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EXCITATORY AMINO ACID RECEPTOR CHANGES INDUCED BY NEONATAL BILATERAL VENTRAL HIPPOCAMPUS LESION IN RATS.

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfilment of the requirements of the degree of Master of Science.

June, 1997

in the hope of a cure for schizophrenics

Table of Contents

Abstract 5
Résumé 6
Acknowledgments 7

1- INTRODUCTION 8
1.1.- Pharmacological Models of Schizophrenia 8
1.2.- The Neurodevelopmental Hypothesis of Schizophrenia 12
1.3.- The Neonatal Bilateral Excitotoxic Ventral Hippocampus Lesion Model 15

2- OBJECTIVES 17

3- METHODS 18
3.1.- Animals 18
3.2- Materials 18
3.3.- Surgical Procedures 18
3.4.- Brain Processing 19
3.5.- Receptor Binding Autoradiography 19
3.6.- Behavioral Test 20
3.6.1.- Locomotor Activity Test 20
3.6.2.- Radial Arm Maze Test 21

4- RESULTS 23
4.1.- Anatomical Evaluation of the Neonatal Lesion 23
4.2.- Behavioral Test 26
4.2.1.- Locomotor Activity 26
4.2.2.- Radial Arm Maze 26
4.3.- Receptor Binding Autoradiography 32
4.3.1.- [^{125}I]-MK-801 Binding 32
4.3.2.- [^{3}H]- AMPA Binding 41
4.3.3.- [^{3}H]-KAINATE Binding 45
4.3.4.- [^{3}H]-mGLUR Binding 50

5- DISCUSSION 55

5.1.- Behavioral Findings in the Neonatal VH Lesion Model 55
5.1.1. Locomotor Activity 55
5.1.2. Cognitive Function in the Neonatal VH Lesion Model 59
5.2- Changes in Excitatory Amino Acid Receptors 61
5.2.1. Ionotropic Glutamate Receptors 61
5.2.2. Changes in mGlu Receptor Binding 63
5.2.3. Prefrontal Cortex and the VH Lesion Model 67
5.3.- The DA/Glutamate Relationship in the Neonatal VH Lesion Model 68

5.4.- EAA and Stress in the Neonatal VH Lesion Model 71
5.5.- Developmental Issues in the Neonatal VH Lesion Model 73

Appendix 75
References 76

ABSTRACT

Bilateral excitotoxic lesions of the ventral hippocampus in neonatal rats result in hyperlocomotion induced by dopamine agonists, and hyperresponsiveness to stress in postpubertal animals. A cortical maldevelopment has been suggested to explain the behavioral changes observed but direct evidence is lacking to support this assumption. We studied possible alterations in excitatory amino acids receptor levels in this animal model of schizophrenia. Ventral hippocampal lesion at postnatal day 7 (PD7) induced significantly increased locomotor activity after a D-amphetamine injection (1mg/kg) in PD56 and 6 month-old (PD 180) rats but not PD35 ones. In addition, behavioral deficits were observed in postpubertal rats in the radial arm maze test. By using a [^{125}I]-MK-801 receptor binding autoradiography protocol, neonatal ventral-hippocampal-lesioned rats depicted significant postpubertal increased levels of N-methyl-D-aspartate (NMDA) receptors in frontal cortical regions at ages PD56 and 6 month, but not prepubertally at PD35. [^{3}H]-AMPA receptor binding levels were also found increased in 6-month old rats, while no significant changes in [^{3}H]-kainate receptor binding levels were found at any of the ages studied. Finally, metabotropic glutamate receptor levels measured by [^{3}H]-glutamate binding were decreased in PD56 and PD180. Taken together our results suggest that cortical glutamate abnormalities observed in hippocampal-lesioned rats are of enduring nature and may underlie some of the behavioral changes observed in these animals.

RÉSUMÉ

Une lésion excitotoxique néonatale de l'hippocampe ventrale chez le rat produit une augmentation de l'activité locomotrice en réponse à l'administration d'agonistes dopaminergiques chez l'animal adulte, ainsi qu'une réponse exagérée au stress. Des déficits développementaux du cortex frontal ont été proposés pour expliquer les changements comportementaux qui l'on observe dans ce modèle animal de la schizophrénie. Dans ce memoire, nous avons donc étudié le rôle possible de la transmission glutamatergique en utilisant une technique d'autoradiographie des récepteurs des acides aminés excitateurs dans le cortex frontal des animaux lésés. Nos resultats ont montré des niveaux élevés de récepteurs au NMDA dans le cortex frontal des animaux lésés quand on avait les mesurés aux jours 56 et 180 après la naissance, mais non au jour 35, juste avant l'atteinte de la maturité sexuelle. Quant aux récepteurs de type AMPA, leur densité est augmentée seulement au jour 180. Pour ce qui est des récepteurs métabotropes, ils sont diminués aux jours 56 et 180 dans notre modèle. Aucun changement des récepteurs au kainate n'a été observé chez les animaux lésés. Par ailleurs, l'activité locomotrice est augmentée suite à l'administration de la D-amphetamine (1mg/Kg) chez les animaux sexuellement matures, mais non chez les animaux immatures. De plus, les rats lésés ont montrés des déficits d'apprentissage dans le labyrinthe radial. Dans leur ensemble, nos résultats pourraient contribuer a mieux comprendre les changement comportementaux et neurochimiques qui se produisent dans notre modèle lors de l'atteinte de la maturité sexuelle.

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1- INTRODUCTION

Schizophrenia is a complex disorder of cognition and affect. The first serious intent of medical description of the illness was made by Emil Kraepelin, a noted German psychiatrist, in 1900. He named '*dementia praecox*' a chronic psychotic disorder with onset in youth and deteriorating social function. In 1910, Eugene Bleuler, a Swiss psychiatrist, changed the name of dementia praecox to '*schizophrenia*' to mean split intellect. He noticed in these patients disturbed thinking and the inability to communicate but preservation of memory and mathematical abilities. The introduction of neuroleptics in the 1950s brought about some improvement in the treatment of these patients marking the beginnings of psychopharmacology, but the observations of Kraepelin of an ultimate deteriorating course still hold true. The disorder is characterized by the presence of hallucinations, delusions, disorganization of spontaneous speech, and loss of normal affective expressiveness and represents perhaps the greatest public health concern in psychiatry, affecting approximately 1% of the general population.

The pathophysiology of schizophrenia is unknown. An anatomic origin of the symptoms has not been determined; however, ventricular enlargement and thalamic abnormalities in schizophrenics have been documented from both neuroimaging [1,2,3] and post-mortem studies [4,5,6,7]. Computed tomography and magnetic resonance imaging studies have shown lateral ventricle and third ventricle enlargement, widened cortical sulci, cerebellar atrophy, cerebral asymmetry, and decreased brain density consistently in many studies of schizophrenic patients [8,9]. Using more dynamic measurements, changes have been reported in the cerebral blood flow in the anterior frontal regions, the temporal cortex, and the globus pallidus, as well as increased dopamine (DA) D2 receptor sites in schizophrenics [10]. Most of the above-described dysfunctions imply an abnormality in the functional integration of sensory and cognitive information in schizophrenia.

1.1.- Pharmacological Models Of Schizophrenia

As we know, there are two predominant drug models of schizophrenia: the stimulant-induced psychoses and phencyclidine (PCP) psychosis. The principal symptoms of stimulant psychosis [11], induced by chronic amphetamine or cocaine abuse, include

stereotypies, paranoid delusion and varied sensory hallucinations, with parasitosis (the delusion that bugs or snakes are present on the skin) being one of the most prevalent. However, these positive symptoms are manifested on a background of considerable anergy and dysphoria. On the other hand, PCP psychosis [27] presents an entirely different and even more comprehensive set of schizophrenic-like symptoms such as (1) perceptual disorders, especially changes in body image and feeling of unreality and depersonalization and an inability to maintain the distinction between reality and fantasy; (2) thinking disorders and intellectual impairment (loose associations, disorganization and concreteness), (3) hallucinatory experiences, (4) flattened or bland affect; and (5) heightened evocation of affectively charged personal experiences.

Compelling evidence suggests that non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine and ketamine may provide a more complete model of psychosis than previously recognized, in that they mimic both persisting symptomatology and neuroanatomical abnormalities. They can induce psychosis in drug addicts and exacerbate the symptoms of chronic schizophrenics. The psychotic symptoms these drugs induce mimic a variety of schizophrenic symptoms including flattened affect, dissociative thought disorder, depersonalization and catatonic states. Dizocilpine (MK-801), with simpler chemical structure than PCP or ketamine, has also showed in animal studies neurotoxic effects on neurons of the posterior cingulate cortex [26]. It can also induce further degeneration in other limbic structures such as pirifom cortex, posterior regions of the entorhinal cortex, dentate gyrus and ventral hippocampus. PCP was originally developed as an anesthetic but it was soon found to induce severe reactions in up to 50% of the patients, characterized by excitation, bizarre behavior, paranoia, depersonalization, concreteness of thought and hallucinations [43]. Ketamine, a dissociative anesthetic with pharmacological effects similar to PCP, also induces alteration in perception and vigilance, disruption of delayed word recall and psychotomimetic schizophrenic-like symptoms [43]. In addition, ketamine significantly increases regional cerebral blood flow (rCBF) in anterior cingulate cortex but reduces flow in visual cortex and hippocampus, suggesting abnormal glutamatergic neurotransmission in schizophrenia [12]. The psychotomimetic mechanism of both compounds have been related with the potentiation of acetylcholine release (inhibiting AchE and actions at muscarinic and nicotinic receptors), increased dopamine release and blockade of dopamine reuptake, increased serotonin levels and various effects at alpha-adrenoreceptor, sigma and GABA/benzodiazepine receptors [13] . However, only three of these sites are recognized with submicromolar affinity: the NMDA ion channel (at the so-called PCP binding site), the sigma binding site, and the dopamine uptake site [14] .

Traditionally, the most widely held hypothesis to explain positive schizophrenic symptoms has been the DA hyperactivity hypothesis. The most compelling evidence for the DA hypothesis of schizophrenia is that DA receptor antagonists are at least partially effective in ameliorating positive symptoms in many patients with schizophrenia. In fact, the observation that in vitro neuroleptic binding to dopamine receptors directly correlates with clinical potency in reducing psychotic symptoms [15a,15b] strengthens this view. The biochemical data available, however, indicate that there may not be absolute dopaminergic hyperactivity in the brain [16]. The biochemical abnormality in schizophrenia may well be located in another neurotransmitter system which is somehow linked to dopamine neurons. The dopaminergic and glutamatergic systems are closely linked to one another [17,18,19], thus possibly explaining the therapeutic action of neuroleptic drugs [20]. Dopamine functionally antagonizes the glutamatergic system; for instance, activation of the dopaminergic system reduces glutamate release from cortico-striatal terminals while neuroleptic drugs restore the glutamate release [21]. Research into the function of L-glutamate in the central nervous system has led to the hypothesis that decreased glutamatergic neurotransmission may play a role in the pathophysiology of psychosis [22,23,16,24,25,26,27]. An important contribution to this hypothesis is the observation that the psychotomimetic compound PCP acts at the NMDA receptor subtype of the receptors activated by glutamate [28]. Moreover, the psychosis evoked by PCP is regarded to be the best current pharmacological model of schizophrenia [29] because this drug produces both positive psychotic symptoms and negative symptoms [30]. It has been reported that PCP blocks responses of central neurons to NMDA. It has become increasingly clear that PCP acts as an open channel blocker of the NMDA receptor-coupled ion channel and is, therefore, a non-competitive glutamate antagonist. Markers for glutamatergic neurotransmission are particularly high in brain areas thought to be involved in the pathogenesis of schizophrenia such as entorhinal cortex, frontal cortex and the hippocampus [31]. Developmental disturbances of the second neuronal layer of the entorhinal region has been found in schizophrenic patients [32] suggesting a dysfunction of the glutamatergic perforant pathway. A decreased release of glutamate has been observed in the frontal and temporal cortex of schizophrenic patients [33] while increased NMDA receptor density has been measured in hippocampus and frontal cortex [34]. With regard to other glutamate receptor subtypes, unchanged quisqualate receptor densities have been found in the frontal, temporal and parietal cortices [35] while kainate receptor binding is increased in the frontal cortex [36] or unchanged [37], and decreased [38] in the hippocampus. In the putamen, increased [23] or unaltered [34,39] NMDA receptor densities have been reported.

In addition to their psychotomimetic properties, these NMDAR-antagonist compounds have neurotoxic effects of their own. Olney et al [40] found a neurotoxic-like effect of dizocilpine in rats manifested as vacuolization of neuronal cytoplasm in posterior cingulate cortex. Interestingly, Farber [41] could block this reaction by administering high doses of several antipsychotic drugs. Other anatomical studies of neurotoxicity of PCP [27] have revealed a pattern of degeneration that involve three interrelated systems: the olfactory tubercle, the parahippocampal and hippocampal regions (especially in ventral CA3 and CA1 regions and virtually no degenerating cells in dorsal hippocampus), and posterior cingulate cortex. Cingulate, retrosplenial and entorhinal cortices were acutely affected while parahippocampal, hippocampal and olfactory regions were so only after chronic exposition to these compounds.

Therefore, the glutamate hypothesis of schizophrenia unifies the structural alterations found in the cerebral cortex of schizophrenic patients, the psychotomimetic effects of phencyclidine and the therapeutic activity of dopamine antagonists in the treatment of schizophrenia. It is possible to improve the explanatory power of either the DA or glutamate hypothesis by incorporating the former into the latter. For example, since one action of DA receptors is to inhibit glutamate release [42], a primary defect in the DA system that causes DA hyperactivity could result in excessive suppression of glutamate release at NMDA receptors, with the consequent hypofunction of the NMDA receptor system as the basis of schizophrenic symptoms. Amelioration of schizophrenic symptoms by DA receptor blockers could be explained in terms of the DA receptor blockade disinhibiting glutamate release, thereby correcting glutamate hypofunction. On the other hand, a role for glutamate on the developmental pathogenia of schizophrenia has been suggested by both clinical evidence [43] supporting the use of ketamine as a safe pediatric anesthetic due to the lack of induction of psychotic symptoms in children, and experimental evidence in rats reporting lack of sensitivity to neurotoxic effects of NMDA antagonist before puberty [44]. Nevertheless, as an increase of dopaminergic activity may contribute to the development of paranoid hallucinatory psychosis in schizophrenic patients, and a dysfunction in glutamatergic activity has been postulated to be involved in both the production of psychotic symptoms and anatomical changes; both dopamine antagonist and glutamate agonist should therefore be of therapeutic benefit in these conditions. By contrast, a loss of dopaminergic activity or glutamatergic hyperactivity may result in akinesia [45] and therefore both neurotransmitter systems appear to be involved in motor function as well.

1.2.-The Neurodevelopmental Hypothesis of Schizophrenia

Feinberg [46] was among the earliest to suggest a developmental etiology of schizophrenia and to propose that the disorder could be explained by a maturational failure in normal pruning processes. Numerous controlled studies by computed tomography (CT) of living schizophrenic patients have found quantitative evidence of brain pathology in the form of enlarged third and lateral ventricles and cortical markings suggestive of reduced gyral mass or atrophy especially in frontal and temporal cortices [47]. The majority of CT studies have not found a relationship between ventricular enlargement and duration of the illness, a correlation that would be expected if the underlying pathological changes were active and progressive. The relationship of the lesion to the pathogenesis of schizophrenia is made even more obscure by the apparent distance between the occurrence of the lesion and the onset of the psychosis. The information that bears most directly on this issue has been obtained from postmortem studies where no signs of an ongoing neuropathologic process such as reactive gliosis, dying neurons, inclusion bodies, or inflammation had been found suggesting an early episode of brain damage. The data from CT studies also strongly suggest that the lesion would be ancient and idle. Moreover, ventricular enlargement has been reported in first-episode schizophrenic patients, a finding that implicates early pathology, since ventricular enlargement would be unlikely to develop acutely during adolescence without concomitant neurologic symptoms [48]. The observation that the ventricular size correlates with poor premorbid social adjustment and reports of a link between perinatal complications also suggest that the pathology exits early in life [49].

There is evidence that brain abnormalities in schizophrenia occur early in development, long before there is any indication of clinical illness, perhaps during fetal development. For example, minor physical abnormalities may be more frequent in schizophrenics than in their healthy sibling. Schizophrenics appear to have early difficulties in motor functioning. Hypotonicity, choreoathetoid movements, and abnormal hand postures were found in the first two years of life [50] of patients who later on developed schizophrenia. In a hallmark epidemiological study of all children born in England in one week in 1946 who were followed prospectively for 40 years, Jones and colleagues [51] reported that the 30 children, who developed schizophrenia in adulthood, demonstrated slightly delayed achievement of motor and linguistic milestones, coupled with slight but significant IQ and academic achievement deficits when compared to a matched sample drawn from the entire cohort, therefore suggesting brain abnormalities early in development.

Using functional neuroimaging techniques frontal lobe hypometabolism [52,53], temporal lobe abnormalities [54] and failures to activate motor regions [55] has been noted in the brain of schizophrenics when compared to control subjects. Indeed, several regions of the brain appear to be affected in the disease, and this suggests a subtle but relatively widespread structural brain abnormality. Thus, Weinberger and colleagues reported hyperactivity of the hippocampus coupled with hypometabolism of the frontal cortex during the performance of the Wisconsin Card Sorting Test [56]. Although some studies have found expected correlation of medial temporal lobe volumes and memory performance [57] and frontal lobe volumes with executive task performance [58], other studies have found mixed results [59], such as temporal lobe structures correlated with frontal lobe task performance, as well as frontal lobe areas predicting memory performance. These paradoxical findings probably reflect the integrative activity of wide areas of the brain; by using this connectional approach, a subtle change of one part of the brain in early development could be tardily altering such integrative activity. This connectionistic perspective has led to novel animal models of schizophrenia that incorporate the concept of maldeveloped cortical connectivity, alteration of dopamine function and disturbances of cognitive functions [60].

The concept of a neurodevelopmental origin of schizophrenia is based in part on the nature of the microscopic data such as disoriented neurons, missing or abnormally sized neurons or abnormal patterns of myelination. Thus, Akbarian and colleagues [61] found a significant decline in NADPH-diaphorase immunoreactive neurons in the cortical gray matter and the superficial white matter of the frontal lobe of five schizophrenics. This cellular displacement pattern has been interpreted as a defect in the normal orderly migration of neurons toward the cortical plate; these changes in cell population distributions considered likely to have serious consequences for the establishment of a normal pattern of cortical connections leading to a potential breakdown of frontal lobe function in schizophrenics. A second study from the same group [62] reported a significantly low numbers of NADPH-d neurons in the hippocampal formation and in the neocortex of the temporal lobe accompanied by a significant increase of these neurons in the white matter of the lateral temporal lobe and a tendency for an increase in parts of the parahippocampal white matter. These data seems to extend the finding to a more pervasive and global developmental defect. According to the timing for the migration of these neurons in the developing brain of the rhesus, one would expect the perturbation to have occurred at some point in the middle to late part of the second trimester of gestation. Current views of postnatal development hold that neurons refine and strengthen those synaptic connections that provide the best fit of their genetically programmed attributes

around that period of life. If the out-of-position NADPH-d neurons were to have become elements of dysfunctional circuits, unable to be appropriately modified by the subsequent events of activity-dependent experience, one could expect signs during development in addition to those that emerge later in the course of the disease. This has been corroborated by series of developmental analysis of children at high risk of schizophrenia assessed by Fish's pandysmaduration index [63] but ignored from usual medical examinations.

Maternal viral infections could account for this apparently global developmental disturbances, but how a virus might produce such delaying event is unclear and the nature of the virus remains undetermined. Other causative mechanisms could be considered, for example, premature switching off of genes responsible for trophic factors or their receptors in response to fever, famine or stress could as well affect the precise temporal orchestration of the steps underlying cell migration, synapse formation and refining. In fact, the genome of a given family tree might simply fail to maintain the expression of genes required to complete the process of cortical neuronal migration, even in the absence of external signals. A variety of cytoarchitectural abnormalities have been described in schizophrenics brains both in the limbic structures and in the frontal cortex. Abnormal orientation of hippocampal neurons [64], disturbed laminar organization of entorhinal [65] and frontal cortices [66] implying a failure of migration and settling of neurons into their appropriate target sites apprises that occurs during the second trimester on intrauterine brain development [67], volume reduction of mesial temporal lobe structures [68], misplacement of NADPH-diaphorase positive neurons in both temporal and frontal cortices have all been related to abnormal cortical development. Neuronal malconectivity resulting from these anatomical defects have been proposed to be key factors in the symptomatology of schizophrenia.

The hippocampal formation is also believed to be deeply involved in the pathophysiology of schizophrenia [69,70]. Recently bilateral reduced expression of synaptophysin in subiculum and parahippocampal gyrus [71] and microtubule-associated protein MAP-2 and MAP-35 has been described in the hippocampus of postmortem schizophrenics brains [72,73]. In addition, a reduction in polysialic acid-neural cell adhesion molecule has been observed in the hilus region of schizophrenic hippocampi when compared with control [74]. Finally, another cell recognition molecule, L1 antigen, has been found decreased in the CSF of schizophrenic patients and a mutation of the molecule has been characterized as a possible cause of mental retardation [75,76]. Furthermore, magnetic resonance imaging (MRI) studies point toward a compromised prefrontal-hippocampal network in schizophrenia [77], and the left parahippocampal gyrus has been the major brain area linked to schizoprenic-symptom production (such as reality distortion and

disorganized thinking) in PET studies of rCBF in schizophrenics [78] playing, maybe, a critical role in the genesis of the symptoms [79,80]. This critically dysfunctional network might be making specific contributions to the symptomatic and cognitive abnormalities in schizophrenia [81].

1.3.- The Neonatal Bilateral Excitotoxic Ventral Hippocampus Lesion Model

In 1993 Lipska and colleagues described postpubertal hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic damage of the ventral hippocampal (VH) formation on 7th day after birth in rats. They suggested that the neonatal VH lesion may affect functional development of the medial prefrontal cortex [60]. They also related the model with having most of the major phenomena associated with schizophrenia such as postpubertal onset, congenital hippocampal damage, cortical functional deficits, limbic dopamine dysregulation, and vulnerability to stress. Their results demonstrated that in rats with neonatally induced excitotoxic VH lesions, behavioral indices consistent with increased mesolimbic DA responsitivity to stressful and to pharmacological stimuli emerge only in early adulthood. They also suggested that homologous mechanisms might underlie certain aspect of schizophrenia.

There have been many attempts to model diverse phenomenological aspect of schizophrenia. Early neurobiological models have emphasized primary perturbations in striatolimbic dopamine activity to account for the therapeutic effects of antidopamine drugs [82,83,84] but without *mimicking* physiopathological mechanisms for the disease such as cortical defects or postpuberal onset. Other models have postulated the prefrontal cortex as a regulatory structure for subcortical dopaminergic activity [85,86,87,88,89]. The VH lesion models emerged later with the work of Lipska et al 1992 [90,91] where they reported that an excitotoxic lesion of ventral hippocampal formation in the adult rat enhanced spontaneous exploration and amphetamine induced locomotion, while inducing opposite changes in DA transmission in cortical (reduced DA activity in prefrontal cortex) and limbic (increased DA activity in nucleus accumbens) fields innervated by the hippocampal formation. Lipska et al. tested, then, the effects of development on the behavioral changes induced by the VH lesion [60]. Particularly they posited that the effects of early hippocampal lesions on limbic DA systems would not be attenuated by maturation but would instead emerge with maturation [60]. In this work the motor activity of different lesioned and sham cohorts of rats, that underwent excitotoxic destruction of the VH formation at postnatal day (PD) 7, was measured at both prepubertal (PD35) and postpubertal (PD56) periods in three different conditions: after exposure to a novel environment, after saline, and after

amphetamine injection. Only the postpubertal group showed an increase in the locomotor activity. One of these cohorts was additionally exposed to a swim test 2 weeks after the last testing (on PD70) to further explore the effects of stress in neonatally lesioned rats. In this cohort only the lesioned group showed an increase in locomotor activity. An additional group lesioned on PD42 was exposed to a swim test to compare their response to stress to the neonatally lesioned group. In this case no difference between lesioned and sham groups was seen. Another group of rats was treated for 3 weeks (from PD35 until PD56) with either vehicle or haloperidol to assess the effects of neuroleptic treatment on hyperlocomotion. Here, neuroleptic treatment blocked the emergence of hyperactivity in the lesioned group, giving thus additional support to this model of schizophrenia based on an early brain injury.

Although the developmental hypothesis of schizophrenia has a representative animal model in Lipska et al.'s work , the effect of VH lesion as a potential animal model of schizophrenia has been explored by other authors as well. Csernansky and col. [92] have postulated an animal model of schizophrenia after i.c.v. injection of kainic acid that combines decreased hippocampal neuronal number, increased dopamine receptor binding in the nucleus accumbens and behavioral hyperactivity. Whishaw and col. [93] have also reported an animal analogue of schizophrenia that involves hippocampal modulation of locomotion.

2- OBJECTIVES:

Since the neonatal VH lesion model has been proposed as an animal model of schizophrenia, we wanted to investigate the levels of ionotropic and metabotropic glutamate receptors in different brain regions in order to search for a linkage between the glutamatergic hypothesis of schizophrenia and the postpubertal behavioral manifestations displayed in neonatal VH lesioned animals. We also wanted to investigate the long-term consequences that the neonatal lesion would have on behavioral and neurobiological markers. In particular we aimed to:

1- Determine whether stress- and dopamine agonist- induced hyperlocomotion, observed at PD56 after bilateral neonatal excitotoxic ventral hippocampus lesion, persists until 6 months of age.

2- Test neonatal VH lesioned animals in paradigms that involve working and spatial memory such as the radial arm maze.

3- Study changes of expression of ionotropic excitatory amino acid receptors (N-methyl-D-aspartate, AMPA and kainate receptors) in frontal cortex in neonatal VH lesioned animals.

4- Measure the expression of metabotropic glutamate receptor as a possible mediator of long-term changes in the neonatal VH lesion model.

3- METHODS

3.1.- Animals.

Pregnant Sprague-Dawley rats were obtained at gestational day 14-15 from Charles River Canada (St.Constant, Quebec, Canada). Animals were individually housed in a temperature- and humidity- controlled environment on a 12 hr light/dark cycle with free access to standard laboratory chow and tap water until time of delivery. The day after birth, litters of 6-10 male pups were formed, and on postnatal day 7 (PD7), corresponding to a body weight of 15-17 gm, each pup was assigned to either the sham or lesioned group.

3.2.- Materials.

Ibotenic acid, MK-801, quisqualate, L-glutamate and kainate were purchased from Research Biochemicals (Natick, MA). D-amphetamine sulphate was obtained from Sigma (St.Louis, MO). [^{125}I]-MK-801, [^{3}H]-glutamate, [^{3}H]-kainate and [^{3}H]-AMPA were obtained from DuPont NEN (Boston, MA). ^{3}H Hyperfilm and microscale tritium and iodine standards were obtained from Amersham (Toronto, Ont, Canada). 2-methyl-butane was purchased from BDH Chemicals (Montreal, Que, Canada) and gelatin was purchased from Fisher Scientific (Montreal, Que, Canada).

3.3.-Surgical Procedures.

The method for performing neonatal ventral hippocampal lesions described by Lipska [60] was followed with minor modifications. First, anesthesia by hypothermia was obtained by placing the pups on wet ice for 10 to 15 min. The pups were then positioned and taped on a platform, fixed to a stereotaxic Kopf instrument. An incision was made over the skull and 0.3 µl ibotenic acid (7 µg/µl) or an equal volume of the vehicle (0.1 M phosphate-buffered saline, pH 7.4) was injected in each ventral hippocampus over a 2 min. period through a 30-ga stainless steel cannula positioned at the following coordinates: AP -3.0 mm, ML ± 3.5 mm and VD -5.0 mm from the dura [60]. The cannula remained in place for 4 min following completion of the infusion. After this procedure, pups were placed under a warming lamp for recovery and then returned to their mothers. At PD21, animals were weaned, grouped two or three per cage and housed as described above. All

surgical procedures described in this study had been approved by McGill University Animal Care Committee in accordance with the guidelines of the Canadian Council for Animal Care.

3.4-Brain processing.

Rats were sacrificed by rapid decapitation after the last day of the locomotor activity test, that is at PD39, PD60 or PD184, except for the PD56 group that underwent the radial arm maze test that was killed at PD80. Brains were rapidly removed, frozen in 2-methyl-butane maintained at between -20 and -40°C and stored at -80 °C until use. Frozen rat brains were sectioned at 20 µm thickness on the coronal plane using a Leitz cryostat. Sections were collected on precleaned, gelatin-coated microscope slides (3 sections/slides), thaw-mounted , desiccated under vacuum at 4 °C overnight and then stored at -80 °C until the day of the experiment. For assessment of lesion size, serial sections at the level of hippocampus were cut, stained with 0.5 % cresyl violet and examined under microscope for lesion and probe placement visualization.

3.5.- Receptor Binding Autoradiography.

Brain sections taken at the level of the frontal cortex (Plates 7 and 8), striatum (Plates 13 and 14) and cerebellum (Plates 35 and 39) according to the atlas of Paxinos and Watson [94] were used in the following protocols (taken from Sakurai et al [95] for [^{125}I]-MK-801; and Clark et al [96] for both [^{3}H]-AMPA and [^{3}H]-Kainate, and used with minor modifications): For *NMDA Receptor binding*, slides were incubated for 60 min. at room temperature with 200 pM (+)-3-[^{125}I]-MK-801 (2,200 Ci/mmol) in 50 mM PBS (pH 7.4) also containing 10 mM glycine and 30 mM glutamic acid. Non-specific binding was defined in adjacent sections by the addition of 5 µM MK-801 to the incubation buffer. Washing once (for 2 min) in room-temperature PBS and twice (for 15 min) in ice-cold PBS followed the incubation period. After a final brief dipping in ice-cold distilled water to remove salts, slides were dried at room temperature and apposed to [^{125}I]-Hyperfilm (Amersham, Toronto, Ontario) for 8 hours. For *Kainate Receptor binding*, brain sections were preincubated in 50 mM Tris-citrate pH 7.0 first for 30 min at 4°C and then for 10 min at 30°C. Sections were then incubated in the same buffer containing 20 nM [^{3}H]-kainic acid (58 Ci/mmol) for 30 min at 4°C. Non-specific binding was determined on adjacent brain sections by adding 50 µM kainic acid to the binding buffer. Dipping slides in ice-cold buffer four consecutive 5-second washes followed the incubation period. After dipping in

ice-cold deionized water to remove salts, slides were dried at room temperature and apposed to [^{3}H]-Hyperfilm (Amersham, Toronto, Ontario) for 5 weeks, in the presence of [^{3}H]-Microscales calibrated tritium standards (Amersham, Toronto, Ontario). For *AMPA Receptor binding*, tissue sections were preincubated in 50 mM Tris-acetate pH 7.2 for 30 min. at 4°C. Sections were then incubated for another 30 min. at 4°C with 50 nM [^{3}H]-AMPA (53 Ci/mmol) in 50 mM Tris-Acetate pH 7.2 containing 100 mM KSCN. Non-specific binding was determined on adjacent brain sections by adding 1 mM glutamic acid. Four 4-second consecutive dippings in ice-cold incubation buffer followed the incubation period. After quickly dipping in ice-cold deionized water, brain sections were dried at room temperature and apposed to [^{3}H]-Hyperfilm for 2 weeks. For *metabotropic glutamate receptor binding* [97], brain sections were preincubated in 50 mM Tris-HCl, 2.5 mM $CaCl_2$, 30 mM KSCN, 100 µM NMDA, 10 µM AMPA 7.2) for 30 min at 4°C. After prewashing, sections were dried under a stream of cool air. Sections were then incubated in the same buffer containing 110 nM L-[^{3}H]-glutamate (41 Ci/mmol) for 45 min at 4°C . Non-specific binding was determined on adjacent brain sections by adding 2.5 µM quisqualate. Four ice-cold buffer washing, followed by two 2.5% glutaraldehyde in acetone, followed the incubation period. Slides were dried under a stream of hot air and apposed to [^{3}H]-Hyperfilm (Amersham, Toronto, Ontario) for 8 weeks, in the presence of [^{3}H]-Microscales calibrated tritium standards (Amersham, Toronto, Ontario).

Following previously defined autoradiography exposure times, films were analyzed with a computerized image analysis system (MCID-4, Imaging Research, Ste-Catherine, Ontario). All frontal and striatal sections were divided in subregions according to Paxinos and Watson atlas [98]. Comparison between groups was achieved by applying two-way repeated measures ANOVA with *post hoc* comparison between groups, $p<0.05$ being considered significant.

3.6.- Behavioral tests.

3.6.1.- Locomotor activity test.

Four weeks (PD35), 7 weeks (PD56) or 6 months (PD180) after surgery, locomotor activity of sham and ibotenic acid-lesioned rats was assessed in 2-photocell activity boxes (30cm X 20cm X 20cm) connected to an IBM computer equipped with a software ("actanal") developed by Concordia University (Montreal, Québec). The locomotor activity of each animal was assessed under three different testing conditions: (1) following exposure to a novel environment: unacclimatized rats were placed in activity

boxes for a 60 min period while the locomotor activity score was recorded, (2) following saline injection: after recording of locomotion in a novel environment, animals were kept in the activity boxes for another 60 min period after being injected with 1 ml/kg of 0.9% NaCl (s.c.), and (3) following amphetamine injection: 1 mg/ml solution of D-amphetamine sulphate dissolved in 0.9% NaCl (1 mg of free base/kg, s.c.) was administered and the locomotor activity was recorded for the next 120 min. Data were analyzed with two-way ANOVA (with repeated measures) test.; $p<0.05$ being considered significant.

3.6.2.- Radial Arm Maze test

The radial maze used in this study had an hexagonal center platform with 8 arms radiating from the center [99]. The arms remained in the same location with respect to extramaze cues, and the same four arms were consistently baited for any one animal. The room was well lighted and there were a number of distinctive extramaze cues (table, door, two different pictures on the walls, panel of fluorescent lights, rack of cages). Any one animal had the same four arm baited from trial to trial. The test was run using a computer algorithm made to set up experiments according to design (number of animals tested: n = 3 sham and n = 3 lesion; total time per trial: initially 10 min, and after criterion 5 min; total number of choices per trial: initially 20 and after criterion 10; total number of arm baited: 4 out of 8, as well as their location in the arm maze).

Preliminary training consisted of giving PD56 rats (3 days after the last locomotor activity test) 15 min/day to explore the apparatus and no food was presented in the apparatus during this time. Then all rat were placed on a food deprivation schedule to bring body weights down to 85 % of ad lib. After one week of restricted feeding, the animals were placed individually on the center platform for a 10-min. period. The animals were placed on the maze on each of 10 days. Since choice accuracy gradually improved during the first days and then remained stable during the second 10 days [100], the data were analyzed in 10-day blocks. During the second ten-day block training, each rat was given two daily trials for 6 days per week. Each session consisted of two three trials separated by intervals of 60s, during which time the subject was removed from the maze to a holding cage. The order in which trials were given to a subject was alternated from day to day. A trial consisted of baiting four of the correct arms with food bits and placing the rat in the center of the platform. The animals remained on the maze until all four reinforcements had been received, until total number of choices per trial were made, or until total time per trial had elapsed, whichever occurred first. Choices of arms and total running times were recorded. The program also calculated reference errors (e.g. when an incorrect arm is

chosen), working memory errors (e.g. when a correct arm is revisited), working memory impairment errors (e.g. when an incorrect arm is revisited) and repeats (e.g. the sum of working memory errors and working memory impairment errors). Correct choices was calculated as the difference between total choices and reference errors. The four correct arms for the place task were randomly chosen for each subject with the restriction that there be no obvious pattern and no more than two adjacent correct. Training trials were continued for 10 days by which time performance were stabilized. The data were analyzed by an independent t-test for each variable; $p<0.05$ being considered significant.

4- RESULTS

4.1.- Anatomical Evaluation of the Neonatal Lesion.

Anatomical evaluation of the neonatal lesion in the adult animals was done by histological examination of cresyl violet-stained serial coronal sections throughout the hippocampal formation from plate 19 till plate 28 (Paxinos and Watson, 1986)[98] of 17 rat brains (3 sham and 14 lesioned). An example of a representative VH lesion is shown in Figure 1. Neuronal loss, atrophy, and some cavitation was observed in the ventral part (VH) of the lesioned group with the sparing of the most anterior (dorsal) aspects of the hippocampal formation. The lesion affected the dentate gyrus and the alveus of the hippocampus. The parasubiculum, presubiculum and subiculum were also damaged to a variable extent. No other extrahippocampal injuries were observed in the examined brains under optical microscopic analysis. In one of the lesioned animals, the lesion boundary extended to the basal amygdaloid nucleus and amygdalohippocampal area. This animal was not excluded from the study since the behavioral data obtained from it showed no difference with respect to those without extrahippocampal damage. The extent of the lesion varied between animals with smaller lesions involving disruption of the cell body layer of CA3 along with a decrease in the size of the ventral hippocampal area and larger lesions showing complete destruction of normal hippocampal tissue along with residual gliosis of the area. In some cases there was a slight asymmetry in the size of the lesion when compared right and left sides. Overall, the extent of ventral hippocampal damage as well as the slight asymmetry of the lesion did not appear to correlate with performance. The lesion produced was comparable to that described in previous reports in which the same surgical procedures were followed [60,101].

Figure 1. Representative histological sections of a VH lesion animal (left) and a sham-operated one (right) for comparison. Note that the lesion produced neuronal loss, atrophy, and cavitation in the ventral part of the hippocampus.

LESIONED

SHAM

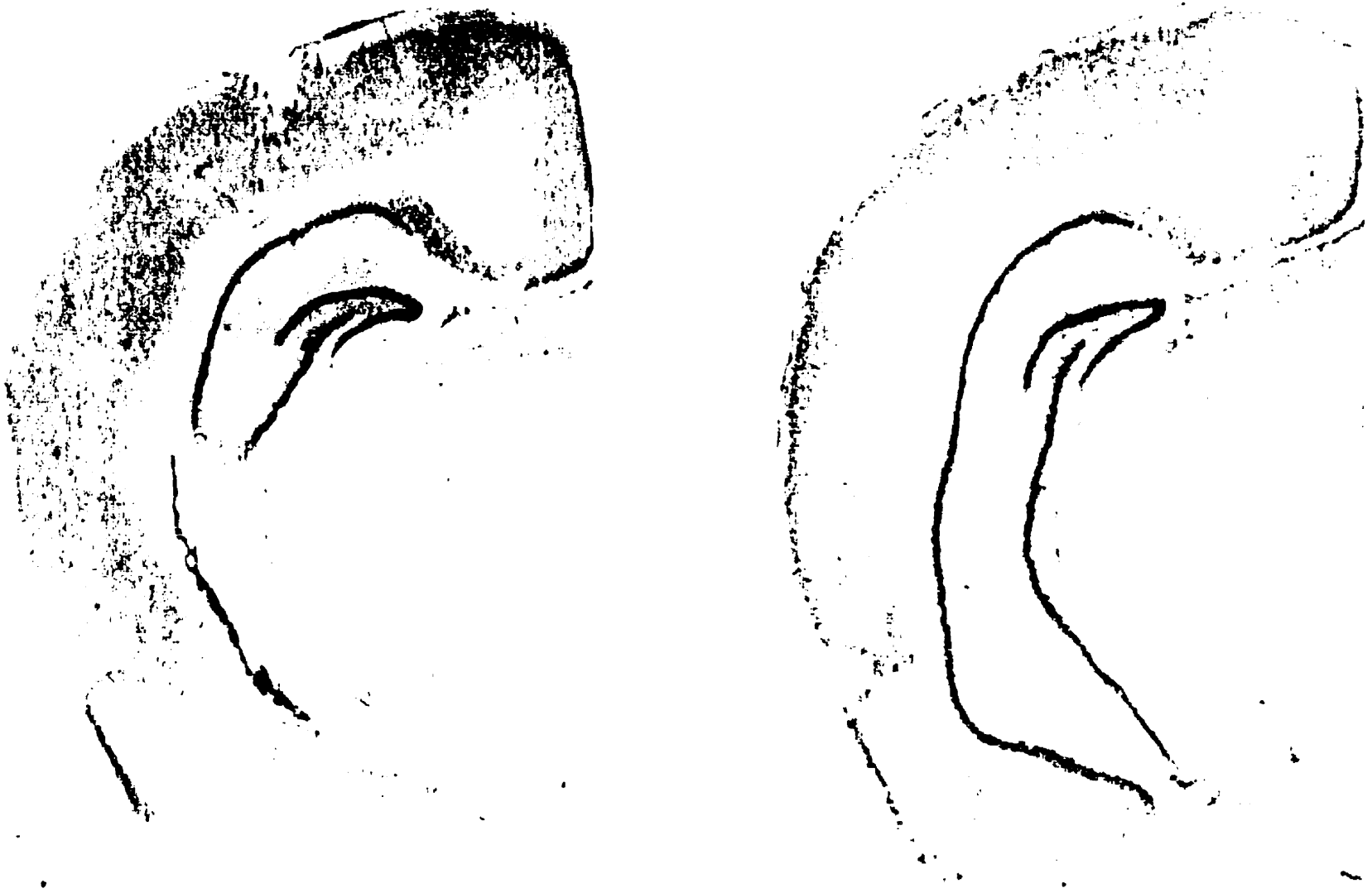


Figure 1

4.2.-Behavioral tests

4.2.1 Locomotor Activity.

Neonatal VH lesion effects on locomotor activity at three developmental stages are illustrated in Figure 2. In all age groups, either in sham or lesioned animals, active exploratory behavior was the initial response of rats to novel environment. In the novel exploration, 35-day old lesioned rats (n = 12) showed no significant difference with respect to the aged matched sham group (n = 7). However, 56-day old lesioned group (n = 11), as well as the 6-month old lesioned group (n = 10), showed statistically significant increase of locomotor activity after novelty when compared to age-matched shams (n = 7). After one hour of novelty, saline injection (1ml/kg s.c.) was administered to rats and locomotor activity was recorded during the following 60 min. Similarly, 56-day and 6-month old lesioned groups showed significant increase of locomotor activity after saline injection when compared to aged-matched shams. The 35-day old lesioned group did not show any significant difference with their age-matched shams. After D-amphetamine injection (1mg/kg s.c.), both the 56-day old lesioned group and the 6-month old lesioned group showed a significant increase in locomotor activity when compared to matched age shams while the 35-day old lesioned group did not differ from age-matched sham controls.

In summary, by using a two-way ANOVA (with repeated measures) test, significant differences were found between lesion vs. sham animals at PD56 and PD180 but not at PD35, but no significant differences among lesioned groups of PD56 and PD180 in any treatment; $p<0.05$ being considered significant. These results are in agreement with other previous studies in the same animal model [60,101].

4.2.2- Radial Arm Maze.

Each rat was placed on a platform (25 cm in diameter) in the middle of an 8-arm radial maze. After being placed on the central platform, the rat visited each arm and ate all the 4 pellets.

Figure 2. Effects of VH lesion on locomotor activity at PD35, PD56 and PD180 (6 m) during 60 min of placement in a novel environment (top), during 60 min after a saline injection (middle), and during 120 min after a D-amphetamine (1mg/kg s.c.) injection (bottom).

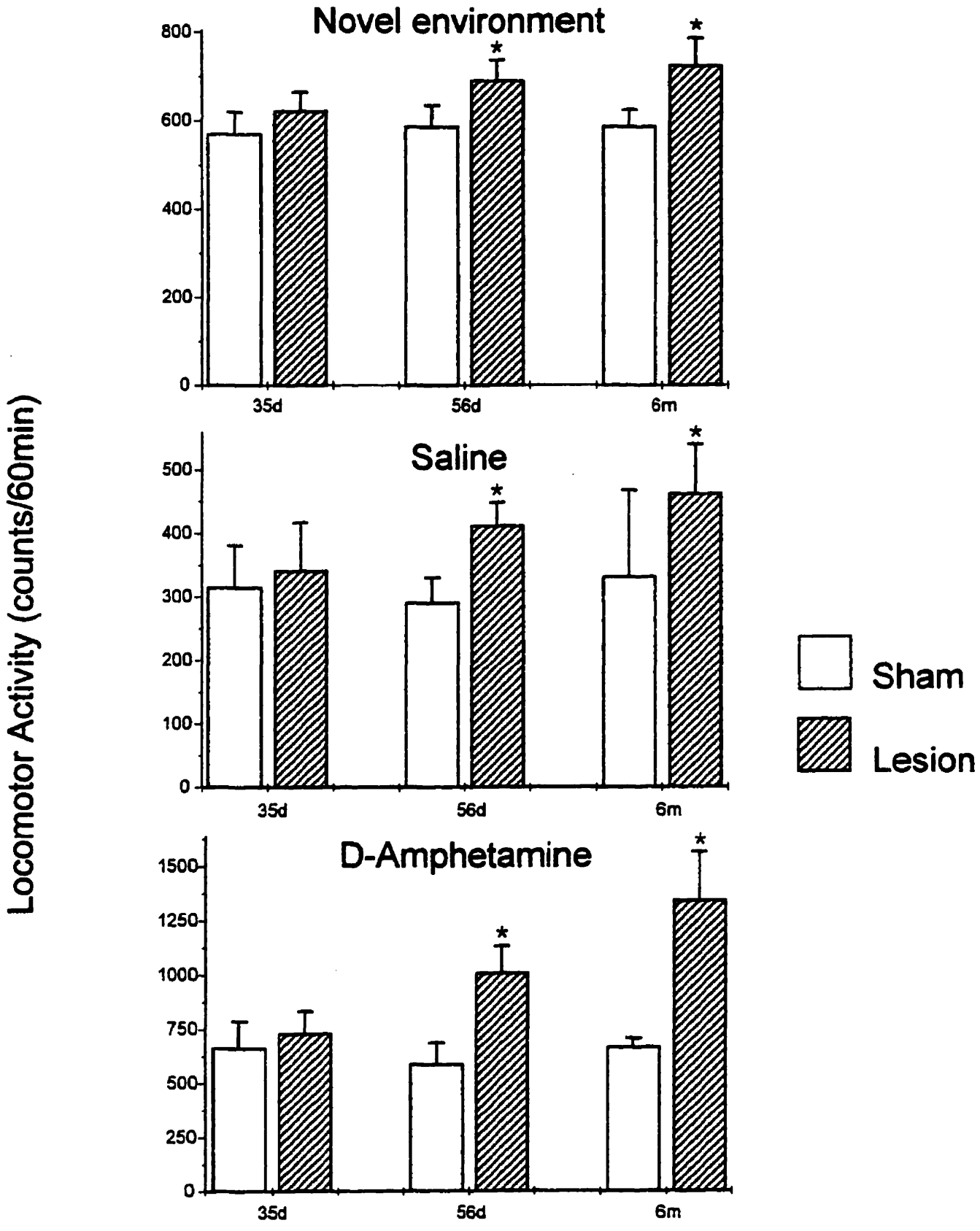

Novel environment
Saline
D-Amphetamine
Locomotor Activity (counts/60min)
800
600
400
200
0
500
300
100
1500
1250
1000
750
250
35d
56d
6m
*
Sham
Lesion

Figure 2

The test animals learned and memorized the 8-arm radial maze task using the spatial relationship between their own location and the various objects in the background environment. They learned not to re-enter an arm that had been previously visited during the same test. A computerized program recorded frequency of arm visits, velocity of walking, errors made and the time required to accomplish the task. The behavioral observation was discontinued after 5 min. even if the animal did not finish the task. The performance of the animal in each session was assessed by the number of correct choices and the number of errors which was defined as a re-entry into an already visited arm.

A learning curve based on the percentage of correct responses showed that the animals continuously improved their performance and that by day 10 both groups had reached 80% criteria which persisted thereafter.

Analysis of total running times revealed a very significant decrease in the time required to accomplish the task in the lesioned group when compared with sham animals as shown in Figure 3. This result is consistent with the data obtained from the locomotor activity boxes and confirms hyperlocomotion in the lesioned group, but using a different paradigm.

It appears that the neonatal ventral hippocampus lesion did not affect the overall performance of lesioned rats in this place-task radial 8-arm maze paradigm. However, it must be noticed that during the first 5 days of training, when novelty was present, lesioned animals showed poorer performance in the paradigm when compared to shams. An inverse trend was observed thereafter. Reference errors were significantly reduced in lesioned rats, when compared to shams, once learning criteria was reached; this indicated that lesioned rats tended to explore less arms and therefore revisited wrong arms.

As shown in Figure 3, correct choices were significantly reduced ($p<0.05$) in the lesioned group. Working memory impairment errors and repeats tended to be higher in the lesioned group. We did not perform the cue task in this paradigm to accurately measure working memory deficits in our model; however, it appears that lesioned animals have more difficulty learning a new task ,and they also tended to revisit the same arms and did not explore other arms.

This study provides additional evidence for the hypothesis that the hippocampus plays a major role in mediating spatially organized behavior. It also indicates that spatial deficits are not solely due to dorsal hippocampus and fimbria lesions but involves many part of the hippocampal system as it is supported in other works [102].

Figure 3. Effects of VH lesion on arm maze performance. Top left: Total running time was reduced ($p<0.05$) in the lesioned group. Top Right: The average number of repeats was slightly higher in the lesioned group. Bottom Left: Lesioned rats had more working memory impairment errors. Bottom Right: Correct choice were reduced ($p<0.05$) in the lesioned.

Radial Arm Maze Performance

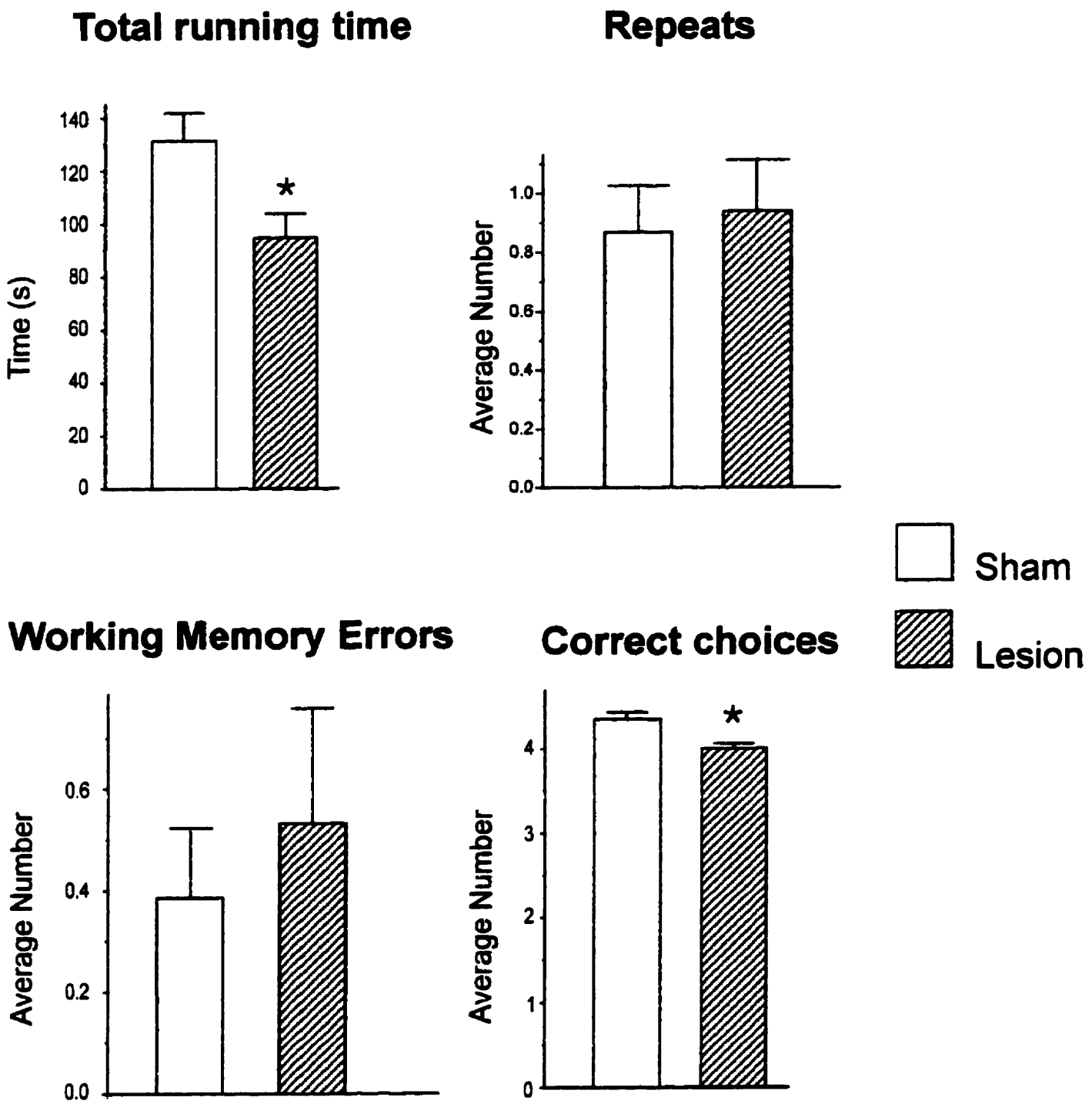


Figure 3

4.3.- Receptor binding autorradiography

4.3.1.- [^{3}H]-MK-801 Binding

Specific [^{125}I]-MK-801 binding sites were widely distributed in the brain of prepubertal (PD35), postpubertal (PD56), and adult (PD180) rats. However, areas in which [^{125}I]-MK-801 binding was concentrated included the hippocampal formation and cortex. Outer layers of the neocortex were richer in specific [^{125}I]-MK-801 labeling than inner layers. Moderate levels of specific [^{125}I]-MK-801 binding were found in subcortical areas such as the striatum. The total percentage of specific binding for [^{125}I]-MK-801 was about 90 %.

As shown in Table Ia, Ib, and Figure 4, PD56 lesioned rats (n = 3) compared with their age-matched shams (n = 3) showed statistically significant increases in [^{125}I]-MK-801 binding in cortical areas such as frontal cortex-1, -2, -3 both inner and outer layers; cingulate cortex 1 and its outer layer; agranular insular cortex both inner and outer layers; and lateral orbital cortex inner layer. The percentage of increase in significant cortical region of lesioned vs sham in this group was about 38% (range=29-46%). Similarly, in PD180 rats (n = 4) significant increases in cortical areas included: frontal cortex-1,2,3 both inner and outer, cingulate cortex-1 inner and outer, cingulate Cortex-3 inner, agranular insular cortex inner, ventrolateral orbital cortex inner, lateral orbital cortex both inner and outer, and medial prefrontal cortex both inner and outer. In this group the percentage of increase in significant cortical areas was about 30 % (range=21-39%). Previous data has shown that no difference was found between lesioned and sham PD35 groups [103], with most of regions showing less than 5 % increase.

Table Ia. Quantitative analysis of [^{125}I]-MK-801 Receptor Binding in 56-day old rats

Regions	SHAM (n = 3) MEAN ± SD		LESION (n = 3) MEAN ± SD		%CHANGE	
FRCX-1	18.63	1.09	33.62	1.56	44.59	*
FRCX-2	18.88	0.97	33.32	2.95	43.35	*
FRCX-3	19.21	1.33	30.46	3.00	36.96	*
FCXINN-1	15.72	1.03	26.39	2.16	40.43	*
FCXINN-2	16.09	1.29	25.89	1.57	37.87	*
FCXINN-3	15.38	0.60	24.76	2.97	37.88	*
FCXOUT-1	20.53	1.55	38.16	1.82	46.20	*
FCXOUT-2	20.08	0.90	36.39	3.88	44.82	*
FCXOUT-3	21.99	2.40	33.77	2.73	34.90	*
CG-1 TOTAL	18.17	1.07	28.31	4.96	35.83	*
CG-3 TOTAL	18.52	1.38	24.86	7.16	25.50	
CG-1 INN	15.90	1.23	20.25	3.55	21.47	
CG-3 INN	17.19	0.38	21.58	6.84	20.34	
CG-1 OUT	19.58	1.50	31.57	5.83	37.99	*
CG-3 OUT	19.28	2.44	27.83	7.80	30.71	
AGINCX	19.96	2.07	31.67	5.21	36.96	*
LOCX	18.08	2.12	25.92	4.07	30.25	
VLO	18.07	1.58	25.09	5.16	27.97	
INFRALIMBICX	17.26	2.32	22.78	5.75	24.24	
DORSALPEDUNCX	18.60	2.99	24.60	4.23	24.37	
INFRALIM-INN	15.80	0.85	21.16	5.48	25.32	
INFRALIM-OUT	18.53	4.07	24.91	5.61	25.59	
DORSALPEDU-INN	17.35	2.79	22.93	5.73	24.35	
DORSALPEDU-OUT	18.98	3.27	23.90	5.05	20.58	
AGINCX-INN	16.57	1.02	26.32	4.60	37.05	*
AGINCX-OUT	21.54	2.93	34.25	5.66	37.10	*
VLO-INN	16.07	0.39	22.30	5.04	27.93	
VLO-OUT	20.68	2.91	28.60	4.40	27.67	
LOCX-INN	15.83	0.78	22.18	3.26	28.65	*
LOCX-OUT	20.37	3.37	29.47	6.12	30.87	
MPFC	18.15	1.71	24.74	7.20	26.64	
MPFC-INN	16.75	0.31	22.00	7.31	23.85	
MPFC-OUT	19.11	2.66	27.14	7.18	29.58	

Receptor levels in fmol/mg of wet tissue are expressed as mean and standard deviation of specific binding from individual pooled values obtained from triplicate. For brain regions see Appendix.

* :$p<0.05$

Figure 4. Quantitative analysis of [^{125}I]-MK-801 receptor binding at PD56 in representative brain regions expressed as average (±SD) values in fmol/mg wet tissue. For illustration representative autoradiograms of specific binding [^{125}I]-MK-801 are shown.

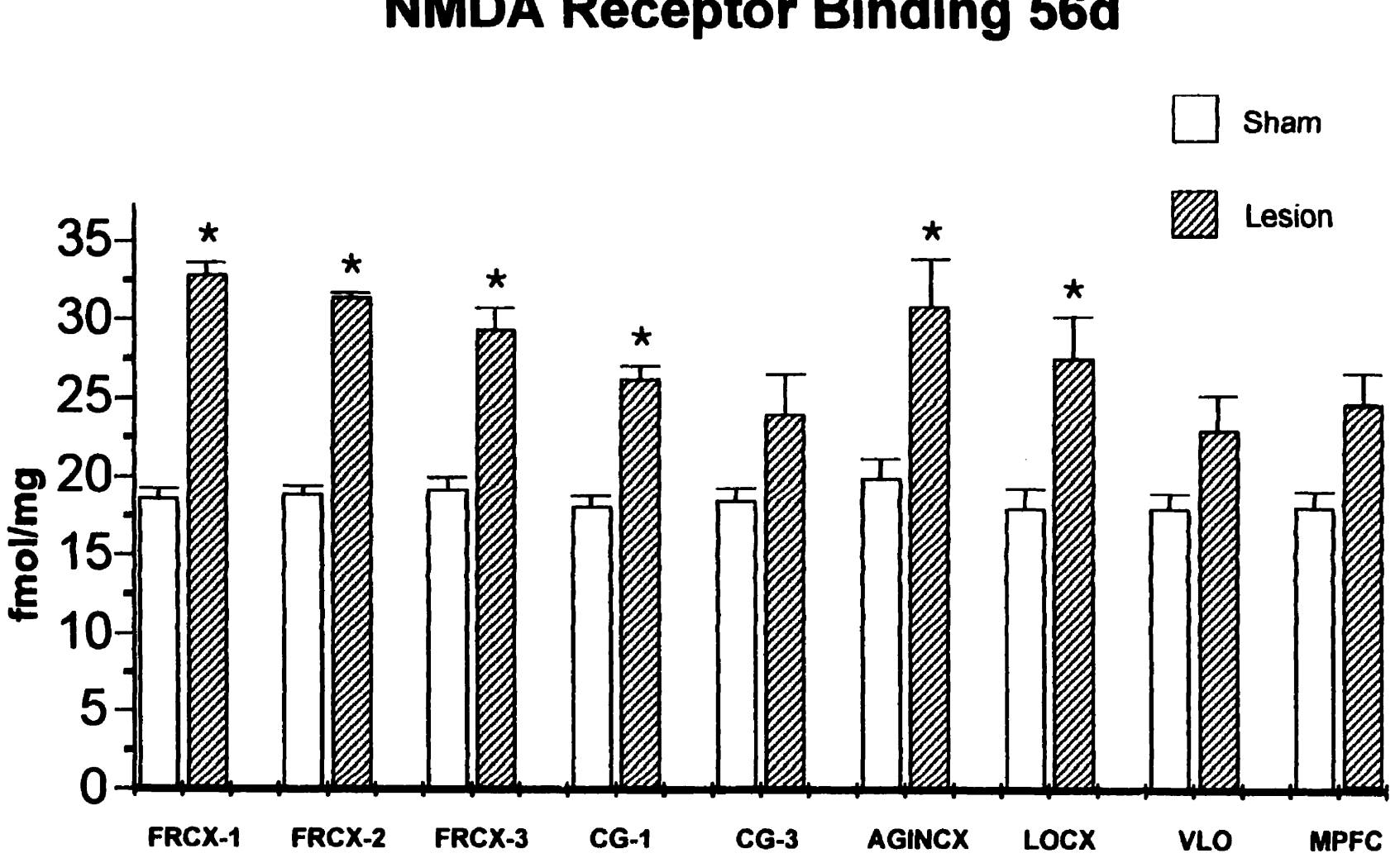
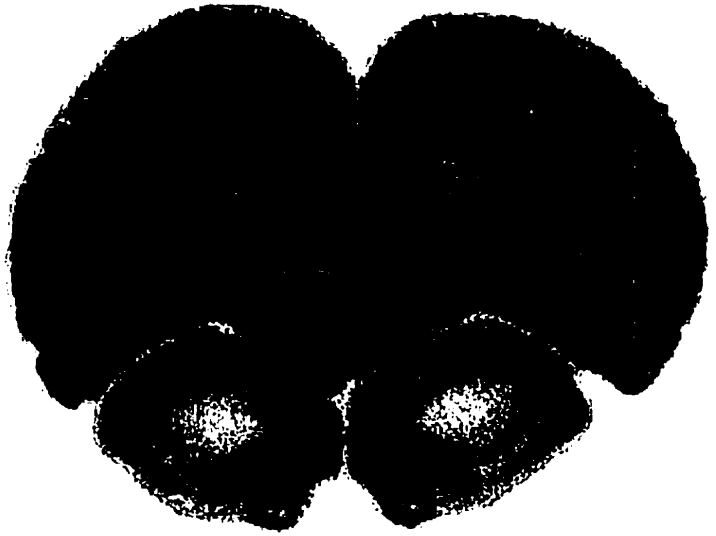
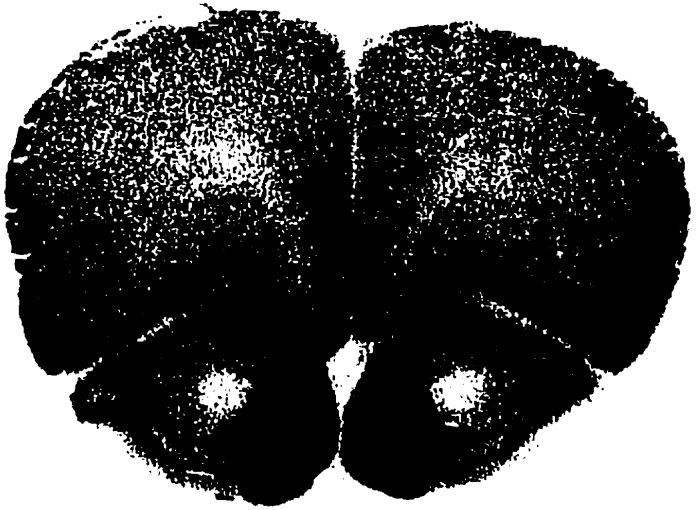

NMDA Receptor Binding 56d
Sham
Lesion
fmol/mg
35
30
25
20
15
10
5
0
*
FRCX-1
FRCX-2
FRCX-3
CG-1
CG-3
AGINCX
LOCX
VLO
MPFC

Figure 4

[I^{125}] MK-801 Receptor Binding



Lesion



Sham

Table Ib. Quantitative analysis of [^{125}I]-MK-801 Receptor Binding in 6-month old rats

Regions	SHAM (n = 4) MEAN ± SD	LESION (n = 4) MEAN ± SD	%CHANGE	
FRCX-1	21.22 2.05	30.16 2.55	29.65	*
FRCX-2	21.74 1.78	30.02 1.39	27.57	*
FRCX-3	20.53 4.14	28.65 1.81	28.34	*
FCXINN-1	16.99 1.86	24.96 1.83	31.94	*
FCXINN-2	17.62 2.38	26.21 2.02	32.77	*
FCXINN-3	16.96 0.39	24.40 1.15	30.51	*
FCXOUT-1	23.57 2.14	32.45 2.39	27.36	*
FCXOUT-2	23.19 2.49	31.87 1.07	27.24	*
FCXOUT-3	23.31 0.69	31.82 2.57	26.76	*
CG-1 TOTAL	21.61 0.88	28.81 1.50	25.00	*
CG-3 TOTAL	21.90 1.75	29.38 1.82	25.48	
CG-1 INN	16.89 1.79	24.97 1.96	32.36	*
CG-3 INN	18.79 0.88	26.68 1.42	29.56	*
CG-1 OUT	23.88 1.48	31.05 1.37	23.09	*
CG-3 OUT	24.65 2.23	31.57 2.16	21.93	
AGINCX	23.05 4.39	30.39 1.56	24.14	*
LOCX	19.17 2.97	28.09 3.69	31.76	*
VLO	20.53 2.85	29.54 2.17	30.50	*
INFRALIMBICX	21.32 3.59	28.72 2.02	25.79	
DORSALPEDUNCX	23.64 4.32	30.33 2.56	22.05	
INFRALIM-INN	18.45 2.68	25.93 2.00	28.83	
INFRALIM-OUT	24.98 4.83	33.09 2.40	24.51	
DORSALPEDU-INN	21.97 3.69	28.66 3.45	23.33	
DORSALPEDU-OUT	24.22 4.59	31.05 2.47	21.98	
AGINCX-INN	18.78 2.97	30.0Ɛ 3.59	37.56	*
AGINCX-OUT	25.24 1.28	32.49 5.97	22.32	
VLO-INN	17.70 1.94	29.08 2.35	39.12	*
VLO-OUT	23.80 1.66	29.79 1.69	20.12	
LOCX-INN	16.55 2.20	27.12 3.31	38.97	*
LOCX-OUT	22.21 2.04	32.12 3.90	30.86	*
MPFC	21.85 1.43	27.67 1.16	21.03	*
MPFC-INN	18.79 1.28	26.76 1.22	29.79	*
MPFC-OUT	24.58 1.72	31.04 1.05	20.81	*

Receptor levels in fmol/mg of wet tissue are expressed as mean and standard deviation of specific binding from individual pooled values obtained from triplicate. For brain regions see Appendix.* :$p<0.05$

Figure 5. Quantitative analysis of [^{125}I]-MK-801 receptor binding at PD180 (6 m) in representative brain regions expressed as average (±SD) values in fmol/mg wet tissue. For illustration representative autoradiograms of specific binding [^{125}I]-MK-801 are shown.

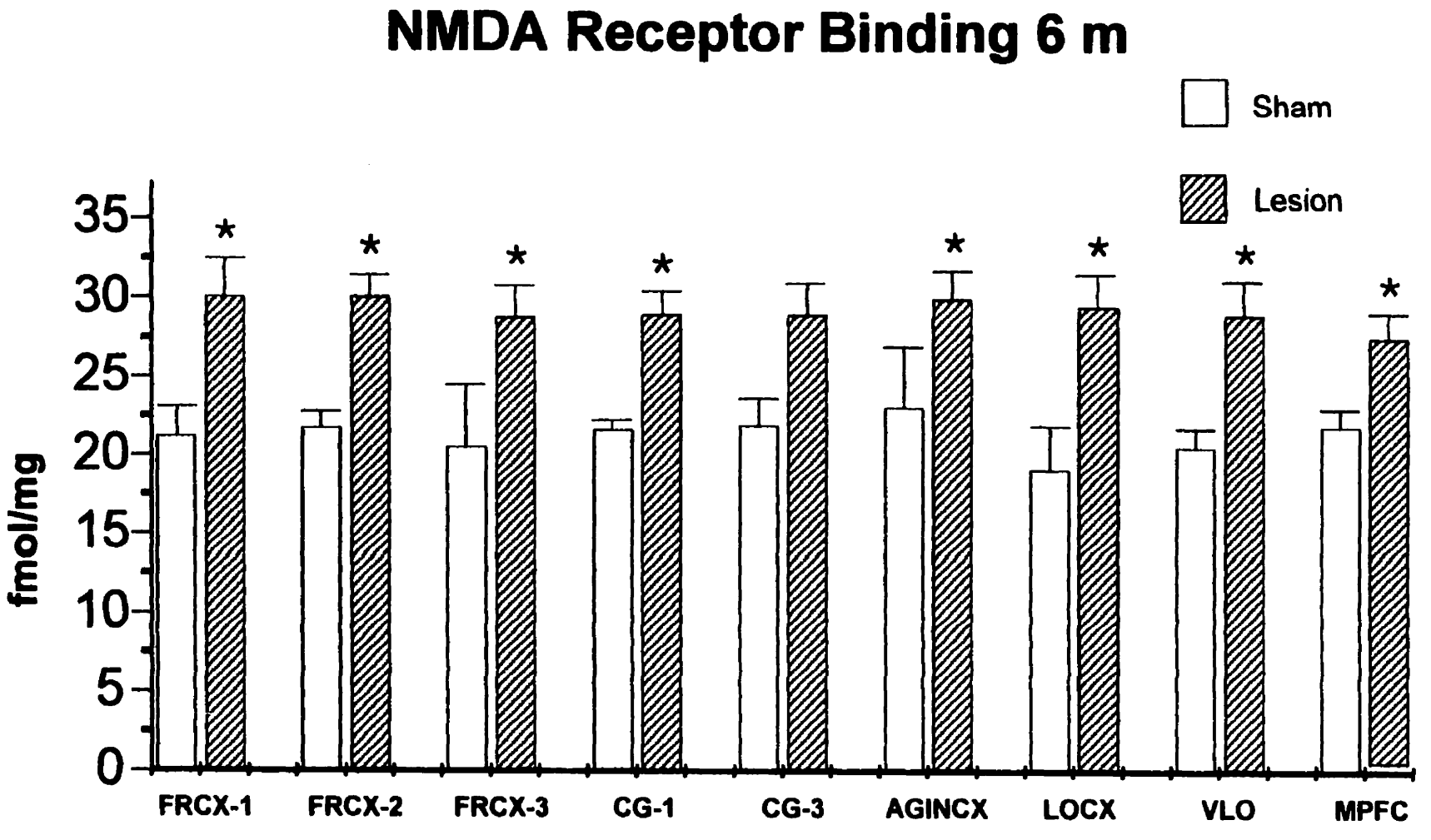
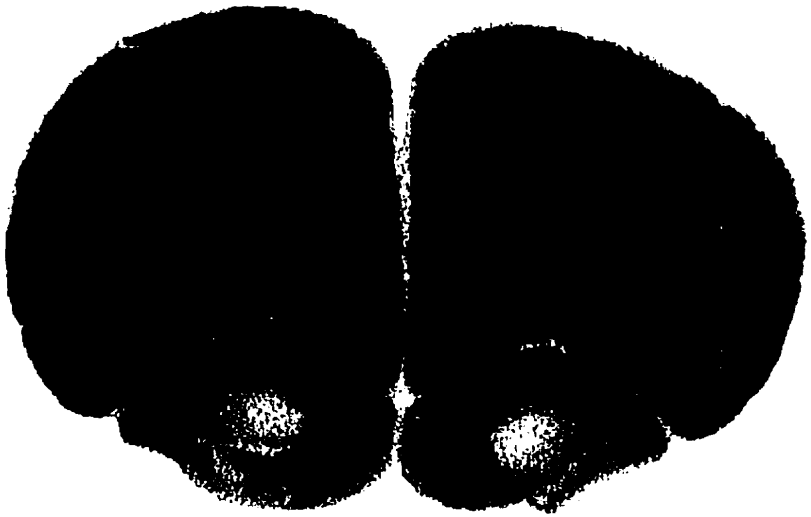
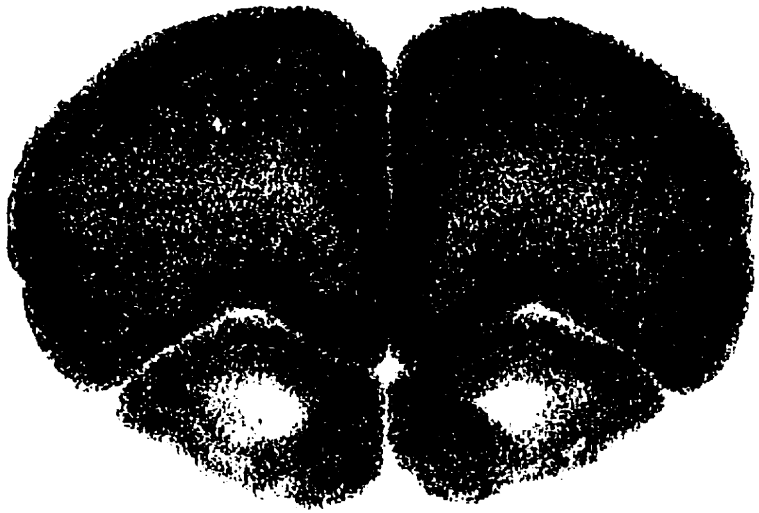

NMDA Receptor Binding 6 m
Sham
Lesion
fmol/mg
35
30
25
20
15
10
5
0
*
FRCX-1
FRCX-2
FRCX-3
CG-1
CG-3
AGINCX
LOCX
VLO
MPFC

Figure 5

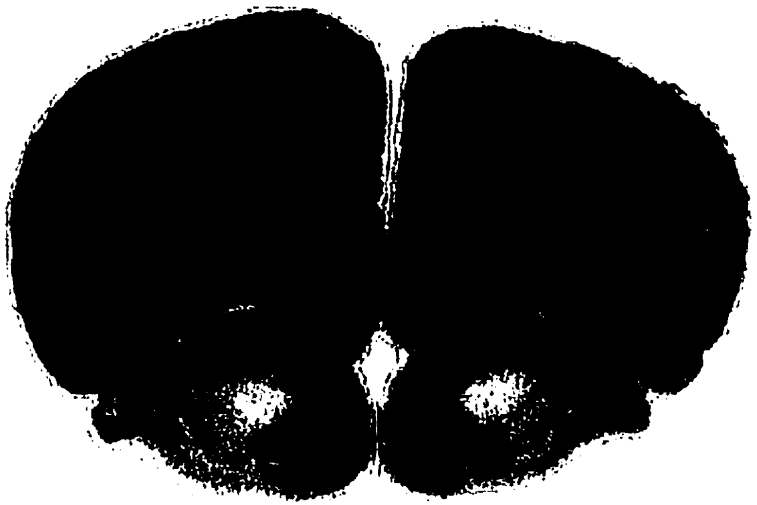
[I^{125}]MK-801 Receptor Binding



Lesion



Sham



Lesion

Sham

4.3.2-[^{3}H]-AMPA Binding

Similar to NMDA receptor binding, specific [^{3}H]-AMPA binding sites were widely distributed in the rat brain with higher densities in the hippocampal formation and neocortex. Subcortical areas such as striatum were not as much enriched with specific [^{3}H]-AMPA binding sites. The total percentage of specific binding for [^{3}H]-AMPA was about 80%. As shown in Table II and Figure 5, lesioned PD180 animals (n = 3) showed significant increases of [^{3}H]-AMPA binding sites in the following cortical areas: frontal cortex-1 inner, frontal cortex-2 outer, agranular insular cortex inner when compared to aged-matched shams. The percentage of increase of binding in significant cortical areas of lesioned animals vs. shams was about 27%. This result in AMPA binding diverges from our preliminary data [103] in which no difference was found in either PD35 and PD56, however no [^{3}H]-AMPA binding data on PD180 group is available for comparison.

Table II. Quantitative analysis of [^{3}H]-AMPA Receptor Binding in 6-month old rats

Regions	SHAM (n = 3) MEAN ± SD	LESION (n = 3) MEAN ± SD	%CHANGE	
FRCX-1	142.76 31.30	190.86 46.83	25.20	
FRCX-2	157.41 41.05	180.14 15.93	12.61	
FRCX-3	128.34 46.54	179.64 42.81	28.55	
FCXINN-1	105.66 20.65	150.80 11.63	29.93	*
FCXINN-2	134.12 42.63	149.50 25.72	10.29	
FCXINN-3	129.31 51.54	151.01 9.79	14.37	
FCXOUT-1	150.81 18.32	211.83 62.07	28.81	
FCXOUT-2	157.75 20.41	197.02 13.84	19.93	*
FCXOUT-3	150.62 65.64	186.70 56.64	19.32	
CG-1 TOTAL	155.43 32.07	174.20 38.28	10.78	
CG-3 TOTAL	177.75 67.02	194.97 43.62	8.83	
CG-1 INN	133.26 59.45	146.29 24.29	8.91	
CG-3 INN	158.10 43.47	178.48 32.91	11.42	
CG-1 OUT	174.18 44.54	185.67 41.25	6.19	
CG-3 OUT	187.74 65.47	206.73 53.08	9.18	
AGINCX	148.90 33.97	184.05 39.60	19.10	
LOCX	143.82 26.58	168.54 39.55	14.67	
VLO	157.82 34.19	170.09 34.21	7.21	
INFRALIMBICX	174.25 33.33	199.54 40.92	12.67	
DORSALPEDUNCX	180.63 32.86	196.34 48.58	8.00	
INFRALIM-INN	133.41 27.57	180.58 34.05	26.12	
INFRALIM-OUT	143.91 32.16	221.29 45.32	34.97	
DORSALPEDU-INN	146.97 30.48	187.55 34.74	21.63	
DORSALPEDU-OUT	141.35 37.75	201.17 51.46	29.74	
AGINCX-INN	120.65 19.65	176.98 24.65	31.82	*
AGINCX-OUT	124.43 14.60	185.97 44.74	33.09	
VLO-INN	118.26 18.84	158.37 23.07	25.33	
VLO-OUT	123.91 27.42	185.24 49.42	33.11	
LOCX-INN	106.37 29.55	149.84 21.60	29.01	
LOCX-OUT	122.57 40.86	183.08 45.81	33.05	
MPFC	134.01 19.79	204.10 44.03	34.34	
MPFC-INN	130.99 20.50	189.86 35.42	31.01	
MPFC-OUT	140.53 23.89	214.88 50.91	34.60	

Receptor levels in fmol/mg of wet tissue are expressed as mean and standard deviation of specific binding from individual pooled values obtained from triplicate. For brain regions see Appendix.* :$p<0.05$

Figure 6. Quantitative analysis of [^{3}H]-AMPA receptor binding at PD180 in representative brain regions expressed as average (±SD) values in fmol/mg wet tissue. For illustration a representative pair of autorradiograms of specific binding [^{3}H]-AMPA is shown in Fig 7.

AMPA Receptor Binding 6 m

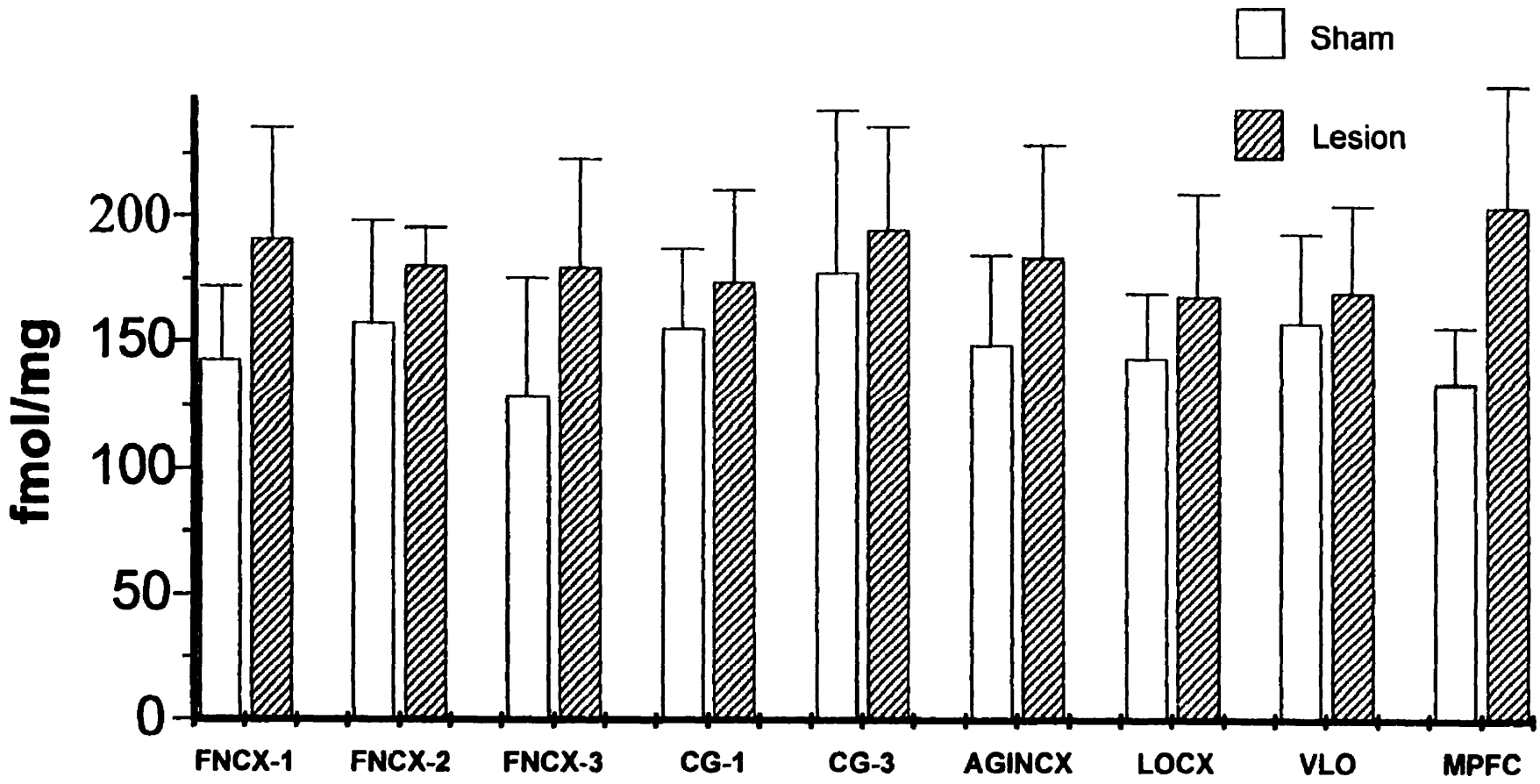


Figure 6

4.3.3-[^{3}H]-Kainate Binding

The regional distribution of specific [^{3}H]-kainate binding sites was restricted to the hippocampus, cortical areas, striatum and granule cell layers of the cerebellum. The total percentage of specific binding for [^{3}H]-kainate was about 70%. As shown in Tables IIIa and Figure 6, no significant difference was found in [^{3}H]-kainate binding in any cortical region between lesioned (n = 3) and sham (n = 3) group at PD180. The percentage of change between lesioned and shams was less than 5 % in this group. In our previous preliminary data no difference was found between lesioned and shams for this receptor [103] at either PD35 or PD56.

Table IIIa. Quantitative analysis of [^{3}H]-KAINATE Receptor Binding in 6-mo old rats

Regions	SHAM (n = 3) MEAN ± SD	LESION (n = 3) MEAN ± SD	%CHANGE
FRCX-1	27.89 0.69	26.59 1.83	-4.66
FRCX-2	28.99 1.01	27.11 0.68	-6.48
FRCX-3	29.30 2.83	26.65 3.18	-9.05
FCXINN-1	27.57 0.64	27.11 2.35	-1.67
FCXINN-2	28.84 1.77	27.40 0.53	-4.98
FCXINN-3	28.03 0.36	27.71 2.58	-1.14
FCXOUT-1	28.15 1.73	26.41 1.73	-6.21
FCXOUT-2	29.10 1.52	27.15 0.92	-6.69
FCXOUT-3	31.67 6.37	27.18 3.33	-14.18
CG-1 TOTAL	31.28 1.58	28.76 0.49	-8.06
CG-3 TOTAL	34.56 2.06	31.14 1.34	-9.92
CG-1 INN	30.04 2.88	28.17 0.68	-6.24
CG-3 INN	33.57 3.51	29.94 2.17	-10.80
CG-1 OUT	31.80 3.11	29.08 0.65	-8.56
CG-3 OUT	35.10 2.35	32.29 0.47	-8.02
AGINCX	29.36 2.82	27.44 3.24	-6.54
LOCX	28.01 0.32	28.45 1.27	1.58
VLO	30.59 2.83	29.75 1.47	-2.73
INFRALIMBICX	35.12 3.37	31.76 1.95	-9.57
DORSALPEDUNCX	34.70 2.90	33.21 1.17	-4.29
INFRALIM-INN	34.02 3.62	30.32 3.21	-10.87
INFRALIM-OUT	36.18 2.82	32.77 0.61	-9.41
DORSALPEDU-INN	35.21 3.93	32.55 1.97	-7.54
DORSALPEDU-OUT	34.82 2.81	34.01 1.02	-2.33
AGINCX-INN	27.71 1.24	26.26 1.85	-5.26
AGINCX-OUT	29.86 4.38	27.42 4.13	-8.18
VLO-INN	30.37 2.90	28.85 1.49	-5.00
VLO-OUT	31.07 2.72	30.92 1.67	-0.51
LOCX-INN	28.25 0.21	28.55 0.81	1.06
LOCX-OUT	28.03 0.72	28.66 1.93	2.25
MPFC	35.13 1.73	31.20 0.63	-11.19
MPFC-INN	33.56 4.07	29.80 0.69	-11.22
MPFC-OUT	36.25 2.15	32.31 0.56	-10.85

Receptor levels in fmol/mg of wet tissue are expressed as mean and standard deviation of specific binding from individual pooled values obtained from triplicate. For brain regions see Appendix.* :$p<0.05$

Figure 7. Quantitative analysis of [^{3}H]-kainate receptor binding at PD180 in representative brain regions expressed as average (±SD) values in fmol/mg wet tissue. For illustration a representative pair of autoradiograms of specific binding [^{3}H]-kainate is shown.

Kainate Receptor Binding 6 m

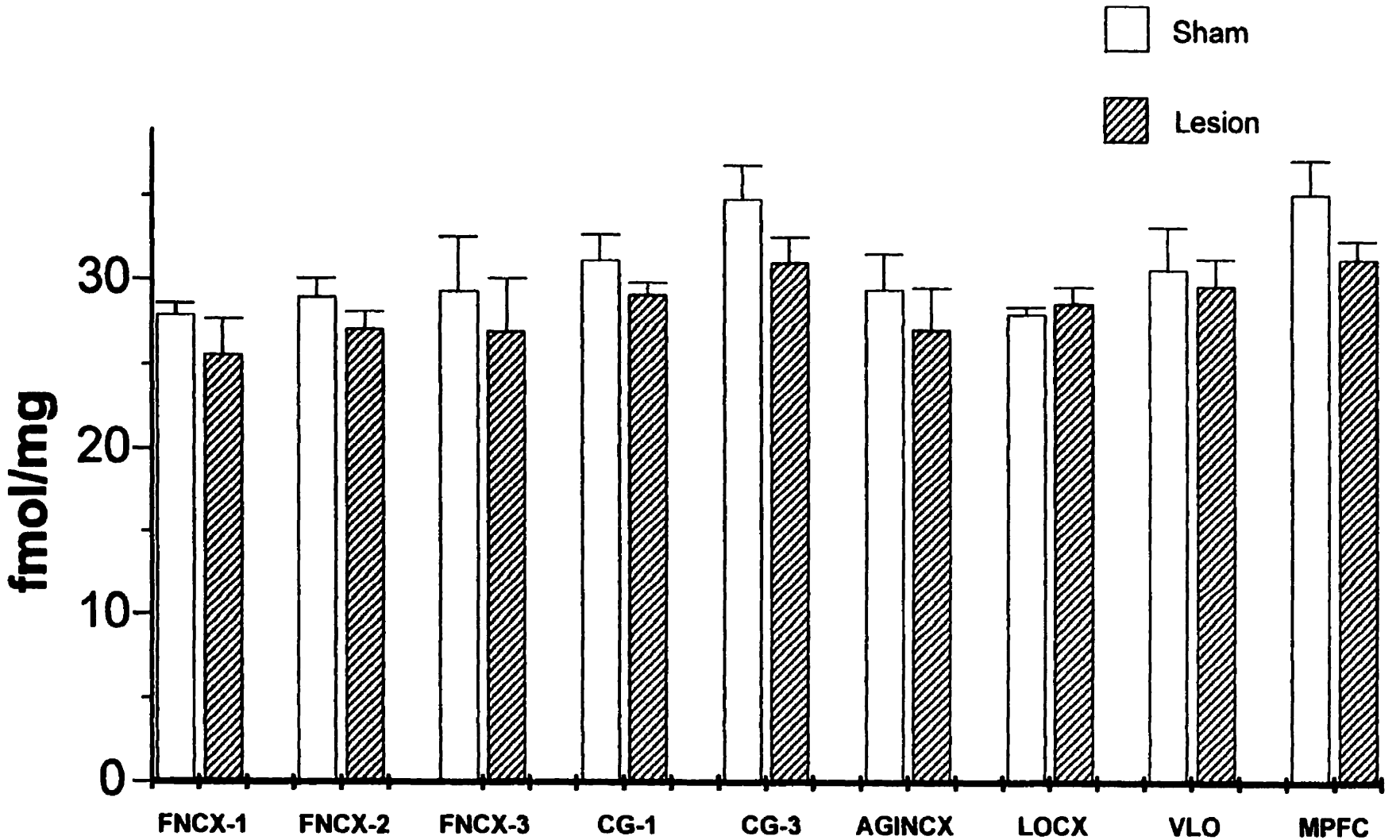


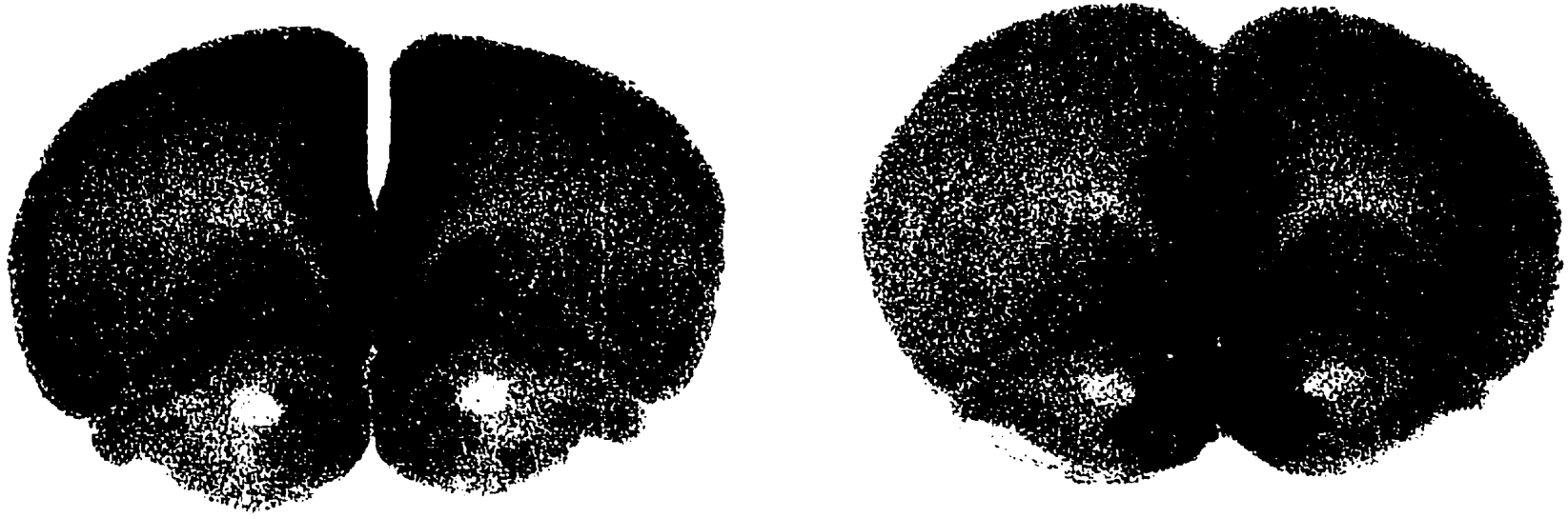
Figure 7

[H^3]Kainate receptor binding

Lesion

Sham

[H^3]AMPA receptor binding



Lesion

Sham

4.3.4- [^{3}H]-mGLUR Binding

As shown in Tables IV and Figure 7, specific [^{3}H]-mGlur binding sites were widely distributed in the rat brain with higher densities in hippocampal formation, outer layers of various neocortical areas, and striatum. Cerebellar layers were not much enriched with [^{3}H]-mGlu binding . The percentage of specific [^{3}H]-mGlu binding was between 43 and 56% in most areas.

A significant decrease of about 60% in [^{3}H]-mGlu binding sites of lesioned animals (n = 3) when compared to shams (n = 3) at PD180 were found in cortical areas such as: frontal cortex-2 (total and outer) and cingulate cortex-1 (total and outer). In our preliminary data for the PD35 group the percentage of change between lesioned and shams was less than 5% and for the PD56 group the percentage of change was about 28% in frontal areas.

Table IV. Quantitative analysis of [^{3}H]-mGlu Receptor Binding in 6-month old rats.

Regions	SHAM (n = 3) MEAN ± SD		LESION (n = 3) MEAN ± SD		%CHANGE	
FRCX-1 - 1	22.34	4.94	11.93	11.28	-46.62	
FRCX-2 - 2	23.18	2.07	8.45	4.59	-63.55	*
FRCX-3 - 3	19.71	2.18	12.99	12.17	-34.09	
FCXINN-1 - 1	8.52	2.07	5.64	2.80	-33.82	
FCXINN-2 - 1	11.25	3.76	4.61	2.90	-59.04	
FCXINN-3 - 1	8.32	2.72	9.75	8.51	17.22	
FCXOUT-1 - 1	28.97	8.41	12.92	12.64	-55.40	
FCXOUT-2 - 1	28.35	2.50	10.11	5.31	-64.35	*
FCXOUT-3 - 1	23.22	4.73	13.97	13.66	-39.84	
CG-1 TOTAL - 2	23.17	5.75	9.37	4.97	-59.56	*
CG-3 TOTAL - 3	23.04	9.99	11.38	10.33	-50.63	
CG-1 INN - 1	12.36	5.57	5.81	4.15	-53.02	
CG-3 INN - 1	17.29	8.35	7.63	10.29	-55.86	
CG-1 OUT - 1	30.95	5.56	11.42	5.74	-63.10	*
CG-3 OUT - 1	27.90	12.06	14.06	10.11	-49.62	
AGINCX - 2	14.57	4.70	9.53	12.13	-34.61	
LOCX - 2	16.19	6.96	12.30	15.02	-24.00	
VLO - 2	25.60	7.78	14.69	9.49	-42.61	
AGINCX-INN - 1	10.60	3.94	6.25	11.86	-41.02	
AGINCX-OUT - 1	16.20	5.29	14.23	16.85	-12.12	
VLO-INN - 1	18.71	7.42	8.19	7.15	-56.25	
VLO-OUT - 1	32.81	8.56	20.33	11.26	-38.04	
LOCX-INN - 1	12.29	8.03	9.63	13.47	-21.64	
LOCX-OUT - 1	20.48	5.10	15.38	17.59	-24.92	

Receptor levels in fmol/mg of wet tissue are expressed as mean and standard deviation of specific binding from individual pooled values obtained from triplicate. For brain regions see Appendix.

* $p<0.05$

Figure 8.. Quantitative analysis of [^{3}H]-mGLU receptor binding at PD 180 (6 m) in representative brain regions expressed as average (±SD) values in fmol/mg wet tissue. For illustration a representative pair of autoradiograms of specific binding [^{3}H]-mGLUr is shown.

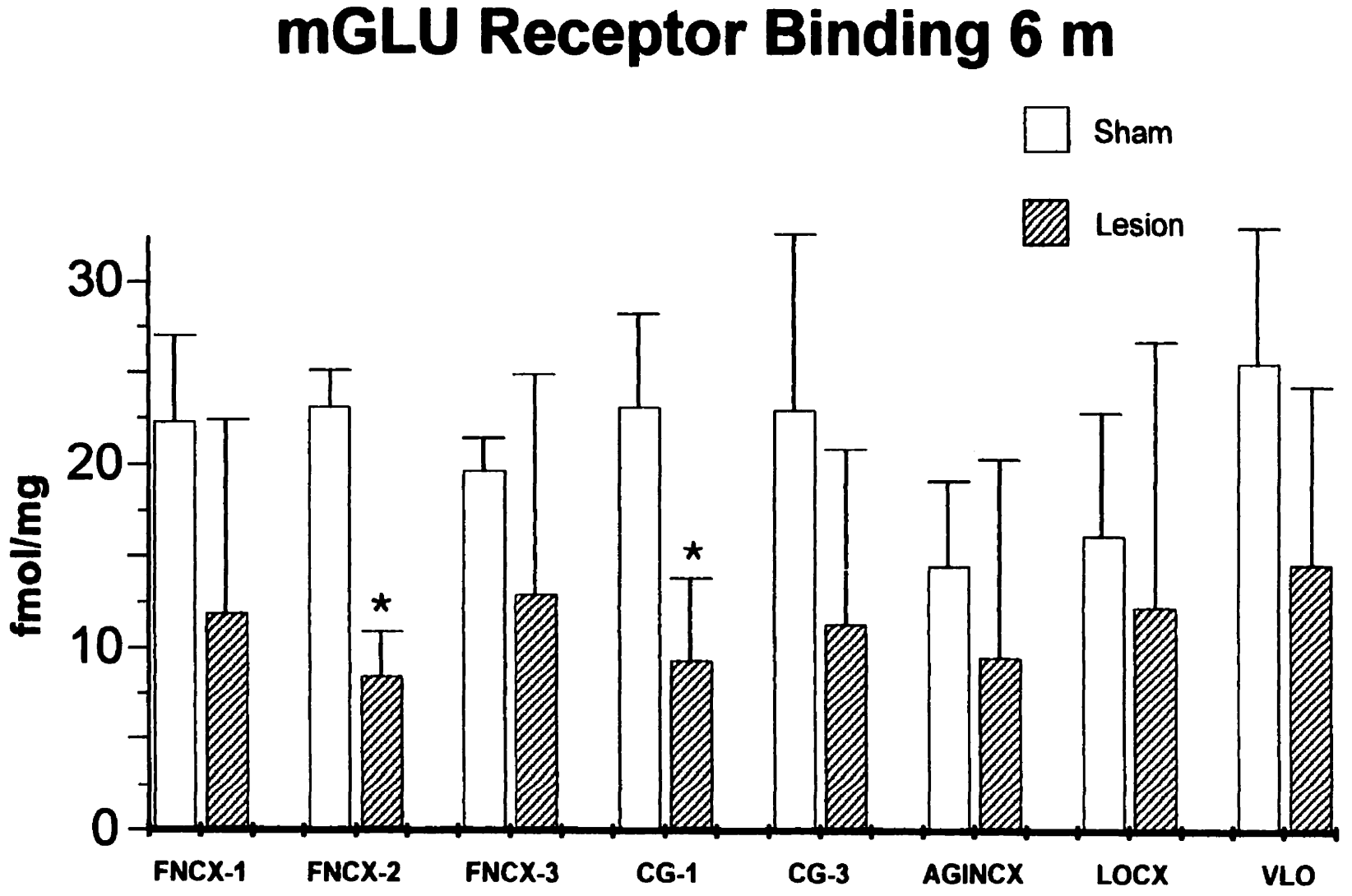
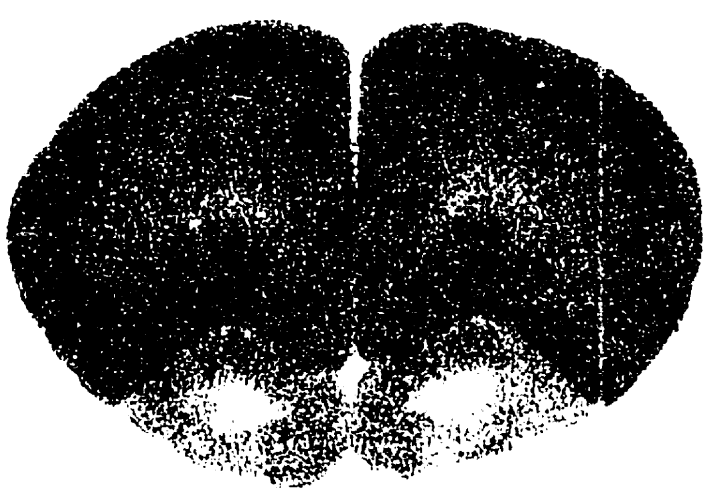

mGLU Receptor Binding 6 m
Sham
Lesion
fmol/mg
30
20
10
0
*
*
FNCX-1
FNCX-2
FNCX-3
CG-1
CG-3
AGINCX
LOCX
VLO

Figure 8

[H^3]Metabotropic Glutamate Receptor Binding



Sham

Lesion

5- DISCUSSION

5.1. Behavioral findings in the neonatal VH lesion model

5.1.1. Locomotor Activity

A new animal model of schizophrenia was developed in 1993 by Lipska et al [60] with the interesting feature of postpubertal emergence of hyperresponsiveness to stress and to D-amphetamine (measured as increased locomotor activity) after lesioning the ventral hipppocampal formation at PD7 in rats. They found increased locomotor activity at PD56 but not PD35 in response to milder stressors such as both exposure to novel environment or saline injection, and to dopamine agonists such as D-amphetamine. Furthermore, these behavioral responses that appear in early adulthood respond to neuroleptics. Hyperlocomotion can be also found after a swim-stress test in neonatal lesioned animals. More recently, using this model, Flores et al. [101] also found hyperlocomotion after a swim-stress test, as well as after D-amphetamine and apomorphine administration, in PD56 lesioned rats. In our present study locomotor hyperactivity in response to milder stressors such as both exposition to novel environment or saline injection, as well as to dopamine agonists such as D-amphetamine, were also found in neonatal lesioned animals at PD56 but not PD35.

One of the objectives of this thesis was to investigate whether these postpuberal changes lasted over a longer period of time. Thus, a neonatally lesioned 6-month old (PD180) cohort was tested in locomotor activity boxes in the three experimental conditions previously applied to the PD35 and PD56 groups: novel environment, after saline injection and after D-amphetamine injection. Indeed, as in the younger postpubertal lesioned group (PD56), hyperlocomotor activity after novelty, saline and D-amphetamine injection were evident in the older (PD180) lesioned group compared to the age-matched sham group. A slight increase in locomotor activity after D-amphetamine was noticed in this 6-month old neonatal lesioned group when compared with the neonatal lesioned PD56 group, however this difference was not statistically significant.

Both novelty- and D-amphetamine-induced locomotion have been linked to mesolimbic DA transmission [104,105]. The fact that the emergence of hyperactivity can be blocked with antidopaminergic drugs, such as haloperidol, is also consistent with an involvement of DA systems [106]. Then, an excitatory lesion of the neonatal VH lesion can provoke delayed emergence of behaviors that are suggestive of increased mesolimbic DA responsiveness to environmental as well as to pharmacological stimuli. There are several

mechanisms by which limbic-cortical regions may modulate mesolimbic DA transmission and locomotion. Thus, glutamatergic projections from the subiculum of the VH formation are believed to be in close apposition to mesolimbic DA axons innervating cell bodies of the nucleus accumbens [107] and have been strongly implicated in exploratory and in D-amphetamine-elicited motor activity in the adult rat [108]. Recently preferential release of dopamine in response to amphetamine has been reported in the shell region of the nucleus accumbens as compared to dorsal striatum in an in vivo microdialysis study [109]. The shell of the nucleus accumbens corresponds to the region where microinjection of D-amphetamine generates the greatest level of locomotion [110]. In addition to the dopaminergic input from the ventral tegmental area, the shell of the nucleus accumbens receives projections from various limbic structures including basolateral amygdala, ventral hippocampus, subiculum and entorhinal cortex as well as frontal cortex [111,112]. All these inputs are likely to be glutamatergic and therefore excitatory in nature.

Isaacson and co-workers [113] were among the first to suggest that the hippocampus is able to influence exploratory locomotion via its connection with nucleus accumbens, and that disruption of this link underlies the enhancement in locomotion [113]. Consistent with this hypothesis, Yang and Mogenson [114] showed that NMDA infused into the ventral subiculum lead to increased locomotion, and this effect could be prevented by the intra-accumbens infusion of dopamine D2 antagonists. In addition, direct injection of glutamate agonists (quisqualate, kainate, AMPA, NMDA) into the nucleus accumbens can also induced hypermotility [115] whereas infusion of the broad spectrum glutamate antagonist kynurenic acid suppress locomotion [116]. Therefore, an up-regulation of NMDA receptors in frontal cortex, like the one we found, would increase glutamatergic transmission toward nucleus accumbens through prefrontal cortex even in the case of low glutamate release from hippocampal afferent fibers.

The role of mesolimbic DA terminals in the nucleus accumbens in the initiation of locomotion in rats has been studied [117]. Locomotor activity was initiated by activation of the excitatory input from the ventral subiculum to the nucleus accumbens using NMDA, with mesolimbic DA terminals being essential to this activation [118]. In addition, anatomical studies have also shown that the hippocampal-accumbens projection terminates on accumbens output neurons that also receives a mesolimbic DA input [107]. The anatomical convergence of these inputs, which is restricted to the shell of the nucleus accumbens, implies that its activity and hence its output is determined , at least in part, by a balance between the excitatory limbic input and the presumably inhibitory dopaminergic input [119].

On the other hand, the nucleus accumbens receives excitatory inputs from a number of other limbic structures. The specification of their relative contribution to the

regulation of locomotion and of their interaction with the ascending mesolimbic dopaminergic projection has been a matter of debate. Wilkinson [120] has speculated that in normal intact animals the subiculum-accumbens projection increases DA release through presynaptic modulation and that hippocampus would inhibit dopaminergic transmission in nucleus accumbens via an overall negative influence on subicular projection neurons. Thus, lesion to the hippocampus would disinhibit the subicular projection to nucleus accumbens thereby increasing DA release over and above the increase caused by amphetamine.

The precise nature of glutamate-dopamine interaction at the limbic circuit still remains controversial. The hippocampal formation also sends excitatory projection to the prelimbic regions of the medial prefrontal cortex [121] which in turn can affect both neurochemical and behavioral indices of mesolimbic dopaminergic transmission [122,123]. Direct projections from Ammon's horn to prefrontal cortex in the rat have been labeled with higher number of cells confined to the temporal (ventral) half of fields CA1 and CA2 which project to the infralimbic area of the prefrontal cortex by way of an unusual subcortical route through the septal region [124].

Differential efferent projections from the ventral and dorsal hippocampus, suggesting that both structures might have different functional roles [125], have also been identified. Thus, ventral hippocampal fibers terminate in massive numbers in the lateral septum and nucleus accumbens (a second afferent pathway from the ventral hippocampus was traced from the alveus to the subiculum) while dorsal hippocampus fibers terminate in the medial septal nucleus, hypothalamic paraventricular nucleus and hypothalamic posterior nucleus.

There are two well-defined locomotor systems in the brain; the first one points out the relationship between basal ganglia structures (dorsal striatum, globus pallidum, substantia nigra, subthalamic nucleus) and the mesencephalic locomotor region (including the pedunculopontine nucleus). This circuit controls cortical motor areas of the cerebral cortex in feedback through the thalamic centromedial, ventrolateral and ventroanterior nuclei [126,127]. The second consist of a parallel afferent system that involves limbic structures such as pyriform and entorhinal cortices, ventral hippocampus, amydgala and ventral tegmental area as an input region to the nucleus accumbens [128] (which function as the parallel ventral structure of the dorsal striatum) and then projecting to ventral pallidum, caudoventral central gray and peribranquial cuneiform, substantia nigra, ventral tegmental area and pedunculopontine nucleus .

The nucleus accumbens has been involved in influencing locomotor activity in parallel with the striatum [127]. The absence of neocortical input to the accumbens, compared

to the striatum, and the predominant limbic projection to the area suggest a role for this nucleus in locomotor behavior geared toward appetitive responses. The accumbens itself is contained within a feedback loop system in which the accumbens exerts control over the ventral pallidum which in turn sends excitatory projections to the dorsomedial (DM) nucleus of the thalamus [129]. The DM has excitatory interconnections with the PFC which supplies excitatory input to the nucleus accumbens.

The neonatal VH lesion model has been described as a model of enhanced mesolimbic DA transmission, behaviorally expressed as hyperlocomotion, and in that sense it could be considered an animal model of schizophrenia. The aim of this study has been to try to characterize and expand this feature in terms of the glutamatergic hypothesis. It relies on the explanation of how an early lesion, which is glutamatergic in nature, would be altering the function of the limbic locomotor circuit. In that respect, hippocampal lesions induced in adults rats are shown to display a DA turnover reduction in MPFC [90]. In other paradigms, primary reductions in DA activity in MPFC have been associated with enhanced limbic DA transmission [85]. In order to explain, in the neonatal VH lesion model, how a primary lesion of this ventral structure which sends input (glutamatergic) to an important locomotor-limbic nucleus (such as nucleus accumbens) could produce long-term changes in the latter structure, we were tempted to postulate the mediation of the prefrontal cortex. The early VH lesion could have disrupted the direct projection from hippocampus to nucleus accumbens as well as the more indirect hippocampus-prefrontal cortex- nucleus accumbens pathway. We postulate, then, that abnormal changes in the latter structure can be perpetuated in the long-term by breaking up the circuit formed by these structures (hippocampus, prefrontal cortex and nucleus accumbens). In that sense, exploring prefrontal cortex changes in this model became a tantalizing idea.

Indeed, the increased locomotor activity displayed by neonatal VH lesioned animals could be explained, first, in terms of specific connectivity to the nucleus accumbens (directly or through the prefrontal cortex), and second, in terms of neurochemical changes such as the decreased expression of D3 dopamine receptors [101] in the shell region of the accumbens that has been recently found in the model. Our results (see section 4.3) in that sense appear to be more as a consequence of a glutamatergic dysfunction than an explanation for hyperlocomotion itself.

What is new in this study, however, is the fact that the 6-month (PD180) old lesioned cohort depicted the same changes showed by the PD56 lesioned cohort. This could imply that once hyperlocomotion appears after puberty this altered mesolimbic behavior remains over time, at least until 6 months.

5.1.2. Cognitive function in the neonatal VH lesion model

Since our work concerned an animal model of schizophrenia, we were interested in testing cognitive functions, especially those that involve frontal cortex and hippocampus. This was the rational for using the radial arm maze in this model [99,100]. We tested a cohort at PD56 mainly because the hippocampus is mature at this stage [130], and any change observed in the paradigm might be attributable only to the neonatal lesion.

Our results in this paradigm strengthen the previously observed hyperlocomotion of neonatal VH lesioned animals since a very significant decrease in total running time per trial were found in neonatal lesioned animals when compared to shams since the beginning of the experiment. This result corroborates hyperlocomotion in the lesioned group by using a different paradigm.

Another interesting result relates to the fact that neonatal VH lesioned animals showed a significant decrease in the number of correct choices. Lesioned animals showed less reference error than shams but kept repeating and revisiting the already explored arms. Also, the analysis of the type of errors made by lesioned animals are in agreement with other studies done on animals with different limbic lesions [102]. These data are consistent with those demonstrating that, following hippocampal destruction, animals have difficulty in inhibiting responses [131]. Therefore, it seems that they have difficulties exploring new environments; so they tend to prefer the already explored arms and that preference interferes with learning the paradigm. Spatial working memory deficits [132], and interference with cognitive tasks [133] have also been found in schizophrenics.

Lesioned animals tended to revisit incorrect arms more than correct ones. This is a very interesting result since it would imply serious deficit in learning at least in the place task. This new task learning deficit may involve failures in either acquisition, retrievement, or both, but also may be due to hyperresponsiveness to stress. Lesioned animals showed statistically significant less correct choices ($p< 0.05$) per trial in the first four trials where novelty to learn a new task would be present. These difficulties in exploring new arms would lead animals to repeat the same choices, and thus prevent them from learning the arms in which the pellet was located. So they initially chose some arms randomly and repeated them whether or not these arms were correct choices. It might suggest a relationship between learning, in this case a spatial task, and hyperresponsiveness to stress. Indeed, stress has been shown to impair long-term potentiation in CA1 area of the hippocampus and to interfere with behavioral tasks; both effects being prevented by NMDA antagonists before experiencing stress [99].

Nevertheless it is important to notice that during the last two days of the experiment, lesioned animals were choosing more correct arms than shams, although this was not statistically significant. Therefore, lesioned animals might be able to learn the paradigm after the stressful situation is gone, but longer trials and reversal trials are needed before drawing this kind of conclusion.

This study also provides additional supporting evidence for the hypothesis that the hippocampus plays a major role in mediating spatially organized behavior [134]. It also indicates that spatial difficulties are not restricted to interference in dorsal hippocampus or fornix but involve many part of the hippocampal system as it is supported in other works [102]. In addition, a selective gate that could be related to the hypothesized role of hippocampus in regulating context sensitive cues has been recently proposed [135]. In this hypothesis the hippocampus may enable transit of information from PFC to nucleus accumbens, playing therefore an important role in all task involving information processing. Indeed, the hippocampus has been proposed to play a role in providing spatial context frame of reference [136] as well as in the motivation to explore novel environments [137]. Recent studies have shown that, as with the hippocampal neurons, the activity of a set of accumbens cells is correlated with the location of the animal in space ("place cells") [138]. A disruption of this system, interfering with context-sensitivity , leading to increased distractibility (inability to screen out irrelevant information) [139] or increasing the incidence of perseverative error [140] would be present in schizophrenia.

As hippocampal afferents to the prefrontal cortex demonstrated NMDA receptor-dependent long term potentiation [141], their disruption might affect learning and memory tasks such as the radial arm maze task. Also deficits in learning and memory tasks (reinforced alternation, radial arm maze and the position habit reversal test) with dizocilpine in rats [142] and prefrontal cortex function impairments have been reported after NMDA receptor antagonists [143]. The latter group implicated the excitatory amino acid transmission at the NMDA receptor as directly related with the capacity to perform working memory-related tasks. Clinical studies have also showed phencyclidine impairing the ability of individuals to use internalized representation for problem-solving strategies [144]. Additional studies have also reported deficits in passive avoidance learning [145], prepulse inhibition of acoustic startle response [146], Morris water maze [147] and 8-arm radial maze [148] after NMDA antagonism. In that sense an alteration in glutamatergic systems, as it is the case in our model, would be in correspondence with those cognitive deficits explored using the radial arm maze task.

5.2. Changes in Excitatory Amino Acids Receptors

5.2.1. Ionotropic Glutamate Receptors

In preliminary experiments we reported an increase in NMDA receptor levels in frontal cortex at PD56 but not at PD35 in animals that underwent a neonatal ibotenic acid lesion of the ventral hippocampal formation [103]. No statistically significant changes for AMPA and kainate receptor binding were then found. We were interested to further explore whether these changes of NMDA receptors in neonatal VH lesioned animals were transient or long-lasting. Thus, a cohort of 6 month (PD180) old neonatally lesioned and sham-operated animals were behaviorally tested for their locomotor activity, and their brains processed to assess possible changes in the expression of excitatory amino acid (EAA) receptors. We also did the same with cohorts at PD35 and at PD56 in order to replicate some of our previous observations.

As in our previous results, we found an increase of about 30 % in NMDA receptor binding in neonatally lesioned (PD56) rats with respect to sham-operated group. Similarly, we also found an increase of about 30% in neonatally lesioned animals of the 6 month old group with respect to the age-matched sham. In spite of the small sample, statistically significant changes in frontal cortex were found for NMDA and AMPA receptors but not for kainate binding sites.

Research into the function of L-glutamate in the central nervous system has lead to the hypothesis that decreased glutamatergic neurotransmission may play a role in the pathophysiology of psychosis [16,22]. NMDA receptor antagonists can induce schizophrenia-like psychosis but the role of NMDA receptor in the pathophysiology of schizophrenia remains unclear. Our results are in agreement with receptor binding studies conducted on human neocortex of schizophrenics that report 30-40% higher NMDA binding sites in frontal cortex compared with parieto-temporal cortex [149]. In addition, Akbarian et al found significant alterations of the NMDA receptor subunits in schizophrenics confined to the prefrontal cortex [150].

However, even though cortical NMDA receptor levels are increased, it does not mean that glutamate as neurotransmitter or the glutamatergic tone are increased in this model. Glutamate concentrations in frontal cortex at the different time points chosen need to be determined. On the other hand, increased NMDA receptor binding level do not necessarily mean increase of fully functional NMDA receptors. Along with increased levels, electrophysiological changes such as those described during development [151] might

have occurred. The gradual replacement of the NR2B by the NR2A subunit during postnatal development provoke changes in functional properties of the NMDA receptor leading to decreased synaptic plasticity during brain maturation. In that sense, as the VH lesion at perinatal period might have modified the later development of the glutamatergic pathway and altered glutamate receptors postpubertally, the early lesion could have also interfered with the normal developmental shift of the modulatory NR2B subunit and altered their electrophysiological correlate. Interestingly, a shift in the relative proportion of the NR2 subunit family, with a 53% relative increase in expression of the NR2D subunit has been found in the frontal cortex of schizophrenic patients [150].

Given that the NMDA receptor levels are increased in frontal cortex in neonatally lesioned animals when compared to shams, we could postulate that the glutamatergic transmission would not be as effective, even in presence of normal levels of glutamate, because the relative proportion of functionally normal receptors might be diminished.

The finding of increased AMPA binding sites observed in the neonatally lesioned animals of the 6-month old group, on the other hand, coincides with electrophysiological data showing AMPA receptor-mediated responses evoked in prefrontal cortex neurones by hippocampal stimulation [152]. Thus, the hippocampus could modulate, at least during acute excitatory transmission, the prefronto-accumbens pathway via AMPA receptors. It also points to the glutamate-dopamine relationship since an inhibitory modulation of the hippocampo-prefrontal path by the mesolimbic dopaminergic system has been postulated [153]. In addition, AMPA receptors appear responsible for the response to environmental stress since stress-induced increases in DA release in the prefrontal cortex appear to be mediated by stress-activated glutamatergic (through activation of AMPA receptors) neurotransmission in this region [154]. However, anatomical work in the field has reported minimal changes in AMPA receptor binding [35] in frontal cortex from postmortem brains of schizophrenics. Other groups have found decreased expression of mRNAs encoding AMPA-preferring non-NMDA glutamate receptor subunits GluR1 and GluR2 in medial temporal neurons in schizophrenia [155]. No significant changes in AMPA receptor binding between lesioned and sham animals were found in either PD35 or PD56 groups in our previous preliminary data [103].

We failed to find any significant change between lesioned and sham groups in kainate receptor binding sites at PD180, and as previously reported [103], no changes were found at PD35 or PD56 either. However, studies with postmortem tissue from schizophrenic patients have shown that kainate receptor binding either increased [36] or remained unchanged [37] in frontal cortex , and decreased [38] in hippocampus.

5.2.2. Changes in mGlu Receptor Binding

We were interested in measuring the metabotropic glutamate (mGlu) receptor since several lines of evidence indicate that mGlu receptors are involved in neuronal degeneration, synaptic plasticity and development [156.157]. mGlu receptor is not present in target organs of the autonomous nervous system and therefore changes in its function might underlie central nervous system disorders such as schizophrenia [158].

In our preliminary results we observed a trend for a decrease in the mGluR binding levels as the animals get older. More interestingly, this decrease was even greater in PD56 and PD180 lesioned animals when compared with aged-matched sham. On average the decrease was about 36% , but especially remarkable was the decrease, up to 60%, in some structures such as FXCX-2 and CG-1.

There is evidence for the role of mGluR in development [158]. Indeed, stimulation of PI hydrolysis by excitatory amino acids is known to be particularly elevated early after birth and then to decline progressively during postnatal development [159,160]. In that sense, we could speculate that mGluR3 and mGluR5 subtypes would be the ones involved in our case, since they are known to play an important role during both synaptogenesis and maintenance of adult synapses, whereas other subtypes such as mGluR1, mGluR4, and mGluR7 are involved in adult neurotransmission [161].

An inhibitory modulation by mGluR has been proposed at the level of the nucleus accumbens where these receptors are known to inhibit dopamine release from both the mesoaccumbens DA terminals and the phasic activation of the PFC [162]. Therefore, decreased levels of mGlu receptor may diminish the physiological inhibition on mesocorticolimbic dopaminergic activation. On the other hand, Rainnie at al. found a role for glutamate as an inhibitory transmitter in basolateral amygdala during periods of mGluR activation [163] . This observation seems to be in partial agreement with our locomotor data since as we saw in session 5.1.1, the basolateral amygdala nucleus is one component of the limbic locomotor circuitry. Therefore the fact of having mGlu receptor levels decreased postpubertally might give additional neurobiological support to behavioral features found in our model. In that sense not only the postpubertally decrease found in D3 receptor levels but in mGluR could be explaining in part the locomotor data.

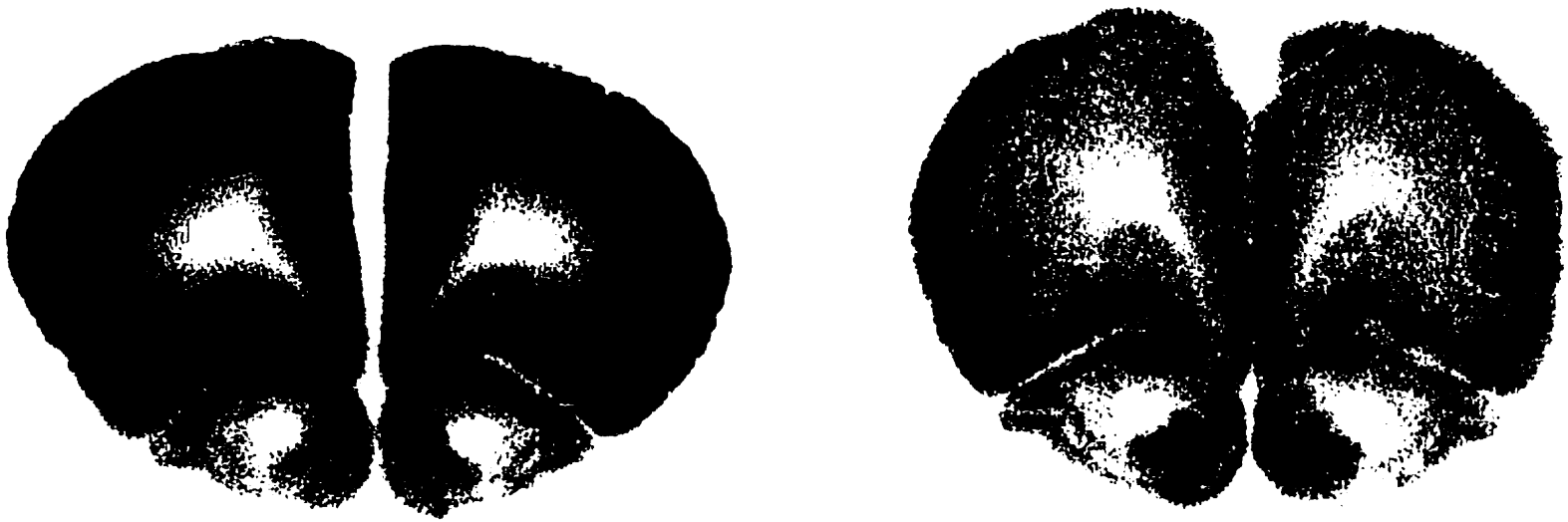
The role of mGluR in frontal cortex is relatively unknown. Nevertheless, as discussed above, there is anatomical data indicating that glutamatergic projections from the prefrontal cortex would regulate the activity of dopaminergic neurons in the nucleus accumbens via metabotropic glutamate receptors[162]. However, mGlu receptors could be located at either postsynaptic dopaminergic terminal in the nucleus accumbens arising from

mesencephalic areas or non dopaminergic neurons such as those of corticostriatal synapses[162]. Therefore, although indirect, some evidence exists for the role of mGluR in hyperlocomotion.

Another key point refers to the interaction between NMDA and mGlu receptors (Figure 8). A negative modulation of NMDA receptors on metabotropic glutamate receptors in human cerebral cortex [165] has been described. That point is actually very interesting since we found in our study an up-regulation of the NMDA receptor together with a down-regulation of the mGlu receptor in the cortex. Furthermore, mGlu receptors are shown to synergize with NMDA receptors in inducing neuronal damage [166]. As mGlu receptor, through PI hydrolysis, leads to mobilization of intracellular Ca^{2+} and activation of PKC, besides trophic support for developing neurons, these receptors would be potentially toxic when combined with additional mechanisms that lead to a sustained increase in intracellular calcium. In that sense, the ability of PKC to relieve the Mg^{2+} blockade of NMDA-gated ion channels has been proposed as one of the fundamental processes by which activation of group I mGlu receptors amplifies NMDA toxicity [167]. Furthermore, the modulation of NMDA toxicity by mGlu receptors depends not only on the relative proportion of facilitatory and inhibitory mGlu receptor subtypes, but also on the subunit composition of NMDA receptors. However, the antagonism of these mGlu receptors has been thought to be potentially neuroprotective. Having lesser levels of mGlu receptors (at least of the type I) could explain a glutamatergic dysfunction without necessarily involving hypofrontality. Similarly, the upregulation of NMDA receptors might not necessarily cause neurotoxicity if their molecular composition is being altered.

Figure 9. Autoradiograms for NMDA and mGLU receptors binding. Note the increase in the density of the NMDA and the decrease in the mGLU in the VH lesion animals.

NMDA Receptor



mGLU Receptor

LESION

SHAM

5.2.3. Prefrontal Cortex and the VH lesion model

Glutamate receptors in addition to mediating fast neurotransmission in the brain, play a key role in many forms of neural plasticity such as learning and memory, neural ontogeny and functional compensation to tissue injury [168]. Due to the fact that in this particular model both hippocampal-accumbal and hippocampal-cortical projections are destroyed early, a putative neurodevelopmental defect secondary to an early destruction of the ventral hippocampus could have occurred. As we discuss above, since glutamate is the principal neurotransmitter of the hippocampal efferents it could play a central role to explain the behavioral changes of this model. Glutamate has been shown to increase dopamine release in the nucleus accumbens [169] and to have an effect on locomotor activity mainly through NMDA receptor antagonism [164,170].

Since the VH lesion model of schizophrenia is a developmental model, the search for a neurobiological basis of the delayed hyperactivity that appear after lesioning a limbic structure in the neonatal period is a difficult enterprise. The developmental program of behavior is far from being elucidated (see Section 5.5.). Postpubertal hyperactivity depicted from neonatally lesion animals is believed to involve an alteration of the mesolimbic and mesostriatal dopaminergic transmission [60], as it is the case for adult lesioned animals, where alterations of dopaminergic transmission at either prefrontal cortex and subcortical structures [90] have been reported. The neonatal VH lesion appears to have an effect on the development of cortical and subcortical connectivity which alters a variety of dopamine related behaviors following puberal eclosion [91]. Furthermore, the neonatal VH lesion appears to combine effects of both adult ventral hippocampus lesion and adult prefrontocortical lesion on subcortical related behaviors [60,94,171].

Anatomical studies have documented direct projections from Ammon's horn to the prefrontal cortex in the rat [172,173]. A glutamatergic hippocampal-accumbens projection has also been described [174]. These projections are both NMDA dependent and LTP enduring [175]. However, since the major inputs to the infralimbic area arise from the ventral subiculum, the basolateral nucleus of the amygdala, the piriform cortex, the paraventricular-parataenial nuclei of the thalamus and the ventral tegmental area [122], it seems likely that this prefrontal region is primarily involved in limbic system function. This is consistent with the evidence that the synaptic organization of the prefrontal cortex is immature prepubertally and continues to develop during adulthood [176]. In addition, Carr et al. recently described a presynaptic triadic complex between hippocampal excitatory afferents, mesencephalic dopaminergic afferents and intrinsic pyramidal cells in the rat prefrontal cortex [177]. On the other hand, lesions of the prefrontal cortex in adult rats have

also been reported to increase DA agonist-induced behavior [178], to affect subcortical dopaminergic transmission [179] and to potentiate swim-stress induced locomotion [180].

Moreover, the rat medial prefrontal cortex exerts a predominantly inhibitory influence on locomotor exploration [181]. Nonspecific ablative medial prefrontal cortex lesions have been reported to augment locomotor activity [179] as well as hyperresponsiveness to novelty [182]. Similarly, neonatally ventral hippocampus lesioned rats display hyperlocomotion and hyperresponsiveness to swim stress when they reach adulthood.

Disruptions of the medial prefontal cortex have been shown to alter subcortical dopaminergic transmission [179] and potentiate swim-stress-induced locomotion [180] . In our study we found that the prefrontal cortex was affected by ventral hippocampal lesions. These findings seem to show that the prefrontal cortex plays an important role in the behavioral effects reported in this model.

5.3. The dopamine/glutamate relationship in the neonatal VH lesion model

The dopaminergic hypothesis of schizophrenia is based on the clinical observation that dopaminomimetics generally worsen the symptoms of schizophrenia but also on the finding that the potency of neuroleptics in binding to striatal dopamine receptors *in vitro* directly correlated with their clinical potency in reducing psychotic symptoms [15a].

Decreased dopamine activity in the prefrontal cortex has been linked to behavioral evidence of prefrontal dysfunction in schizophrenia [52]. On the other hand, animals studies shows increase in PFC glucose metabolism following the administration of DA agonists [20]. A reciprocal relationship between cortical hypodopaminergia and subcortical hyperdopaminergia had been proposed to underlie the pathophysiology of schizophrenia. In the rat this peculiar state has been produced by a specific lesion of the prefrontal cortex [21]. Thus, Pycock et al. showed that after selectively destroying DA afferents within the PFC, chronic subcortical DA hyperactivity develops. Their results included both the increased DA turnover (as judged by the levels of the metabolite homovanilic acid) and up-regulation of postsynaptic receptors, findings that are similar to the postmortem neurochemical data in schizophrenia. This landmark experiment suggested not only that such a peculiar state can exits but also that mesocortical DA neurons affect PFC neurons that exert feedback control over mesolimbic dopamine activity. Whatever the precise physiology of the system, prefrontomesocortical projections seem to be essential to its regulation. Additionally, the prefrontomesocortical pathways appear to play an important role in response to stress (see Section 5.4).

On the other hand, glutamatergic projections have increasingly been regarded important for the function of behavioral limbic circuitry in the rat. A differential regulation of DA receptor expression in limbic areas after MK-801 administration in rats has been reported [183]. Also, MK-801 produces hyperlocomotion in mice [184], and this locomotor stimulation can be inhibited by dopamine D1 and D2 antagonists although to lesser extent than that induced by amphetamine [164]. Moreover, it has also been reported that dopamine D1 receptors seem to be more important than D2 receptors for MK-801-induced hyperactivity [185]. In this line, DA has been regarded as having a less crucial role than formerly supposed in the regulation of psychomotor functions. Carlsson et al. have shown a pronounced behavioral activation in mice following suppression of glutamatergic neurotransmission even in the DA depleted mouse brain [184]. This observation has given rise to the possibility that a deficient activity within the corticostriatal glutamatergic pathway may be an important pathophysiological component in some cases of schizophrenia [186]. In a broader perspective, schizophrenia may be looked upon as a syndrome induced by a neurotransmitter imbalance in a feedback-regulated system, where DA and glutamate both play crucial roles in controlling arousal and the processing of signals from the outer world to the cerebral cortex via the thalamus [187].

In addition, non-NMDA glutamate receptor activation in the nucleus accumbens has been shown to facilitate presynaptic DA function in that structure, and this dopamine-glutamate interaction in nucleus accumbens is thought to control prepulse inhibition in rats, a behavior that has been associated with schizophrenia [188].

Indeed, glutamate is an important modulator of subcortical DA function; the loss of glutamatergic pathways have been shown to affect subcortical dopaminergic transmission in both the VTA and the nucleus accumbens [189].

Additionally, antipsychotics influence forebrain systems that utilize glutamate [190]. Fitzgerald et al. showed that cortical and subcortical glutamate receptor subunit expression could be regulated by antipsychotic drugs. In this study, haloperidol increased NMDAR1 subunit expression in the striatum while SCH23390, an D1-antagonist, had the opposite effect. In contrast, clozapine had no effect at that level. On the other hand, both haloperidol and clozapine increased GluR1 levels in the medial prefrontal cortex while SCH23390 treatment decrease GluR1 levels. Clozapine but none of the other treatments increased GluR2 levels in the frontal/parietal cortex, nucleus accumbens and hippocampus in that study. This regionally distinct effect of various antipsychotic on the level of some glutamate receptor subunits is thought as an important mechanism to exert some of their long-term effects on brain function. In addition, Yamamoto and Cooperman [191] have

shown that the levels of DA and glutamate in striatum and prefrontal cortex are differentially regulated by chronic treatment with typical or atypical neuroleptics.

The recently cloned dopamine D3 receptor has been proposed to play an important role in schizophrenia and its treatment. Its predominant localization in limbic brain areas such as nucleus accumbens, olfactory tubercle and islands of Calleja [192,193] as well as its high affinity for antipsychotics suggest this role. In contrast to prototypic D2 antagonists, D3 receptor-preferring antagonists increase spontaneous locomotor activity and potentiate locomotion induced by D-amphetamine or apomorphine in rats [194]. Similarly the selective D3 agonist 7-OH-DPAT decreases spontaneous locomotor activity [195].

The D3 receptor inhibits locomotor activity. Indeed , Flores et al. [101] have suggested that at least part of the hyperdopaminergic behavior displayed by postpubertal neonatally VH-lesioned animals could be related with the pattern of expression of D3 receptors in motor limbic areas. In this study, dopamine D3 receptor binding levels were measured in various limbic brain regions, and marked reductions of D3 receptor binding at PD62 but not at PD35 were documented in the shell of nucleus accumbens, olfactory tubercle and islands of Calleja, along with an increase in D1 receptor in caudate-putamen. Even though this postpubertal hypersensitivity is believed to involve an alteration of the mesolimbic and mesostriatal dopaminergic transmission, the reduction of D3 binding can hardly explain on its own such an intriguing postpuberal behavioral abnormality. Thus, adult VH lesion in rats affect dopaminergic transmission at either prefrontal cortex or subcortical structures [90]. Neonatal VH lesion seems to have developmental effects on cortical and subcortical connectivity, therefore, a variety of DA related behaviors may appear after puberal eclosion [81]. Additionally, neonatal VH lesions appear to combine effects of both adult ventral hippocampus lesion and adult prefrontal cortex lesion in subcortical related behaviors [91,60]. We think, though, that changes in dopamine D3 receptors may be related with the changes found in this study in EAA receptors. Thus, as an excitatory amino acid projection from the medial prefrontal cortex to the anterior part of the nucleus accumbens (considered to be the major prefrontal input to the nucleus accumbens) has been described [196], it is therefore conceivable that a glutamatergic alteration in prefrontal cortex could affect the expression of D3 receptors in the nucleus accumbens. Indirect support for the role of glutamate in this expression is the recent finding that MK-801, a non-competitive NMDA antagonist, significantly decreases the mRNA encoding for D3 dopamine receptors in the nucleus accumbens [183].

5.4. EAA and stress in the neonatal VH lesion model

Another interesting feature of this model relates to the influence of stress in the emergence of the observed increased locomotor activity. It is noteworthy that behavioral changes after neonatally lesioning the ventral hippocampus appear as hyperresponsitivity to stress. Indeed, adult VH lesioned animals do not display this hyperresponsiveness to stress [60,91]. Neonatal endotoxin exposure has been suggested to increase hypothalamic-pituitary-adrenal responsiveness to stress [197]. Interestingly, Lipska et al. [60] have mentioned the possibility that the neonatal VH lesion could be affecting the development of other neural systems implicated in the mesolimbic response to stress, such as the MPFC [86,87]. Prefrontal function has been regarded as particularly important in organizing responses to aversive stimuli and in regulating mesolimbic DA activity during stress [86,122]. In this regard, lesions of the medial prefrontal cortex in adult rats produce enhanced locomotion after stressful situations (such as a saline injection or swim stress) [122,123]. Prefrontal cortical DA depletion enhances the responsiveness of mesolimbic dopaminergic neurons to stress [198]. Furthermore, the prefrontocortical-accumbens projection has been regarded as the one being stress-sensitive in the whole mesocorticolimbic circuitry [91].

Henry et al. have recently shown that prenatal stress during late pregnancy (from day 14 till day 21) induces long-lasting changes in DA receptors of the nucleus accumbens as well as in the capacity to develop amphetamine-induced sensitization in adulthood [199]. Adult offspring at PD90 showed DA receptor changes such as significant decrease in D3 receptor binding in the nucleus accumbens, a significant increase in D2 receptor binding in the nucleus accumbens, and no significant change in D1 receptor binding in either striatum or accumbens. This result agrees with the D3 dopamine receptor changes observed in adult rats which underwent a neonatal lesion of the ventral hippocampus [101]. Interestingly, maternal stress effects on the dopaminergic system of the offspring is discussed in terms of an impaired control of corticosterone secretion. They postulated corticosterone released during stress sessions in the mother affecting the development of the hypothalamo-pituitary-adrenal (HPA) axis in the fetus. Also prenatal maternal stress can also have an effect on swim-stress test of the offspring [200]. Decreased hippocampal glucocorticoid receptor levels and prolonged corticosterone secretion have been found in prenatal-stressed rat. Glucocorticoids promote sensitization to amphetamine in rats, mainly through type II receptors. Since glucocorticoid receptors are present in dopamine neurons of the ventral tegmental area projecting to the nucleus accumbens [201], and glucocorticoids modulate DA release in the mesolimbic system [202], stress can have an effect on the mesolimbic system.

On the other hand, structures such as the hypothalamic paraventricular nucleus together with the hippocampus have been considered to be the prominent brain areas affected by stress; changes in the hippocampus include increased mRNA expression of the NMDA subunits NR2b in the CA3 region [151] and decreased RNA expression of GluRA subunit of AMPA receptor in CA3 and CA1 [203]. NR2b subunits seems to correlate with the high plasticity of the developing brain. It has been hypothesized that a single stress experience may result in an NMDA receptor assembly which is characteristic [151] for earlier developmental stages of the brain. Whether these changes underlie an increased synaptic efficiency, or alternatively, an increased vulnerability of CA3 pyramidal neurons to glutamate neurotoxicity, remains to be determined. In another study, stress was found to induce atrophy of apical dendrites of hippocampal CA3 neurons, and this required corticosterone secretion together with activation of NMDA receptors [204].

An early lesion could affect the response to stress in adulthood by both developmentally altering prefrontal pathways (or receptors) involved in the stress response, and/or inducing changes in the hippocampus (through glucocorticoid receptors type II). Prenatal administration of amphetamine (also regarded as a model of schizophrenia) has been reported to increase locomotor activity in adult offspring [205], and prenatal stress affects D3 receptor expression and locomotion in adult offspring. It is noteworthy that changes in the level of glucocorticoids simulate changes otherwise seen in amphetamine sensitization or DA-mediated behaviors. Since the hippocampus (through glucocorticoid receptor type II) has been regarded as the central structure governing endocrinologic changes in response to stress, it might be that the early lesion of the hippocampus could be diminishing the total number, the functional receptor capabilities, or simply the developmental expression with maturation of glucocorticoid receptors in the hippocampus in our model, and therefore the onset of DA-related behavior.

An interesting inducer of behavioral changes in our model refers to swimming stress. Independently, both Lipska et al. [60] and Flores et al. [101] have observed statistically significant increased locomotor activity in neonatal-lesioned animals after 15 min. of a swim-stress test. Furthermore, Norwak at al. have found that this type of stress increases the potency of glycine at the NMDA receptor [206] and, therefore, glutamatergic pathways seems to be involved in the neurobiological response to stress. Indeed, swimming stress appears to affect extracellular glutamate release in PFC [207] not in hippocampus or basal ganglia. Moreover, Jedema et al. [154] have demonstrated stress-induced increase in DA release in the prefrontal cortex; this was mediated by stress-activated glutamate neurotransmission (through the activation of AMPA/kainate receptors) in this region. Therefore, NMDA receptors changes in frontal cortex might explain some aspects of stress

in this model. During stress, a elevated DA metabolism occurs in both MPFC and nucleus accumbens, but the stress-induced increase in DA metabolism was solely observed in frontal cortex after administration of a glycine/NMDA receptor antagonist [208]. Goldstein at al. also found that the NMDA receptor complex and their associated glycine modulatory sites play an important role in the afferent control of the mesoprefrontal cortical DA system during stress [209]. Stress also increases GluR1 and NMDAR1 subunit levels in the VTA, an important molecular mechanism by which long-term effects on mesolimbic DA function would be exerted [210]. In this sense, local changes of EAA receptors in frontal cortex could be altering feedback pathways of this structure through its projection back to VTA. Since a cellular convergence occurs in the prefrontal cortex from dopaminergic projections coming from the VTA, which are known to be inhibitory, and the excitatory projections coming from CA1 and the subiculum [121], an early change or malconnection of the hippocampal projection could change via prefrontal cortex the responsiveness to stress as it has been suggested by Weinberger [81].

Recently it has been shown that stress induced behavioral sensitization depends on the secretion of glucocorticoids. Suppression of stress-induced corticosterone secretion abolished locomotor effects of intraccumbens amphetamine injection. These results suggest that glucocorticoids control stress-induced sensitization by changing the sensitivity of the mesencephalic dopaminergic transmission [211]. But indeed a sort of feedback configuration must exist since plasma levels of ACTH and corticosterone are increased by amphetamine and amphetamine is known to release CRF from median eminence [212]. As we have described above conditioned stress-induced increase in plasma corticosterone levels can be regulated by the glycine site of the NMDA receptor complex [209].

5.5. Developmental issues in the neonatal VH lesion model

The absence of prepubertal motor changes is one of the most puzzling features of this model. Changes in the pattern of neural projection, sprouting or rerouting after lesioning the ventral hippocampal formation can not explain by their own the failure of these mechanisms after puberty. Another aspect is the possibility of later developmental influence of hippocampal formation in the consolidation of mesolimbic functions. Therefore, the early lesion could be switching on or off some functional genes related with mesolimbic behaviors later on during development. Indeed, dopaminergic systems and hippocampal physiology are influenced by sexual maturation and hormonal changes [213]. Moreover, testosterone and its neural metabolites estradiol and 5-alpha-

dihydrotestosterone enhance functional activity of dopaminergic neurons [214]. In addition, estrogen and androgen receptors have been found on nerve terminals of the mesolimbic system including septum, amygdala, olfactory tubercle and nucleus accumbens. It has also been reported that estrogen increases locomotor responses to amphetamine and to apomorphine in castrated rats [215]. Nevertheless, gonadal hormones appear not to influence the emergence of postpubertal changes in enhanced DA-related behaviors in this model as Lipska at al. found by castrating neonatal VH lesion animals at PD21 [216].

Another very interesting finding made by Farber et al. [41] is that fetal rats and postnatal rats younger than 1 1/2 months (roughly puberty in the rat) were totally insensitive to the neurotoxic action of NMDA antagonists. Thus, between puberty (age, 1 1/2 months) and full adulthood (ages, 3 and 4 months), rats gradually become fully sensitive to this toxic mechanism, and remain so until at least 10 months of age. Similarly, the incidence of emergence reactions associated with ketamine-induced anesthesia, is age-dependent with the reaction occurring rarely, if ever, in prepubertal children but is manifested in nearly 50% of young to middle-aged adults. For this reason , ketamine is currently used much more frequently in pediatric than in adult medicine. Case reports that have indicated a lack of susceptibility of children to the psychotic effects of phencyclidine [67] suggest a similar age-dependency profile for this entire class of psychogenic agents. So, rats have a gradual onset of susceptibility to NMDA receptor hypofunction in late adolescence [25, 44], just as humans become susceptible to NMDA receptor (ketamine or phencyclidine)-induced psychosis and to the symptomatic expression of schizophrenia in late adolescence.

It logically follows from the above age-dependency that a lesion that has a NMDA receptor hypofunction potential could be present in the developing human brain at birth, but the psychopathological or neuropathological processes would not occur until late adolescence, at which time the lesion presumably would spontaneously begin to trigger schizophrenia-like psychotic symptoms.

APPENDIX

The anatomical nomenclature that appears throughout this text was taken from the atlas of Paxinos and Watson [94],

FRCX-1	Frontal cortex, area 1
FRCX-2	Frontal cortex, area 2
FRCX-3	Frontal cortex, area 3
CG-1	Cingulate cortex, area 1
CG-3	Cingulate cortex, area 3
AGINCX	Agranular insular cortex
LOCX	Lateral orbital cortex
VLO	Ventrolateral orbital cortex
INFRALIMB	Infralimbic cortex
DORSALPEDUNC	Dorsal peduncular cortex
MPFC	Medial prefrontal cortex
INN	Inner layers
OUT	Outer layers

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IMAGE EVALUATION
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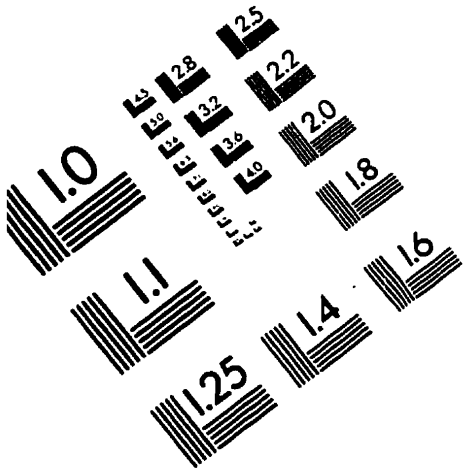

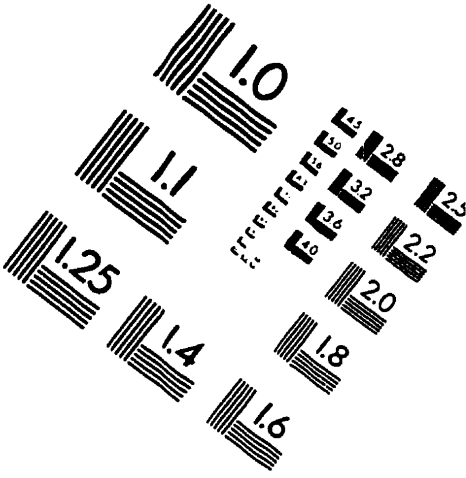

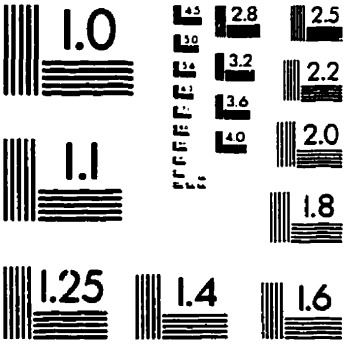

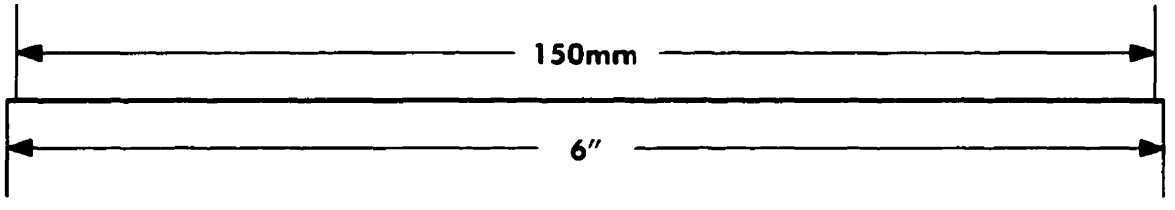

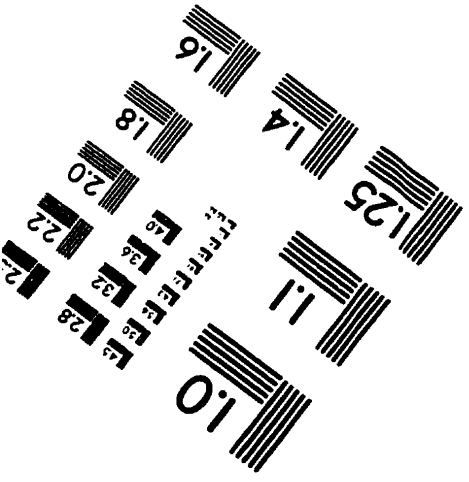

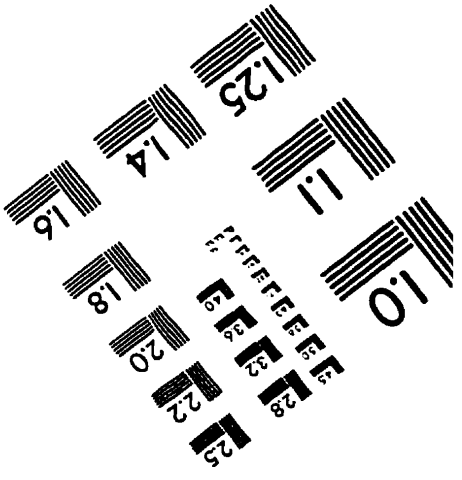

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