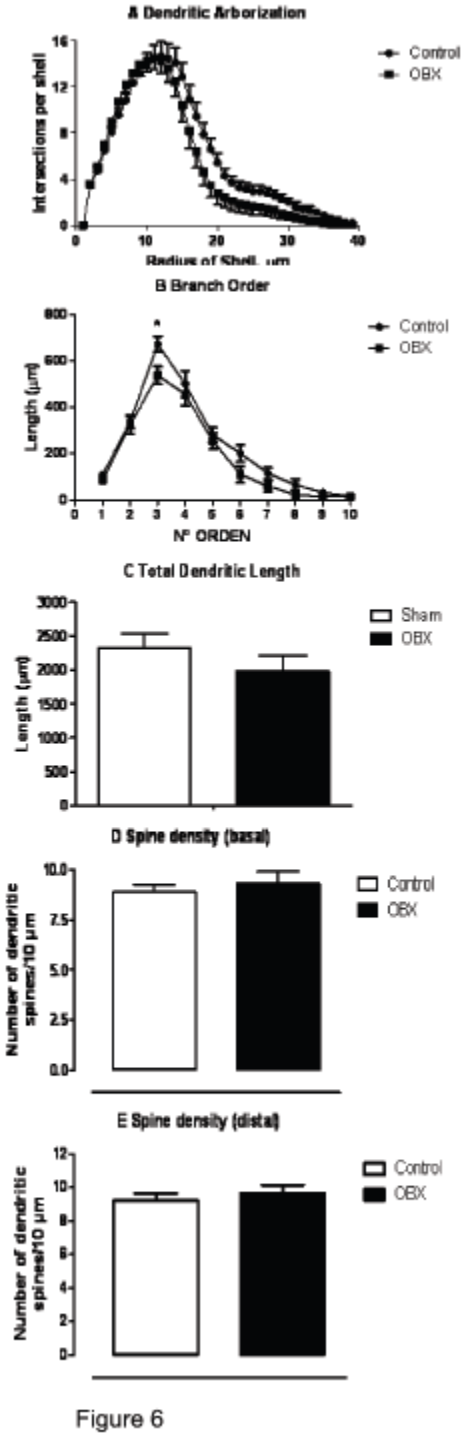

Figure 6

*Figure III.6. Neuronal arborization is reorganized in CA1 pyramidal neurons after olfactory bulbectomy (OBX). The Sholl analysis shows that dendritic remodelling occurs in this population of neurons in OBX animals (A). OBX lesion causes rearrangement of dendritic length, particularly at the third order level (B). This procedure produces no apparent differences in the total dendritic length (C). The basal (D) or distal (E) spine density remains unchanged after OBX lesion. Data are expressed as mean ± SEM with n=10 rats per group, 8-10 neurons per dendritic segment and 8-10 sections of basal and distal dendrites were drawn for each animal. *p<0.05.*

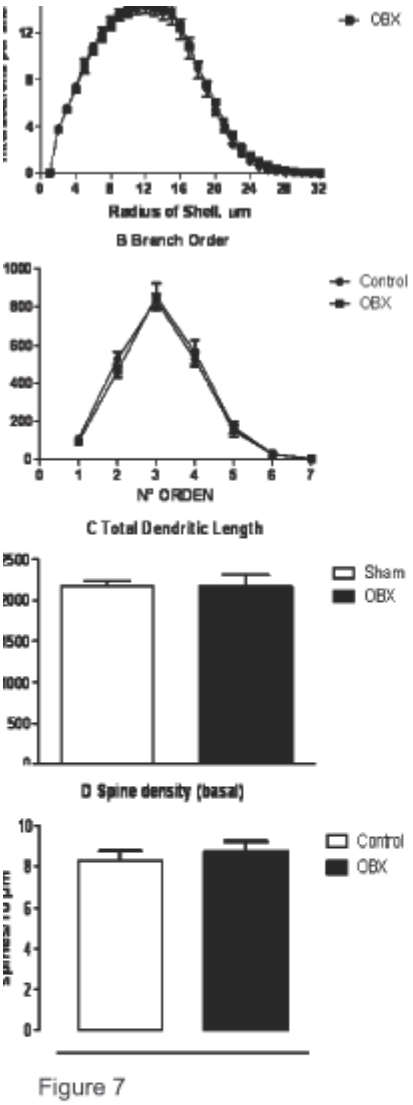

Figure 7

Figure III.7. Olfactory bulbectomy (OBX) has no effect on neuronal plasticity of pyramidal neurons of medial prefrontal cortex in OBX compared to sham animals (A). The total dendritic length (B), branch order (C) and spine density (D) remained unchanged in the OBX rat. Data are expressed as mean ± SEM with n=10 rats per group, 8-10 neurons per dendritic segment and 8-10 sections of basal and distal dendrites were drawn for each animal.

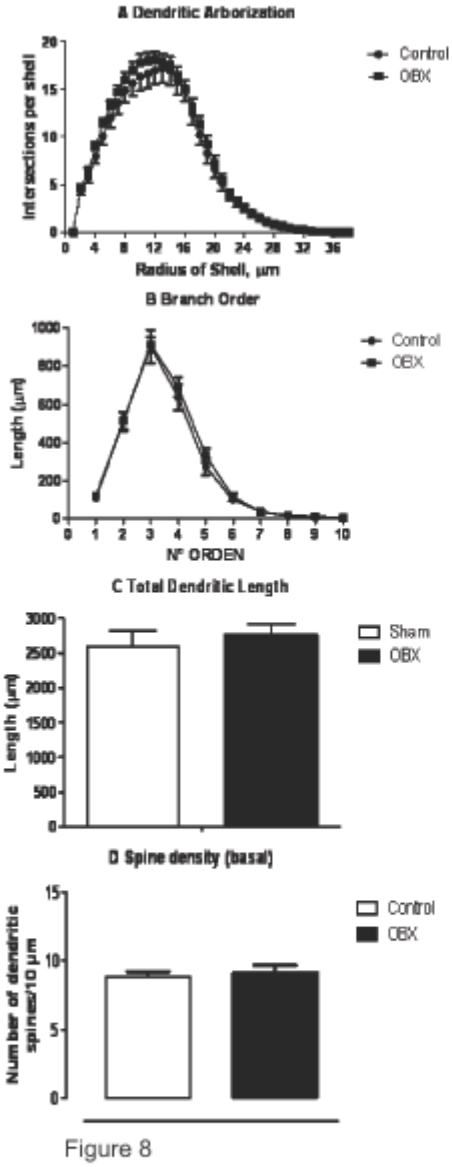

Figure 8

Figure III.8. Pyramidal neurons of orbito-lateral cortex are not affected by olfactory bulbectomy (OBX). The dendritic arborization (A), total dendritic length (B), branch order analysis (C), and spine density analysis (D) show that OBX has little impact on this population of neurons. Data expressed as mean ± SEM with n=10 rats per group, 8-10 neurons per dendritic segment and 8-10 sections of basal and distal dendrites were drawn for each animal.

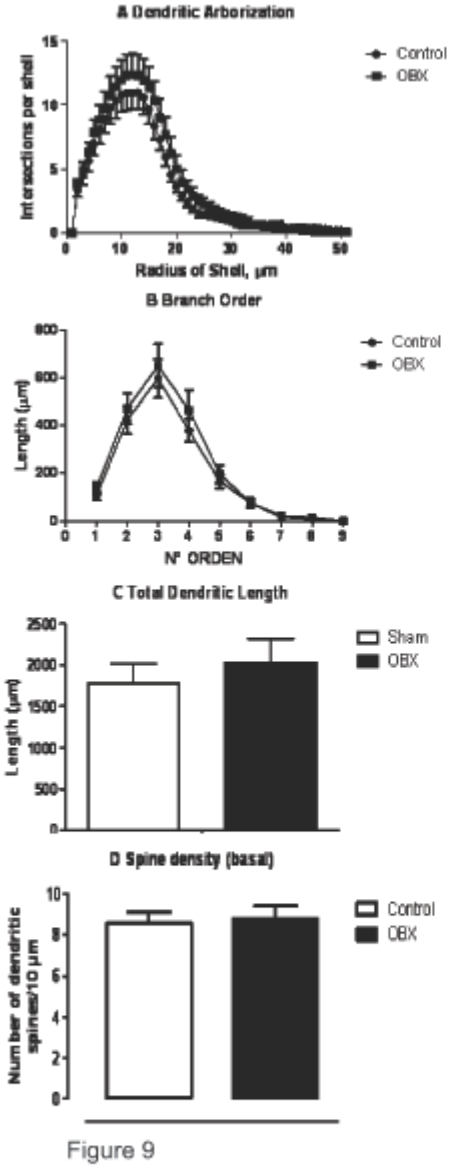

Figure 9

Figure III.9. Olfactory bulbectomy (OBX) has no effect on infralimbic cortex pyramidal neurons. The dendritic arborization (A), total dendritic length (B), the branch order (C) and spine density (D) analyses indicate that this population of neurons remains unchanged after OBX. Data expressed as mean ± SEM with n=10 rats per group, 8-10 neurons per dendritic segment and 8-10 sections of basal and distal dendrites were drawn for each animal.

4. Discussion

Removal of the olfactory bulbs is widely used as a model for depression-related behavior based on a wide range of abnormalities, and its response to antidepressants only after repeated administration similar to the data obtained in the human condition (Kelly et al., 1997; Mar et al., 2002). In the present study, we observed an impaired adaptation to novel environment, lack of motivation to escape, memory-deficits, dendritic remodeling and disrupted cell proliferation in hippocampus of OBX rats, all hallmarks observed in depressed subjects.

4.1 OBX induced hyperlocomotion in the open field, and increased immobility time in the forced swim test

We first assessed the behavioral effects of OBX in OFT and FST in male Sprague Dawley rats. OBX animals are widely known to display hyperactivity in the OFT, a behavior associated with impairment to adapt and to evaluate risk assessment (Harkin et al., 2003; Kelly et al., 1997; Song and Leonard, 2005). In contrast, the effect of OBX lesion in the FST, a depression-like behavior test, widely used to screen antidepressants (Lucki, 1997; Porsolt et al., 1977) was only recently established. It has been previously reported that OBX increases immobility time in the FST in mice (Han et al., 2009) and Wistar rats (Tasset et al., 2008; Vieyra-Reyes et al., 2008) but not in Long Evans rats (Vieyra-Reyes et al., 2008). As observed in the present study, OBX in Sprague Dawley rats also increased immobility time. Despite the fact that the hyperlocomotion in the OF test is widely replicated across laboratories and reversed after repeated administration of antidepressants, this abnormal behavior is not observed in other animal models of depression-like behavior. Therefore, to better understand the possible antidepressant-like

effect of promising drugs, the OF test followed by the FST should be used in future studies when using this robust animal model.

4.2 Memory deficits in the OBX rat correlate to a full spectrum of behaviors observed in depressed patients

In rodents and humans, learning and memory are strongly influenced by stress and depression (Castaneda et al., 2008; D'Hooge and De Deyn, 2001). In this regard, OBX animals consistently have shown deficits in passive-avoidance behavior (Kelly et al., 1997; Nakagawasai et al., 2003; Sieck, 1972), a behavior associated with learning deficits. However, Primeaux and Holmes (1999) suggested that this terminology is inadequate for such behavior and should be interpreted only as "a dysfunction in the defensive freezing capacity of the OBX rat." The OBX rat also displayed learning- and memory-deficits in the 3-lever operant task, the 3-panel runaway apparatus, the 3-choice serial time task, and the 2-lever apparatus (Yamamoto et al., 1997). However, none of these tests are broadly used to evaluate memory-related tasks in rodents.

In contrast, the MWM is one of the most frequently used tools to evaluate spatial learning and memory in rodents (D'Hooge and De Deyn, 2001). In the present study, we observed that the spatial memory acquisition in the OBX rat was altered during days 2 and 3 of the MWM training while in the shorter version of this test, Redmond et al. (1994) reported memory deficits on the second day of training. Altogether, our findings support the usefulness of the OBX model not only to evaluate depression-related behaviors but memory deficits as well.

4.3 Olfactory bulbectomy selectively rearranges neuronal arborization in hippocampal and cortical pyramidal neurons

The PirC has been associated with olfaction processes (Barkai and Saar, 2001). In this regard, Wilson and Stevenson (2003) propose that "the olfactory bulb circuitry creates odor-specific spatial-temporal patterns synthesized and stored in the PirC through Hebbian synaptic plasticity". In the present study, we report for the first time that OBX induces quantitative neuronal dendritic reorganization in the PirC. Such an OBX-induced dendritic atrophy of the PirC neurons could result in decreased excitability. In support of this hypothesis, Wang et al. (2007) have shown that PirC pyramidal neurons are degenerated following the removal of the olfactory bulbs. Additionally, Heimer and Kalil (1978) observed cell death in the primary olfactory cortex of OBX rodents with the PirC belonging to this cortex. Interestingly, olfactory-discriminating learning induces increased spine density and enhanced neuronal excitability in the PirC pyramidal neurons (Barkai, 2005; Brosh and Barkai, 2009). Our findings further support that PirC neurons are sensitive to the changes in gain or loss of input from the olfactory bulbs.

We also observed debranching in arborization in the CA1 hippocampal neurons in the OBX rat, especially at the third level. In support of these findings, Nesterova et al. (2008) recently reported neuronal degeneration of the hippocampal CA1/CA3 regions one year after the ablation of olfactory bulbs. Different stressors or CORT treatments induce neuronal atrophy (Alfarez et al., 2008; Morales-Medina et al., 2009; Sousa et al., 2000) or decrease spine density (Hajszan et al., 2009) in the CA1 pyramidal neurons as well. This neuronal atrophy also is observed early in the development of the learned-helplessness rat (Hajszan et al., 2009) and the adult offspring of the low maternal care rat

(Bagot et al., 2009; Champagne et al., 2008), animals that present emotional dysfunctional behaviors. However, we did not observe changes in distal or proximal spine density which is often disturbed in animal models of depression-related behavior. In contrast, Norrholm and Ouimet, (2001) observed a marginal decrease (6.5%) in spine density in CA1 hippocampus in the OBX rat. These differences could be mostly methodological. Interestingly, this group transported the animals for a long distance between OBX and sacrifice. In this regard, stress-related processes are well known to decrease spinogenesis in hippocampus (Magarinos and McEwen, 1995; Magarinos et al., 1996). Taken together, these findings suggest that CA1 hippocampal neurons are very sensitive to any form of stress or challenged conditions in the brain.

We also evaluated the neuronal state in the mPFC, ILC and OLC of the OBX rat. The mPFC (anterior-cingulate) mediates a range of higher order executive functions including working memory and decision making, which is severely affected by stress (Holmes and Wellman, 2009). Cumulative evidence has also shown that long-term CORT administration or repeated stress leads to mPFC (anterior-cingulate) pyramidal neurons hypotrophy (Brown et al., 2005; Cerqueira et al., 2005; Wellman, 2001). The ILC, the most ventral part of the mPFC, receives strong inputs from the suprachiasmatic nucleus, the master circadian pacemaker (Perez-Cruz et al., 2009). Since both the circadian (Perret et al., 2003; Vinkers et al., 2009) and endocrine (Marcilhac et al., 1997) rhythms are disturbed in OBX animals, we hypothesize that these changes are caused by alterations in ILC pyramidal neurons. A physical stress of one week (Perez-Cruz et al., 2009) or 1-3 days (Izquierdo et al., 2006) induces hypotrophy of the ILC pyramidal neurons. Moreover, Izquierdo et al. (2006) suggested that the ILC is an initial target for stress that

might later expand to other brain regions. The OLC plays a key role in olfactory and emotional processes (Gottfried and Zald, 2005; Gur et al., 2000; Ramus and Eichenbaum, 2000). Lesions of this cortex are associated with deficits in olfactory-discriminating learning (Otto and Eichenbaum, 1992) as well as liability, mania, and changes in personality (Mega and Cummings, 1994). In addition, the density of the pyramidal neurons of this cortex is decreased in the brains of subjects with major depression (Rajkowska et al., 2005). Since the removal of olfactory bulbs impairs smelling, modifies emotional-like responses, and induces retracted dendritic arborization in the PirC pyramidal neurons, a brain region that sends inputs to the OLC (Ramus and Eichenbaum, 2000), we evaluated the role of OBX in this cortical region. However, none of these three cortices was affected by OBX. These apparent discrepancies can be explained by the fact that different sources of stress, as well as the length of exposure, affect these neurons differentially or a neuronal rearrangement of these cortices in other models could be produced by pathways not disturbed by bulbectomy in the rat.

4.4 Adult hippocampal neurogenesis is compromised in the OBX rat

Cumulative evidence suggests that adult hippocampal neurogenesis is compromised in various animal models of depression-related behavior (David et al., 2009; Oomen et al., 2009). However, the effect of OBX in this process is not clear. To further investigate this process, we used endogenous (Ki67) and exogenous (BrdU) cell division markers to respectively evaluate cell proliferation and survival in the DG of this animal model. By using the endogenous marker Ki67, we found that the number of proliferating cells was decreased in the subgranular zone of the DG. In the same manner, OBX in the Wistar rat -when administered 24 hours prior to sacrifice—produces a

decrease in the number of BrdU+ cells in the DG (Jaako-Movits and Zharkovsky, 2005; Jaako-Movits et al., 2006). The administration of BrdU two to four weeks prior to sacrifice is routinely used to assess new born cell survival in the DG (David et al., 2009). Here, we found no differences in DG cell survival between OBX and sham rats two weeks after BrdU administration. In contrast, the number of BrdU+ cells in the DG was previously found to be decreased in the Wistar rat (Jaako-Movits et al., 2006; Keilhoff et al., 2006) and mice after the removal of the olfactory bulbs (Shioda et al., 2010). These discrepancies could be due to methodological differences and the use of different strains and species. Interestingly, repeated CORT administration or chronic stress produces decreases in cell proliferation without disturbing cell survival in the DG (David et al., 2009; Heine et al., 2004). Thus, the removal of olfactory bulbs could mimic the deficits in adult neurogenesis observed in models of stress-induced depression. In this regard, two independent studies suggest that CORT levels are increased in this animal model (Cairncross *et al.*, 1977; Uriguen *et al.*, 2008).

4.5 Olfaction and its role in emotional dysfunctional processes

Several studies have suggested that depressed subjects often experience olfactory dysfunction; however, those studies are not conclusive (Lombion-Pouthier et al., 2006; Postolache et al., 1999; 2002). Odors stimulate olfactory receptors from the nasal olfactory epithelium, which in turn sends projections to the olfactory bulbs. The information is then sent to different brain regions, including the PirC, OLC, frontal cortex, and amygdala (Atanasova et al., 2008). The inputs from the PirC and entorhinal cortex, as well as amygdala connect with the hippocampal formation. In the present study, we found decreased dendritic arborization in the PirC and CA1 hippocampal

neurons. A possible mechanism of neuronal CA1 hippocampal atrophy could be based on the finding that the PirC pyramidal neurons (mainly glutamatergic) project to the granular cells of the hippocampal DG (Wang et al., 2007). The DG projects to CA1 pyramidal neurons. Thus, CA1 hippocampal neuronal remodeling likely occurs due to a decreased input coming from the PirC neurons.

4.6 Association between behavioral deficits and compromised hippocampal plasticity in the OBX rat

In the present study, we report disturbances in two well-known tests of depression-like behaviors, a memory paradigm and two forms of hippocampal plasticity. Hence, we hypothesize that these hippocampal dysfunctions may contribute to the abnormal behaviors observed following OBX.

In this regard, repeated stress or CORT induces depression-like behaviors in rodents in various paradigms (David et al., 2009; Gregus et al., 2005; Kalynchuk et al., 2004). These animals also display deregulated neurogenesis (David et al., 2009; Heine et al., 2004) and hippocampal atrophy (Morales-Medina et al., 2009; Sousa et al., 2000). Recently, an antidepressant treatment has shown to reverse several, but not all behavioral deficits, in a CORT-induced model of depression-like behavior (David et al., 2009). These authors suggested that antidepressants treatment reversed behavioral deficits through neurogenesis-dependent and neurogenesis-independent mechanisms. This phenomenon is particularly important, since we observed two independent processes being disturbed in the hippocampus in the OBX rat. Therefore, these data further support the role of at least two hippocampal-dependent mechanisms disturbed in emotional dysfunctional conditions.

In addition, increasing evidence points to a crucial link between CA1 hippocampal atrophy and spatial memory. For example, repeated stress or CORT treatment induced both hippocampal hypotrophy and deficits in navigation memory (Conrad et al., 1996; de Quervain et al., 1998; Sousa et al., 2000). In humans, hippocampal-dependent memory is also compromised after treatment with glucocorticoids (Newcomer et al., 1999). Sapolsky et al. (2000) suggested that the dendritic remodeling likely occurs through enhanced glutamatergic transmission. Exogenous CORT administration increases glutamate release in the CA1 hippocampus (Venero and Borrell, 1999). Interestingly, Wang et al. (2007) reported altered hippocampal glutamatergic transmission in the OBX rat. Thus, in this model, hippocampal neuronal remodeling likely occurs due to an altered glutamatergic transmission and disrupted PirC neuronal input, resulting in memory deficits.

5.0 Conclusions

Taken together, these findings suggest that OBX triggers depression-like behavior and memory deficits accompanied by hippocampal dendritic remodeling and disrupted cell proliferation. The present study also further supports the usefulness and validity of the OBX lesion as a model of depression-like behavior.

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Chapter IV

Role of neuropeptide Y Y_1 and Y_2 receptors on behavioral despair in a rat model of depression with co-morbid anxiety

4.1 Preface

In the previous chapter, we characterized the OBX animal model used in this thesis. We concluded that this model is suitable to be used to evaluate depression- and anxiety- like behaviors in the rat. We also found neuronal rearrangement as well as disrupted neurogenesis in the hippocampus in this model. We are now proposing to investigate the role of NPY Y_1 and Y_2 receptors in anxiety- and depression- related behaviors using this animal model.

We studied first NPY Y_1 and Y_2 receptors on the basis of their broad distribution in the CNS (Dumont et al., 1998b) with particularly high levels seen in cortex, hippocampus and amygdala, three brain regions critically involved in emotional processes. In addition, previous findings using KO animals as well as acute administration of ligands of these receptors revealed anxiolytic- and antidepressant-related effects.

We observed that the continuous administration of the Y_1-like agonist [$Leu^{31}Pro^{34}$]PYY reversed the hyperlocomotion observed in the OF, decreased immobility in the FST and induced social contacts in SI test in OBX rats, whereas decreased grooming in the OF was noted in sham animals.

Moreover, the Y_2 antagonist BIIE0246 reduced immobility time in the FST in OBX rats while decreasing grooming and increasing sociability in the SI test in sham animals. The Y_2 agonist PYY3-36 increased immobility time in the OBX rat and grooming in sham animals.

Since these NPY related compounds do not cross the blood brain barrier, we also evaluated the effect of another Y_2 antagonist, the JNJ compound, a drug that can cross the blood brain barrier in the OBX rat (supplemental data).

Finally, we also assessed the role of these receptors in adult neurogenesis in the hippocampus of this animal model (supplemental data). We evaluated the role of a NPY Y_1 agonist, a Y_2 agonist and a Y_2 antagonist in cell proliferation and cell survival in dentate gyrus, since these processes are dysregulated in depression as well as in animal models of depression-related behaviors (Boldrini et al., 2009, David et al., 2009, Oomen et al., 2009). Moreover, recent data strongly suggest that antidepressants mediate their effects at least partially, by modulating adult neurogenesis in the dentate gyrus (Santarelli et al., 2003).

To further characterize the role of NPY Y_1 and Y_2 receptor subtypes in emotional dysfunctional conditions, we also compared the effects of continuous administration of agonists and antagonists of these receptors in the corticosterone (CORT)- induced anxiety model (Supplemental data). This model is characterized by a simple administration of CORT induced anxiogenic behavior, 12 days after the injection as well as the hypertrophy in the basolateral amygdala.

4.2 Manuscript

Role of neuropeptide Y Y_1 and Y_2 receptors on behavioral despairs in a rat model of depression with co-morbid anxiety

Julio César Morales-Medina[a,b], Yvan Dumont[b] Charles-Etienne Benoit[b], Stéphane Bastianetto[b], Gonzalo Flores[c] Alain Fournier[e] and Rémi Quirion,[a,b*]

[a]*Dept. of Neurology & Neurosurgery,* [b]*Douglas Mental Health University Institute*

[b]*Dept. of Psychiatry, McGill University, Montréal, QC, Canada, H4H 1R3*

[c]*Laboratorio de Neuropsiquiatría, Instituto de Fisiología, Universidad Autónoma de Puebla. 14 Sur 6301, Puebla, México, 72570*

[d]*INRS-IAF, Université du Québec, 245 Boul. Hymus, Pointe-Claire, QC, Canada H9R 1G6*

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1. Introduction

Neuropeptides such as corticotrophin releasing factor (CRF) (Rotzinger et al., 2009), VGF (non-acronymic) (Thakker-Varia and Alder, 2009) and neuropeptide Y (NPY) (Dumont et al., 2009, Morales-Medina et al., 2010b, Morales-Medina and Quirion, 2011) have emerged as neuromodulators of physiological and pathological dysfunctions associated with emotional disorders. Among them, NPY, a 36 amino acid peptide (Tatemoto et al., 1982), is both highly conserved and widely distributed in the central nervous system (CNS) (Dumont and Quirion, 2006a). This abundant peptide modulates its biological effects by the activation of at least four G protein-coupled receptors known as Y_1, Y_2, Y_4 and Y_5 (Michel et al., 1998). Interestingly, the Y_1 and Y_2 receptors are highly expressed in the CNS (Dumont et al., 1998b, 2000) with high levels in the cortex, hippocampus and amygdala (Dumont et al., 1998b) which are brain regions known to be involved in emotional processes (Kask et al., 2002).

Clinical studies have shown that NPY levels may be decreased in depressed subjects (Heilig et al., 2004, Hou et al., 2006, Widerlov et al., 1988b). In addition, cumulative data has suggested a role for NPY and NPY related molecules in emotional processes in rodents (Dumont et al., 2009, Morales-Medina et al., 2010b). For example, Heilig et al. (1989) reported that NPY displays anxiolytic properties in the elevated plus maze in naïve normal rats. Meanwhile, our group and others have shown that acute, exogenous administration of NPY (Redrobe et al., 2002, Stogner and Holmes, 2000) and the Y_1–like receptor agonist, [Leu^{31}Pro^{34}]PYY (Redrobe et al., 2002), induces

Abbreviations. central nervous system (CNS), corticotrophin releasing factor (CRF), forced swim test (FST), Krebs Ringer Phosphate (KRP), nanomolar (nmol), neuropeptide Y (NPY), olfactory bulbectomized (OBX), open field test (OFT), social interaction test(SIT)

antidepressant-like effects in naïve normal animals. Moreover, the antagonism in naïve normal rats (Bacchi et al., 2006, Redrobe et al., 2002) or genetic ablation in mice of the Y_2 receptor subtype (Carvajal et al., 2006a, Redrobe et al., 2003b) induced antidepressant- and anxiolytic- like behaviors. However, these earlier studies were conducted in naïve normal animals. A more relevant strategy should be based on the use of well-known animal models of emotional disorders to further characterize the possible anxiolytic- and antidepressive-like effects of NPY and related molecules.

Animal models represent powerful tools to explore the pathology of depression as well as to screen for novel treatments (Cryan and Slattery, 2007, Holmes, 2003, Neumann et al., 2010). The olfactory bulbectomized (OBX) rat displays behavioral, neurochemical, endocrine and immunological alterations similar to those observed in human mood disorders (Holmes et al., 1998, Kelly et al., 1997, Song and Leonard, 2005). Interestingly, NPY levels have also been shown to be altered in the OBX rat (Holmes et al., 1998, Primeaux and Holmes, 2000). Additionally, Wang et al. (2007) suggested that OBX animals display both depressive- and anxiogenic- like behaviors. Recently, we proposed the use of a battery of behavioral tests in OBX animals to better establish the antidepressant- and anxiolytic- like properties of candidate molecules (Morales-Medina et al., 2010a). Since this animal model presents unique disturbances in anxiety- and depression- related behaviors as well as known alterations in the NPY system, this model is most suitable to evaluate the role of this neuropeptide and its receptors in emotional processes.

Accordingly, the present study aimed to demonstrate the respective role of the Y_1 and Y_2 receptor subtypes in depression- and anxiety- related behaviors in OBX rats and if the apparent levels of Y_1 and Y_2 receptor binding are altered in this model providing additional support for the role of NPY in emotional behaviors.

2. Methods

A series of behavioral and autoradiographic studies were carried out to evaluate the role of NPY Y_1 and Y_2 receptors in OBX rats. Generally, animals were allowed to recover from OBX or sham surgery for two weeks (Song and Leonard, 2005). Since antidepressant drugs are administered for two weeks, the behavioural experiments and receptor autoradiography were performed four weeks after the removal of olfactory bulbs.

2.1 Animals

Male Sprague Dawley rats (Charles River Canada, Montréal, QC, Canada) weighing 150-170g at the beginning of the treatment were housed two per cage and maintained on a 12 h light/dark cycle (lights on at 8:00 AM) with *ad libitum* access to food (Purina Lab Chow) and water. Animals were used either for behavioral or quantitative receptor autoradiographic studies. For behavioral studies, all animals underwent two surgeries: (1) OBX or sham lesions performed on day 1 and (2) minipump implantation carried out on day 14. The animals were weighed once a week (until behavioral tests on days 28-30) and monitored constantly to ensure their health. For autoradiographic studies, animals underwent either sham or OBX surgery, and were sacrificed four weeks post-lesion and brains collected four weeks post-lesion. Figure 1 shows a flow chart of the procedural order of these experiments.

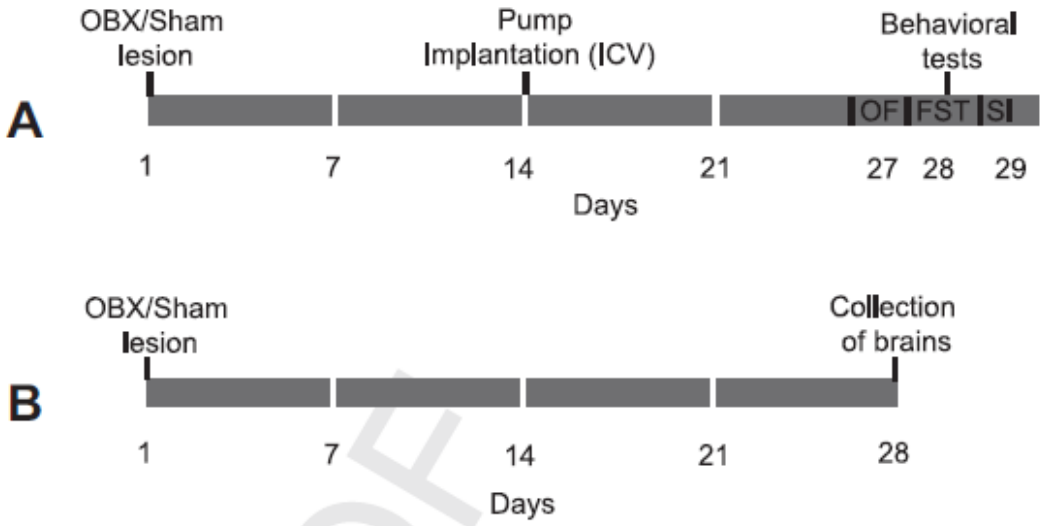


Figure IV.1. Flow chart depicting the procedural order used in this study.

All procedures were approved by the McGill Animal Care Committee and according to the guidelines of the Canadian Council on Animal Care and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2 OBX surgery

Bilateral olfactory ablation was performed according to previous reports (Hasegawa et al., 2005, Watanabe et al., 2003). Briefly, 5% isoflurane (Baxter, Mississauga, ON) was used to induce anesthesia and subsequently maintained at 2.5% during the surgical procedures. A cranial window 5.2 mm anterior to the bregma was created in the frontal bone and the olfactory bulbs were cut and aspirated out. Sham

operations were performed in the same manner but the bulbs were left intact. Prevention of blood loss from the cranial window was achieved by filling the open space with a haemostatic sponge (Gelfoam, Pfizer Canada Inc., Montréal, QC). Following surgery, rats were administered with carprofen (0.1 mL/100g animal) (Pfizer Animal Health, Montréal, QC) and saline solution (0.9% NaCl) (Hospira Healthcare Corporation, Montréal, QC) and left in pairs in their respective cages to recover for two weeks. Only data from animals with complete removal of olfactory bulbs and no damage to the frontal cortex (determined by examination following brain removal) were included in the analysis.

2.3 Osmotic minipump implantation

For behavioral tests, an osmotic minipump (Alzet, model 2002, Durect Cupertino, CA, USA) connected to an indwelling ICV cannula was implanted to deliver NPY analogs (flow rate 0.5 µl/hr) for two weeks in OBX or sham rats (Tanaka et al., 2005). Previous studies have shown that NPY related molecules are stable in Alzet pumps for up to 28 days (Adams et al., 2006, Moriya et al., 2009). Briefly, 5% isoflurane was used to induce anesthesia and subsequently maintained at 2.5% during surgical procedures. For osmotic pump implantation, animals were placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) and a cannula was implanted into the left lateral ventricle (anterior-posterior: -0.8 mm from bregma, lateral: -1.3 mm from bregma, and vertical: -4.5 mm from dura) (Paxinos and Watson, 1986). The cannula was sealed with dental cement and connected to an osmotic pump by medical grade vinyl tubing. The pump was placed into a subcutaneous pocket in the dorsal region. Animals from the same treatment and condition group were left in pairs until behavioral testing. We additionally examined

the lateral ventricles as well as the dentate gyrus to explore possible differences in those areas as a result of the long term infusion. No differences were seen between the ventricle that received the cannula and the contralateral ventricle or between dentate gyri (data not shown).

NPY or NPY Y_1 agonists and Y_2 antagonists have usually been used in the picomolar range when administered into specific nuclei and in the nanomolar (nmol) range for ICV infusions (Rotzinger et al., 2009). In the present study, the vehicle was 0.9% NaCl for agonists or 1% DMSO/0.9% NaCl for antagonists. For OBX animals, osmotic pumps were filled with either vehicle or BIBO3304 (3 nmol/day), [$Leu^{31}Pro^{34}$]PYY (0.1, 0.3 & 1 nmol/day), BIIE0246 (1 & 10 nmol/day) and PYY3-36 (1 nmol/day) for 14 days. For sham animals, the pumps were filled with BIBO3304 (3 nmol/day), [$Leu^{31}Pro^{34}$]PYY (1.0 nmol/day), BIIE0246 (10 nmol/day), PYY3-36 (1 nmol/day) or vehicle for 14 days. Pumps were filled a day prior to surgery and incubated at 37°C overnight in a sterile saline solution for priming. Human (h)[$Leu^{31}Pro^{34}$]PYY and hPYY3-36 were synthesized at Institut National de la Recherche Scientifique-Institut Armand-Frappier, (Montréal, Canada) as previously reported (Forest et al., 1990). BIBO3304 and BIIE0246 were generously provided by Boehringer Ingelheim (Ingelheim, Germany).

2.4 Behavioral tests

A series of three behavioral tests was performed on three consecutive days, as previously reported in another model of depression-related behaviors (Kalynchuk et al., 2004). Similar protocols have also been used in the OBX model (Pandey et al., Rajkumar et al., 2009, Wang et al., 2007). Each test was carried out separately.

The open field test (OFT) was carried out on day 27, the forced swim test (FST) was performed on day 28, whereas the social interaction test (SIT) was done on day 29 post OBX or sham surgery, respectively. All three behavioral tests were carried out during the light phase of the light–dark cycle (9:00-13:00). Rats were kept in the same housing conditions throughout all tests. Eight to twenty animals per group were assessed.

2.4.1 Open field test

This test was carried out in a large arena, originally introduced for the study of emotionality in rats. The test was performed in an OF apparatus (100x100x45 cm) made of a black wooden box with a grey floor and no top lid and the field divided into 64 equal-size squares at 300 LUX. The behavior of animals was recorded for a 10 minute period including locomotion during the first exposure (horizontal behavior) and frequency of rearing and grooming (vertical behaviors) by an observer blind to the treatment. The testing apparatus was cleaned with peroxigard solution (Bayer Healthcare, Toronto, ON) after each trial. Behavior was determined as depression-related by hyperlocomotion (Song and Leonard, 2005) as well as enhanced rearing and grooming which is associated with stress coping behaviors (Kalueff and Tuohimaa, 2004, Song et al., 1996) observed in OBX animals and its reversal by repeated antidepressant treatment.

2.4.2 Forced swim test (FST)

FST is a well characterized tool to screen potential antidepressants (Lucki, 1997, Porsolt et al., 1977). In the present study, we used a modified version of the test where animals perform this task only once (Gregus et al., 2005, Kalynchuk et al., 2004, Redrobe et al., 2002). This behavioral test was performed in a white cylindrical tank (29 cm diameter X 43 cm height) with no top lid and was filled with water (25°C ± 2°C). Rats

were placed in the swimming tank for a 10 min period. A camera was mounted 1m above the tank and immobility was evaluated by an observer blind to the experimental conditions. Immobility time was recorded when the animal made minimum movements necessary to keep its body afloat. Increased time spent immobile to escape from water was determined as depression-related behavior due to lack of motivation (Cryan et al., 2005a). Following the test, rats were removed from the cylinder, cleaned with a towel and placed under a red lamp until the fur was dried.

2.4.3 Social interaction test (SIT)

SIT has been pharmacologically validated as an experimental paradigm to measure anxiety (File, 1980, File and Seth, 2003). This test was performed in a familiar situation (apparatus used was the same as for the OF test) under bright light. A pair of rats was placed in opposing corners of the OF apparatus and was allowed to explore the arena for 10 minutes. Active behavior was recorded when animals were running towards, grooming, mounting, and crawling under the other rat. Pairs of rats used for this test had previously undergone the same treatments but were housed in different cages. The OF apparatus was cleaned with peroxigard after each trial. The total duration of active contacts and number of contacts were measured for each pair of animals. The analysis was performed by a person blind to the treatment. An anxiogenic behavior was inferred from the reduced social contacts in OBX animals.

2.5 Quantitative receptor autoradiography

NPY Y_1 and Y_2 receptor levels in OBX relative to sham rats were determined using receptor autoradiography as previously described (Dumont et al., 1998a, 2005). Radiolabeling of [^{125}I][Leu^{31}Pro^{34}]PYY and [^{125}I]PYY3-36 was performed using

chloramine T method as previously described (Dumont et al., 1995, Dumont and Quirion, 2000). The specific activity was assumed to be of the theoretical value (2000 Ci/mmol).

Rats were sacrificed by decapitation on day 28 after either ablation of olfactory bulbs or sham surgery. Brains were removed from the skull, frozen in 2-methylbutane (-40°C) for 15 seconds and then kept at -80°C. Sections (16 µm) were obtained using a cryomicrotome at -17°C, mounted on super frost plus slides, dried overnight in a desiccator at 4°C and kept at -80°C until use. On the day of the experiment, adjacent coronal sections were pre-incubated for 60 min at room temperature in Krebs Ringer Phosphate (KRP) buffer at pH 7.4, followed by incubation in a fresh preparation of KRP buffer containing 0.1% BSA, 0.05% bacitracin, [^{125}I]PYY3-36 and [^{125}I][Leu^{31}Pro^{34}]PYY, in the presence or absence of 100 nM BIBO3304 or 1000 nM BIIE0246 to block Y_1 or Y_2 receptors, respectively (Dumont et al., 2004)). After an incubation period of 2h, sections were washed four times, 1 min each in ice-cold KRP buffer, and subsequently dipped in deionized water to remove salts followed by rapid drying. Nonspecific binding was determined using 1 µM [Leu^{31} Pro^{34}]PYY (Y_1), or 1 µM PYY3-36 (Y_2). Sections were placed alongside radioactive standards on Kodak Biomax MR films (Eastman Kodak, Rochester, NY) for 5 to 14 days and subsequently quantified (Dumont et al., 1998a).

2.6 Statistical analysis

Data from behavioral tests were analyzed by one-way ANOVA followed by Dunnett test for post hoc comparisons. Results from autoradiographic experiments were analyzed by two-way ANOVA followed by Bonferroni post-test. $P < 0.05$ was considered significant.

3. Results

OBX lesion and treatment with NPY related compounds had no effect on body weight during the four weeks of observation. Horizontal and vertical activities in the OF apparatus; immobility in the FST; and active social contacts in the SI paradigm were measured following sustained exposure to agonists and antagonists of Y_1 and Y_2 receptors using osmotic minipumps in OBX and sham rats. Moreover, the levels of Y_1 and Y_2 receptor binding were also investigated in OBX and sham animals.

Effect of sustained administration of agonists and antagonists of NPY Y_1 and Y_2 receptors in hyperlocomotion in open field in OBX rats

Chronic ICV administration of [Leu^{31}Pro^{34}]PYY significantly decreased hyperlocomotion ($F(5, 63) = 9.957$, $p < 0.0001$, 0.3 nmol, $p< 0.001$; 1.0 nmol, $p<0.05$) in OBX animals (Fig 2A). Long term infusion of BIBO3304, PYY3-36 or BIIE0246 did not modulate locomotion in OBX rats in the OF (Fig 2B, C & D). Moreover, chronic infusion of NPY Y_1 and Y_2 agonists and antagonists did not affect locomotion in sham animals (Fig. 2).

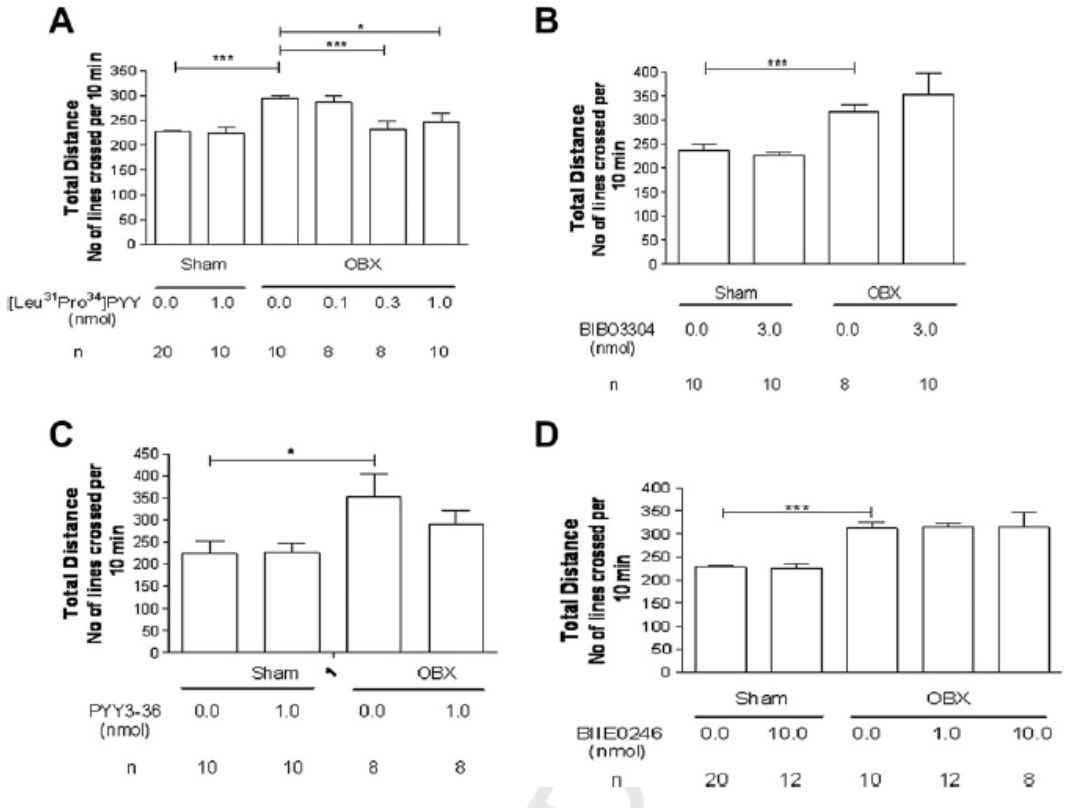
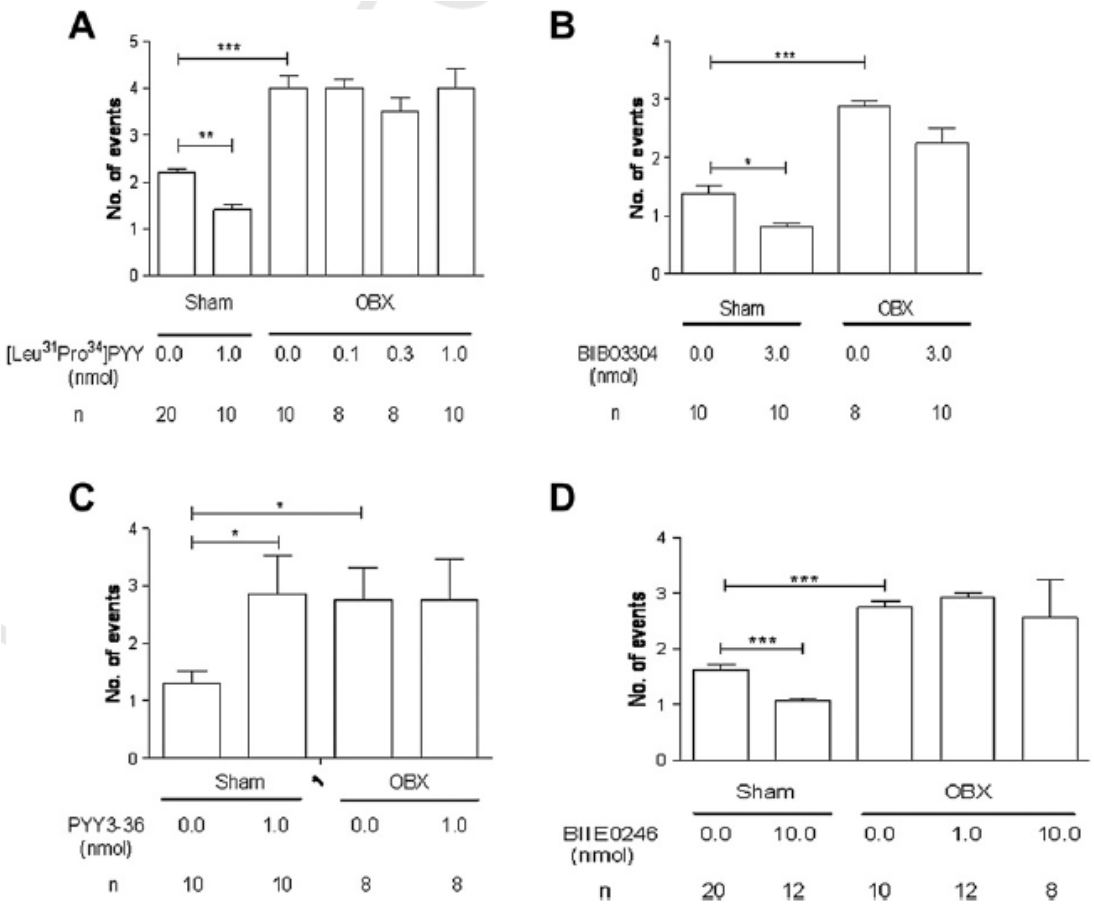


*Figure IV 2. Effect of sustained administration of agonists and antagonists of Y_1 and Y_2 receptors on locomotion in the open field test. Intracerebroventricular administration of [Leu^{31}Pro^{34}]PYY reduced hyperlocomotion observed in OBX animals. Results are expressed as mean ± S.E.M. (n=8-20 animals per group), * p<0.05, ** p<0.01, *** P<0.001.*

Effect of sustained infusion of agonists and antagonists of NPY Y_1 and Y_2 receptors on rearing and grooming in open field in OBX rats

Removal of olfactory bulbs consistently resulted in an increase in grooming and rearing events compared to sham animals (Figs 3 and 4, respectively). We observed that [Leu^{31}Pro^{34}]PYY (F(5, 66) =31, p < 0.0001, 1.0 nmol, p<0.01) (Fig 3A), BIBO3304 (F(3, 27) =62, p < 0.0001, 3.0 nmol, p<0.05) (Fig 3B) and BIIE0246 (F(4, 59) =53, p < 0.0001,

10 nmol p<0.001) (Fig 3D) decreased grooming activity, while PYY3-36 ($F(3, 34)=58$, $p < 0.01$, 1 nmol, p<0.05) (Fig 3C) increased this activity in sham animals in the OF. Neither of the NPY related molecules had an effect on grooming in OBX rats (Fig 3). On the other hand, BIIE0246 ($F(4, 59) =60.57$, $p < 0.0001$, 10.0 nmol, p<0.05) attenuated the enhanced rearing produced by OBX lesions (Fig 4D), while [Leu^{31}Pro^{34}]PYY (Fig 4A), BIBO3304 (Fig 4B) and PYY3-36 (Fig 4C) had no effect on rearing in either sham or



OBX rats.

Figure IV.3. Effect of sustained administration of agonists and antagonists of Y_1 and Y_2 receptors on grooming behavior in the open field test. Intracerebroventricular administration of [Leu^{31}Pro^{34}]PYY, BIIE0246 and BIBO3304 reduced grooming

*behavior in sham animals. PYY3-36 does not modulate grooming behavior in either sham or OBX animals. Results are expressed as mean ± S.E.M. (n=8-20 animals per group), * p<0.05, ** p<0.01, *** P<0.001.*

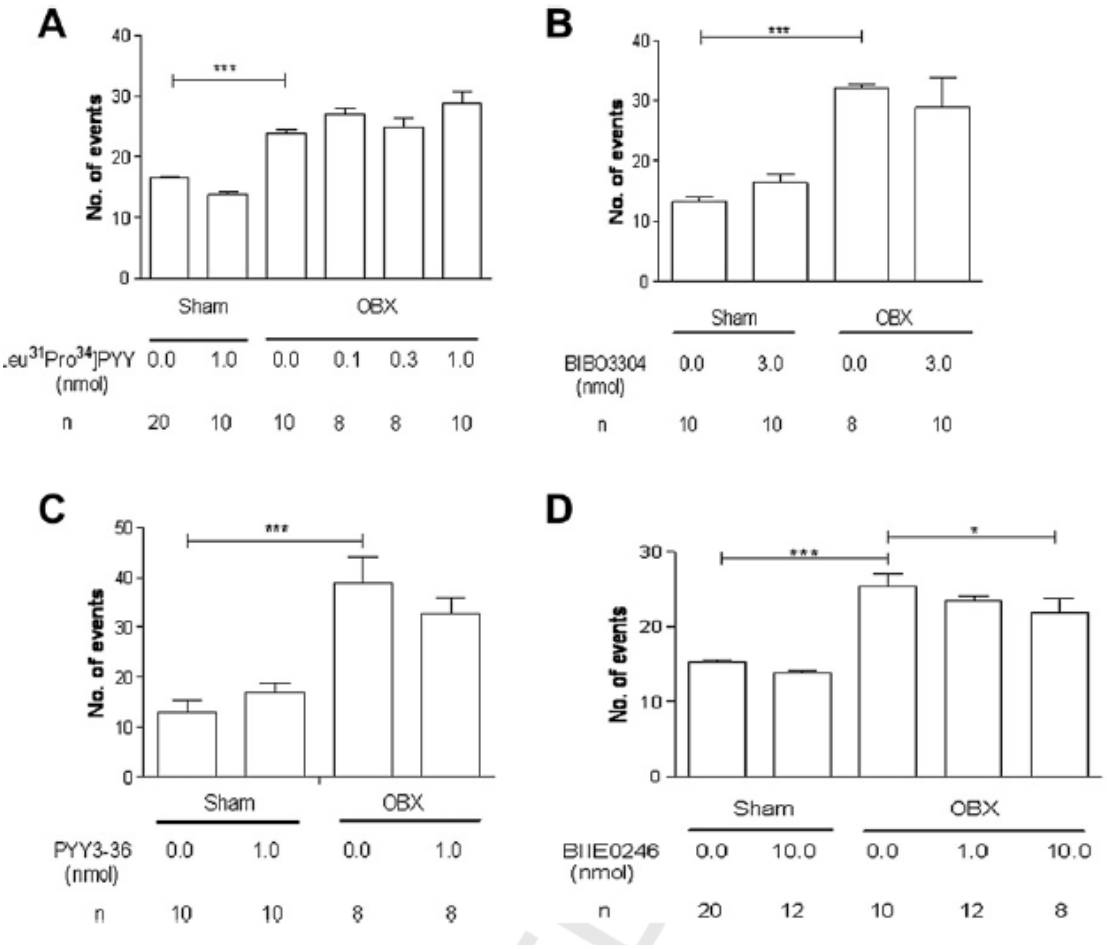


*Figure IV.4. Effect of sustained administration of agonists and antagonists of Y_1 and Y_2 receptors on rearing behavior in the open field test. Intracerebroventricular administration of BIIE0246 reduced rearing behavior in OBX rats. Neither [Leu31Pro34]PYY, BIBO3304 nor BIIE0246 has an effect on rearing behavior in sham or OBX rats. Results are expressed as mean ± S.E.M. (n=8-20 animals per group), * p<0.05, ** p<0.01, *** P<0.001.*

Effect of sustained administration of NPY Y_1 agonist and Y_2 antagonist on immobility in the forced swim test in OBX rats

As shown in Fig 5, ICV administration of [Leu^{31}Pro^{34}]PYY (F(5, 55) =78, p < 0.0001, 0.3-1.0 nmol, p<0.05; 3.0 nmol, p<0.01) (Fig 5A) and BIIE0246 (F(4, 51) =61, p < 0.0001, 1.0 nmol, p<0.05; 10.0 nmol, p<0.001) (Fig 5C) decreased immobility time in the FST in OBX rats. In contrast, PYY3-36 (F(3, 35) =65, p < 0.0001, 1.0 nmol, p<0.001) (Fig 5D) increased immobility in this paradigm while BIBO3304 had no effect (Fig. 5B) in the OBX rat. Furthermore, neither of the NPY related molecules tested here had an effect in the FST in sham animals (Fig. 5).

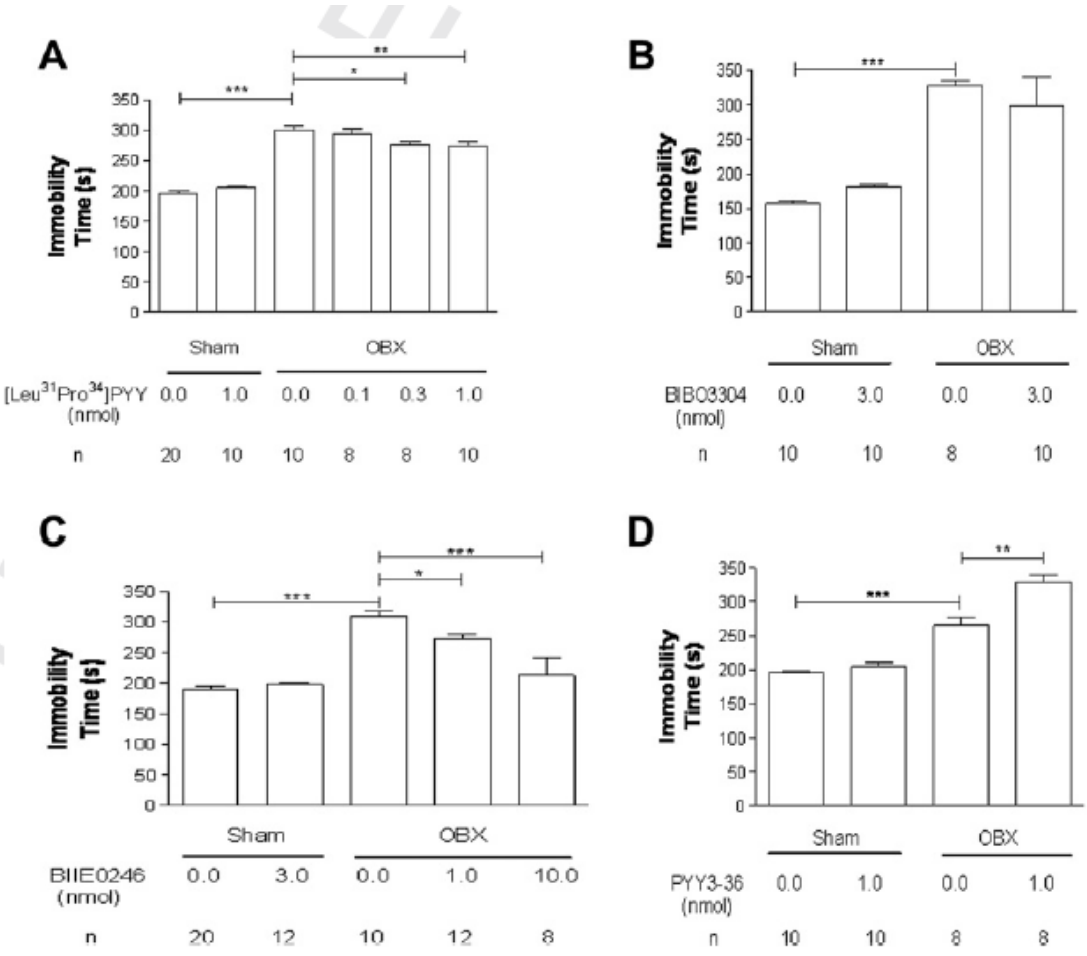


Figure IV.5. Effect of sustained administration of agonists and antagonists of Y_1 and Y_2 receptors in the forced swim test (FST). Intracerebroventricular administration of [Leu^{31}Pro^{34}]PYY and BIIE0246 decreases immobility time in the FST in the OBX rat. Results are expressed as mean ± S.E.M (n=8-20 animals per group), * $p<0.05$, ** $p<0.01$, *** $P<0.001$.

Role of sustained administration of NPY Y_1 agonist and Y_2 antagonist in active contacts in the SI test in OBX rats

In the SI test, OBX animals showed a deficit in social contacts (Fig. 6), which was reversed by a 2 week infusion of [Leu^{31}Pro^{34}]PYY ($F(5, 31) = 6.057$, $p=0.0008$, 0.3-1.0

nmol, $p<0.05$; 3.0 nmol, $p<0.01$) (Fig 6A). In contrast, treatment with BIBO3304 (Fig 6B), PYY3-36 (Fig 6C) or BIIE0246 (Fig 6D) had no effect on active social contacts in OBX rats. While, BIIE0246 ($F(4, 33) = 15.79$, $p < 0.0001$, 10 nmol; $p<0.05$) increased social contacts, [Leu^{31}, Pro^{34}]PYY, BIBO3304 and PYY3-36 had no effect in this test in sham animals (Fig 6).

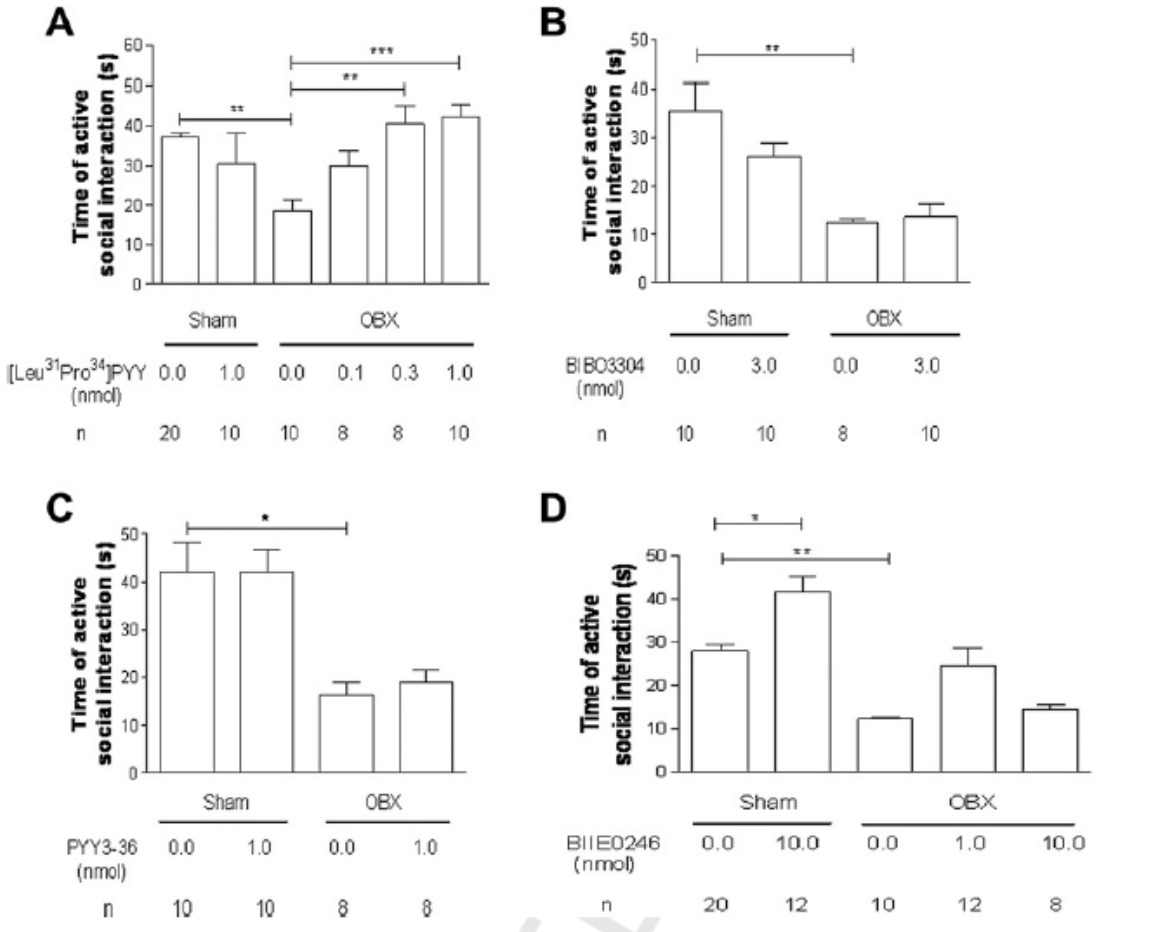


*Figure IV.6. Effect of sustained administration of agonists and antagonists of Y_1 and Y_2 receptors in the social interaction test. [Leu^{31}Pro^{34}] PYY consistently increases social contacts in OBX relative to vehicle treated animals. BIIE0246 increased social contacts in sham animals. Results are expressed as mean ± S.E.M. (n=8-20 animals per group), * p<0.05, ** p<0.01, *** P<0.001.*

OBX lesion alters the level of NPY Y_2 receptor binding in hippocampus and amygdala

The level of specific [^{125}I][Leu^{31}Pro^{34}]PYY binding in the presence or absence of BIBO3304 was not altered in OBX rats relative to sham animals (Fig 7A and B, respectively). After subtraction of the non-specific binding with BIIE0246, we observed an increase in specific [^{125}I]PYY3-36 binding in the CA1/CA2 subfield of the

hippocampus and amygdala in OBX rats (Fig 7C). A two-way ANOVA (OBX lesion * region) revealed that the levels of Y2 receptor sites were affected significantly by region (F7,40=119, P <0.0001), OBX lesion (F1, 40=6.17, P < 0.0172) as well as OBX lesion and region interaction (F7, 40 =6.13, P < 0.0001). In figures 7D and E, we show the autoradiographic sections of dorsal and ventral hippocampus treated with [^{125}I]PYY3-36, respectively.

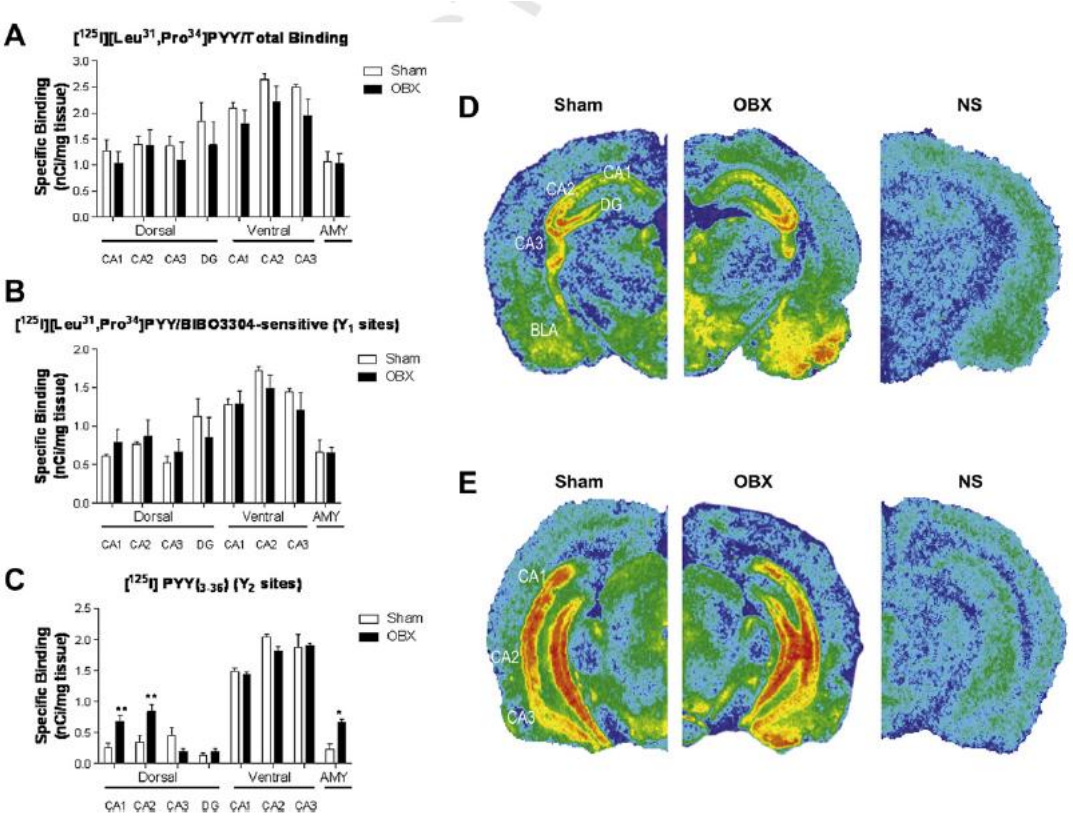


*Figure IV.7. Effect of OBX lesion on the expression of Y_1 and Y_2 receptor subtypes in the hippocampus and amygdala. Comparative quantitative receptor autoradiographic data show that ^{125}I[PYY3-36] binding (Y2 sites) is increased in dorsal hippocampus and amygdala in the OBX rat. Results are expressed as mean ± S.E.M. (n=4-5 animals per group), * p<0.05, ** p<0.01*

4. Discussion

The NPY system is certainly involved in emotional processes. Here, we further characterized the role of NPY Y_1 and Y_2 receptors in a well documented model of depression with co-morbid anxiety. Our results show that treatment with [$Leu^{31}Pro^{34}$]PYY, a Y_1-like agonist, reversed hyperlocomotion in the OF, decreased immobility in the FST and increased active interactions in the SIT in OBX rats. Treatment with BIIE0246, a Y_2 antagonist, decreased the immobility time observed in FST in OBX animals and increased active contacts in the SIT in sham animals. Additionally, autoradiographic receptor studies revealed increased levels of Y_2 receptor binding in the dorsal hippocampus and amygdala in OBX rats. Taken together, these data suggest that in the OBX rat exogenous administration of a Y_1 agonist reverses the deficits associated with depression- and anxiety- like behaviors while a Y_2 antagonist improves depression-like behaviors yet induces an anxiolytic effect under normal conditions (for summary see table 1).

Test	Y_1 agonist		Y_1 antagonist		Y_2 agonist		Y_2 antagonist	
	Sham	OBX	Sham	OBX	Sham	OBX	Sham	OBX
OF locomotion	=	+	=	=	=	=	=	=
OF rearing	=	=	=	=	=	=	=	+
OF grooming	+	=	+	=	=	=	+	=
FST	=	+	=	=	=	−	=	+
SI	=	+	=	=	=	=	+	

Table IV.1 Summary of comparative effects of i.c.v. administration of [$Leu_{31}Pro_{34}$] PYY, BIBO3304, PYY3-36 and BIIE0246 in three behavioral assays. (+) means a recovery effect of the phenotype and (-) indicates an augmentation of the behavioraldeficit. = indicates no change in the phenotype.

4.1 Relevance of a battery of behavioral tests in the OBX rat

We recently proposed the use of a large battery of behavioral tests in animal models of depression and anxiety to better characterize novel candidate molecules (Morales-Medina et al., 2010a). Various groups have used similar approaches in different animal models of depression-related behaviors, including the OBX animal model (Gregus et al., 2005; Kalynchuk et al., 2004; Pandey et al.; Rajkumar et al., 2009; Wang et al., 2007). Moreover, Mitra and Sapolsky (2008) showed that acute stressors applied only for one day did not induce long lasting behavioral changes. Therefore, performing a second behavioral test one or two days after the first test should have limited (if any) anxiogenic effect as evidenced by Wang et al., (2007).

4.2 The Y_1 *receptor subtype in depression and anxiety related behaviors*

We are the first to show that [Leu^{31}Pro^{34}]PYY at 1 nmol/day for 14 days reverses the hyperlocomotion in OBX rats while had no effect in sham animals. Hyperlocomotion seen in the OF is a common trait of the OBX animal model (Song and Leonard, 2005). Similarly, repeated exogenous ICV administration of the Y_1-like agonist, [Leu^{31}Pro^{34}]NPY has been shown to decrease hyperlocomotion in OBX rats (Goyal et al., 2009). [Leu^{31}Pro^{34}]PYY (1nmol/day) also decreased grooming behaviors in OF in sham animals in the current study. Abnormal rearing and grooming observed in the OF test could be due to aversive stimuli, strain differences as well as OBX lesions (Goyal et al., 2009; Kalueff and Tuohimaa, 2004; Kantor et al., 2000; Song et al., 1996; To et al., 1999). We also observed decreased rearing and grooming at a high dose of [Leu^{31}Pro^{34}]PYY (3 nmol), which also decreased locomotion in OF in sham animals, suggesting a sedative-like effect (data not shown). ICV administration of NPY (Song et

al., 1996) and [Leu^{31}Pro^{34}]NPY (Goyal et al., 2009) has also been reported to decrease grooming in OBX rats.

[Leu^{31}Pro^{34}]PYY significantly reversed, in a dose dependent manner, the deficits in SI test seen in OBX rats (as well). This test is broadly used to screen both anxiolytic and anxiogenic effects of environmental and physiological factors (File and Seth, 2003). In accordance with these results, the administration of NPY into the basolateral amygdala (Sajdyk et al., 1999) and the dorso-caudal lateral septum (Kask et al., 2001a) produced anxiolytic-like effects in the SIT in naïve normal animals. Taken together, chronic infusion of [Leu^{31}Pro^{34}]PYY display an anxiolytic as well as antidepressant effect in the OBX rat.

In contrast to [Leu^{31}Pro^{34}]PYY, treatment with BIBO3304 (3 nmol) only had limited effect in the behavioral tests used in the present study. In agreement with these results, neither BIBO3304 nor BIBP3226 (first generation Y_1 antagonist) had a behavioral effect in the OF or FST in control mice (Redrobe et al., 2002). However, Goyal et al. (2009) reported that BIBP3226 (0.1 nmol) enhanced hyperlocomotion in the OF in OBX rats. This result is rather surprising since BIBO3304 has a tenfold higher affinity for the Y_1 receptor compared to BIBP3226 (Hipskind et al., 1997). This effect may be associated with the well known neurotoxic properties of BIBP3226. Interestingly, in earlier reports the behavioral effects of NPY were abolished by a pre-treatment with BIBO3304 in the FST (Redrobe et al., 2002) and SIT (Sajdyk et al., 1999) in control animals, which further suggested a role for this receptor subtype in emotional processes. In the present study, we could not investigate the effect of a co-treatment with an agonist and an antagonist of the Y_1 receptor subtype with the tools currently available. Indeed,

both agonists and antagonists of NPY Y_1 and Y_2 receptor subtypes can hardly penetrate into the brain following a peripheral injection. Moreover, the solvent vehicle required for peptide agonists is distinct from that needed for antagonists. Therefore, their simultaneous use in an infusion pump is unlikely possible at present. It is also noteworthy to mention that only very scarce literature exists on the co-administration of an agonist and an antagonist for a given receptor in the same minipump (Chavez and LaManna, 2002; Liu and Wang, 2003; Zhang et al., 2007). Further studies using molecules that cross the blood brain barrier are required in the future but are not yet developed.

4.3 Y_2 *receptor subtype in depression and anxiety related behaviors*

In the present study, BIIE0246 reversed immobility time in the FST as well as grooming in the OF test in OBX rats. In addition, this antagonist decreased grooming and SI contacts in sham animals. The Y_2 receptor subtype modulates the release of NPY and other neurotransmitters (King et al., 1999; 2000). Since an antidepressant activity has been associated with elevated levels of NPY (Redrobe et al., 2003a) and a depressive phenotype with low levels (Heilig et al., 2004; Widerlov et al., 1988), the blockade of Y_2 receptors has been hypothesized as an indirect way to increase NPY levels and improve emotional processes (Morales-Medina et al., 2010b). In accordance with these data, germinal Y_2 KO mice show a strong antidepressant- and anxiolytic-related phenotype independent of sex, age or behavioral paradigm (Carvajal et al., 2006b; Painsipp et al., 2008a; Redrobe et al., 2003b; Tschenett et al., 2003). Acute BIIE0246 treatment also increased swimming time in the FST in naïve control rats (Redrobe et al., 2002).

In contrast, PYY3-36 increased the immobility time in the FST in OBX rat as well as grooming in OF in sham animals. We have shown previously that the acute

administration of the Y_2 agonist, NPY13-36, had no effect in the OF or FST in naïve animals (Redrobe et al., 2002). However, the Y_2 agonist, C2-NPY elicited an anxiogenic response in the basolateral amygdala in the SI test in control animals (Sajdyk et al., 2002b). While additional research is certainly warranted, these findings suggest that the Y_2 receptor subtype may play different roles under normal versus challenged conditions.

Recent data suggest a potential role for Y_4 (Painsipp et al., 2008b; Tasan et al., 2009) and Y_5 (Kask et al., 2001, Sorensen et al., 2004, Walker et al., 2009) receptor subtypes in emotional processes in naïve and KO animals. Therefore, future research should evaluate their possible role in various animal models of depression-related behaviors.

4.4 Effect of OBX lesion on the level of NPY Y_1 and Y_2 receptors

Since we observed changes in behaviors following treatments with [Leu^{31}Pro^{34}]PYY and BIIE0246 in OBX animals, we hypothesized that these differences could be associated with changes in the levels of NPY receptors. Indeed, specific Y_2 binding was significantly increased in hippocampus and amygdala. These regions were chosen since preliminary data from our group suggested that these two regions display disrupted neuronal dendritic arborization in the OBX rat (Morales-medina et al., 2010a). In apparent agreement with our data, Caberlotto and Hurd (2001) have previously observed increased Y_2 mRNA levels in depressive suicide completers. Additionally, earlier studies investigated the expression of NPY in the OBX rat and observed increased levels of prepro-NPY mRNA in the piriform cortex (PIR), hippocampus (Holmes et al., 1998) and amygdala (Rutkoski et al., 2002) after the removal of the olfactory bulbs. These findings suggest that decreased levels of NPY observed in the OBX rat may be

induced by the activation of negative presynaptic Y_2 autoreceptors regulating the subsequent release of this neuropeptide.

Indeed, the increased levels of Y_2 receptor binding in the hippocampus may account, at least partially, for the negative effect of the Y_2 agonist in the FST in OBX rat as well as the positive action of the Y_2 antagonist in the SI test in sham animals. The observed beneficial effects of the Y_1 agonist may therefore relate to the postsynaptic compensation of NPY signaling due to hyper-activation of Y_2 negative autoreceptors. Our results suggest that the changes in the level of expression of the Y_2 receptor in the hippocampus and amygdala may be linked with alterations in NPY levels in the OBX rat.

Conclusion

In summary, the present study explored the possible contribution of the Y_1 and Y_2 receptor subtypes in depression- and anxiety- related behaviors in the OBX rat. Our data extend earlier studies by suggesting that the NPYergic system is disrupted in depression and anxiety -related processes with a likely differential role for the Y_1 and Y_2 subtypes.

5. Acknowledgements

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Figure IV.8 (supplementary). Effect of sustained administration of Y_2 antagonist JNJ31020028 in various behavioral tests in the OBX rat. This molecule did not modulate locomotion (A) but decreases rearing (B) and grooming (C) in the open field tests. In addition this Y_2 antagonist reversed the immobility time in the forced swim test with no effect on the social interaction test. Results are expressed as mean ± S.E.M. (n=10-20 animals per group), * $p<0.05$

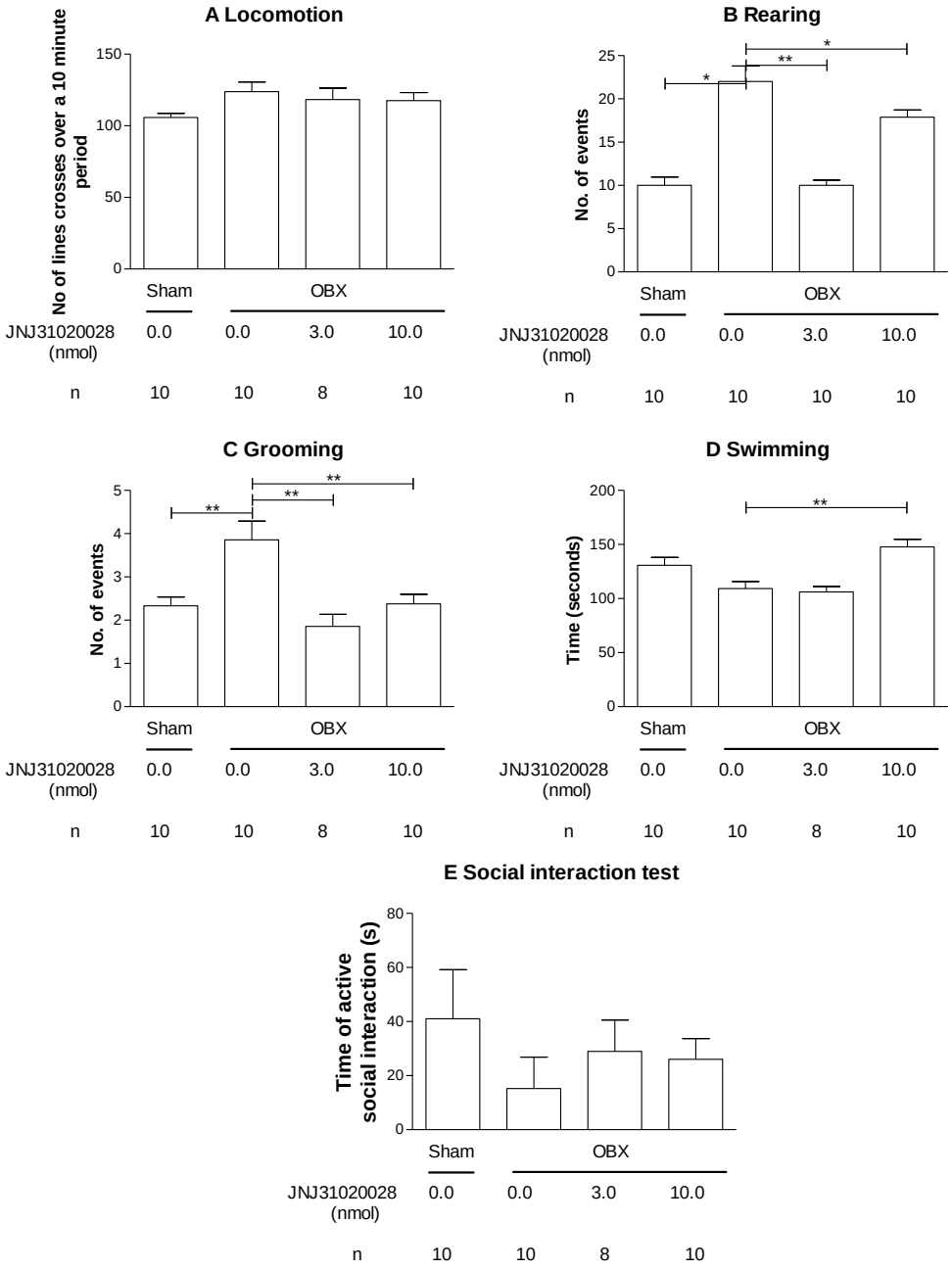


Figure IV.9 (supplementary). Effect of sustained administration of a Y_1 agonist on adult cell proliferation in the OBX rat. Results are expressed as mean ± S.E.M. (n=4-5 animals per group per triplicate), * $p<0.05$

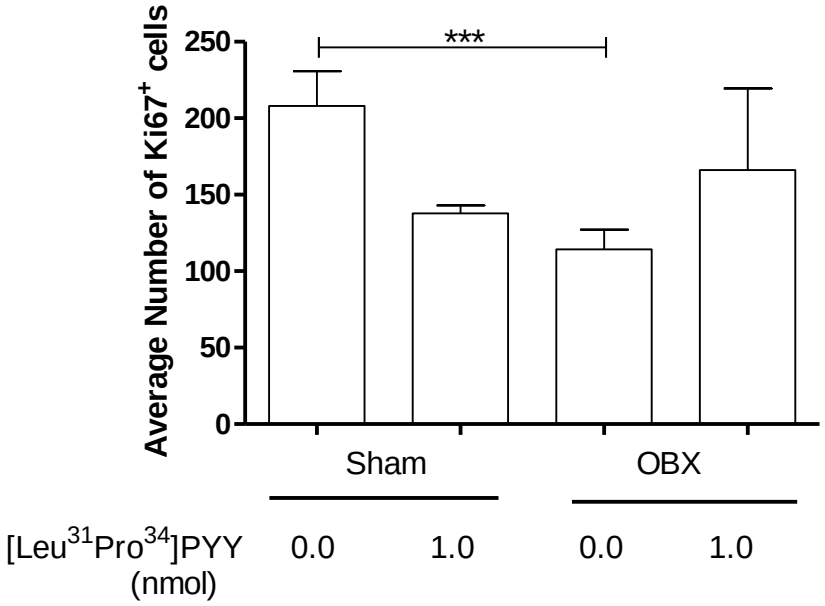


Figure IV.10 (supplementary). Effect of sustained administration of a Y_1 agonist on adult cell survival in the OBX rat. Results are expressed as mean ± S.E.M. (n=4-5 animals per group per triplicate), * $p<0.05$, ** $p<0.01$

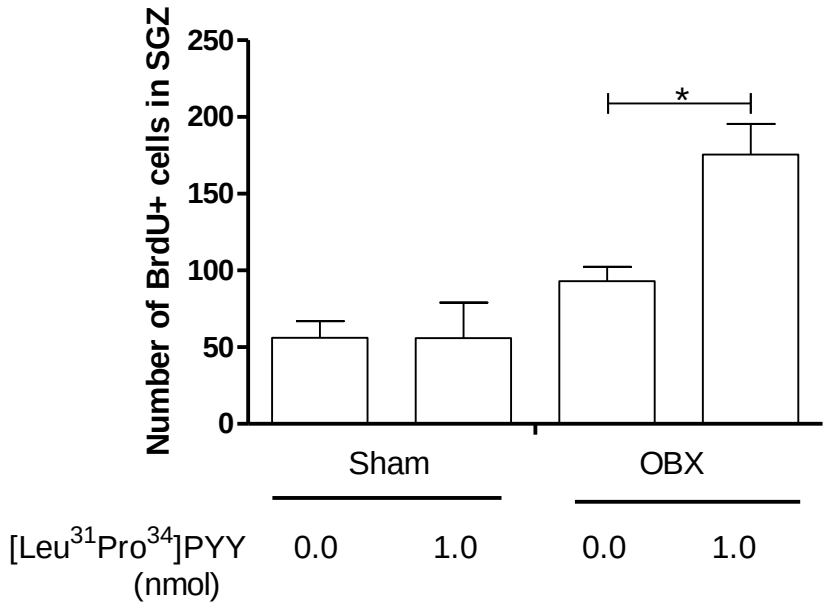


Figure IV.11 (supplementary). Effect of sustained administration of Y_1 agonist in the corticosterone-induced anxiety model. The Y_1 agonist did not modulate entries in open arm (A), percentage of entries in open field (B) or entries in close arm in the elevated plus maze. Results are expressed as mean ± S.E.M. (n=11-19 animals per group), * $p<0.05$

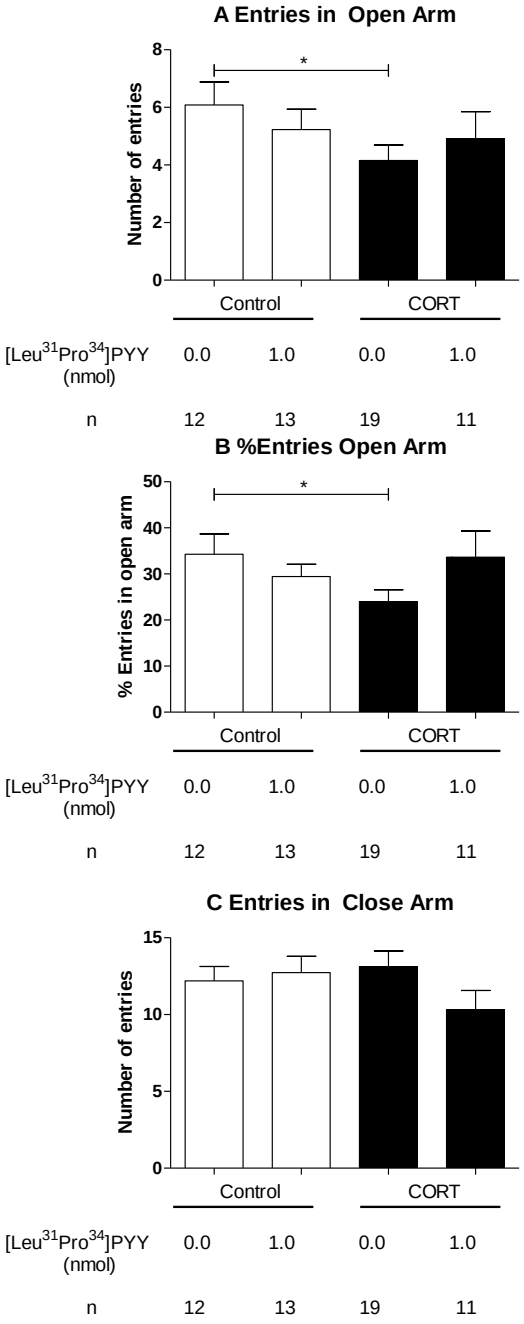


Figure IV.12 (supplementary). Effect of sustained administration of Y_2 agonist in the corticosterone-induced anxiety model. The Y_2 agonist decreased the number of entries in the open arm of the elevated plus maze (EPM). However, this molecule did not affect the percentage of entries in open field (B) or entries in close arm (C) of the EPM. Results are expressed as mean ± S.E.M. (n=10-15 animals per group), * $p<0.05$

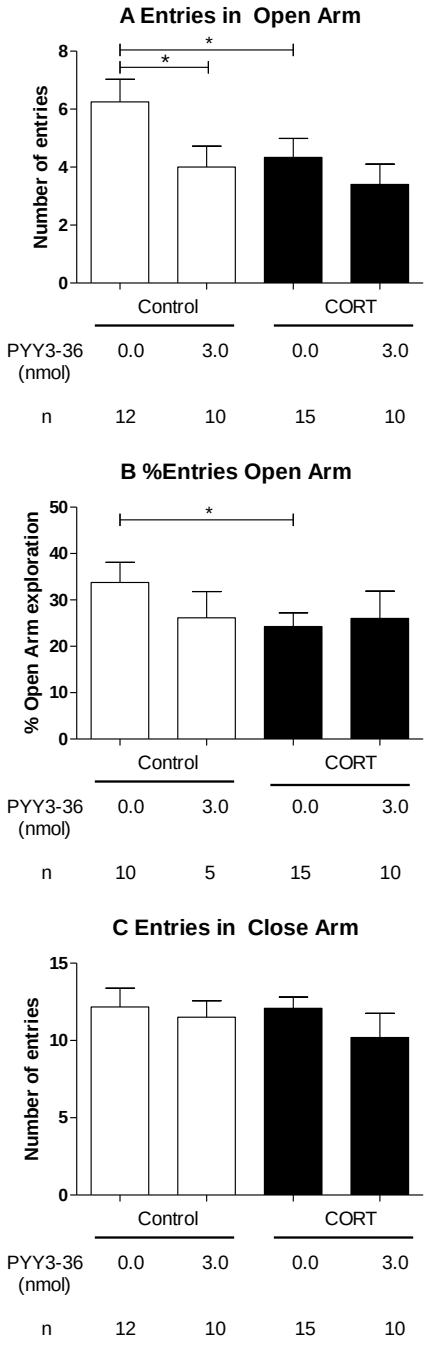
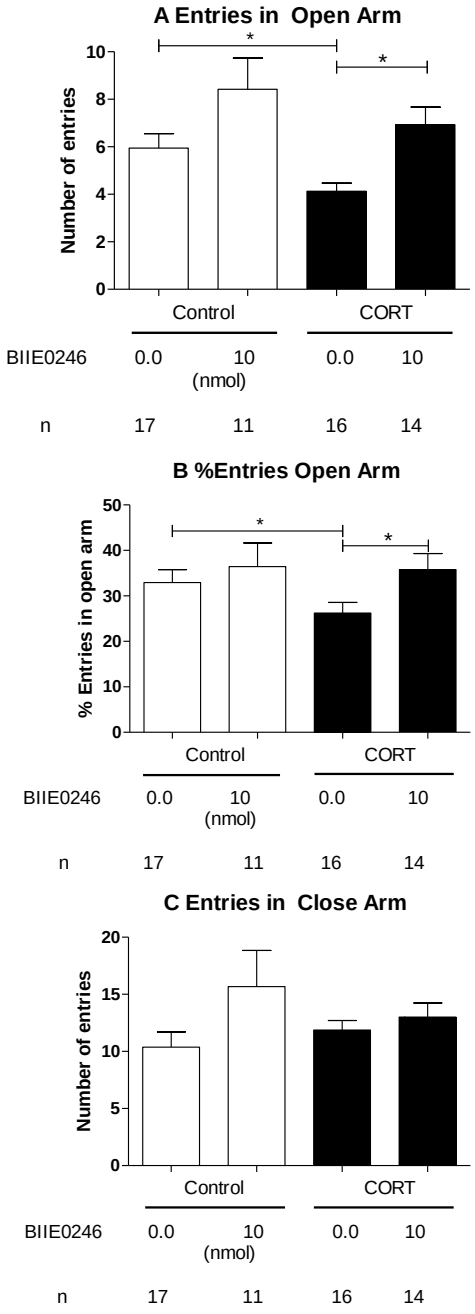


Figure IV.13 (supplementary). Effect of sustained administration of Y_2 antagonist in the corticosterone-induced anxiety model. This antagonist increased the number of entries in the open arm (A) as well as the percentage of entries (B) but not the number of entries in the close arm of the elevated plus maze (C). Results are expressed as mean ± S.E.M. (n=11-17 animals per group), * $p<0.05$



Chapter V

The selective neuropeptide Y Y_5 agonist [cPP$^{1–7}$,NPY$^{19–23}$,Ala^{31},Aib^{32},Gln^{34}]hPP differently modulates emotional processes and body weight in the rat

5.1 Preface

The NPY system plays a key role in emotional processes, however the contribution of each receptor subtype is not fully understood. The possible role of NPY Y_1 and Y_2 receptor subtypes was evaluated in emotional dysfunctional conditions as described in the chapter three of this thesis.

The Y_5 receptor subtype has been largely known as the "feeding receptor" (Marsh et al., 1998). Anorexic properties have been associated with Y_5 antagonists, such as CGP71683A in naïve as well as obese animals (Kask et al., 2001) and MK-0557 after a long term treatment in humans (Erondu et al., 2006). In contrast, food intake is enhanced after the administration of the NPY Y_5 agonist [cPP^{1-7}, NPY^{19-23}, Ala^{31}, Aib^{32}, Gln^{34}]hPP in rats (Cabrele et al., 2000). The Y_5 receptor subtype is apparently only expressed in low levels in hypothalamic nuclei, lateral septum, locus coeruleus, brainstem and amygdala at least in the rat brain (Dumont et al., 2000). Recent data also suggested that this receptor subtype may be involved in mood-related behaviors in animal models (Kask et al., 2001, Sajdyk et al., 2002a, Sorensen et al., 2004, Walker et al., 2009). However, these latter findings are still rather controversial.

Thus in the present chapter, we investigated the effects of the administration of a selective Y_5 agonist, [$cPP^{1–7}$,$NPY^{19–23}$,Ala^{31},Aib^{32},Gln^{34}]hPP, on body weight as well as on anxiety- and depression-related behaviors, using the two animal models described in chapters 2 and 3 of this thesis.

5.2 Manuscript

The selective neuropeptide Y Y_5 agonist [cPP$^{1–7}$,NPY$^{19–23}$,Ala^{31},Aib^{32},Gln^{34}]hPP differently modulates emotional processes and body weight in the rat

JC Morales-Medina[a,b], S Dominguez-Lopez[a], G Gobbi[c], AG Beck-Sickinger[d] and R Quirion[b,c*]

[a]*Dept. of Neurology & Neurosurgery,* [b]*Douglas Mental Health University Institute*

[c]*Dept. of Psychiatry, McGill University, Montréal, QC, Canada, H4H 1R3*

[d] *Institute of Biochemistry, University of Leipzig, Leipzig, Germany, D 04103*

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Summary

The neuropeptide Y (NPY) has been suggested to act as a major regulator of emotional processes and body weight. The full spectrum of biological effects of this peptide is mediated by at least four classes of receptors known as the Y_1, Y_2, Y_4, and Y_5 subtypes. However, the respective contribution of each of these receptor subtypes, especially the Y_5 subtype, in emotional processes is still mostly unknown. In the present study, we investigated the effect of long term administration of a selective Y_5 agonist [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP on emotional processes and body weight using two rat models of emotional dysfunctions, the corticosterone (CORT)-induced anxiety model as well as the olfactory bulbectomized (OBX) model of depression and anxiety in Wistar and Sprague-Dawley rats, respectively. The administration of the Y_5 agonist reversed the high levels of locomotion, rearing and grooming in the open field test and the impaired social activity induced by OBX, while increased the percentage of entries and time in the open arm of the elevated plus maze in CORT-treated rats. Furthermore, this Y_5 agonist increased body weight in both strains of naïve, control rats. These data further demonstrate that Y_5 receptors are not only involved in the control of body weight but also mediate anxiolytic- and antidepressant-like effects under challenged conditions. Thus, the pharmacotherapeutic administration of a Y_5 agonist could be considered as a potentially novel strategy to alleviate some forms of anxiety and depression in humans.

Keywords: anxiety, corticosterone, depression, neuropeptide Y, olfactory bulbectomy.

Introduction

Anxiety comprises multiple disorders with an excessive social burden and a very high prevalence (Kertz and Woodruff-Borden, 2011). These disorders also present high comorbidity with other emotional and physical disabilities including depression (McLean et al., 2011; Roy-Byrne et al., 2008). Current treatments of choice include various benzodiazepines but their long term administration induces several side effects including cognitive deficits as well as the development of tolerance (Rudolph and Knoflach, 2011). Thus, the search for novel approaches for the treatment of anxiety-related disorders is one of the foremost challenges in mental health research.

The neuropeptide Y (NPY), an abundant peptide expressed in various regions of the central nervous system (CNS), is known to play various roles in emotional processing (Morales-Medina et al., 2010; Morales-Medina and Quirion, 2011). Cumulative evidence has shown that the NPY levels are decreased in animal models of depression-like behaviors (Caberlotto and Hurd, 1999; Jimenez-Vasquez et al., 2001; Zambello et al., 2010) and in concordance, the exogenous administration of NPY exerts anxiolytic- and antidepressant- like behaviors in naive rodents (Heilig et al., 1989; Redrobe et al., 2005; Stogner and Holmes, 2000). Furthermore, numerous studies have reported the presence of lower levels of NPY in depressive subjects (Heilig *et al.*, 2004; Hou *et al.*, 2006; Olsson *et al.*, 2004) and a negative correlation has been shown between NPY gene expression and emotional processing in humans (Heilig *et al.*, 2004; Sjoholm *et al.*, 2009; Zhou *et al.*, 2008). These data support the significance of the NPY system in emotionally dysfunctional conditions in humans.

The biological effects of this peptide are mediated by the activation of at least four classes of receptors known as the Y_1, Y_2, Y_4 and Y_5 subtypes (Michel *et al.*, 1998). Most of the actions of NPY in the CNS have been linked to the activation of the broadly expressed Y_1 and Y_2 subtypes (Dumont et al., 2009; Morales-Medina and Quirion, 2011). However, comparatively little information is available on the role of Y_5 receptor subtype in the brain and Dumont et al., (1998b) have reported low levels of this receptor in discrete brain regions. However, recent data suggest a broader distribution in areas such as the olfactory bulbs, hippocampus, amygdala and hypothalamus (Campbell *et al.*, 2001; Quarta *et al.*, 2011).

Early on, Cabrele et al., (2000) reported that the Y_5 agonist, [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP, had minimum three times higher affinity than the natural ligand (NPY) for the Y_5 receptor subtype and a significant selectivity for this receptor versus other NPY receptor subtypes *in vitro*. In this regard, this compound had an IC50 of 0.24 for Y_5 receptors compared to >500 for Y_1 and Y_2 receptors *in vitro* (Cabrele et al., 2000). Lecklin et al., (2003) also observed that this compound had a Ki value of 1.32 nmol for Y_5 receptors compared to 85 nmol for Y_1 receptors *in vitro* in guinea pigs. Acute ICV administration of this Y_5 agonist increased food intake in control rats. This agonist also showed a strong modulatory role in hippocampal excitatory transmission (Guo *et al.*, 2002). Thus, this modified hPP analog clearly represents a suitable tool to investigate further the role of the Y_5 receptor *in vivo*.

Recent data suggested that the Y_5 receptors are involved in anxiety and sedation (Sajdyk *et al.*, 2002; Sorensen *et al.*, 2004). However, these earlier studies were performed using only a single dose of peptides in control naive animals and the selective

agonist mentioned above has never been investigated in animal models of emotionally dysfunctional conditions. Hence, here we studied the effects of a long term administration of this Y_5 agonist on anxiety- and depression-like behaviors in two well studied rat models, namely the corticosterone (CORT)-induced anxiety model (Mitra and Sapolsky, 2008) and the olfactory bulbectomized (OBX) lesion (Kelly et al., 1997) since OBX induces a variety of emotional disturbances resembling symptoms of anxiety and depression in rat (Morales-Medina et al., 2012; Wang et al., 2007). We also evaluated the effect of this modified peptide on the body weight of these animals.

2.0 Material and methods

2.1 Animals

Male Wistar rats and Sprague Dawley rats weighing 150-170g (Charles River Canada, Montréal, QC, Canada) at the beginning of the treatment were housed two per cage and maintained on a 12 h light/dark cycle (lights on at 8:00 AM) with *ad libitum* access to food (Purina Lab Chow) and water. The animals were weighed once a week until behavioral tests and constantly monitored to ensure their health.

All procedures were approved by the McGill Animal Care Committee and according to the guidelines of the Canadian Council on Animal Care and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2 Corticosterone administration

Wistar rats were administered with a single injection of 10 mg/mL/Kg of CORT (subcutaneously, s.c., Sigma-Aldrich, Montreal, Canada) as previously described (Mitra

and Sapolsky, 2008). CORT was dissolved in peanut oil as vehicle on the day of injection. Control animals were administered with the same volume of vehicle. The dose of CORT injected resembles several hours of physiological stress (Stein-Behrens et al., 1994). The behavioral tests were performed 12 days after the injection of CORT or vehicle as previously reported (Mitra and Sapolsky, 2008).

2.3 OBX surgery

Bilateral olfactory ablation was performed in Sprague-Dawley rats as described earlier (Hasegawa et al., 2005; Morales-Medina et al., 2012). Briefly, 5% isoflurane (Baxter, Mississauga, ON, Canada) was used to induce anesthesia and subsequently maintained at 2.5% during the surgical procedure. A cranial window 5.2 mm anterior to the bregma was created in the frontal bone and the olfactory bulbs were cut and aspirated out. Sham operations were performed in the same manner but the bulbs were left intact. Prevention of blood loss from the cranial window was achieved by filling the open space with a haemostatic sponge (Gelfoam, Pfizer Canada Inc., Montréal, QC). Following surgery, rats were administered with carprofen (0.1 mL/100g) (Pfizer AnimalHealth, Montréal, QC) and saline solution (0.9% NaCl) (Hospira Healthcare Corporation, Montréal, QC) and were placed in pairs in their respective cages to recover for two weeks. Only data from animals with complete removal of olfactory bulbs and no damage to the frontal cortex (determined by examination following brain removal) were included in the analysis.

2.4 Osmotic minipump implantation

An osmotic minipump (Alzet, model 2002, Durect Cupertino, CA, USA) connected to an indwelling ICV cannula was implanted to deliver the Y_5 agonist, [cPP^{1-7}, NPY^{19-23},

Ala[31], Aib[32], Gln[34]]hPP or vehicle (0.9% NaCl) at a flow rate of 0.5 µl/hr for 12 days in CORT and 14 days in OBX rats (Morales-Medina et al., 2012; Tanaka et al., 2005). The pumps were implanted one day after the administration of CORT or vehicle in the CORT-induced anxiety model and two weeks after the OBX or sham lesion. This treatment delivers 1nmol/day in CORT-treated and OBX rats. Pumps were filled a day prior to surgery and incubated at 37°C overnight in a sterile saline solution for priming**.** The peptide [cPP[1-7], NPY[19-23], Ala[31], Aib[32], Gln[34]]hPP was synthesized as described previously with some modifications. In brief, it was obtained by automated multiple-solid-phase peptide synthesis on a Syro II peptide synthesizer (MultiSynTech, Bochum, Germany) by Fmoc/tBu-strategy using Rink amide resin (resin loading = 0,045 mmol/g) to yield C-terminally amidated peptides (Cabrele *et al.*, 2000; Findeisen *et al.*, 2011). Side chain protecting groups included tBu (Ser, Tyr, Asp, Glu), Trt (Asn, Gln, and His), Pbf (Arg) and Boc (Lys). The peptide was purified to homogeneity (>95 %) by preparative RP-HPLC (Phenomenex Jupiter Proteo C-18 column, 22 mm × 250 mm, 4 µm/90Å). The identity of the peptide was verified by MALDI-ToF mass spectrometry (Ultraflex III MALDI-ToF/ToF, Bruker Daltonics).

For osmotic pump implantation, rats were anesthetized with 5% isoflurane to induce anesthesia and subsequently maintained at 2.5% during the extent of the surgical procedure. In brief, animals were placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) and a cannula was implanted into the left lateral ventricle (anterior-posterior: -0.8 mm from bregma, lateral: -1.3 mm from bregma, and vertical: -4.5 mm from dura) as previously reported (Morales-Medina et al., 2012). The cannula was sealed with dental cement and connected to an osmotic pump by medical grade vinyl

tubing. The pump was placed into a subcutaneous pocket in the dorsal region. Animals from the same treatment and condition group were left in pairs until behavioral testing. The lateral ventricles as well as the dentate gyrus were examined for any possible changes in those areas as a result of the long term infusion after behavioral test were finished. No differences were seen between the ipsilateral and the contralateral ventricles or dentate gyri (data not shown).

2.5 Behavioral tests

Behavioral tests included the elevated plus maze (EPM) for the CORT-treated rats and the open field test (OFT), the forced swim test (FST) and the social interaction test (SIT) for OBX rats. The EPM was conducted on CORT treated rats and its controls 12 days after the treatment as previously described (Mitra and Sapolsky, 2008). In addition, a series of three behavioral tests was performed on three consecutive days in the OBX rat as reported earlier (Morales-Medina et al., 2012). The OFT test was carried out on day 27, the FST was performed on day 28, whereas the SIT was done on day 29 post OBX or sham surgeries. All three behavioral tests were carried out during the light phase of the light–dark cycle (9:00-13:00). Rats were kept in the same housing conditions throughout all tests. Eight to twenty animals per group were assessed.

2.5.1 Elevated plus maze

The EPM apparatus consisted of a maze with two open arms (50×10 cm) opposite each other crossed by two walled (closed) arms (50×10×40 cm) raised 80 cm above ground with central area (10×10 cm) forming the intersection of the four arms. The rats were placed in the central area facing one of the open arms. The number of entries, the time, the percentage of entries and time in each of the arms were analyzed as well as the

distance traveled in the close arm for a ten minute period. All sessions were video recorded and analyzed off-line using the Videotrack system (Viewpoint Life Science, Montreal, Quebec, Canada) via an automatic differential movement analysis. Behaviors were determined as anxiogenic when the number of entries, the percentage of entries and/or the percentage of time in the open arms is decreased compared to control animals (Mitra and Sapolsky, 2008; Pellow et al., 1985). The percentage of entries in open arms was evaluated as [# of entries in open arm/(# entries in open arm + # entries in close arm)]. The percentage of time in open arms was evaluated as [time in open arm/(time in open arm + time in close arm)]. The locomotion was also evaluated with the number of entries, distance traveled and time spent in the closed arms of the apparatus as previously described (Pellow *et al.*, 1985; Redrobe *et al.*, 2003).

2.5.2 Open field test

The OFT is commonly used to measure anxiety-related behavior. Particularly, the hyperlocomotion in the OBX rat in this test is associated with the failure to adapt to novel environment (Kelly et al., 1997), but no changes in anxiety since there is no difference in the time that the animals stay on the center of the arena between OBX and sham animals (Mar et al., 2002). This test was performed under bright light conditions in a large arena of an OFT apparatus (100x100x45 cm) made of a black wooden box with a grey floor and no top lid and the field was divided into 64 equal-size squares. The behavior of animals was recorded for a 10 minute period including locomotion during the first exposure (horizontal behavior) and frequency of rearing and grooming (vertical behaviors) by an observer blind to the treatment. The testing apparatus was cleaned with peroxigard solution (Bayer Healthcare, Toronto, ON) after each trial. Behavior was

determined as depression-related by hyperlocomotion (Song and Leonard, 2005) as well as enhanced rearing and grooming which is associated with stress coping behaviors (Kalueff and Tuohimaa, 2004; Song et al., 1996) observed in OBX animals and its reversal by repeated antidepressant treatment.

2.5.3 Forced swim test

FST is a well characterized tool to screen potential antidepressants (Lucki, 1997; Porsolt *et al.*, 1977). In the present study, we used a modified version of the test exposing the animals to the FST only once (Gregus *et al.*, 2005; Kalynchuk *et al.*, 2004; Redrobe *et al.*, 2002). This behavioral test was performed in a white cylindrical tank (29 cm diameter X 43 cm height) filled with water (25°C ± 2°C) and no top. Rats were placed in the swimming tank for a 10 minute period. A camera was mounted 1m above the tank and immobility was evaluated by an observer blind to the experimental conditions. Immobility time was recorded when animal makes minimum movements necessary to keep its body afloat. Depression-related behavior was inferred from increase in the time spent immobile to escape from water, as a lack of motivation to escape from the water (Cryan et al., 2005). Following the test, rats were removed from the cylinder, cleaned with a towel and placed under a red lamp until the fur was dried.

2.5.4 Social interaction test

SIT has been pharmacologically validated as an experimental paradigm to measure anxiety (File, 1980; File and Seth, 2003). This test was performed in a familiar situation (apparatus used was the same as for the OFT) under bright light. A pair of rats was placed in opposing corners of OF apparatus and was allowed to explore the arena for 10 minutes. This pair of animals belongs to the same experimental group but the animals

were not cage mates. Active behavior was considered when animals were running towards, grooming, mounting, and crawling under the other rat. Each pair of rats tested had previously undergone the same treatment but not exposed previously. The OFT apparatus was cleaned with peroxigard after each trial. The total duration of active contacts and number of contacts were measured for each pair of animals by a person blind to their treatment. An anxiogenic behavior was inferred from the reduced social contacts in the treated animals.

2.6 Statistical analysis

. Body weight results and data from behavioral tests were analyzed by a two-way ANOVA followed by Bonferroni post hoc test. p values < 0.05 were considered significant.

3.0 Results

In this study, we investigated the effect of continuous ICV administration of a Y_5 agonist on anxiety- and depression-related behaviors. Body weight was also monitored.

Effect of continuous administration of a Y_5 agonist on horizontal and vertical behaviors in open field in OBX rats.

The removal of the olfactory bulbs induced an increase in the number of square crossing, grooming and rearing events in the OFT compared to sham animals (Figs 1A, 1B and 1C, respectively). Treatment with the Y_5 agonist decreased square crossing (OBX $F(1,42)=13.15$, $p= 0.0008$, drug $F(1,42)=9.86$, $p=0.0031$, interaction $F(1,42)=3.06$, $p=0.0876$, Fig 1A), reduced rearing $F(4,62)=2.3$, $p < 0.05$, (Fig 1B) and decreased grooming $F(4.50)=3.77$, $p < 0.05$, (Fig 1C) in this paradigm in OBX rats. This agonist had no effect on these behaviors in sham control animals.

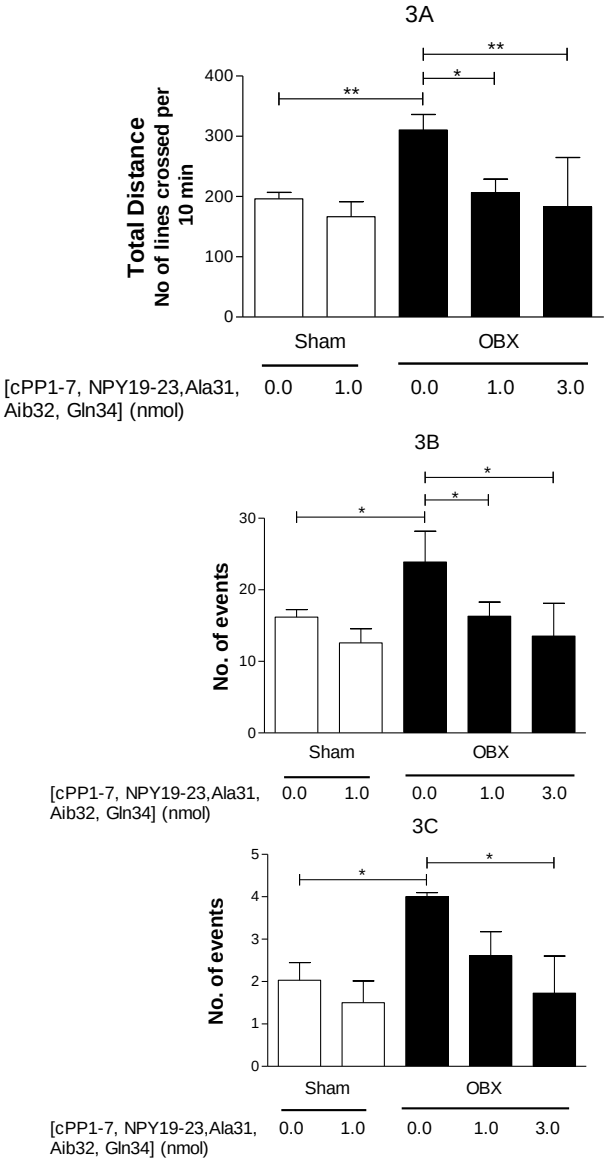


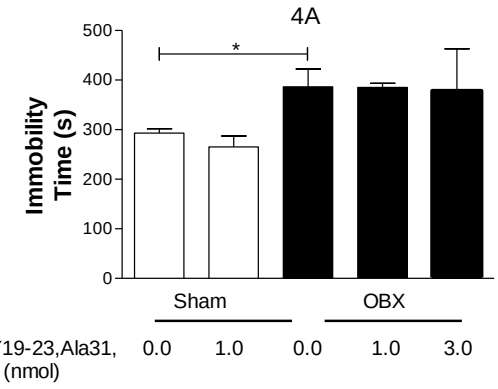
Figure 1. Sustained administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP decreased vertical and horizontal behaviors in the open field test (OFT) in the olfactory bulbectomized (OBX) rat. Administration of this compound

*decreased the number of square crossing (Fig 1A), the number of grooming (Fig 1B) and the number of rearing (Fig 1C) in the OBX rat. n=10-14 rats per group, results are expressed as mean ± S.E.M. * p<0.05, ** p<0.01.*

Effect of continuous administration of a Y_5 agonist on immobility in the forced swim test in OBX rats.

In OBX rats, an increase in immobility time was observed in the FST compared to sham animals (Fig 2). The treatment with this Y_5 agonist did not modify immobility time in either control or OBX rats (Fig 2).

Figure 2. Sustained administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP did not modulate immobility activity in the forced swim test in the olfactory bulbectomized rat. n=10-14 rats per group, results are expressed as mean ± S.E.M.



Role of continuous administration of a Y_5 agonist on active contacts in the SI test in OBX rats.

As shown in Fig 3, treatment with [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP significantly increased the number of active interactions in OBX rats $F(4,33)=4.260$, $p < 0.01$, but had no effect in sham animals.

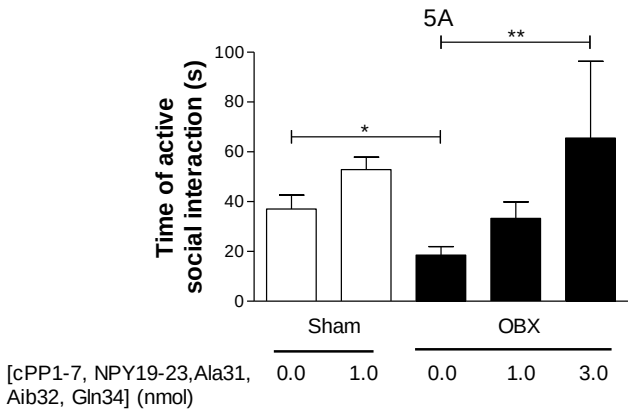
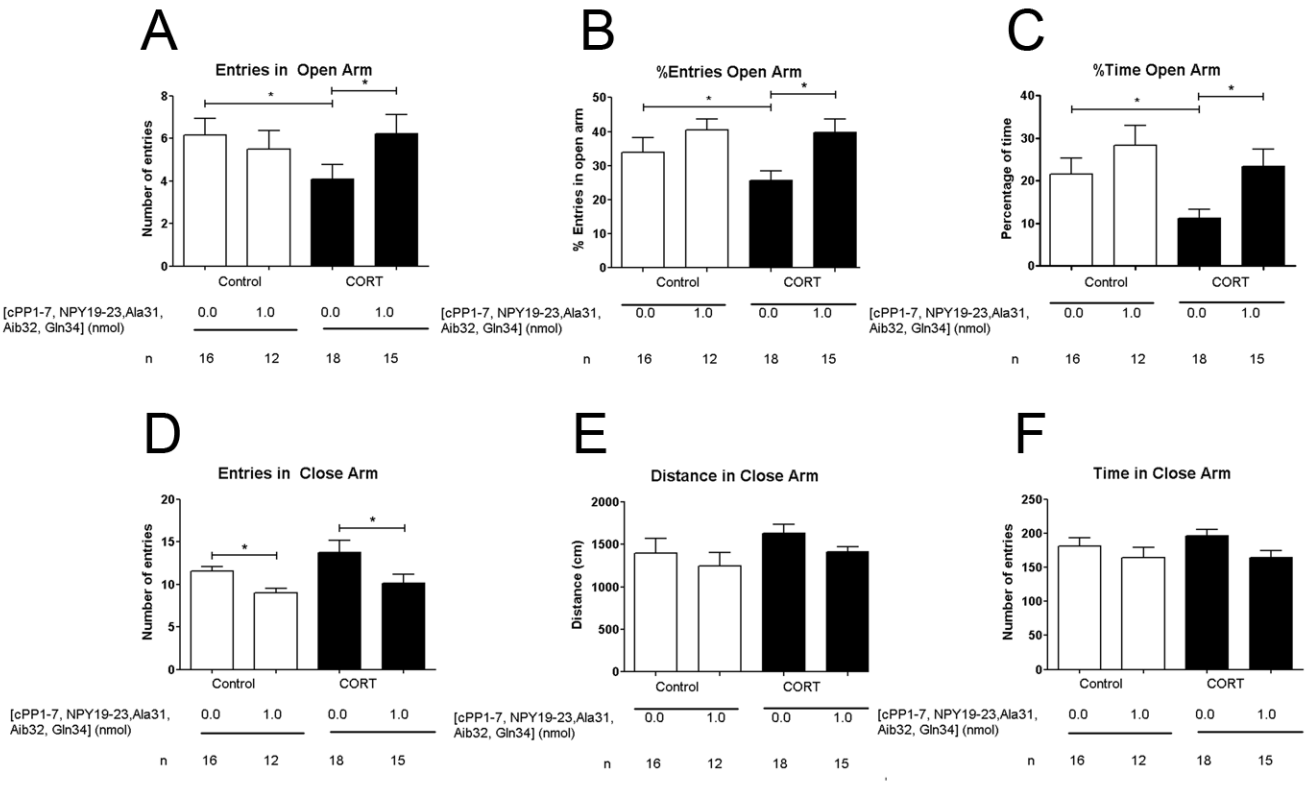


*Figure 3. Sustained administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP increased active social contacts in the social interaction test (SIT) in the olfactory bulbectomized rat. This Y_5 agonist increased the sociability in the SIT only selectively in the OBX rat. n=10-14 rats per group, results are expressed as mean ± S.E.M., * p<0.05, ** p<0.01.*

Role of continuous administration of the Y_5 agonist in the elevated plus maze in CORT-treated rats

As shown in Fig 4, treatment with [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP increased the number of entries $F(3, 58)=3.726$, $p< 0.05$ (Fig 4A), the percentage of entries $F(3,49)=3.552$, $p< 0.05$, (Fig4B) as well as the percentage of time $F(1, 55)=6.750$ $p=0.0120$ (Fig 4C) in the open arm. In addition, the number of entries $F(3, 45)=4.332$, $p< 0.05$, (Fig 4D) in the closed arm of the EPM was decreased in CORT-treated rats. However, neither the distance traveled (Fig 4E) or the time spent (Fig 4F) in the close arm was affected by either CORT-treatment or Y_5 agonist administration



Effect of continuous administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP on body weight in Wistar and Sprague Dawley rats.

One-way ANOVA revealed that the continuous administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP for twelve days significantly increased body weight of control Wistar rats compared to CORT treated animals (treatment $F(1, 47)=12.57$ $p< 0.001$, CORT $F(1, 47)=1.33$ interaction $F(1, 47)=0.89$, post hoc $p< 0.05$, (Fig5A). Simmilarly, the treatment with this Y_5 agonist increased body weight at 7 and 14 days after the beginning of the treatment in SD naive animals, treatment $F(16,172)=24.84$ $p< 0.001$, time $F(4, 172)=500.73$ $p< 0.001$, interaction, $F(4,172)=5.532$, $p< 0.001$, but had no effect in OBX rats (Fig5B).

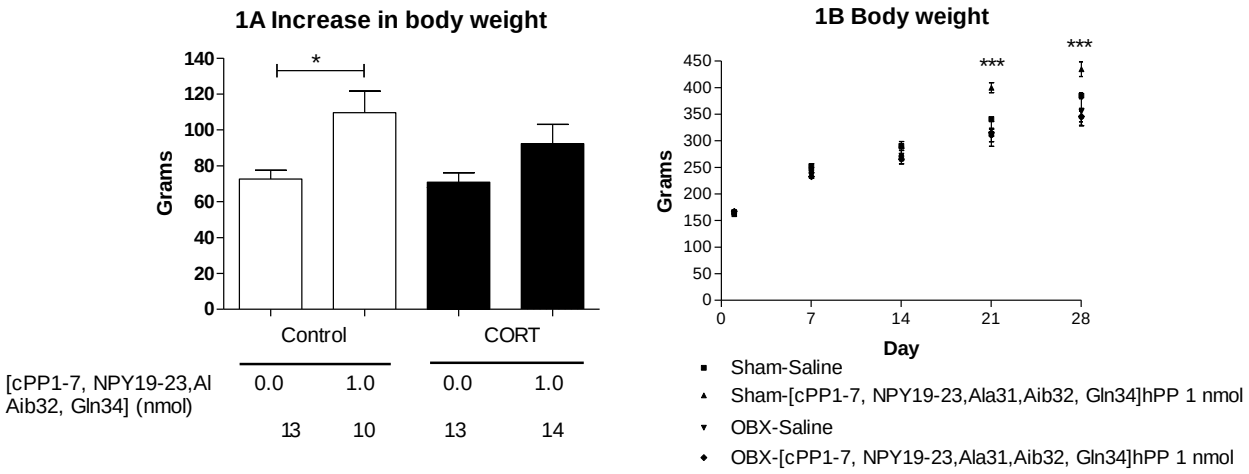


*Figure 5. Sustained administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP increased body weight in control naïve rats. The effect of this Y_5 agonist on the increase of body weight in control Wistar (Fig1A) and Sprague-Dawley (Fig 1B) rats. n=10-18 rats per group, results are expressed as mean ± S.E.M. * p<0.05, *** p<0.001.*

4.0 Discussion

This study investigated the effect of long term ICV administration of a Y_5 agonist, [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP, on anxiety- and depression-like behaviors in rats exposed to manipulations that induce emotionally dysfunctional behaviors. Concomitantly, we evaluated the effects of the treatment with this Y_5 agonist on body weight. Our results show that this highly selective agonist induced an anxiolytic-like effect in the SI test in OBX rats while decreasing a depressive-like behavior was observed in the OF, but not in the FST in OBX rats. This treatment induced an anxiolytic-related effect in the EPM in CORT-treated rats and increased body weight in control naive animals. Taken together, our results suggest that the stimulation of Y_5 receptors can

selectively modulate emotional processing in animal models of emotional dysfunctional behavior all those without increasing body weight.

4.1 The selective Y_5 agonist, [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP modulates specific behaviors in the OBX rat.

OBX is well known to induce hyperlocomotion (Song and Leonard, 2005) as well as high levels of rearing and grooming (Goyal et al., 2009; Morales-Medina et al., 2012) in the OFT, to decrease immobility in the FST (Morales-Medina et al., 2012) and to impair social activity in the SIT (Morales-Medina et al., 2012; Wang et al., 2007). The hyperlocomotion observed in this model can only be reversed after a long term administration of antidepressants (Kelly et al., 1997; Song and Leonard, 2005) and these animals do not present anxiogenic-related behavior in this test (Mar et al., 2002). Thus the modifications observed in locomotion in the OFT in the OBX rat can be only interpreted as antidepressant-related effect.

Our results show here for the first time that the continuous administration of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP decreased the hyperlocomotion as well as rearing and grooming in the OFT in OBX rats. The reduction in hyperlocomotion is therefore associated with antidepressant-related effect in this animal model. Previously, Goyal et al., (2009) reported that ICV administration of NPY decreased locomotion and rearing and grooming in this test in OBX rats. The Y_1–like agonists [Leu^{31}Pro^{34}]PYY (Morales-Medina et al., 2012) and [Leu^{31}Pro^{34}]NPY (Goyal *et al.*, 2009) decrease locomotion in the OFT in OBX rats as well. Interestingly, [Leu^{31}Pro^{34}]PYY and a Y_2 antagonist decreased rearing and grooming but only in control naive animals in this paradigm (Morales-Medina et al., 2012). Whereas, Sorensen et al (2004) reported a

decrease in the number of rearing for the Y_5 receptor subtype in the OFT after acute administration of a Y_5 agonist. Thus, the modulation of Y_5 receptor produces various behavioral effects that clearly are distinct from those transduced by the Y_1 and Y_2 receptors.

The Y_5 agonist used in our study decreased the hyperlocomotion seen in the OF but did not effect the immobility time in the FST in OBX rat. The FST is a widely used tool to screen for antidepressants-like actions (Lucki, 1997; Porsolt *et al.*, 1977) but is apparently strain-dependent in the OBX rat (Morales-Medina et al., 2012; Vieyra-Reyes et al., 2008). The rationale of this test is based on the lack of motivation to escape, as observed in various models of depression-related behavior, a trait commonly seen in depressed subjects. We previously showed that a Y_2 antagonist has a strong effect on this test in OBX rats (Morales-Medina et al., 2012). Thus, the stimulation of Y_5 receptors induces a selective antidepressant effect in the OBX rat.

In addition, the Y_5 agonist used in our study increased active contacts in the SI test in OBX rats. In agreement with our results, an acute administration of this Y_5 agonist in the lateral ventricle (Sorensen *et al.*, 2004) and basolateral amygdala (BLA) (Sajdyk *et al.*, 2002) increased the percentage of entries in the open arm of the EPM and facilitated social contacts in the SI test, respectively. Moreover, Sorensen et al. (2004) did not observe any changes in locomotion in the EPM after the administration of this agonist. Thus, it would appear that the sustained activation of the Y_5 receptor can modulate some traits of depression-related behaviors and induce an anxiolytic-like effect in the OBX rat.

4.2 Effects of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP in CORT-treated rats

In the present study, we observed that the Y_5 agonist induced an anxiolytic -like effect in the EPM in CORT-treated rats. This effect is strong since the number of entries, the percentage of entries as well as the percentage of time was increased in CORT-treated animals that received the Y_5 agonist. In addition, the parameters of locomotion remained unchanged, therefore, we conclude that the effect produced by the agonist was anxiolytic and not sedative. Earlier on, Sorensen et al. (2004) had reported that the acute ICV administration of the Y_5 agonist used in our study induced an anxiolytic-like effect in the EPM and sedation in the OFT. Accordingly, the activation of the Y_5 receptor subtype was associated with sedation in one of the test. In that regard, it is well known that OBX induces disturbances in NPY levels (Holmes et al., 1998; Primeaux and Holmes, 2000) as well as over-stimulations of Y_2 receptors in dorsal hippocampus and BLA and Y_5 receptors in the BLA (Morales-Medina et al., 2012) while acute CORT administration induces hypertrophy in pyramidal neurons of BLA (Mitra and Sapolsky, 2008), a brain region with high amounts of Y_5 receptors (Dumont et al., 1998a; Quarta et al., 2011). In addition, Cambell et al., (2001) suggested that "the majority of Y_5 receptors migth be stored, but under certain physiological conditions the receptors are mobilized to the outher membrane". Thus, the anxiolytic- and antidepressant-like effect of Y_5 agonists could require increased levels of plasma membrane Y_5 receptors leading to imbalance in NPY levels as observed in OBX rats and possibly in CORT-treated rats but not in control animals. In support of this hypothesis, Karssen et al., (2007) observed an upregulation of Y_5 receptor gene expression in primates after chronic stress. In addition, the Y_5 but not Y_1

or Y_2 receptor genes, is associated with panic disorder risk in humans, an anxiety-related disorder (Domschke et al., 2008).

4.4 [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP increases body weight in control naive animals

Cumulative evidence suggests that the Y_5 receptor subtype is involved in the modulation of feeding behaviors (for a recent review, Morales-Medina et al., 2010). Our data show that the sustained ICV infusion of [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP increased body weight in normal control Wistar and Sprague Dawley rats, respectively. In agreement with this finding, an acute ICV administration of this agonist has been shown to increase food intake in control rats (Cabrele *et al.*, 2000; Walker *et al.*, 2009) and guinea pigs (Lecklin et al., 2003). Similar results were reported following a 7 day treatment with the Y_5 agonist D-Trp^{34}NPY in control mice (Moriya *et al.*, 2009) and after acute ICV administration in rats (Parker *et al.*, 2000). Interestingly, there is a lack of orexigen effect of this Y_5 preferring agonist on body weight in both the CORT and OBX rats. As previously indicated there is hyperactivation of Y_5 receptors in the BLA in the OBX rat and hypertrophy in pyramidal neurons of this region in CORT-treated animals. This disturbed processes could either modify the activity of these receptors in other brain areas including the hypothalamus or the larger number of available Y_5 receptors require a greater amount of this agonist to induce an orexigenic effect.

In contrast to Y_5 agonists, the inhibition of Y_5 receptor transduction apparently decreases body weight. For example, the administration of Y_5 receptor antisense nucleotides into the third ventricle induced a loss in body weight (Campbell *et al.*, 2001). Similarly, an acute injection of the Y_5 receptor antagonist CGP71683A decreased body

weight in control rats as well as food intake in fasting animals (Kask *et al.*, 2001). However, Della Zuang et al. (2001) reported that this molecule possesses nanomolar affinities for muscarinic receptors and serotonin uptake sites questioning the specificity of the effects reported in other studies. Additionally, a Y_5 antagonist blocked D-Trp^{34}NPY-induced increase in body weight in normal mice (Moriya *et al.*, 2009) while it had no effect by itself on body weight in control animals and only produced a minimal reduction (4%) in obese mice (Moriya *et al.*, 2009). Another Y_5 receptor antagonist, Lu AA33810, also suppressed [cPP$^{1-7}$, NPY$^{19-23}$, Ala^{31}, Aib^{32}, Gln^{34}]hPP-induced food consumption (Walker *et al.*, 2009). However, the effect of Lu AA33810 on its own has yet to be investigated. Finally, and rather surprisingly, the germinal deletion of Y_5 receptors in mice had no effect on body weight in young animals but increased after fasting or with age (Higuchi *et al.*, 2008; Marsh *et al.*, 1998). In addition, Marsh et al., (1998) reported that the deletion of this receptor failed to prevent obesity in the OB/OB mouse model. Taken together, it thus appears that the inhibition of Y_5 receptors does not obligatory result in altered body weight, at least in rodents.

The role of NPY in body weight has been well demonstrated. However, previous studies suggested the involvement of Y_1 and Y_2 receptor subtypes in this process as well. In that regard, we recently reported that the sustained administration of the Y_1–like agonist, [Leu^{31}Pro^{34}]PYY, the Y_1 antagonist, BIBO3304, the Y_2 agonist PYY3-36 and the Y_2 antagonist BIIE0246 failed to induce any changes in body weight in either control or OBX rats (Morales-Medina et al., 2012).. Thus, it would appear that the appetite-related properties of the NPY system can at least partly be mediated by the activation of Y_5 receptors in normal, naive animals but that effect is either blocked or blunted under

emotionally dysfunctional conditions. Thus this could turn out to be most advantageous clinically.

5.0 Conclusions

We have previously reported that the Y_1 agonists and Y_2 antagonists can modulate several emotional processes in the OBX rat. Here we show that the continuous administration of a selective Y_5 agonist also induces anxiolytic-like effects as observed following an acute administration. Most interestingly, this Y_5 agonist also possesses antidepressant-like properties in OBX animals without producing changes in body weight or sedation. These findings certainly warrant further investigation as to the possible usefulness of Y_5 agonists for the treatment of emotionally dysfunctional conditions without apparent alterations in body weight.

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Statement of conflicts of interest

None

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CHAPTER VI

General discussion and conclusion

In this thesis, we further confirmed that the NPY system play a key rolein emotional processing. In addition, we dissected the specific contribution of NPY receptors, the Y_1, Y_2 and Y_5 subtypes known to be expressed in the CNS, in control as well as animal models of emotional dysfunctional conditions.

6.1 Relevance of the OBX model as a model of emotional dysfunctional condition

In the chapter III, we evaluated the OBX rats as a model of depression-related behavior and proposed a mechanism of action on how this lesion produces a constellation of disturbances in the rat CNS. We performed several behavioral tests to better characterize candidate molecules not only for depression but also for anxiogenic-related behaviors in the rat. We carried out these tests, OF, FST and SIT on three consecutive days in this animal model. These behavioral tests have been previously performed in this and other animal models of emotional dysfunctional conditions (Kalynchuk et al., 2004, Wang et al., 2007). In addition, it has been suggested that neurotransmission imbalance in the brains of these animals is caused at least partially by cellular disturbances in the hippocampus namely, neuronal rearrangement and dysregulated adult neurogenesis. These processes are compromised in other animal models of depression related behavior as well as in depressed human subjects (Jaako-Movits and Zharkovsky, 2005, Jaako-Movits et al., 2006, Magarinos et al., 1998, Manji et al., 2001, Watanabe et al., 1992).

6.2 Role of Y_1 receptor subtype in emotional dysfuctional conditions

Subsequent to the use of the battery of behavioral tests as well as the strong validity of OBX as a model of depression and anxiety-related behavior, we evaluated the role of the Y_1 and Y_2 receptor subtypes in this animal model as described in chapter IV. The role of the NPY system in emotional processing has been studied and replicated in

various behavioral tests (Heilig et al., 1989, Redrobe et al., 2005, Stogner and Holmes, 2000). The imbalance in the level of this peptide has been observed in animal models of depression-related behavior (Caberlotto et al., 1999, Jimenez-Vasquez et al., 2001, Rutkoski et al., 2002) and some of the behavioral effects of antidepressants are associated with an increase in NPY levels in the CNS (Caberlotto et al., 1998, Stenfors et al., 1989, Zambello et al., 2010). However, the contribution of each of NPY receptor subtypes in emotional processing is not yet clear.

Recent studies have attempted to dissect how NPY modulates its effects. The Y_1 receptor subtype is densely expressed in brain regions associated with emotionality and memory processing (Dumont et al., 1998b). In addition, this receptor has high affinity for NPY and its analogs including [Leu^{31}, Pro^{34}]NPY and [Leu^{31}, Pro^{34}]PYY (Dumont et al., 2000). The Y_1 receptor subtype was the first to be characterized, and is abundant in the CNS, particularly in the cortex. This receptor has been hypothesized as the mediator of most of the postsynaptic effects of NPY. In the present study, we observed that the continuous administration of a Y_1 agonist induces strong antidepressant and anxiolytic-like effects in the OBX rat (Chapter 3). However, this treatment did not modulate the anxiogenic-like behavior observed in CORT-induced anxiety in the rat (Chapter 3). In accordance, our group previously observed that the acute administration of Y_1 agonists induced anxiolytic- and antidepressant- effects in naïve rodents and that the co-administration of the Y_1 antagonist, BIBO3304, abolished the behavioral effects induced by [$Leu^{31}Pro^{34}$]PYY (Redrobe et al., 2002). However, controversial data were generated from Y_1 KO mice studies. Painsipp et al. (2010) observed that the female Y_1 KO mice showed an antidepressant phenotype in the TST but not in the FST. In contrast, Karlsson

et al. (2008) found a depressive phenotype in these animals while Karl et al. (2006) observed that Y_1 KO mice displayed normal behaviour in the EPM, but stress produced an anxiolytic-like effect in these animals. Additionally, Painsipp et al. (2010) detected an antidepressant effect after stress in Y_1 KO mice in various depression-related paradigms. Interestingly, recent studies using Y_1 KO mice have suggested that Y_1 receptors may play a role in the stress response. For example, Karl et al. (2006) reported that male Y_1 KO mice display an important anxiolytic-like phenotype only after physical restraint while female Y_1 KO mice show augmented antidepressant-related activity in both the FST and the tail suspension test after being tested in the EPM one week earlier (Painsipp et al., 2009). In both cases, the HPA axis was previously activated. In addition, Thorsell et al. (2000) had also suggested that the stressful event produces an anxiolytic-like effect in NPY transgenic rats. Taken together, present and previous findings suggest that the Y_1 receptor subtype modulates emotional processes following stressful stimuli.

6.3 Role of Y_2 receptor subtype in anxiety- and depression- related behavior

Subsequent to the study of Y_1 receptor, we evaluated the role of the Y_2 receptor subtype in the OBX rat. The Y_2 receptor modulates the release of NPY and some classical neurotransmitters, including monoamines and excitatory amino acids such as glutamate (King et al., 1999, 2000, Qian et al., 1997). Since antidepressant-related activity has been associated with elevated levels of NPY, the inhibition or deletion of Y_2 receptors has been hypothesized to indirectly increase NPY and produce antidepressant- and anxiolytic-like behaviors (Redrobe et al., 2003a, Weiser et al., 2000). A second possible mechanism of action of the Y_2 receptor subtype includes the presynaptic modulation of glutamate release, particularly in the hippocampus. In this regard, high

level of CORT induces depression-related behaviors and neuronal hypotrophy (Kalynchuk et al., 2004, Morales-Medina et al., 2009). This process seems to be mediated, at least partially, by glutamate (Magarinos and McEwen, 1995). Thus, the modulation of the Y_2 receptor subtype would regulate the various presynaptic effects of NPY on the release of transmitters in the synaptic cleft.

Here, we observed that the exogenous administration of Y_2 antagonist, BIIE0246 induces a strong antidepressant-like effect in the OBX rat while a strong anxiolytic-related effect was observed in control naïve animals. These findings were further replicated by the brain penetrant Y_2 antagonist, JNJ-31020028 and a strong antidepressant-like effect was observed in the OBX rat. In addition, BIIE0246, produced a potent anxiolytic-like effect in the animal model of CORT-induced anxiety and also showed a tendency toward an anxiolytic-related effect in control naïve animals in the CORT model (shown in supplementary data).

We also observed that the activation of Y_2 receptors further contributes to the deterioration of emotional dysfunctional conditions. For example, the continuous administration of a Y_2 agonist, PYY3-36, further increased the immobility time in the FST in OBX rats and increased grooming and rearing in the OF test in control Sprague-Dawley rats. In addition, this molecule induced an anxiogenic effect in control Wistar rats. Interestingly, this Y_2 agonist also increased behavioral despairs and the positive/negative effect of the Y_2 receptor was not observed when modulating the Y_1 receptor subtype which is mostly post-synaptically located. Y_1 agonists have been shown to have a positive effect on these behaviors while Y_1 antagonists are mostly devoid of effects by themselves.

In addition, we observed an overstimulaiton or overproduction of Y_2 receptors in the hippocampus and basolateral amygdala in the OBX rat. This may contribute to the differential effects in control and OBX rats. In agreement with these findings, a reduced anxiety and depressive profile has been consistently observed in $Y_2^{-/-}$ mice independent of sex and age (Carvajal et al., 2006a, Painsipp et al., 2008a, Redrobe et al., 2003b, Tschenett et al., 2003). Most interestingly, Tasan et al. (2010) reported an increase in the time spent in the open arms of EPM in animals with a targeted deletion of Y_2 receptors in the BLAand CeA. Additionally, a decrease in immobility time in the FST was specifically observed after the deletion of Y_2 receptor subtype in the CeA. In the "learned helplessness" model, a rat model of depression, the acute infusion of BIIE0246 in the CA3 region of the hippocampus also produced an antidepressant-like effect (Ishida et al., 2007). Thus, we observed that the blockade of Y_2 receptors produced an anxiolytic-related effect under control conditions as well as after mild stress while it induced an antidepressant-like effect in OBX rats, an animal model displaying an imbalance in NPY and other neutoransmitter systems as well as in response to stress.

Thus, the Y_2 receptor subtype mediates a strong modulatory effects of NPY and related peptides on emotional processes. The pharmacological blockade of this receptor induces a strong positive effect while its activation contributes to the deleterious effects of emotional dysfunctional conditions. These unique characteristics suggest that this receptor subtype modulates both pre and postsynaptic NPYergic system as well as presynaptic glutamatergic and monoaminergic systems. Such profile of activities likely has therapeutic potential which remains to be fully exploited.

6.4 Role of Y_5 receptor subtype in emotional dysfuctional conditions

In chapter V, we evaluated the role of a Y_5 receptor agonist in anxiety and depression related behaviors. Compared to the Y_1 and Y_2 receptor subtypes, little is known about the role of the Y_5 receptor subtype in the brain. The Y_5 receptor subtype has been known as the "feeding receptor" (Marsh et al., 1998) and for its possible role in sedation (Sorensen et al., 2004). Dumont et al., (1998b) reported low levels of expression of this receptor in discrete brain regions. Recent data suggest a broader distribution in various areas including the olfactory bulbs, hippocampus, amygdala and hypothalamus (Campbell et al., 2001, Quarta et al., 2011), all brain regions involved in the stress response as well as in emotional processes. In addition, since the blockade of postsynaptic Y_1 receptors failed to modulate emotional processes in the rat (Morales-Medina et al., 2011, Redrobe et al., 2002), another postynaptically located receptor, the Y_5 subtype, has gained interest as a key transducer of some of the effects of NPY. We hypothesized that the activation of this receptor subtype could mediate some aspects of NPY-associated emotional processing in the brain.

In this thesis, we also evaluated the effect of a selective Y_5 agonist, [cPP^{1-7}, NPY^{19-23}, Ala^{31}, Aib^{32}, Gln^{34}]hPP, in the OBX model as well as the CORT-induced anxiety model in two different strains of rat. Indeed, this agonist reversed hyperlocomotion (antidepressant-like effect) and decreased grooming and rearing in the OF as well as had an anxiolytic-related effect in the SI test in the OBX rat. In addition, this molecule reversed the anxiogenic-like effect of CORT treatment in rats. Earlier on, Sorensen et al. (2004) reported that the administration of this Y_5 agonist induced an anxiolytic-like effect in the EPM and sedation in the OF tests. The activation of the Y_5

receptor subtype was thus associated with a sedative effect in the rat. To explain the mild antidepressant- and strong anxiolytic-like effects seen in the OBX rat, we proposed that this could be due to alterations in NPY levels (Holmes et al., 1998, Primeaux and Holmes, 2000) as well as an overstimulation of Y_5 receptors in the BLA (Morales-Medina et al., 2011, Morales-Medina et al., 2010b). Previously, Campbell et al., (2001) suggested that "the majority of Y_5 receptors might be stored, but under certain physiological conditions the receptors are mobilized to the outer membrane". Thus, the positive effects observed after the administration of NPY or a Y_5 agonist could require the translocation and increased levels of plasma membrane-bound Y_5 receptors as observed in OBX rats but not in control or CORT-treated rats. Our results are also in agreement with earlier studies showing that the acute administration of our Y_5 agonist in the lateral ventricle (Sorensen *et al.*, 2004) and BLA (Sajdyk et al., 2002a) induced an anxiolytic-like effect but not sedation in the rat. Karssen et al. (2007) also observed an upregulation of Y_5 receptor gene expression in primates after chronic stress. In addition, the Y_5 but not Y_1 or Y_2 receptor genes, is associated with panic disorder risk in humans (Domschke et al., 2008). In the present study, we observed for the first time that the activation of Y_5 receptors induces antidepressant- and anxiolytic-related effects under a challenged condition and increase of body weightin control animals.

6.5 Role of Y_1, Y_2 and Y_5 receptor subtype in body weight

Concomitantly to emotional processes, we also evaluated the effect of the modulation of these three NPY receptors on body weight, since NPY is well known to regulate body weight. We observed that the sustained administration of a Y_1–like agonist, a Y_1 antagonist, a Y_2 agonist and a Y_2 antagonist failed to induce any changes in

body weight of both strains of control naïve, CORT or OBX rats. However, the sustained ICV infusion of a Y_5 agonist increased body weight in control Wistar and Sprague Dawley rats. Thus, it appears that the appetite-related properties of the NPY system may at least partly be mediated by the activation of Y_5 receptors in normal naïve animals but that this effect is either blocked or blunted under emotionally dysfunctional conditions.

As observed in table VI.1, we dissected the role of NPY Y_1, Y_2 and Y_5 receptors in emotional processes and body weight. The activation of Y_1 receptors produced a strong anxiolytic- and antidepressant-like effects only under stressful conditions or following changes in NPY levels. The blockade of Y_2 receptors produced an anxiolytic-like effect in control animals and an antidepressant-related effect in animal models of emotional dysfunctional conditions. In addition, we observed a strong anxiolytic-like effect and a mild antidepressant-related effect after the activation of Y_5 receptors under challenged conditions as well as increases in body weight in control naïve animals. Thus, the modulation of NPY Y5 receptor produces diverse behavioral effects which are clearly distinct from those transduced by the other two receptor subtypes evaluated here.

NPY receptor subtype	Control	CORT model	OBX model
NPY Y_1	No effect	No effect	Antidepressant- and Anxiolytic-related effect
NPY Y_2	Anxiolytic-like effect	Anxiolytic-like effect	Antidepressant- related effect
NPY Y_5	Orexogenic effect	Anxiolytic-like effect	Antidepressant- and Anxiolytic-related effect

Table VI.1. Key effects mediated by NPY Y_1, Y_2 and Y_5 in control, corticosterone-induced anxiety (CORT) and olfactory bulbectomized (OBX) rat.

6.6 Effect of Y_1 receptor subtype in adult hippocampal neurogenesis

Following the behavioral characterization of the various effects mediated by the three subtypes under study, we evaluated next the possible cellular mechanism(s) involved. We focused our attention on Y_1 receptors on neurogenesis in the hippocampus since these these receptors are abundantly expressed in this brain region and recent data have strongly suggested the role of neurogenesis as critically important in the effects of antidepressant drugs.

In the present thesis, we observed that a Y_1 agonist at a dose that reversed behavioral despairs also induced cell survival in the OBX rat. These findings suggest that the antidepressant effects observed as a result of the administration of a Y_1 agonist could be mediated, at least in part, by the modulation of neurogenesis-related events in the CNS. This is certainly a most exciting avenue for further research.

6.7 Some Future Directions

In the present thesis, we dissected the role of NPY receptors in anxiety- and depression-related behaviors. This peptide has two major mechanisms of action, modulating its own release and those of other transmitters, presynaptically (mostly via the Y_2 subtype; Haas et al., 1987, Higuchi et al., 1996) and acting through a family of NPY receptors (mostly Y_1 and Y_5) located postsynaptically (Morales-Medina and Quirion, 2011). A general consensus indicates that a variety of stimuli modulates NPY levels including increases in neuronal activity (Higuchi et al., 1996), glutamate (Gemignani et al., 1997), brain derived nerve growth factor (BDNF) (Jones et al., 1994) and the transcription factor known as cAMP response element binding protein (CREB) (McClung and Nestler, 2003). However, the detailed molecular mechanism of action(s) of NPY and its receptors remains to be fully established. Several intracellular pathways have been proposed as possible targets. Few will be breifly discussed below.

1. Classical Neurotransmitters

Glutamate and to a more minor extent its synthetic analogues AMPA and NMDA as well as its homolog kainate, increase hippocampal NPY levels *in vitro* (Gemignani et al., 1997, Reibel et al., 2000) and this effect seems to be mediated by a Ca^{2+} dependent mechanism. Interestingly, these actions are also modulated by presynaptic Y_2 receptors and a Y_2 agonist can inhibit the release of both NPY and glutamate *in vitro* (Greber et al., 1994). Interestingly, cumulative evidence suggests that NPY is co-localized with GABAergic interneurons in the hippocampus and that these neurons can inhibit glutamatergic transmission (Aoki and Pickel, 1990, Carnahan and Nawa, 1995). Thus, NPY (acting on Y_2 receptors) seems to play a negative feedback role in the regulation of

glutamatergic neurotransmission, this possibly having significant implication in emotional processes. Future studies should aim to clearly establish the link between glutamateric neurotransmission and NPY receptors using a variety of tools including inducible gene knock-out animals of the various NPY and glutamatergic receptors, in addition to optogenetic approaches aiming at demonstrating the brain pathways involved.

In addition to glutamate, serotonin (5HT) has also been shown to be involved with NPY in emotional processing. For example, our group has shown that *p*-chlorophenylalanine, a tryptophan hydroxylase inhibitor known to deplete synaptic 5-HT levels, prevents the antidepressant properties of NPY (Redrobe et al., 2005). In addition, the administration of fluoxetine, a selective serotonin reuptake inhibitor, increases NPY levels in the hippocampus (Christiansen et al., 2011), suggesting that its antidepressant properties are mediated, at least in part by NPY. Also, in the FLS rats, a model of depression-related behaviors, the levels of NPY and serotonin are decreased (Husum et al., 2006). However, changes in NPY and serotonin appear to be concomitant, and thus causality will have to be fully established in future studies.

2. Possible role of BDNF

BDNF belongs to a familiy of neurotrophins including nerve growth factor, NT-3 and NT-4/NT-5 (Leibrock et al., 1989). BDNF is particularly known to promote adult neurogenesis and is involved in depression-related behaviors (Monteggia et al., 2007). Moreover, glucocorticoids decrease BDNF expression in the normal hippocampus and high levels of glucocorticoids induce neuronal hypotrophy (Morales-Medina et al., 2009) and depression-related behaviors (Kalynchuk et al., 2004). Interestingly, disrupted levels of this neurotrophin are also associated with changes in NPY levels in the brain

(Carnahan and Nawa, 1995). For example, endogenous deletion of BDNF resulted in decreased levels of NPY in the hippocampus and cerebral cortex in mice (Jones et al., 1994) and these animals displayed depression-related behaviors (Monteggia et al., 2007). Interestingly, the exogenous administration of BDNF increased NPY levels in the hippocampus and cortex *in vitro* (Carnahan and Nawa, 1995) and *in vivo* (Croll et al., 1994, Nawa et al., 1994, Reibel et al., 2000). In addition, cumulative evidence suggests that increasing neuronal activity through various mechanisms (Carnahan and Nawa, 1995, Husum et al., 2004) and glutamate (Zafra et al., 1991) can increase BDNF levels followed by higher amounts of NPY *in vitro* and *in vivo*. Concomitant decrease or increase of BDNF levels resulted in similar changes in NPY levels. Thus under certain circumstances, NPY and BDNF can apparently share common pathways leading to similar behavioral effects. Future studies should aim to clarify this relationship in particular in regard to its sequence of events at the molecular and system levels.

3. Possible role for the transcription Factor CREB

There are various Ca^{2+} dependent transcriptional factors including CaM kinase which modulates CREB, calmodulin-dependent and C/EBP β levels (Higuchi et al., 1996). Among these, the CREB pathway is one of the most studied transcription factors given its possible involvement in various disorders. Inducible transgenic mice with increased CREB gene expression induced a slight increase in NPY levels in the nucleus accumbens (McClung and Nestler, 2003). In addition, the CaM kinase inhibitor KN-62 inhibited the induction of NPY gene expression while the NPY gene was activated following an increase in cAMP levels *in vitro* (Higuchi et al., 1996). All these are involved in the CREB parthway. Interestingly, Gpr26 KO mice, lacking a protein that regulates cAMP

levels, and with decreased CREB levels in the amygdala, display an anxiogenic and depressive phenotype (Zhang et al., 2011). Interestingly, CREB deficient (+/-) mice show a more anxious phenotype as well as decreased BDNF and NPY levels in the brain (Pandey et al., 2004). Taken together, these results reveal that CREB can regulate NPY and BDNF levels in the brain.

Accordingly, I propose that the NPY system acts in a negative feedback loop: ↑glutamate/neuronal excitation → ↑CaMKII → ↑CREB → ↑BDNF → ↑NPY → ↓glutamate. However, most of the studies discussed above have been performed *in vitro*, in KO mice or in normal control naive animals. Thus, a novel strategy would investigate whether this pathway is indeed at play in animal models of depression and anxiety such as those used in my thesis.

For nearly two decades, scientists have studied the role of NPY and its receptors in emotional processing. In this thesis, we dissected the differential contribution of NPY receptor subtypes in anxiety- and depression- related behaviors in relevant animal models. Behavioral results on Y_1 and Y_2 receptors have provided robust data as to their respective role in emotional responses and stress. The possible role of the Y_5 receptor subtype needs further investigation to more clearly establish its unique contribution to emotional processing. In addition, we provided some preliminary results as to the role of adult hippocampal neurogenesis as a possible mechanism of action involved in the effects of NPY in emotional processing. Further studies are certainly warranted in that regard.

6.8 General conclusion

The NPY Y_2 receptor subtype is the strongest modulator of emotional processing possibly through both presynaptic and postsynaptic mechanisms. Meanwhile, the Y_1 and Y_5 receptors modulate different traits of emotional processes under particular conditions. Therefore, by using relevant animal models of emotional dysfunctional conditions, novel approaches should reveal possible mechanisms of action as well as opportunities for long-term clinical treatments using Y_1 agonists, Y_2 antagonists and Y_5 agonists as novel treatments for distinct features of anxiety and depression.

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