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Behavioural Brain Research 107 (2000) 71–83

BEHAVIOURAL BRAIN RESEARCH

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Research report

Changes in adult brain and behavior caused by neonatal limbic damage: implications for the etiology of schizophrenia

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Received 2 April 1998; received in revised form 30 December 1998; accepted 30 June 1999

Abstract

We tested the hypothesis that limbic damage in early development can cause aberrant maturation of brain structures known to be abnormal in adult schizophrenics: the hippocampus, prefrontal cortex, ventricles, and forebrain dopamine systems. We measured brain morphology, locomotor response to apomorphine, and cognitive processes in adult rats which received electrolytic damage to amygdala or hippocampus 48 h after birth. The behavioral measurements involved tasks which depend upon the integrity of the hippocampus or prefrontal cortex, and a task sensitive to forebrain dopamine system activation. The tasks included place navigation, egocentric spatial ability, and apomorphine-induced locomotion. The rats with lesions showed poor performance on the place navigation and egocentric spatial tasks and more apomorphine-induced locomotion after puberty than the sham lesion group. Regardless of lesion location, the adult rats showed smaller amygdalae and hippocampi, and larger lateral ventricles. Analyzing the lesion and sham rats together, adult amygdala volume was found to be positively correlated with cerebral cortex, prefrontal cortex, and hippocampal volumes and place navigation performance, and was negatively correlated with lateral ventricle volume. This study contributes to our understanding of the pathogenesis of schizophrenia by showing that early damage to limbic structures produced behavioral, morphological, and neuropharmacological abnormalities related to pathology in adult schizophrenics. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Neonatal; Amygdala; Hippocampus; Schizophrenia; Rat; Apomorphine; Dopamine; Frontal cortex

1. Introduction

Schizophrenia, a disease with dramatic emotional and cognitive symptoms, is associated with subtle neuropathology. The brains of adult schizophrenics often are found to have smaller amygdalae [11,33], hippocampi [11,33,38], and cerebral cortex, especially frontal lobes [4], and larger lateral and third ventricles [13,33,38,52]. In addition to these structural abnormalities, there are neurochemical abnormalities, although there is not unanimous agreement on this issue, particularly some studies have found an elevated amount of dopamine or dopamine receptors [26,34–36,49].

Schizophrenia is thought by many to result from early specific brain damage which adversely affects maturation in other parts of the brain [4,45,47]. Lipska, Jaskiw, and Weinberger [22] have developed a predictive animal model [32] of schizophrenia based upon the idea that the hippocampus is the early-damaged structure. They have shown that early hippocampal damage in rat can cause some abnormalities in behavior related to forebrain dopamine and prefrontal cortex functioning. Also, this early hippocampal damage has been found to cause deficits in hippocampal functioning before and after puberty, suggesting that the effects of this early lesion are not spared through development [14]. In addition, Bachevalier et al. [7,8] have tested this early lesion hypothesis in monkeys with selective neonatal hippocampal lesions using assessments of their social interactions. At 2 months of age they show minor

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PII: S0166-4328(99)00114-X

deficits in initiation of social interactions, and at 6 months, these changes were less apparent. However, in adulthood, the time spent in social contacts with their normal peers was less than that in normal control monkeys. Although early specific damage to the hippocampus is a dominant hypothesis, it is probably premature to focus only on the hippocampus. It is important to examine other structures known to be abnormal in adult schizophrenics. One such structure is the amygdala (Fig. 1).

There are several pieces of evidence suggesting that the amygdala could be the locus of early damage. The amygdala clearly has extensive connections with brain structures affected in schizophrenia and it matures relatively early [2,6]. The amygdala has reciprocal projections with the hippocampal formation. The major interaction of the two structures is through the entorhinal cortex [2]. The amygdala also has direct, reciprocal connections with the orbital and medial regions of frontal cortex [2]. Thus, without the normal interaction between the amygdala and the frontal cortex and hippocampus via these connections, maturation of the frontal cortex and hippocampus could be disrupted. We speculated that the size of these other forebrain structures would be correlated with the extent of amygdala (or hippocampus) damage.

Importantly, a recent study done by Yeo et al. [51], provides support for amygdala pathology in very young schizophrenics. Using MRI measurements they found that schizophrenia-spectrum children (average age = 11 years) have small amygdalae. The hippocampus, frontal lobe, and ventricular volumes were all normal at this age. Furthermore, Bachevalier and Merjanian [5] have shown that bilateral damage to the amygdala in newborn monkeys can produce deficits in social behaviors at 2 and 6 months of age. Thus, a bilateral amygdala lesion, similar to a bilateral hippocampal lesion, at birth is associated with a loss of social behaviors.

The second trimester is thought to be the most likely time for the initial damage in schizophrenics [12,27]. At birth rat neurodevelopment resembles the human second trimester [19]. Because of this in the present study, neonatal rats were used to assess if limbic damage, including amygdala or hippocampus, would cause lifelong deficits in morphology, neuropharmacology, and behavior. For this to be tested, a variety of behavioral tasks with established dependence upon the integrity of the hippocampus, prefrontal cortex, and forebrain dopamine systems were conducted. The first task is the Morris water task [28]. The hidden platform version of this task is sensitive to hippocampus damage [29,40,41,48], as well as, medial prefrontal damage [20,40]. The visible platform version of this task is not sensitive to either kind of damage, but requires the same motor coordination and motivation to escape from the water. An egocentric spatial task was also used. This task requires rats on a radial arm maze to repeat a specific movement sequence to find the goal arm from different start arms. It is sensitive to medial prefrontal damage [20]. The last task is an apomor-

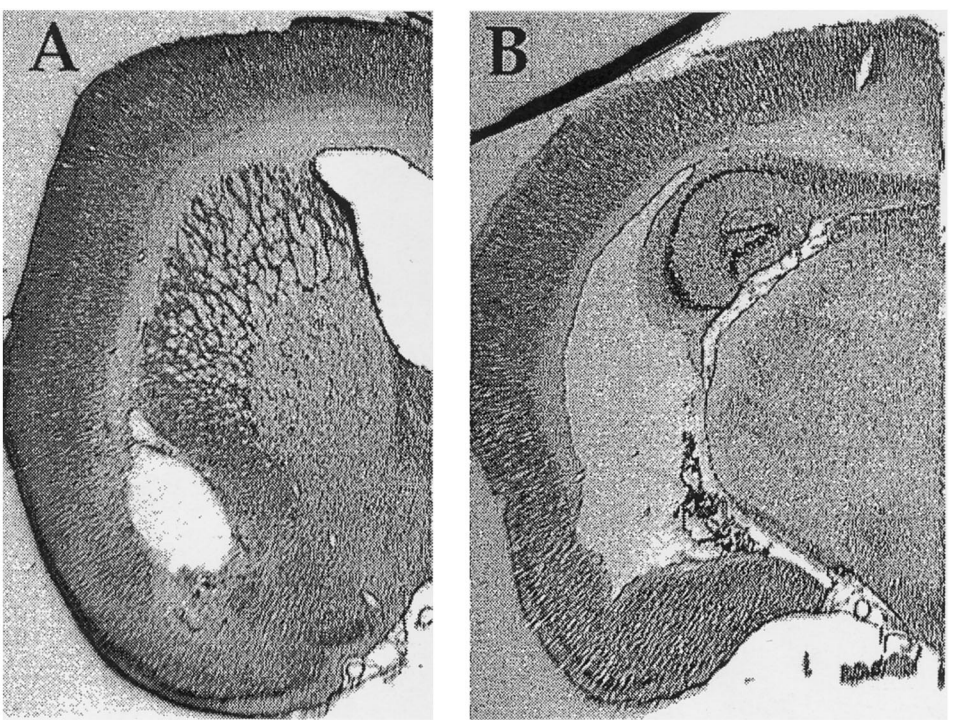

Fig. 1. A is representative of an amygdala lesion. B is representative of a hippocampal lesion.

Table 1
Deficits seen in schizophrenics, predicted deficits in the rats of the present study, and techniques used to test for these deficits

In	Techniques used to test rats	Predicted deficits for the lesion group
Brain region affected		
Frontal lobe	Morris water task	
	Hidden platform version	Yes
	Visible platform version	No
	Egocentric spatial task	Yes
Hippocampal	Morris water task	
	Hidden platform version	Yes
	Visible platform version	No
Neuroanatomical deficits		
Reduced amygdalar volume	Volumetric analysis	Yes
Reduced hippocampal volume	Volumetric analysis	Yes
Reduced frontal lobe volume	Volumetric analysis	Yes
Reduced cerebral cortex volume	Volumetric analysis	Yes
Increased lateral ventricle volume	Volumetric analysis	Yes
Increased third ventricle volume	Volumetric analysis	Yes
Neuropharmacological deficits		
Increased number or sensitivity of DA postsynaptic receptors	Apomorphine-induced locomotion	Yes

phine-induced locomotion task which measures receptor sensitivity in forebrain dopamine systems. Apomorphine stimulates dopamine receptors and causes stereotyped motor behavior [44]. We chose to measure locomotor response after apomorphine administration rather than stereotypic behaviors so that we could reliably investigate developmental changes. Lipska et al. have reliably shown exaggerated locomotor response to a dopamine agonist in their rat model involving early damage to ventral hippocampus after puberty, but not before puberty, in a number of studies [22,24,50]. Whereas this developmental change in stereotypic behaviors after apomorphine administration has only been found by them in one study [23].

In keeping with our hypothesis, impaired performance by the rats with lesions is expected on all of the behavioral tasks, except the visible platform water task. In the apomorphine-induced locomotion task, an increase in locomotion in the rats of the lesion group is expected. It is possible that the increase will be apparent only after puberty, because the early limbic lesion is hypothesized to impair development of the frontal cortex, which is involved in controlling subcortical dopamine function [21]. The frontal cortex does not mature until after puberty [42], so the sensitivity of forebrain dopamine systems may not appear until after puberty.

The purpose of this study is to examine whether early limbic damage, including amygdala or hippocampus, causes abnormal maturation of other portions of the limbic system, prefrontal cortex (the anterior portion of the cerebral cortex), cerebral cortex (the entire cerebral cortex including the prefrontal portion), ventricles, and forebrain dopamine systems, thus causing adult behavioral deficits. These are structures that are abnormal in adult schizophrenia. Table 1 includes deficits seen in schizophrenics, the predicted deficits in this study, and the techniques used to test these predictions.

2. Materials and methods

2.1. Subjects

Thirty-four Long–Evans hooded rats, 19 males and 15 females, were used. The rats were bred and born in-house with an average of 10 rats per litter. All animals were housed in plastic cages with bedding and with their dams until weaned ($\approx$25 days old), after which they were housed independently. The vivarium is maintained on a 12:12 h light–dark schedule.

2.2. Procedure

2.2.1. Surgery

Surgery was performed on 48 h old rat pups. Seventeen rats received bilateral electrolytic stereotaxic lesions and the other 17 were sham lesion rats. Hypothermia was used for general anesthesia for all rats. The rat pups were placed on ice for 10–20 min. Anesthesia was maintained by keeping the pups chilled using cold packs. A stainless-steel electrode (0.25-mm diameter, 0.5-mm uninsulated tip) was lowered into the brain (1.3 mm posterior to bregma, 2.6 mm lateral to the midline, 4.3 mm below the dura) and a 1.5-mA current was passed for 15 s. Silk sutures (5-0) were used to close the scalp incisions. The rats of the sham group were exposed to the same procedure except that the stainless-steel electrode was not lowered.

2.2.2. Morris water task

Rats were tested on two different versions of this task. In both versions the apparatus was a circular, white pool (1.5 m diameter, 0.46 m high) filled with water. Instant powdered skim milk was added to cloud the water. The first version of this place navigation task required the rats to learn to swim to a hidden platform.

The hidden platform was made of clear Plexiglas (13 × 13 cm) and was submerged 1.3 cm below the water surface. The second version required the rat to swim to a visible platform, which sits 2.54 cm above the water. An HVS Image Analysis target scanner (VP112) was used to calculate the position of the rats' head 50 times per second via a video camera. For swim path analysis, the pool's area was divided into four quadrants. Testing began at postnatal day 150.

In the hidden platform version the rats learned to locate a new hidden platform location every day, which was determined pseudorandomly. Swim time and swimming position within the four quadrants of the pool were collected. Each rat received eight trials per day for 14 days, with a 60 s maximum trial duration. The sequence of starting positions at the four cardinal compass points was chosen pseudorandomly, such that each starting position was sampled once in each block of four trials. A probe trial was carried out on the 14th day after the eighth trial. The platform was removed for 20 s. Swim distance in each quadrant was calculated [28,39,48].

In the visible platform version, the platform was equal in size to the hidden platform, but was colored black on the top and on all sides. This visible platform was in a new location each day determined pseudorandomly. Each rat received eight trials per day for 5 days, with a 60 s maximum trial duration. Starting positions were chosen with the same pseudorandom sequence as the hidden platform version. Time to board the platform and swim distance were recorded.

2.2.3. Egocentric spatial task

The apparatus was an 8-arm radial maze with food wells at the end of each arm. The central platform and arms were 95 cm above the ground. The maze was placed in the center of a room where all cues were kept constant. Rats were food-deprived to 85% of their free-feeding body weight before testing began. Habituation consisted of placing two rats at a time for 10 min in the maze for 3 consecutive days. During the habituation phase, the maze had fruit loops placed throughout the center, arms, and food wells. Beginning on the fourth day, fruit loops were placed only in one food well and each rat received eight trials each day until a criterion of seven correct responses out of eight was met or 30 days of testing were completed. The maximum trial duration was 3 min. The testing procedure began by placing the rat at the end of one of the eight arms, facing the center of the maze. The rat was released from a different arm each trial according to a pseudorandom sequence. One fruit loop was placed in the well at the end of the adjacent right arm [20]. If the rat placed all four paws on the correct arm, it was allowed to go to the end of the arm and eat the fruit loop. If the rat placed all four paws onto one of the seven wrong arms, the rat was immediately removed from the maze and returned to a holding cage until the next trial. The intertrial interval was ≈ 5 min. The number of correct responses, the number of trials to meet criterion, and the number of rats that failed to reach criterion were recorded. Testing began at postnatal day 240.

2.2.4. Apomorphine-induced locomotion task

The apparatus was the same as that used for the Morris water task, except that the pool remained empty. The rats' position was tracked using video input to an HVS Image Analysis target scanner (VP112), which calculated the rats' head position 50 times per second. Due to computer complications data were lost for four rats, three rats in the lesion group and one rat in the sham group for this task. Therefore, we conducted our analysis on fourteen rats (seven males and seven females) who were tested at 35 days of age, seven rats with neonatal lesions and seven with sham lesions, and 16 rats (nine males and seven females) who were tested after day 56, seven rats with neonatal lesions and nine with sham lesions. Thus, performance was assessed before and after puberty, to test for developmental changes in apomorphine-sensitivity. Habituation to the apparatus was carried out for 1 h on the day before testing with apomorphine. Each rat was tested with two different doses of apomorphine and vehicle-alone injected intraperitoneally. The doses were 0.1 and 0.75 mg kg^{-1}, and 0.9% saline vehicle. The first two doses were mixed with 0.9% saline and all three doses were mixed with 0.01% ascorbic acid [18,23]. All injections were made 5 min before testing. The order of dosages was randomly chosen to control for order effects. Distance traveled by the rat was stored by the computer for 1 h after injections and for statistical analyses, the hour was divided into three blocks of 20 min intervals.

2.2.5. Histology and volumetric analysis

After all the tasks were completed, histology and brain regional volumetric analyses were performed. The rats were sacrificed by administration of inhaled halothane and were transcardially perfused with 0.9% saline followed by formaldehyde. The brains were removed and stored in 10% formal saline–30% sucrose solution. The whole brains were frozen-sectioned, coronally, at 40 μm and every fifth section through the cerebral hemispheres, including the hippocampus, amygdala, and frontal cortex were mounted on glass slides and stained with cresyl violet. Sections were then captured on a Power Macintosh computer using a Cohu video-camera (580 H/TVL and 350 V/TVL resolution) and a Zeiss Microscope using the imaging processing program, Scion Image 1.59. Volumes of the amygdala, hippocampus, cerebral cortex, frontal cortex, and lateral and third ventricles were assessed by manu-

ally tracing each. For all measurements both hemispheres were measured.

A stereotaxic map was used to define each structure, its boundaries, and the distance from bregma at which we were measuring [31]. Measurement of the amygdala started when it first appeared, −0.8 mm from bregma, and the last measurement was taken when it ended at its posterior boundary, −5.8 mm from bregma. The lateral and anterodorsal boundary for the anterior portion of the amygdala was the endopiriform nucleus. The medial and dorsal boundary was the striatum. The medial boundary was the optic tract. The lateral and ventral boundary was the primary olfactory cortex. The boundaries for the posterior portion of the amygdala were medial and dorsal, the lateral ventricles and subiculum. The lateral boundaries were the endopiriform nucleus, the primary olfactory cortex, and the entorhinal cortex. The posterior boundary was the entorhinal cortex. Measurement of the hippocampus started at its anterior margin, −1.8 mm from bregma, and the last measurement was taken at −8.3 mm from bregma. We included all of the hippocampus and the subiculum. The anterior boundary of the anterior portion of the hippocampus was the ventral hippocampal commissure. The dorsal and lateral boundary was the corpus collosum. The dorsal boundary was the dorsal hippocampus commissure. The lateral boundary was the fimbria. The ventral boundaries were the third ventricle and thalamic nucleus. The dorsal boundary of the posterior portion of the hippocampus was the dorsal hippocampus commissure. The dorsal and lateral boundary was the corpus collosum. The ventral boundary of the posterior portion of the hippocampus was the amygdala and entorhinal cortex. The medial boundary was the ventricle. The posterior boundary was the entorhinal cortex. The entire cerebral cortex was measured, starting at 3.2 mm from bregma and ending at −8.3 mm from bregma. At 3.2 mm from bregma the cerebral cortex was measured with the ventral boundary being the rhinal fissure. A line was drawn straight across from where the rhinal fissure started to the midline, and then around the cortex, to complete the circle at the start of the rhinal fissure. Starting at 2.7 mm from bregma the straight line drawn from the rhinal fissure, was drawn over to the corpus callosum, and then all the way around the corpus callosum to its medial side, and a straight line was drawn over to the midline. The line was then drawn around the outside of the cerebral cortex to complete the circle at the start of the rhinal fissure. When the rhinal fissure was no longer seen, at −7.8 mm from bregma, the measurement of the cerebral cortex consisted of the tissue lateral to the ventricles, except for the posterior part of the subiculum. The frontal cortex measurements were conducted at 3.2 mm from bregma and 2.7 mm from bregma. The procedure for measuring these two sections were the same as for the cerebral cortex described above. The lateral and third ventricles were measured separately. Measurement for the lateral ventricles began when they first appeared, 2.2 mm from bregma, and ended when they no longer were seen, −4.3 mm from bregma. Measurement for the third ventricle began when it first appeared, at −0.8 mm from bregma, and ended at −4.8 mm from bregma. Lesions were distinguished from ventricles and were not included in the measurements of the ventricles. A lesion was defined as a region without tissue, if it was not continuous with a ventricle, and if it did not have a distinct, darkly-stained ependymal lining. Localization of the lesion for each rat took place during this process.

Once all the measurements were collected for each section, volume for each structure was calculated by averaging consecutive measurements on each pair of sections captured and multiplying them by the number (4, as we took every fifth section) and width of slices (40 μm) which were not captured between them. These amounts and the initial measurements (based on every fifth section) were then added for each rat.

2.2.6. Statistical analyses

For both the hidden and visible platform versions of the Morris water task, a repeated-measures ANOVA was used to look at group and day effects. Also, one-tailed *t*-tests for independent samples were conducted to examine whether a group difference was found for escape latencies on the last day of testing for both versions of the Morris water task. On the hidden platform version, a one-tailed *t*-test for independent samples was conducted to analyze whether a group difference was seen for the percent of swim distance in the correct quadrant during the probe trial. For the apomorphine-induced locomotion task, performance of rats was analyzed separately according to whether testing was before or after puberty. For each of the three doses, a repeated-measures ANOVA was used to look at group and time effects. We also conducted a 3-way ANOVA including group, gender, and age as independent factors for each of the three doses. For the egocentric spatial task a repeated-measures ANOVA was done to look at group and day effects. A one-tailed *t*-test for independent samples was also conducted to examine whether a group difference was seen for the percent that failed to reach criterion on the egocentric spatial task. Volume for each structure was compared for the rats of the lesion and sham groups using one-tailed *t*-tests for independent samples. Correlation coefficients were calculated, adjusting for sex, on the volume of each structure for the lesion and sham groups as a whole. Also correlation coefficients for volume of each structure and measures from the behavioral tasks were calculated, adjusting for sex, for both the lesion and sham groups as a whole. Sex differences on the behav-

ioral tasks and volumetric analysis were analyzed using two-tailed t-tests for independent samples. For all statistical tests we used Levene's test for equality of variances to adjust degrees of freedom where appropriate.

3. Results

Thirty-four rats were used for the behavioral tasks, 17 received neonatal lesions (12 males and five females) and 17 received sham lesions (seven males and 10 females). Histology was conducted on 24 rats, 16 with neonatal lesions (11 males and five females) and eight shams (four males and four females). Eight of the sham rats were randomly selected as representative of normal brains for volumetric analyses. We randomly assigned our 48 h old rat pups, thus the lesion and sham groups are not matched for sex. No sex differences were found in the apomorphine-induced locomotion task nor the egocentric spatial task for the sham rats. However, a marginal sex difference was found in the Morris water task, with the male sham rats performing better than the female sham rats. The male sham rats had lower total escape latency on the hidden platform version [$t(5) = -2.53$, $P = 0.053$] and a greater percent swim distance in the correct quadrant during the probe trial [$t(5) = 2.49$, $P = 0.055$] than the female sham rats.

3.1. Localization of the lesions

Of the 16 rats with neonatal lesions 15 were used for volumetric analysis. One neonatal lesion rat was excluded because it sustained bilateral striatal damage which is beyond the scope of the present experiment. We found the following electrolytic lesions: ten rats sustained amygdala lesions, three bilateral (all males) and seven unilateral (three males and four females); ten rats sustained hippocampal lesions, three bilateral (two males and one female) and seven unilateral (five males and two females) (Fig. 1). The number of rats listed above does not sum to 15 because in some rats one side has a hippocampal lesion and the other side has an amygdala lesion. Both the hippocampal and amygdala lesions were subtotal and variable, involving several subcomponents of both structures. This fact precludes conclusions about correlations with specific nuclei or subfields. Finally, three of the neonatal lesion rats had minor additional unilateral damage to ventral striatum and two to entorhinal cortex.

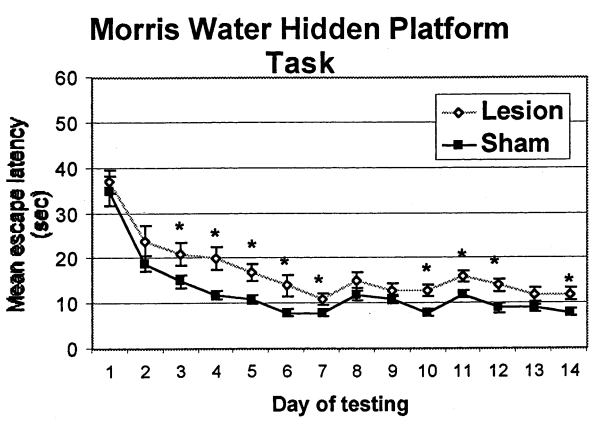

Fig. 2. Mean escape latencies over the 14 days of testing for the lesion and sham groups on the hidden platform version of the Morris water task. The lesion group showed significantly longer escape latencies than the sham group, $F(1, 31) = 9.67$, $P = 0.004$.

3.2. Morris water task

On the hidden platform version, a repeated-measures ANOVA showed a significant main effect of group [$F(1, 31) = 9.67$, $P = 0.004$; Fig. 2] and a significant main effect of day [$F(1, 13) = 46.63$, $P < 0.001$; Fig. 2]. A significant group by day interaction was not found for escape latencies [$F(1, 13) = 0.77$, $P = 0.697$; Fig. 2]. Also, the lesion group had longer escape latencies on the last day of testing, day 14 [$t(22) = -2.12$, $P = 0.023$; Fig. 2]. A significant group difference was not found for percent of swim distance in the correct quadrant during the probe trial [$t(31) = 0.15$, $P = 0.440$]. In the visible platform version, a repeated-measures ANOVA did not show a significant main effect of group [$F(1, 31) = 0.39$, $P = 0.538$] nor main effect of day [$F(1, 4) = 2.22$, $P = 0.071$]. There was not a significant group by day interaction for escape latencies [$F(1, 4) = 1.64$, $P = 0.167$]. Also, no group differences were seen for escape latencies on the last day of testing, day 5 [$t(31) = 0.49$, $P = 0.315$].

When we separately compare the behavior of rats sustaining amygdala damage, either bilateral or unilateral, to sham lesion rats we found that the difference in total escape latency summed over the 14 days of testing was statistically significant [$t(15) = 2.11$, $P = 0.026$]. The same was true if we separately compare the behavior of rats sustaining hippocampal damage, either bilateral or unilateral, to sham lesion rats [$t(15) = 2.00$, $P = 0.032$].

In terms of possible sex differences in performance in this task, the fact that we have a higher proportion of males in the lesion group than in the sham group (68.75 and 41.18%, respectively) cannot account for the lesion deficit we observe, since normally males have a marginal advantage over females in Morris water task performance (see above). However, within the lesion group, the females tended to have significantly longer escape latencies on the last day of hidden platform training [$t(13) = 2.54$, $P = 0.025$] and across all days of visible platform training [$t(13) = 2.89$, $P = 0.013$]. There was no sex difference in quadrant preference during the

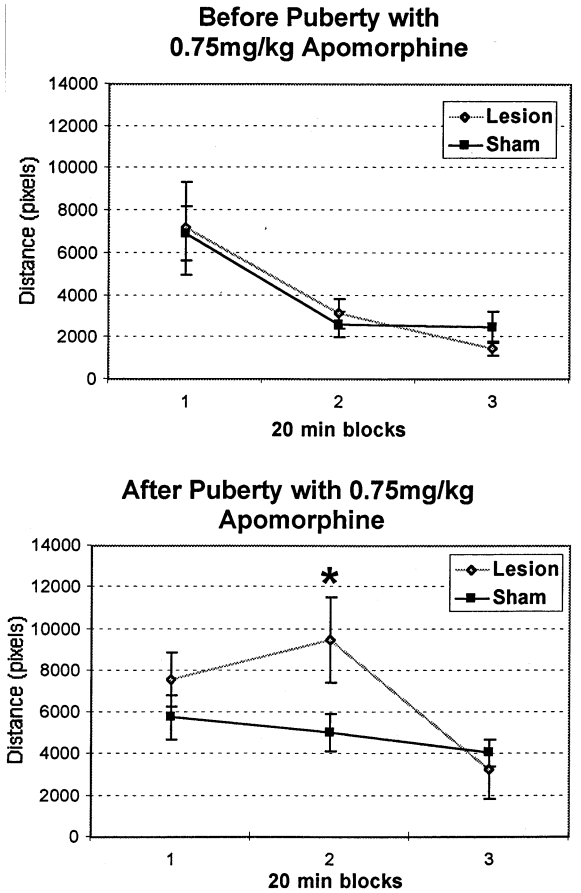

Fig. 3. Top graph: mean distance traveled, before puberty, for the lesion and sham groups during each of three consecutive 20 min blocks after 0.75 mg kg^{-1} apomorphine. No significant group by time interaction in distance traveled was seen before puberty, $F(1, 2) = 0.23$, $P = 0.795$. Bottom graph: mean distance traveled, after puberty, for the lesion and sham groups during each of three consecutive 20 min blocks after 0.75 mg kg^{-1} apomorphine. A significant group by time interaction in distance traveled was seen after puberty, $F(1, 2) = 4.46$, $P = 0.021$.

no-platform probe trial, making the latency differences likely to be attributable to slower swimming speeds by females.

3.3. Apomorphine-induced locomotion

Rats were analyzed separately according to whether they were tested before or after puberty with the 0.75 mg kg^{-1} dose using a repeated-measures ANOVA. No significant main effect of group was found before [$F(1, 2) = 0.01$, $P = 0.933$] or after [$F(1, 2) = 1.64$, $P = 0.221$] puberty. However, a significant main effect of time was seen before [$F(1, 2) = 9.97$, $P = 0.001$] and after [$F(1, 2) = 9.87$, $P = 0.001$] puberty. No significant group by time interaction in distance traveled was seen before puberty [$F(1, 2) = 0.23$, $P = 0.795$; Fig. 3], yet this interaction was statistically significant after puberty [$F(1, 2) = 4.46$, $P = 0.021$; Fig. 3]. Therefore, after puberty the rats of the lesion group locomoted more than the rats of the sham group after 0.75 mg kg^{-1} of apomorphine over the three consecutive 20 min blocks. With the 0.1 mg kg^{-1} dose of apomorphine no significant group main effect before [$F(1, 2) = 0.23$, $P = 0.644$] or after ($Fs < 1.0$) puberty was found. Nor was there a significant main effect of time before [$F(1, 2) = 1.12$, $P = 0.343$] or after [$F(1, 2) = 3.18$, $P = 0.057$] puberty. No significant group by time interaction in distance traveled was seen before puberty [$F(1, 2) = 1.03$, $P = 0.374$], or after puberty [$F(1, 2) = 0.33$, $P = 0.720$]. Interestingly, after puberty there was a significant difference between male lesion rats and female lesion rats in apomorphine-induced locomotion with both the high [$t(5) = 11.75$, $P < 0.001$] and the low doses [$t(5) = 4.15$, $P = 0.009$], with females locomoting more than males. There was no sex difference before puberty at either dose ($Ps > 0.45$).

With the 0.9% saline vehicle no significant group main effect before [$F(1, 2) = 0.35$, $P = 0.565$] or after [$F(1, 2) = 0.14$, $P = 0.710$] puberty was found. Although, a significant time main effect was seen before [$F(1, 2) = 14.37$, $P < 0.001$] and after [$F(1, 2) = 39.54$, $P < 0.001$] puberty. No significant interaction was seen before puberty [$F(1, 2) = 0.08$, $P = 0.922$], or after [$F(1, 2) = 2.53$, $P = 0.097$] puberty.

For the 0.75 mg kg^{-1} dose of apomorphine, a 3-way ANOVA including group, gender, and age as independent factors showed a significant age main effect [$F(1, 21) = 21.03$, $P < 0.001$], sex main effect [$F(1, 21) = 17.89$, $P < 0.001$], and group main effect [$F(1, 21) = 10.51$, $P = 0.004$]. There was not a significant age by sex interaction [$F(1, 21) = 2.19$, $P = 0.153$]. Yet, there was a significant age by group interaction [$F(1, 21) = 5.25$, $P = 0.032$] and sex by group interaction [$F(1, 21) = 9.11$, $P = 0.007$]. A significant age by sex by group interaction was found [$F(1, 21) = 10.76$, $P = 0.004$]. With the 0.1 mg kg^{-1} dose of apomorphine a significant age main effect [$F(1, 21) = 4.79$, $P = 0.040$] and sex main effect [$F(1, 21) = 6.78$, $P = 0.017$] were found, yet no group main effect [$F(1, 21) = 0.30$, $P = 0.588$]. No significant two-way interactions were found. There was also not a significant age by sex by group interaction [$F(1, 21) = 0.11$, $P = 0.740$]. With the 0.9% saline vehicle a significant sex main effect [$F(1, 21) = 22.67$, $P < 0.001$] and group main effect [$F(1, 21) = 6.89$, $P = 0.016$] were found, yet no age main effect [$F(1,21) = 3.08$, $P = 0.094$). There was not a significant age by sex interaction [$F(1, 21) = 0.24$, $P = 0.628$], nor age by group interaction [$F(1, 21) = 0.01$, $P = 0.941$] found. Yet, there was a significant sex by group interaction [$F(1, 21) = 9.56$, $P = 0.006$]. We did not find a significant age by sex by group interaction [$F(1, 21) = 0.16$, $P = 0.695$].

3.4. Egocentric spatial task

A repeated-measures ANOVA showed no significant group main effect [$F(1, 29) = 3.40$, $P = 0.139$], yet did show a significant day main effect [$F(1, 29) = 3.16$, $P < 0.001$]. A significant group by day interaction was found for the percent of correct responses [$F(1, 29) = 1.61$, $P = 0.040$; Fig. 4]. The rats of the lesion group showed a lower percent of correct responses than the rats of the sham group late in training, but not early. A one-tailed t-test for independent samples revealed a significant group difference for percent that failed to reach criterion with the rats of the lesion group having a higher percentage [$t(22) = -1.90$, $P = 0.035$; Fig. 4].

3.5. Volumetric analyses

The rats of the lesion group had lower amygdala volume than the rats of the sham group [$t(20) = 2.21$, $P = 0.02$]. A marginally significant difference was seen for hippocampal volume, with the rats of the lesion group having a smaller volume [$t(20) = 1.61$, $P = 0.062$]. No group difference in frontal cortex volume was found [$t(20) = 1.28$, $P = 0.108$]. The rats of the lesion group had larger lateral ventricles than the rats of the sham group [$t(14) = -2.63$, $P = 0.01$]. The volume of the third ventricle was not significantly different for the two groups [$t(20) = -1.00$, $P = 0.164$]. There was also no difference between groups in cerebral cortex volume [$t(20) = -0.16$, $P = 0.437$].

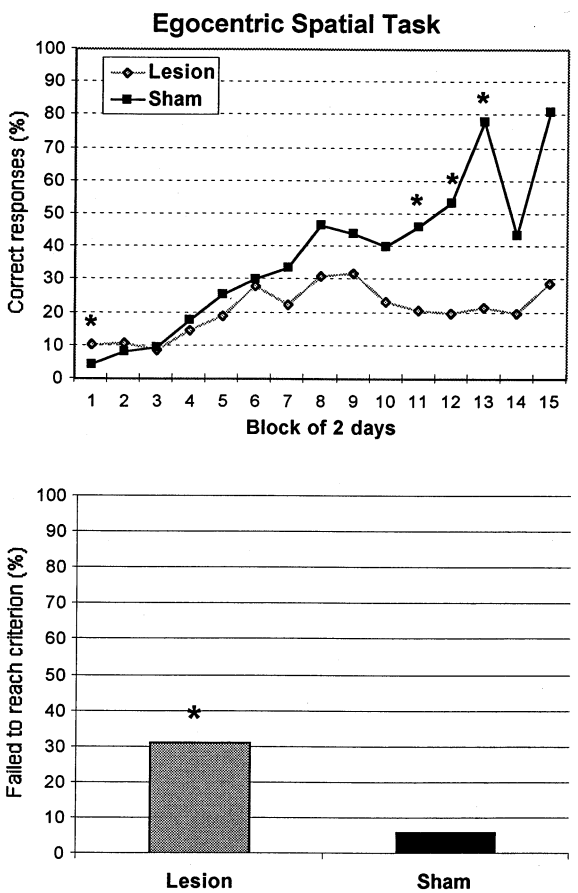

Fig. 4. Top graph: percentage of correct responses for the lesion and the sham group in blocks of 2 days over the 30 days of testing on the egocentric spatial task. The rats of the lesion group showed a lower percentage of correct responses than the rats of the sham group late in training, $F(1, 29) = 1.61$, $P = 0.040$. Bottom graph: percentage of rats that failed to reach criterion after 30 days (240 trials) of testing for each group. The lesion group has a higher percentage of rats that failed to reach criterion than the sham group, $t(22) = -1.90$, $P = 0.035$.

To explore further the association between damage to the amygdala and the volumetric measures, two-tailed t-tests were conducted comparing the 13 cerebral hemispheres with amygdala damage to the 14 cerebral hemispheres with sham lesions. The amygdala lesion hemispheres contained significantly smaller amygdalae [$t(27) = 3.72$, $P = 0.001$] and hippocampi [$t(25) = 2.77$, $P = 0.010$], but not frontal cortex or cerebral cortex (Ps > 0.15). They also had significantly larger volume of the lateral ventricles [$t(25) = 3.6$, $P = 0.001$]. Two-tailed t-tests for independent samples were also conducted on volumetric measures, comparing the 13 cerebral hemispheres with hippocampal damage to the 14 cerebral hemispheres with sham lesions. No significant differences were found for the volumes of cortical or limbic structures (Ps > 0.05), but lateral ventricle volume was marginally significantly larger in the lesion hemispheres [$t(12.01) = 2.09$, $P = 0.058$].

In rats females brains tend to be smaller than male brains, therefore we compared our males and females on each of the volumetric measures. Males of the sham group had greater volume of cerebral cortex ($P = 0.009$), frontal cortex ($P = 0.015$), hippocampus ($P = 0.007$), amygdala ($P = 0.041$), and lateral ventricle ($P = 0.028$). Importantly, none of these volumetric comparisons between males and females was statistically significant in the neonatal lesion rats (all Ps > 0.33). Thus, in general there is a trend for the neonatal lesions to have a greater impact on volumetric measures in males than in females.

A correlation analysis on lesion and sham rats was conducted, adjusting for sex, to measure the strength of the associations among volumes of the relevant structures. These data are illustrated in Table 2. Amygdala volume is positively correlated with cerebral cortex, frontal cortex, and hippocampal volumes, and is negatively correlated with lateral ventricle volume. These same correlations are seen when analyzing the hippocampal volume, with the hippocampus positively correlating with amygdala volume.

3.6. Volumetric analysis and behavior

Correlation coefficients for both the lesion and sham groups for volume of the amygdala, hippocampus,

Table 2
Correlation coefficients for the lesion and sham group's volume of the amygdala (Amg), hippocampus (Hpc), frontal cortex (FC), cerebral cortex (CC), lateral ventricles (LV), and third ventricles (TV)[a]

	Amg	Hpc	FC	CC	LV
Hpc	0.6481 $P<0.01$				
FC	0.3731 $P<0.05$	0.3906 $P<0.05$			
CC	0.6415 $P<0.01$	0.6653 $P<0.01$	0.5577 $P<0.01$		
LV	−0.5234 $P<0.01$	−0.5238 $P<0.01$	−0.3598 NS	−0.4966 $P<0.05$	
TV	−0.1933 NS	−0.0267 NS	−0.2215 NS	−0.2236 NS	0.1234 NS

[a] NS = not significant, $P>0.05$.

frontal cortex, cerebral cortex, lateral ventricles, and third ventricle and measures from the behavioral tasks were calculated adjusting for sex (see Table 3). Data from the apomorphine-induced locomotion task was excluded from this analysis due to unacceptably low N per cell when adjusting for sex. The volume of several structures correlated significantly with Morris water task performance. In the hidden platform version of the Morris water task, larger volume of the hippocampi is associated with lower total escape latencies over the 14 days of testing. Thus, larger hippocampi were associated with better performance. Lateral ventricle volume is positively correlated with total escape latencies over the 14 days of testing. Amygdala, hippocampal, frontal cortex, and cerebral cortex are all positively correlated with swim distance in the correct quadrant during the probe trial. Lateral ventricle volume is negatively correlated with swim distance in the correct quadrant. For the visible platform version of the Morris water task, hippocampal volume is negatively correlated with escape latencies over the 5 days of testing. Lateral ventricle volume is positively correlated with the escape latencies. Escape latencies for the last day of testing, day 5, are positively correlated with the lateral and third ventricle volumes. Overall, larger amygdala, hippocampus, cerebral cortex, and frontal cortex volumes, and smaller lateral ventricle volume were associated with better place navigation performance.

The correlation, based upon all subjects, between volume of frontal cortex and success in the egocentric spatial task is marginally significant (see Table 3). However, if the neonatal lesion group is analyzed separately, the frontal cortex volume is significantly correlated with success in this task ($P=0.05$). This means that the smaller the frontal cortex volume the poorer performance on the egocentric spatial task.

4. Discussion

A neonatal lesion in limbic structures results in: (1) adult deficits in behavioral tasks known to be affected by frontal cortex and hippocampus damage; (2) an increase in locomotor sensitivity to a dopamine agonist which emerges after puberty; (3) reduced adult amygdala and hippocampus volume; and, (4) larger lateral ventricles. Each of these findings is consistent with the

Table 3
Correlation coefficients for the lesion and sham group's volume of measured brain structures and measures from behavioral tasks[a]

	Amg	Hpc	FC	CC	LV	TV
HD total lat	−0.3416 NS	−0.5006 $P=0.01$	−0.2324 NS	−0.2598 NS	0.7265 $P<0.01$	−0.1596 NS
HD last day	0.0275 NS	−0.2233 NS	−0.0280 NS	0.2152 NS	0.1324 NS	−0.2075 NS
HD probe	0.4494 $P<0.05$	0.3766 $P<0.05$	0.4652 $P<0.05$	0.4849 $P<0.05$	−0.5879 $P<0.01$	−0.2672 NS
VL total lat	−0.2423 NS	−0.3769 $P<0.05$	−0.1461 NS	−0.1986 NS	0.6613 $P<0.01$	0.1017 NS
VL last day	−0.0567 NS	−0.1649 NS	−0.2338 NS	−0.0737 NS	0.5607 $P<0.01$	0.4807 $P<0.05$
Ego trials criterion	−0.1012 NS	0.0029 NS	−0.3425 $P=0.06$	−0.0809 NS	0.1807 NS	0.3312 NS

[a] Amg: amygdala volume; Hpc: hippocampal volume; FC: frontal cortex volume; CC: cerebral cortex volume; LV: lateral ventricle volume; TV: third ventricle volume; HD total lat: Morris water task hidden platform — Total escape latency over 14 days; HD last day: Morris water task hidden platform — Escape latency for last day; HD probe: Morris water probe trial — Distance swam in correct quadrant; VL total lat: Morris water task visible platform — Total escape latency over 5 days; VL last day: Morris water task visible platform task — Escape latency for last day, day 5; Ego trials criterion: egocentric spatial task — Number of trials to meet criterion. NS = not significant, $P>0.05$.

early limbic hypothesis of schizophrenia and pathology.

The prenatal neuropathology thought to exist in human schizophrenia is hypothesized to cause adult behavioral deficits due, at least in part, to impaired development of the hippocampus and prefrontal cortex [45]. Our neonatal lesion rats' performance in the Morris water task is consistent with hippocampal and/or medial prefrontal cortex dysfunctioning. Rats with large adult hippocampal lesions or medial prefrontal cortex lesions have longer latencies than control rats to find the hidden platform and during probe trials the rats do not swim more in the correct quadrant as control rats do [20,29,40,41,48]. In the present study, the neonatal lesion rats exhibited longer total latencies to find the hidden platform over the 14 days of testing (see Fig. 2) and longer latencies on the last day of testing, day 14 (see Fig. 2). A significant group difference was not found for percent of swim distance in the correct quadrant during the no-platform probe trial. Clearly, the present deficit is not as severe as if the hippocampus or medial prefrontal cortex were extensively damaged; the rats with neonatal lesions did eventually learn the platform location. This deficit is most likely not due to the rats' motor coordination or sensory abilities involved in swimming to a platform, or motivation to escape from the water, as no group differences were seen for latencies to find a visible platform summed over the 5 days of testing nor for latencies on the last day of testing, day 5. Yet, this does not exclude the contribution of other sensorimotor changes to the deficit seen on this task by the neonatal lesion rats. Rats with either neonatal amygdala or hippocampal damage were significantly worse than controls in the hidden platform version. Thus, a neonatal lesion including damage to the amygdala or hippocampus can cause deficits that resemble adult hippocampal or medial prefrontal lesion effects.

A correlational analysis showed that the volume of several forebrain structures predicts Morris water task performance (see Table 3). Overall, smaller amygdala, hippocampus, cerebral cortex, and frontal cortex volumes, and larger lateral ventricle volume were associated with worse place navigation accuracy during a no-platform probe trial at the end of training, but only hippocampal and lateral ventricle volumes predicted total escape latency across all training days.

An adult deficit in egocentric learning which is consistent with prefrontal cortex (and not hippocampal) dysfunction was seen in our neonatal lesion rats. Kolb et al. [20] found that rats with adult prefrontal cortex lesions were impaired on this egocentric spatial task. They suggest that learning and remembering kinesthetic information, not visual information, is important for solving this task and this ability is disrupted after a prefrontal cortex lesion. In the present study, the rats of the neonatal lesion group made fewer correct responses than the rats of the sham group (see Fig. 4). Also a significantly greater proportion of the lesion rats failed to reach criterion (see Fig. 4). The correlation, based upon all subjects, between volume of frontal cortex and success in the egocentric spatial task is marginally significant (see Table 3). However, if the neonatal lesion group is analyzed separately, the frontal cortex volume is significantly correlated with success in this task. We found that the smaller the frontal cortex volume, the poorer performance on the egocentric spatial task. Thus, our neonatal lesions cause deficits which resemble those caused by an adult prefrontal cortex lesion and the magnitude of the impairment after amygdala or hippocampal damage is predicted by frontal cortex volume.

Lipska et al. [22] came to a similar conclusion about frontal cortex studying rats with neonatal hippocampal lesions. Their rats with neonatal hippocampal lesions show behavioral hyperresponsiveness to stress, similar to rats with adult lesions of the medial prefrontal cortex. The behavioral hyperresponsiveness to stress seen in these rats is taken by them as confirming the hypothesis that a hippocampal neonatal lesion will cause abnormalities in prefrontal cortex functioning related to abnormalities in schizophrenics. When comparing the findings of the current study to Lipska et al.'s findings, it is important to mention that their lesioning technique is different than that of the current study. They conduct excitotoxic lesions, which primarily damage cell bodies, whereas in the current study we conducted electrolytic lesions, which destroy cell bodies as well as fibers of passage. Bertolino et al. [10] provides some support for this idea in monkey. They investigated the effects of early mesial temporal lobe lesions, including both the amygdala and hippocampus, on adult prefrontal cortex using magnetic resonance spectroscopic imaging. They found that the early limbic damage, but not similar adult damage, caused a reduction in *N*-acetyl-aspartate in the neurons of the adult prefrontal cortex, implying that the effect is mediated by altering development, not by a direct lesion effect on prefrontal cortex functioning.

We also found that our neonatal limbic lesions were associated with altered responsiveness to a dopaminergic agonist. Administration of apomorphine generated more locomotion in neonatal lesion than sham rats. A similar finding was reported by Lipska and Weinberger [23] with neonatal hippocampal lesions, using the same dose of apomorphine. More research is needed to discriminate neonatal amygdala or hippocampal lesion effects on sensitivity of forebrain dopamine systems. Also, in line with other data [22,24,50], in the present study the effect was only observed after the rats had reached puberty, not before (see Fig. 3). Therefore, the neonatal lesion caused rats to show enhanced dopamine receptor-induced locomotor activation after puberty. This may reflect an increased number or sensitivity of dopamine postsynaptic receptors [44]. We found that neonatal lesion females showed reliably greater postpubertal locomotor response

to apomorphine than males. Greater normal female responsiveness to a different dopaminergic agonist, amphetamine, has been noted by Beatty and Holtzer (cited in [25]), but our sham lesion rats did not show a sex difference in apomorphine-induced locomotion.

The presence of an early limbic lesion should have important developmental consequences for the structure of connected brain regions. In part this should reflect the loss of inputs to those regions and subsequent compensatory reorganization of competing spared inputs and intrinsic circuitry. Further, some regions normally sending outputs to the lesion tissue will have lost their postsynaptic targets and will experience an enhancement of the process of early neuron loss. In this light we measured the adult volumes of the amygdala, hippocampus, prefrontal cortex, and cerebral cortex, and the lateral and third ventricles. We found that volumes of the amygdala and the hippocampus were significantly smaller in the rats of the lesion group and the lateral ventricles were significantly larger. It should be noted that a lesion was defined as a region without tissue, if it was not continuous with a ventricle, thus the lateral ventricles could possibly include a portion of the lesion which we could not distinguish. The volumes of the cerebral cortex and frontal cortex were not smaller in the neonatal lesion rats, and the third ventricle was not larger. The predicted group difference in frontal and cerebral cortex volume was based on Andreasen et al.'s [4] study of schizophrenic patients. In a subsequent study, Andreasen et al. [3] repeated these measures but did not find the previously reported decrease in frontal, cerebral, and cranial size. Weinberger et al. [46] suggest that evidence for the frontal lobe being an abnormal size in schizophrenics is inconclusive. Instead, neural elements may be disorganized or dysfunctional in the frontal cortex causing the prefrontal cortex abnormalities in schizophrenics [1,9].

Because our neonatal lesion group included some rats with amygdala and other rats with hippocampus damage, we are in a position to address whether one of these is more strongly associated with the adult behavioral and volumetric abnormalities. First, in terms of behavioral effects, both the size of amygdala and hippocampus only predicted adult performance in the Morris water task. However, since the volumes of the amygdala and hippocampus were significantly correlated, we cannot dissociate between the two structures in their correlations with behavioral performances. Yet, similar results were found when the behavior of rats with either amygdala damage or hippocampus damage is compared to sham control rats; namely, only Morris water task performance was significantly different. Thus, although the lesion group was different from the sham group on many behavioral measures, a clear association with amygdala or hippocampus was found only for the same task. In terms of morphology, we found that when all rats are included (see Table 2), the volume of the amygdala positively correlates with the volume of the hippocampus, frontal cortex, cerebral cortex, and negatively correlates with the volume of the lateral ventricles. Correlates of hippocampus volume are very similar to those seen for amygdala volume. Despite the similarity in the overall correlational analysis between amygdala and hippocampus volumes, we found important differences when we performed inferential statistical tests between rats with these amygdala or hippocampal lesions and the sham lesion controls. Specifically, cerebral hemispheres with amygdala damage had smaller amygdalae and hippocampi, and had larger lateral ventricles; cerebral hemispheres with hippocampal damage were not significantly different from sham lesion hemispheres on these measures (although there was a marginally significant trend in the volume of the lateral ventricle). Thus, although we cannot assert this with certainty, there is a trend for amygdala damage to have greater impact on some aspects of adult brain morphology. More work is necessary to discriminate among the possibilities that damage to a single, critical limbic structure, a combination of limbic structures, or any one of several limbic structures is necessary to produce the behavioral and anatomical abnormalities related to schizophrenic pathology.

One unexpected result of our study is the finding that male and female rats show a different magnitude of response to neonatal limbic damage. Specifically, males show greater reduction in volume of forebrain structures and a greater increase in lateral ventricle volumes after neonatal damage. In our study, the adult behavioral impairments can be thought of as analogous to some negative symptoms in human schizophrenia and importantly the size of the behavioral impairments is related to these volumetric changes. There is a body of information about sex differences in humans indicating the males show greater severity of schizophrenic symptoms [16,17], especially negative symptoms [37], earlier onset [15], longer window of fetal vulnerability to stressor effects [43], and greater severity of structural brain abnormalities [30]. Our results with rats are in line with these observations. There are several possibilities for explaining the observation that males are more susceptible to these early lesion effects. One is our limbic lesions could be disrupting gonadal hormone regulation. If estrogen and testosterone differentially affect forebrain maturation then we could have differential effects of the same lesion in males and females. A second possibility is that the same chronological age actually represents a different point in neural maturation in male and female rats. If male brains are less mature at the time of our neonatal lesion, they may show an exaggerated effect on subsequent development.

An exception to this pattern in our data of males being more susceptible to these early lesion effects, is the greater responsiveness of females with neonatal lesions to dopaminergic locomotor activation. A greater responsiveness by normal female rats to dopaminergic

locomotor activation has been reported by Beatty and Holtzer (cited in [25]), but only our lesion, not our sham, rats showed a sex difference. Dopaminergic hyperresponsiveness may be more akin to positive human schizophrenic symptoms and may not reflect the same processes as the severity of negative symptomatology.

The experimental demonstrations by Lipska and others and the present results that neonatal amygdala or hippocampus damage can have adult consequences for cognition, dopaminergic sensitivity, and forebrain morphology lends support to the hypothesis that damage to limbic circuitry in the second trimester of pregnancy is a critical element in the etiology of schizophrenia. Since schizophrenia affects so many people and is such a disabling disease its pathogenesis must be further studied. The development of useful animal models will open important new possibilities for the treatment and preventions of schizophrenia.

Acknowledgements

The authors thank Ron Yeo, Kathy Stansbury, and Bob Astur for comments on an earlier version of this manuscript. We also thank Mike Weisend for his help on performing the lesions. This work was partially supported by the Department of Psychology, QUAD-L foundation, SRAC, and RPT.

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