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Research report

Neonatal ventral hippocampal lesions in male and female rats: Effects on water maze, locomotor activity, plus-maze and prefrontal cortical GABA and glutamate release in adulthood

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ABSTRACT

Schizophrenia is characterized by diverse behavioural and neurochemical abnormalities that may be differentially expressed in males and females. Male rats with neonatal ventral hippocampal lesions (nVHL) have commonly demonstrated behavioural and neurochemical abnormalities similar to those in schizophrenia. Fewer studies have used female rats. We investigated the hypothesis that male and female nVHL rats will demonstrate behavioural abnormalities accompanied by decreased GABA and L-glutamate release in the prefrontal cortex (PFC). On postnatal day (P) 7 rats received VH injections of ibotenate (3.0 μg/0.3 μl/side; $n = 18$) or saline ($n = 21$) or no injections ($n = 22$). On P56, rats began water-maze, locomotor activity and elevated plus maze testing, and were then sacrificed for potassium-evoked GABA and L-glutamate release from PFC slices. nVHL rats showed impaired performance in water maze acquisition and match-to-sample tasks, increased spontaneous and amphetamine-induced locomotor activity and increased percent open-arm time. These behavioural changes were similar in males and females. These effects were accompanied by significantly reduced potassium-evoked L-glutamate release in male and female nVHL rats relative to controls, and non-significantly lower GABA release. Findings support the notion that behavioural abnormalities in post-pubertal male and female nVHL rats are associated with decreases in PFC neurotransmitter release.

1. Introduction

Schizophrenia is a debilitating neuropsychiatric disorder that affects approximately one percent of the population across cultures. It is characterized by positive, negative and cognitive symptoms that are thought to result from alterations in prefrontal cortical (PFC)-temporolimbic cortical connectivity [75]. The neurodevelopmental hypothesis posits that schizophrenia results from a perinatal brain insult that remains relatively silent during early development but manifests itself in early adulthood [46]. At that time, when the PFC undergoes substantial reorganization of connectivity [33,53], the damage that occurred perinatally putatively disrupts the mechanisms of reorganization [75].

Alterations in L-glutamate (glutamate) and γ-aminobutyric acid (GABA) neurons are associated with schizophrenia [18–20,34]. In respect to glutamate, *N*-methyl-D-aspartate (NMDA) receptor antagonists taken by normal subjects induce positive, negative and cognitive symptoms like those seen in schizophrenia [29,45,66] and exacerbate existing symptomatology in patients with schizophrenia [27]. Magnetic resonance spectroscopy imaging studies revealed reduced glutamate and glutamine in the anterior cingulate cortex of chronic schizophrenic patients [69] and increased glutamate in the PFC of first-episode schizophrenics [57]. Post-mortem studies revealed decreased concentrations of glutamate and aspartate in the PFC, and reduced glutamate in the hippocampus of schizophrenic patients [71]. Schizophrenic patients exhibited reduced hippocampal mRNA expression for the excitatory amino acid transporter 2 [55] and the NR1 subunit of the NMDA receptor [17], increased PFC protein expression for metabotropic glutamate receptor (mGluR) 1a, mGluR2/3 [21] and mGluR5 [55] and reduced thalamic mRNA expression for the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor units gluR1 and gluR3 [26]. Neuregulin 1 and RGS4, two candidate susceptibility genes for schizophrenia, regulate glutamate receptor subunit expression and inhibit G protein signaling through the mGlu5 receptor, respectively [24,51]. Thus, converging

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lines of evidence point to an association of altered glutamatergic function in the pathophysiology of schizophrenia.

Abnormal GABAergic transmission has also been found in several brain regions, including the hippocampus [5,60] and PFC [6,56]. The cortical density [3] and mRNA expression per neuron [25] of the parvalbumin (PV)-containing subtype of GABA neurons were found to be reduced in the PFC of schizophrenic patients compared to controls, with an associated decrease in the density of neurons containing the GABA synthesizing enzyme glutamic acid decarboxylase (GAD_{67}) [25]. Correspondingly, $GABA_A$ receptors were upregulated in the PFC, caudate nucleus, cingulate gyrus, subiculum, and hippocampus [7,22] and $GABA_B$ receptors were down-regulated in the hippocampus of post-mortem tissue from schizophrenic patients [50,74]. Results suggest a concurrent dysfunction of GABA and glutamate function in schizophrenia.

Some neurodevelopmental animal models mimic behavioural and neurophysiological abnormalities of schizophrenia. Rats with neonatal ventral hippocampal lesions (nVHL) carried out on postnatal day (P) 7 showed hyperlocomotion, social withdrawal [4], increased sensitivity to pro-dopaminergic agents [38,41,43] and stress [38], and deficits in sensorimotor gating [30] and working memory [30,37] when tested in adulthood. Similar behavioural deficits are seen in schizophrenic patients [43]. nVHL rats show decreased D-aspartate release in the frontal cortex and hippocampus [63] and increased glutamate binding in the frontal cortex [63]. They also show decreased mRNA expression for the GABA synthesizing enzyme GAD_{67} in the PFC [40], increased number of $GABA_A$ receptors in the PFC [14], and increased mRNA expression for the $GABA_A$ receptor subunits $\alpha 1$ [49] and $\beta 2$ [14] in the PFC. As reviewed above, similar neuronal changes are seen in post-mortem schizophrenic brains. Results link nVHL lesions to altered PFC function.

Gender differences have been found in schizophrenia. Males show an earlier age of onset and poorer premorbid functioning, and gender differences have been reported in symptom expression and neurophysiological abnormalities [31]. In nVHL rats, no gender differences were reported in the magnitude of enhanced spontaneous locomotion in early adulthood [65] or in acquisition of the win-shift task in the radial arm maze [10] but gender differences have been found in other studies. Enhanced spontaneous locomotion appeared earlier in adult males than females [9] and nVHL males showed greater deficits in water maze acquisition [65]. Thus, additional research is needed to determine the effects of nVHL on each sex.

The current study examined the hypothesis that male and female nVHL rats will show enhanced amphetamine-induced locomotor activity and impaired water maze learning. Amphetamine-induced locomotion was used to test sensitivity to a pro-dopaminergic agent and water maze learning was used to test working memory. Few studies have investigated gender differences in nVHL rats and results have been inconsistent; therefore, we did not make a specific prediction about possible gender effects. As GABA transmission is implicated in behaviour in the elevated plus maze [12,52,59], we investigated the hypothesis that nVHL rats will be abnormal in the elevated plus maze. We also investigated the hypothesis that nVHL in the rat will be associated with reduced stimulus-evoked glutamate and GABA release from PFC tissue, as previous studies have shown changes in PFC neuronal function following nVHL.

2. Methods

2.1. Subjects

Seventy Sprague–Dawley rat pups (38 male and 32 female) were born from 7 timed pregnant dams purchased from Charles River Canada (St. Constant, QC). The pregnant dams were housed on hardwood laboratory bedding (Beta Chips, Northeastern Products Corp., Warrensburg, NY) in clear polycarbonate cages (48 cm × 38 cm × 20 cm) and food and water were available *ad libitum*. Pups were weaned at P24 when they were separated from their mother and housed in same-sex, same-group (see below) pairs or triplets in smaller polycarbonate cages (46 cm × 24 cm × 20 cm). Food (5001 Rodent Diet, Lab Diet, Brentwood MO) and water were available *ad libitum* in the home cages. Treatment of the animals was in accordance with the Animals for Research Act and the Guidelines of the Canadian Council on Animal Care, and was approved by the Queen's University (Kingston) Animal Care Committee.

2.2. Surgery

On P7 pups were randomly assigned to either control (11 males, 11 females), nVHL (15 males, 12 females) or sham groups (12 males, 9 females) with pups from each of the 7 litters assigned to each group. Surgery was carried out as described by Lipska and Weinberger [41]. Pups were placed on ice for 15–20 min to induce anesthesia, then immobilized in a customized block of plastic in a stereotaxic frame (Scipro Inc., Sunborn, NY). After making an incision along the midline of the scalp and retracting the skin the VH was targeted for injections using co-ordinates from bregma of 3.0 mm posterior, 3.5 mm bilateral and 5.0 mm ventral to the surface of the skull. Using a needle of 0.4 mm diam attached via tubing to a Hamilton microsyringe mounted on an infusion pump (Harvard Apparatus Canada, St. Laurent, QC), injections of 3.0 μg/0.3 μl/side of ibotenic acid solution were administered to nVHL rats over a period of 2 min. Needles were removed 4 min after the end of injections to allow for diffusion. The wound was immediately sutured and pups were warmed under a heat lamp before being returned to their mother's cage. Rats in the sham group underwent similar surgeries but received injection of PBS (0.3 μl/side); control rats were also anaesthetized, immobilized, incised and sutured but received no injections.

2.3. Drugs

D-amphetamine sulfate (Health Canada, Therapeutic Products Directorate, Ottawa, ON) was dissolved in saline (0.9% NaCl). Ibotentic acid solution was prepared using 1.0 mg ibotenic acid (Sigma–Aldrich Canada Ltd., Oakville, ON) in 99 μl PBS and 1.0 μl NaOH, neutralized with HCl.

2.4. Apparatus

2.4.1. Water maze

A circular pool (180 cm diam × 60 cm high) was filled with water (21 °C) to a depth of 40 cm and non-toxic tempera white paint (2 l) was added to make the water opaque. Four release points, equally spaced around the pool, were designated by the four cardinal compass positions that also specified four quadrants. A movable platform (20 cm diam) was hidden approximately 2 cm below the surface of the water in the center of one of the quadrants. Objects were placed on the walls around the pool to provide the rats with spatial cues. A video camera was situated directly above the centre of the pool and all trials were taped using a videocassette recorder.

2.4.2. Locomotor activity

Plexiglass chambers (41 cm × 50 cm × 37 cm) housed in black, Styrofoam-insulated, sound attenuating wooden boxes were each ventilated by a small fan that produced a constant background noise and were illuminated by an overhead incandescent bulb (2.5 W). Chambers were equipped with 14 infrared emitters and detectors (photocells) situated along two heights (5 and 15 cm) above the stainless-steel rod floor. At each height, four photocells were spaced at 10 cm intervals along the length of the chamber and 3 cm along the width. The lower tier of beams reflects horizontal activity including walking and running; the upper tier reflects vertical activity including rearing and jumping. Six separate boxes were connected to a central computer used for data collection from the photocells. For further details see Beninger et al. [8].

2.4.3. Elevated plus maze

A platform was made from urethane-sealed wood constructed into two opposing open arms (50 cm × 10 cm) crossed by two opposing closed arms (50 cm × 10 cm × 40 cm) and was elevated 50 cm above the floor. The maze was situated in a quiet, dimly lit room with a video camera located 2.0 m away from the closest closed arm and an observer seated in a chair behind the camera 2.5 m away from the maze. All sessions were recorded on a videocassette.

2.5. Procedure

Experimentation began on P56 with 5 days of handling for 5 min/day followed by, in chronological order, water maze (5 days), activity (1 day), elevated-plus maze (2 days), GABA and glutamate release studies and histology. Handling and individual behavioural tests were separated by 1–2 days and all animals were subjected to all tests.

2.5.1. Water maze

Day 1 consisted of an acquisition task that included two sessions separated by 20 min. During the first session the platform was positioned in a randomly selected quadrant and 4 consecutive trials were given; for each trial the rat was released from a different cardinal compass point. The order was counterbalanced across rats. A trial consisted of releasing a rat into the pool facing the wall, whereupon the rat swam until it escaped onto the hidden platform; if it did not reach the platform in 60 s it was manually guided to it. The rat was then allowed to rest on the platform for 15 s. At the end of the session, the rat was dried with a towel and placed under a warming lamp for 5 min before returning to its homecage. The second session proceeded similarly except that the platform position was changed and the release point order was different.

Over the next four days, two match-to-sample tasks consisting of paired sample-test trials were given each day. During each of the paired trials, the platform was in a different position, making each match-to-sample a novel task; platform position varied randomly from trial to trial and from rat to rat. For the sample trial, the rat was released facing the wall from a randomly selected release point, whereupon the rat swam until it reached the hidden platform, or if it did not escape in 60 s it was manually guided to it. The rat was then allowed to rest for 15 s on the platform. For the no-delay task, the test trial followed immediately; it was the same as the sample but began from a different release point. For the delay task, the rat was removed from the pool after the sample and placed under a warming lamp for 30 s before continuing to the test trial. The dependent measure for acquisition and match-to-sample was time-to-platform. Manually guided rats were given a maximum score of 60 s.

2.5.2. Swim speeds

For one trial of both the acquisition and match-to-sample water maze tasks, swim speeds were calculated by viewing the videotapes. The first trial of the second session of acquisition testing and the first sample trial on the second day of match-to-sample testing were analyzed as representative samples from each water maze task.

2.5.3. Locomotor activity

Three sessions, habituation, saline and amphetamine were 60, 60 and 90 min long, respectively. For the habituation session, rats were transferred from their homecages to the activity boxes. At the end of the habituation session, the rats were briefly removed from their testing boxes for the administration of an i.p. injection of saline (1 ml/kg). Rats were then placed back into the boxes. After the saline session, rats were removed for an i.p. injection of amphetamine (1.5 mg/kg), and then placed back into the testing boxes. The dependent variable was the number of photocell beam breaks for either the lower or upper beams during 10-min bins.

2.5.4. Elevated plus maze

One day prior to testing, rats were brought into a dimly lit and quiet room next to the maze room for 30 min; rats were then brought into the maze room for a 5-min habituation. On the day of testing, rats were again taken to the pre-experiment room to wait for 30 min before they were individually brought into the testing room. A rat was placed on the plus maze on the central platform with its head facing down the closed arm farthest from the camera. Each rat was allowed to explore the maze for 5 min. In between trials the maze was thoroughly wiped off with a moist towel and then dried with dry paper towel. An observer noted the number of closed- (CE) and open-arm entries (OE), which were only scored if all four of the paws crossed into the appropriate arm. Number of rears, bouts of grooming and fecal boluses were also noted. Percent open-arm time (%OT) was calculated by reviewing the videocassette recording and measuring the closed-arm time (CT) and open-arm time (OT); the calculation was $\%OT = OT/(OT + CT) \times 100$.

2.5.5. GABA and glutamate release

At least 48 h after the end of behavioural testing, all male and female rats (now aged approximately P77) were euthanized, without prior anesthetic, by decapitation. The brain was then quickly excised and placed in ice-cold sucrose substituted Krebs'. The PFC was dissected on an ice filled Petri dish and transverse slices of 400 μm were made with a McIlwain Tissue Chopper. Three to four slices were taken

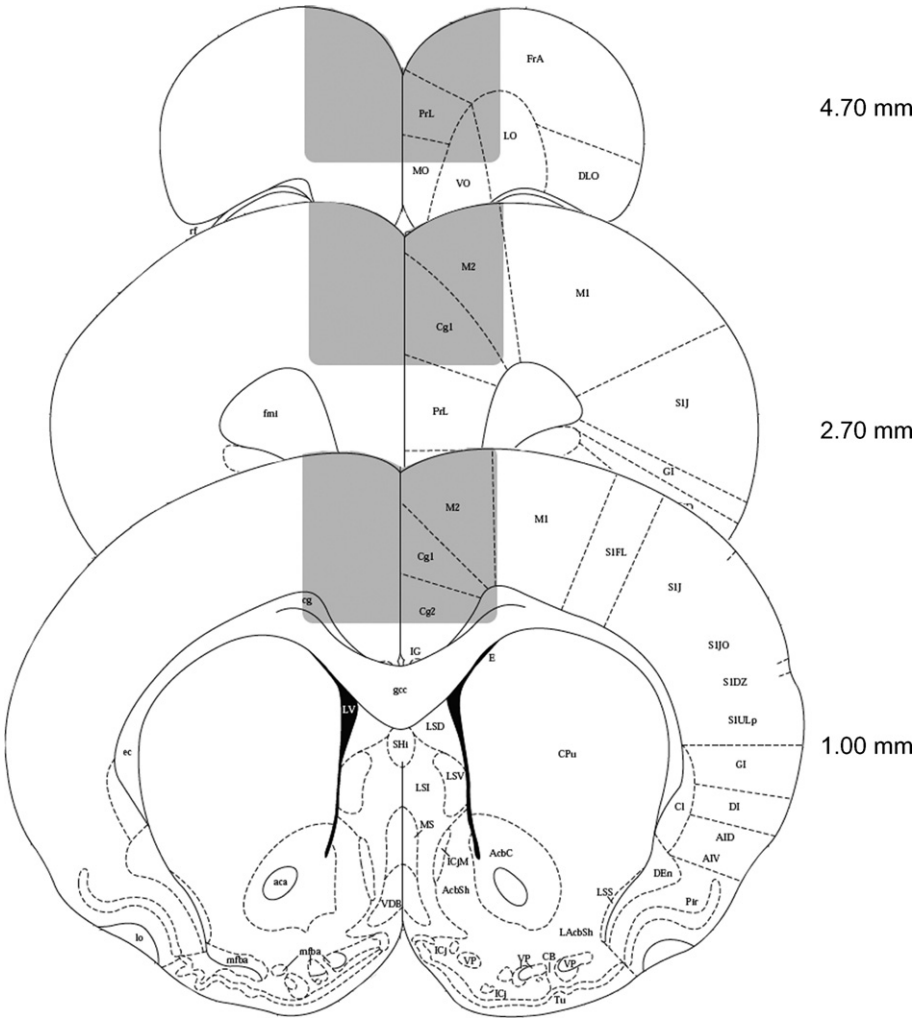

Fig. 1. Rat prefrontal cortex (PFC) coronal sections depicting region sampled (light shading) for analysis of GABA and glutamate release. Numbers are millimeters rostral to bregma for each of the sections. Adapted from the atlas of Paxinos and Watson [58].

from both hemispheres between the co-ordinates of 5.0 and 1.0 mm anterior to bregma, 1.6 mm lateral and 3.0 mm ventral to the surface of the skull (Fig. 1) [58]. Slices were gently separated in a petri dish containing ice-cold, oxygenated Krebs' solution with fine camel hair brushes. PFC slices obtained from each animal were placed in a superfusion chamber (0.5 ml volume, three slices per chamber) that was maintained at 37 °C by submersion in a water bath. The slices were superfused with Krebs' solution warmed to 37 °C and gassed with 95% O_2, 5% CO_2 at a flow rate of 0.3 ml/min.

After 1 h equilibration, superfusate fractions were collected over successive 10-min intervals. Four baseline samples were collected before the slice was exposed to a modified Krebs' Ringer Buffer with high potassium (30 mM) for 5 min, used to induce transmitter release, and then returned to the normal medium for the collection of 3 additional samples at 5-min intervals. At the end of the superfusion the tissue was homogenized in 500 μl of nanopure water and centrifuged at 5000 × *g* for 30 min.

The superfusate and tissue levels of GABA were assayed using HPLC as described in our previous study [23]. Briefly, each superfusate sample (200 μl) was mixed with 200 μl of o-phathaldialdehyde reagent solution before application to a reverse phase contained column (LC-19-Supelcosil, 15 cm × 4.6 mm, 5 μm particle size). The mobile phase contained 0.04 M sodium acetate buffer and 30% methanol (pH 7.5). GABA concentrations were observed using a Shimadzu RF-590 fluorescence detector with excitation and detection wavelengths of 345 and 470 nm, respectively, and compared to a standard curve constructed for each assay. Glutamate concentration was similarly observed with the same wavelengths. The detection limit of this assay was 1 pmol. The same homogenized slices were used to assess both the GABA and glutamate release and were assayed using HPLC for each neurotransmitter twice. Release was expressed as ng/ml/mg protein. Average baseline GABA and glutamate release were each calculated by averaging across the four initial 10-min fractions. GABA and glutamate release recorded at the four 5-min intervals following exposure to high external potassium were then expressed as percent increase over average baseline, according to the calculation: % increase = ((K^+-evoked release − baseline release)/baseline release) × 100. Across the four 5-min intervals following potassium exposure, continued increased neurotransmitter release over baseline was summed to yield scores of total percent GABA and glutamate release increase.

2.5.6. Histology

The brains of nVHL rats were fixed in formalin, and were sliced (40 μm) through the area of the lesion using a freezing cryostat; slices were mounted onto slides and stained with cresyl violet. An observer who was blind to the behavioural and neurochemical results of individual rats rated the extent of the VH lesions on both the left and right side of the brain using a scale from zero (no damage) to four (near complete lesion). Rats of the nVHL group with at least a score of two on both sides of the brain were used for data analysis.

2.6. Data analysis

For the water maze, two 3-way mixed-design analyses of variance (ANOVA; trial × gender × group) were used to analyze the time-to-platform during acquisition. For the match-to-sample tasks a 4-way ANOVA (delay × trial × gender × group) also was used to assess time-to-platform. A 2-way mixed-design ANOVA (gender × group) found no main effects or interactions for time-to-platform on sample trials of the match-to-sample task ($ps > .05$) and validated the use of performance scores (difference between sample and test trial times). A 2-way ANOVA (gender × group) was used to analyze swim speeds for trial 1 of session 2 of the water maze acquisition task. Similarly, a 2-way ANOVA (gender × group) was used to analyze swim speeds on the first sample trial of day 2 of the water maze match-to-sample task. For activity, data from habituation, saline and amphetamine were investigated by separate 3-way mixed-design ANOVA (bin × gender × group) for both upper and lower level photocell beam breaks. The elevated plus maze measures grooming and fecal boluses were analyzed using separate 1-way ANOVA (group) and CE, OE, %OT and rearing were analyzed using separate 2-way ANOVA (gender × group). For neurotransmitter release, total percent GABA and glutamate release increase were analyzed using separate 3-way ANOVA (gender × group × HPLC test). Correlations between total percent GABA and glutamate release increase and time-to-platform for each trial of the acquisition task averaged over sessions was calculated for each group separately to assess possible relationships between PFC GABA and glutamate release and cognitive scores. All main effects were further investigated using Tukey HSD post hoc analyses. Interactions were further investigated using simple effects ANOVA followed by post hoc comparisons where appropriate.

3. Results

Nine rats from the nVHL group had lesions scored with damage less than 2 (half the maximal lesion) on at least one side of the brain and were eliminated from analyses leaving eighteen (11 male and 7 female) nVHL rats. The size of the ventral hippocampal lesions is represented in Fig. 2.

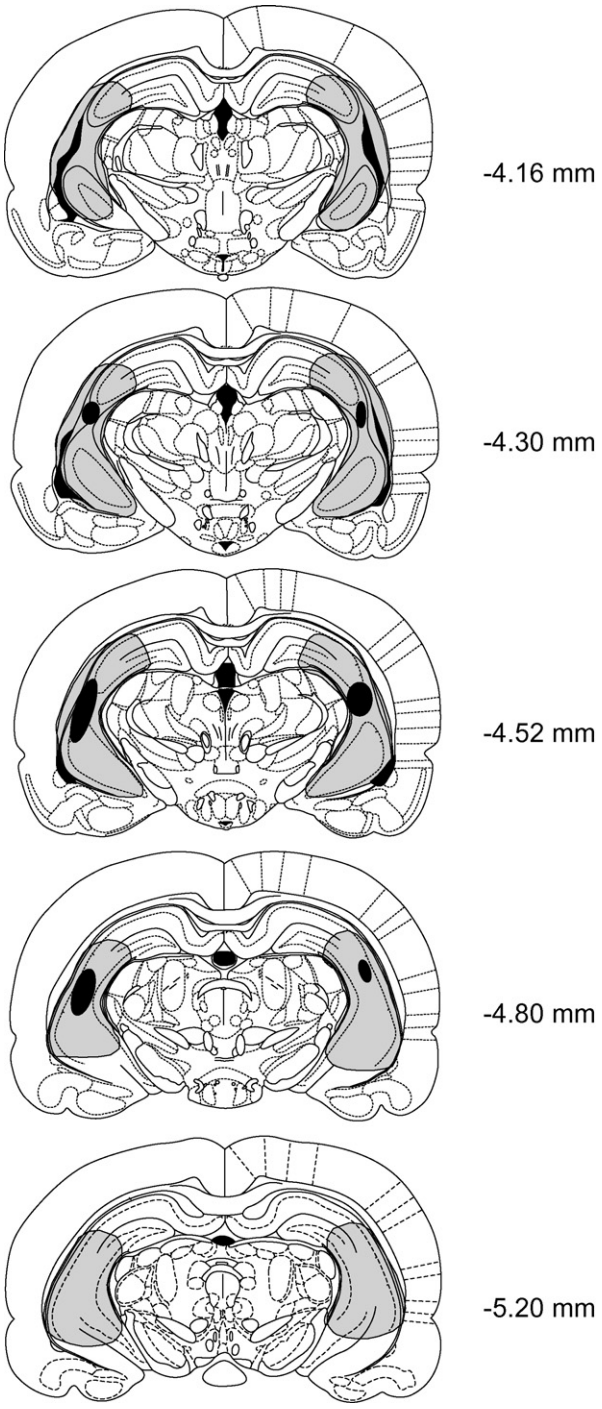

Fig. 2. Rat brain sections from 4.16 to 5.20 mm posterior to bregma [58] showing the total (transparent grey) and common (solid black) excitotoxic damage of all rats in the neonatal ventral hippocampal lesion group ($n = 18$) after the elimination of 9 rats with lesions that were considered to be too small (score of less than 2 on at least one side).

3.1. Water maze

3.1.1. Acquisition

In general, the time-to-platform during session 1 and session 2 (Fig. 3) decreased less across trials for the nVHL groups than for the control and sham groups, suggesting that the nVHL groups were impaired. For session 1 (Fig. 3, upper panels), group differences depended on gender and trial (significant 3-way interaction, $F(6, 165) = 2.70$, $p < .016$). For the females, the nVHL group differed

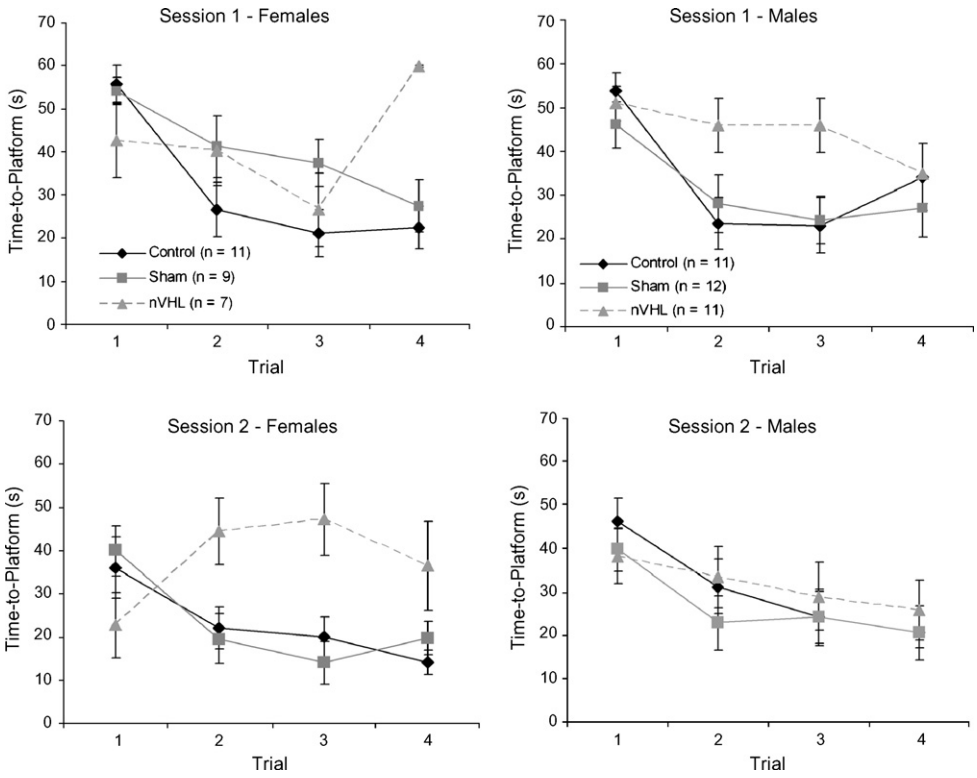

Fig. 3. Upper: Mean (±SEM) time-to-platform (s) during the water maze acquisition task for session 1 for males (right) and females (left). For males, the neonatal ventral hippocampal lesion (nVHL) group was impaired compared to the sham group [analysis of variance (ANOVA) followed by pairwise comparisons]; for females the nVHL group differed from control and sham on trial 4 (pairwise comparisons after simple effects ANOVA after significant interaction in omnibus ANOVA). Lower: Mean (±SEM) time-to-platform (s) during the water maze acquisition task for session 2 for males (right) and females (left). The 3-way ANOVA found a significant trial × group interaction. Collapsing over gender, simple effects analysis found a main effect of group for trials 2 and 3, with the nVHL group taking significantly longer than the control and sham groups.

from the control and sham on trial 4 [significant 2-way interaction, $F(6, 72) = 3.89$, $p = .002$, followed by simple main effects of groups on trial 4, $F(2, 27) = 14.70$, $p < .001$, followed by pairwise comparisons, $ps < .001$]. For the males, groups differed, $F(2, 31) = 4.15$, $p < .025$, and pairwise comparisons showed that nVHL rats had non-significantly longer latencies than controls ($p < .09$) and significantly longer latencies than sham rats ($p = .028$). Results show that although the specific trial on which a significant impairment was observed differed for females and males, overall, nVHL rats of both genders were impaired.

For session 2 (Fig. 3, lower panels), although inspection of the figure suggests that group differences depended on gender and trial, only the trial × group interaction was significant, $F(6, 165) = 3.32$, $p = .004$. Thus, when genders were combined simple effects analysis found a significant main effect of group for trial 2, $F(2, 58) = 3.185$, $p = .049$ and trial 3, $F(2, 58) = 3.36$, $p = .042$. Pairwise comparisons revealed that the nVHL group had significantly longer time-to-platform latencies than the sham group for both trials ($p = .041$, $p = .048$). Results showed that initial time-to-platform scores were similar for all groups; however, as trials progressed, the control and sham groups of both genders improved but the nVHL rats generally did not.

3.1.2. Match-to-sample

Mean (±SEM) performance scores (time-to-platform differences from sample to test trial) were calculated for each trial for each rat. Groups combined over gender and trials differed at both delays (Fig. 4), the nVHL group showing poorer performance than the sham and control groups. A 4-way ANOVA (delay × trial × gender × group) revealed only a main effect of group, $F(2, 55) = 19.30$, $p < .001$. Tukey pairwise comparisons show the nVHL rats had significantly lower performance scores than either the control or sham rats ($ps < .001$).

3.1.3. Swim speeds

2-way ANOVA for swim speeds (Table 1) during trial 1 of session 2 for acquisition revealed no significant group effect, $F(2,54) = 1.31$, $p = .278$, but a significant gender effect, $F(1,54) = 13.71$, $p = .001$, with males demonstrating higher swim speeds than females. 2-way ANOVA for swim speeds for the first sample trial on day 2 of the match-to-sample task revealed no significant group, $F(2,54) = .99$, $p = .38$, or gender, $F(1,54) = 1.13$, $p = .29$, effects. Thus, group dif-

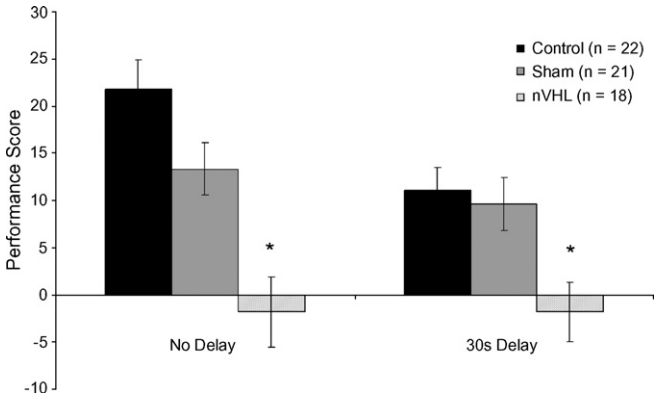

Fig. 4. Mean (±SEM) performance scores for the no-delay and 30-s delay match-to-sample tasks averaged over 4 days of testing. A 4-way analysis of variance (delay × trial × gender × group) revealed a main effect of group with neonatal ventral hippocampus lesion (nVHL) rats having significantly ($p < .05$) lower performance scores than either control or sham rats (indicated by *).

Table 1
Mean (±SEM) swim speeds (cm/s) for all groups during trial 1 of session 2 of acquisition and for the first sample trial of day 2 of the match-to-sample task.

Task	Control		Sham		nVHL	
	Male	Female	Male	Female	Male	Female
Acquisition	50.88 ± 4.32	39.84 ± 8	49.28 ± 7.52	48.48 ± 8.64	53.6 ± 9.76	43.2 ± 6.24
Match-to-Sample	55.5 ± 8.75	51.75 ± 15	49.75 ± 15.25	57.25 ± 7.5	45.75 ± 8.5	51.75 ± 12.75

ferences in the water maze tasks cannot be accounted for by differences in swim speed among groups.

3.2. Locomotor activity

3.2.1. Upper

During habituation, activity appeared to decrease across bins for both genders of each group (Fig. 5, left panels) and a 3-way (bin × gender × group) ANOVA revealed a significant bin effect, $F(5, 275) = 47.623$, $p < .001$. The ANOVA also revealed a significant bin × group interaction, $F(10, 275) = 2.608$, $p = .005$. This interaction occurred with genders combined and resulted from higher levels of activity in the nVHL group compared to the other two groups in bins 2–6 but not bin 1.

During saline, activity remained fairly stable across bins (Fig. 5, middle panels) and ANOVA revealed no significant effects of bin or group or interactions. There was a significant gender effect, $F(1, 55) = 5.211$, $p = .026$, reflecting higher activity in the combined female groups compared to the combined male groups.

During the amphetamine session, the nVHL males and females were more active than their associated sham or control groups and overall, females were more active than males (Fig. 5, right panels). ANOVA revealed a significant main effect of group, $F(2, 55) = 6.605$, $p = .003$, and gender, $F(1, 55) = 6.661$, $p = .013$, supporting this description of the data. Tukey HSD pairwise comparisons showed the lesion group to differ significantly from the control, $p = .03$, and sham groups, $p = .01$. Thus, the upper activity level of nVHL rats did not differ significantly from control or sham groups during saline sessions but was higher during the habituation and amphetamine sessions.

3.2.2. Lower

During habituation, activity decreased across bins for both genders of each group (Fig. 6, left panels) and a 3-way (bin × gender × group) ANOVA revealed a significant bin effect, $F(5, 275) = 92.45$, $p < .001$. The ANOVA also revealed a significant gender effect, $F(1, 55) = 15.14$, $p < .001$ reflecting higher activity by females and a group effect, $F(2, 55) = 8.12$, $p = .001$. Tukey HSD post hoc analyses showed that nVHL rats were significantly more active than control ($p = .006$) and sham ($p = .003$) rats.

During saline (Fig. 6, middle panels), activity generally decreased across bins and the 3-way ANOVA revealed a significant effect of bin, $F(5, 275) = 7.77$, $p < .001$, as well as a significant gender effect, $F(1, 55) = 13.72$, $p < .001$, reflecting higher activity in the combined female groups compared to the combined male groups. The ANOVA also showed a significant group effect, $F(2, 55) = 3.73$, $p = .003$ possibly reflecting higher activity in the nVHL group. However, in Tukey HSD post hoc tests, none of the group differences was significant.

During the amphetamine session, the nVHL males and females were more active than their associated sham or control groups and overall, females were more active than males (Fig. 6, right panels). The 3-way ANOVA revealed a significant main effect of bin, $F(8, 440) = 20.49$, $p < .001$, gender, $F(1, 55) = 77.02$, $p < .001$ and group, $F(2, 55) = 6.40$, $p = .003$ supporting this description of the data. Tukey pairwise comparisons of groups combined over gender and bin showed the lesion group to differ significantly from the control group, $p = .02$. The ANOVA revealed a significant bin × gender interaction, $F(8, 440) = 8.34$, $p < .001$ as well as a significant gender × group interaction, $F(2, 55) = 3.07$, $p = .05$. Simple effects 1-way ANOVA showed a significant effect of group for both females, $F(2, 24) = 5.06$, $p = .015$ and males, $F(2, 34) = 3.71$, $p = .04$. Tukey HSD post hoc analysis of males found that the nVHL group differed significantly from the sham group ($p = .03$). Similar analyses of females showed that the nVHL ($p = .02$) and sham ($p = .05$) groups significantly differed from the control group. The observation that the sham group showed elevated levels of lower activity similar to those seen in the nVHL group was unexpected. Overall, the lower-tier activity level of nVHL rats generally was significantly greater than the control or sham groups during all three sessions.

3.3. Elevated plus maze

One-way ANOVA for grooming and fecal boluses yielded no significant effects (data not shown).

3.3.1. Number of open- and closed-arm entries

A 2-way ANOVA (gender × group) on closed-arm entries revealed a main effect of gender, $F(1, 55) = 16.77$, $p < .001$, with females ($M = 11.15 \pm \text{SEM} = 0.48$) having more closed-arm entries than males ($M = 8.79 \pm \text{SEM} = 0.34$). Similar ANOVA for open-arm entries (Fig. 7, top panel) revealed a significant main effect of gender, $F(1, 55) = 10.88$, $p = .002$, with females having more open-arm entries than males, and group, ($F(2, 55) = 6.46$, $p = .003$), reflecting significantly more open-arm entries for the nVHL group compared to the sham ($p = .02$) and control ($p = .04$) rats. ANOVA also revealed a significant gender × group interaction, $F(2, 55) = 4.87$, $p = .011$. One-way simple effects ANOVA showed a significant effect of group for females, $F(2, 24) = 5.68$, $p = .01$ but not males, $F(2, 31) = 2.06$, $p = .144$. Tukey pairwise comparisons of females revealed the nVHL rats significantly differed from both sham ($p = .05$) and control ($p = .008$) rats.

3.3.2. Percent open-arm time

A 2-way ANOVA of percent open-arm time revealed a significant main effect of group, $F(2, 55) = 3.99$, $p = .024$. Tukey HSD post hoc analysis showed that nVHL rats differed significantly from sham ($p = .03$) and near significantly from control ($p = .057$) rats (Fig. 7, middle panel).

3.3.3. Number of rearings

A 2-way ANOVA revealed a significant effect of gender, $F(1, 55) = 6.09$, $p = .017$, due to more rearings in females than males, and group, $F(2, 55) = 5.25$, $p = .008$ (Fig. 7, lower panel). The Tukey HSD post hoc analysis showed that nVHL rats had significantly less rearings than the sham rats ($p = .008$).

3.4. Neurochemistry

3.4.1. GABA and glutamate release

Release data were lost for a number of rats because of technical difficulties resulting in final *n*'s [males (m), females (f)] for control, sham and nVHL groups of 16 (8, 8), 13 (7, 6) and 14 (8, 6), respectively. Percent glutamate release increase (±SEM) for each group was as follows: control m = 50.9 ± 14.7,

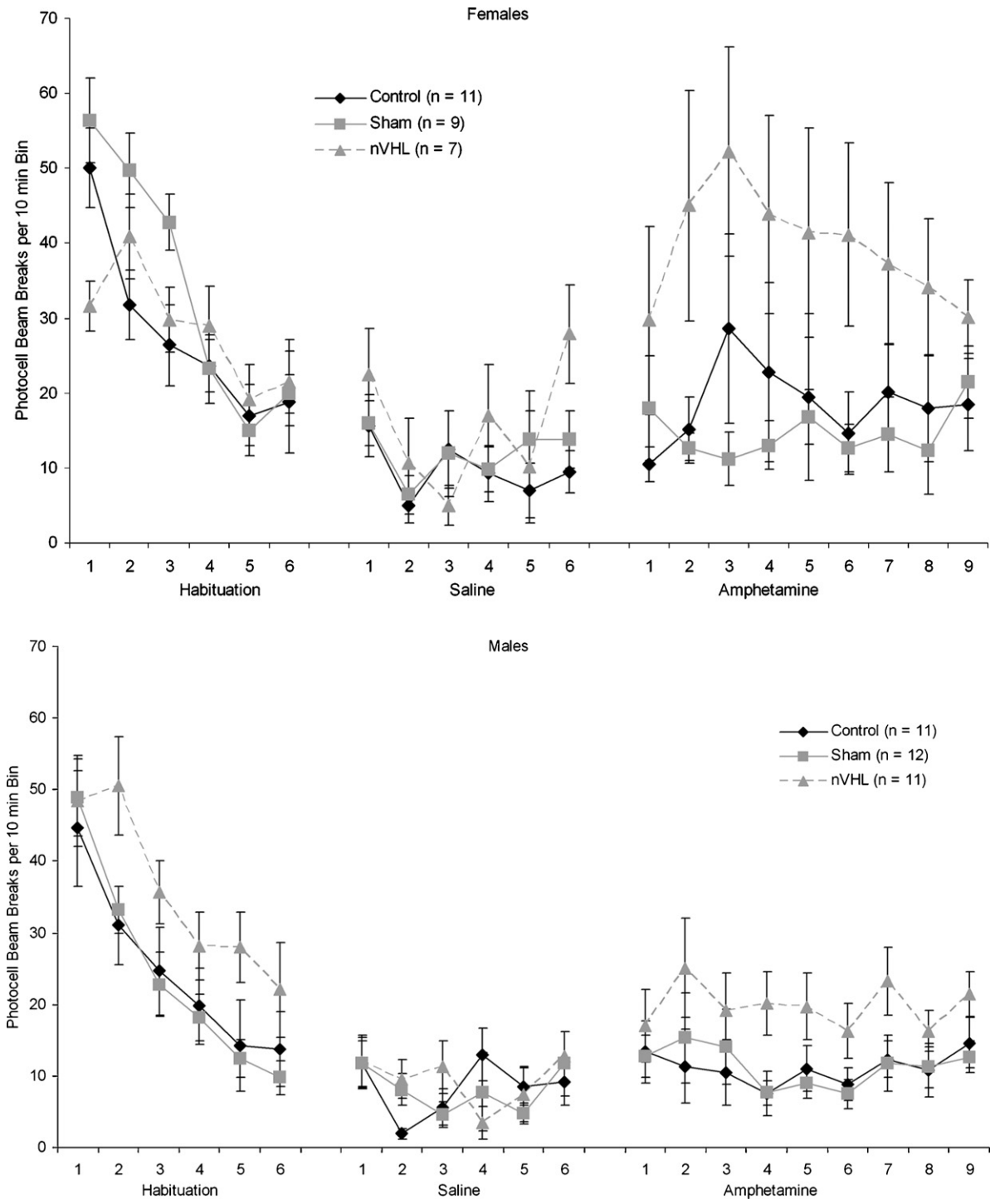

Fig. 5. Mean (±SEM) number of upper level photocell beam breaks per 10-min bin during habituation, saline and amphetamine sessions. Neonatal ventral hippocampal lesion (nVHL) rats showed higher activity during the habituation session compared to the other two groups during bins 2–6. Females displayed higher activity during the saline session compared to the combined males groups indicated by the significant gender effect in the 3-way analysis of variance (ANOVA). nVHL males and females were significantly more active than the sham or control groups with females being more active overall during the amphetamine session.

f = 68.2 ± 20.7, sham m = 55.9 ± 13.0, f = 105.0 ± 46.3 and nVHL m = 43.3 ± 8.6, f = 34.2 ± 4.4. The3-way (gender × group × HPLC test) ANOVA revealed a significant effect of group, $F(2,37) = 3.12$, $p = .047$ and no significant main effect of gender or HPLC test or interactions. Although nVHL rats showed the lowest total glutamate release, Tukey HSD post hoc analysis revealed that nVHL rats differed significantly from sham lesion rats only ($p = .02$). However, sham lesion and control rats did not differ significantly from each other and ANOVA comparing glutamate release with sham lesion and control rats combined still revealed a significant group effect, with nVHL rats showing significantly less glutamate release increase than combined sham lesion and control rats, $F(1,39) = 4.66$, $p = .037$. Percent glutamate release increase averaged across HPLC tests for each 5-min interval for each group is shown in Fig. 8A.

nVHL rats showed the lowest total GABA release. However, a 3-way ANOVA for total percent GABA release increase revealed

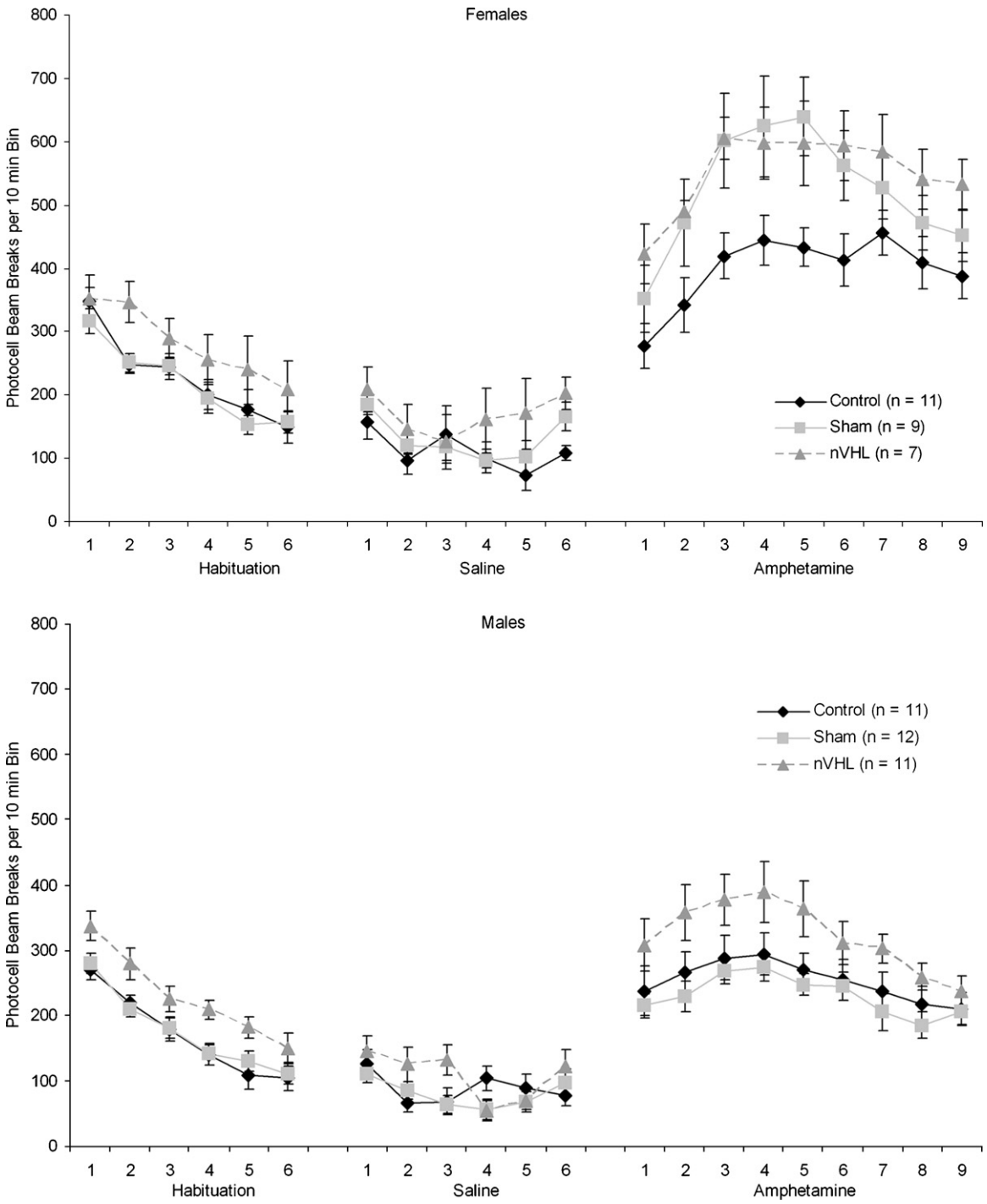

Fig. 6. Mean (±SEM) number of lower level photocell beam breaks per 10-min bin during habituation, saline and amphetamine sessions. During habituation females were more active than males and neonatal ventral hippocampal lesion (nVHL) rats were significantly ($p < .05$) more active than control and sham rats. Collapsing over groups, females showed greater lower activity during the saline session than males. During the amphetamine session, lesioned and sham females showed significantly greater activity than controls from pairwise comparisons following omnibus analysis of variance (ANOVA) following a significant gender × group interaction. nVHL males differed significantly from the sham group following similar analyses.

no significant differences between groups, $F(2,48) = .192$, $p = .826$ (Fig. 8B).

Correlation analyses for each group for total percent glutamate and GABA release increase and average time-to-platform for each acquisition trial averaged across sessions revealed no significant relationships for sham lesion or control rats. For nVHL rats, total percent glutamate release increase was significantly related to time-to-platform on trial 4, $r = -.55$, $p = .03$, and total percent GABA release increase was significantly related to time-to-platform on trial 1, $r = -.61$, $p = .01$ (Fig. 9, A + B). To clarify this relationship between GABA and trial 1 in nVHL rats, separate correlations were run for trial 1 of sessions 1 and 2. The relationship might be expected to be stronger for session 2 which takes place after some learning has occurred. These revealed that this relationship was driven by time-to-platform scores following relocation of the platform on trial 1 of session 2, $r = -.63$, $p = .007$, rather than by time-to-platform scores on trial 1 of the first session in the water maze, $r = -.20$, $p = .45$ (Fig. 9, C + D).

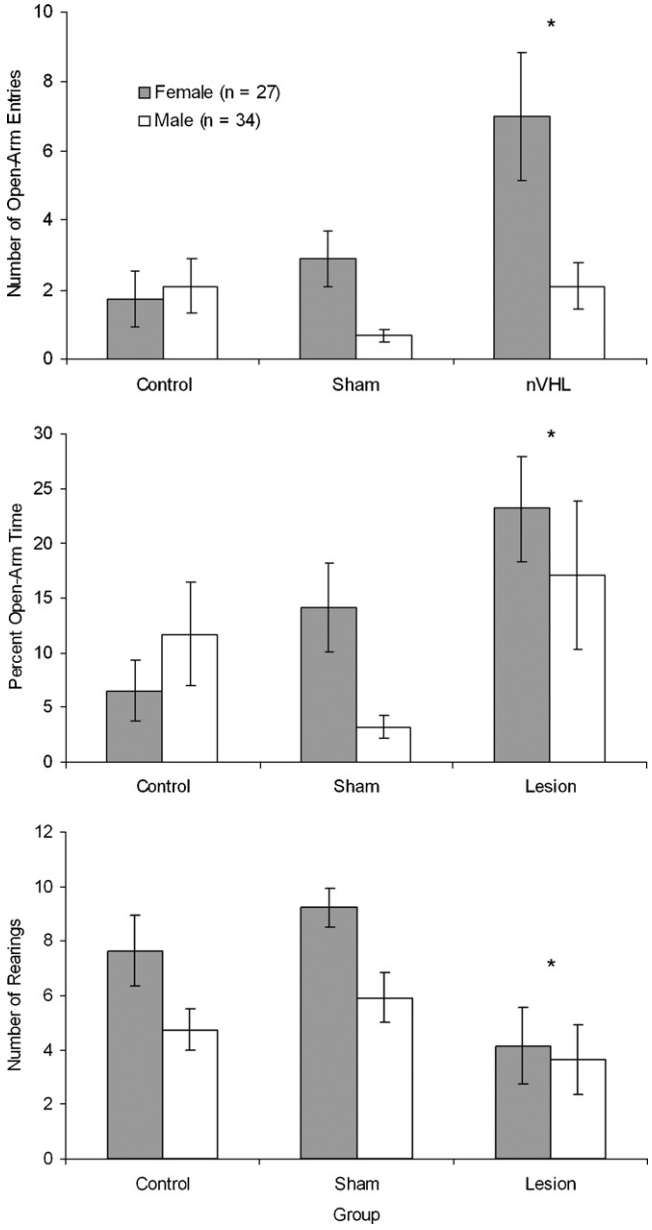

Fig. 7. Mean (±SEM) open-arm entries, percent open-arm time and number of rearings during 5 min exploration of the elevated plus-maze. For mean open-arm entries, 2-way analysis of variance (ANOVA) revealed a main effect of group and pairwise comparisons showed that the neonatal ventral hippocampal lesion (nVHL) group (combined over gender) differed significantly from the other two groups (*). The ANOVA also revealed a group × gender interaction with the nVHL female group differing from the control and sham female groups but the male groups not differing from one another. For percent open-arm time, ANOVA revealed a main effect of group (combined over gender) and the nVHL group differed from the control and sham groups (*). For number of rearings, ANOVA revealed a significant main effect of group (combined over gender) and pairwise tests showed the nVHL group to differ from the sham but not the control group (*).

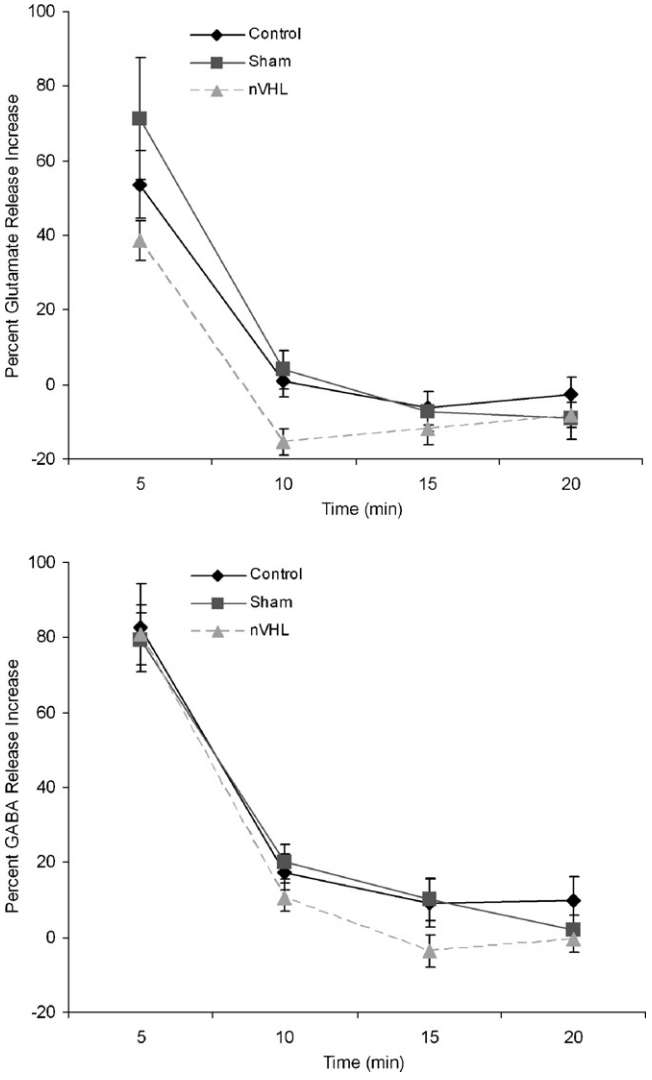

Fig. 8. Top: Mean (±SEM) percent glutamate release increase over baseline for control ($n = 19$), sham ($n = 15$) and nVHL ($n = 16$) groups (combined over gender) at 5-min intervals following exposure to K^+. Analysis of variance (ANOVA) for total percent glutamate release increase (summed over time; see Section 2) revealed a main effect of group with Tukey post hoc analysis yielding a significant (<.05) difference between neonatal ventral hippocampal lesion (nVHL) and sham rats, and nVHL and combined control and sham rats. Bottom: Mean (±SEM) percent GABA release increase over baseline at 5-min intervals following exposure to K^+. ANOVA for total percent GABA release increase revealed no significant main effects or interactions.

4. Discussion

The main findings in this study showed that when tested in adulthood the nVHL rats, compared to their control or sham lesioned counterparts, were impaired in the water maze acquisition and match-to-sample tasks, had greater spontaneous and amphetamine-induced locomotor activity, and spent more time in the open arms of the elevated plus maze. Females overall had greater activity than males; this was reflected in upper and lower locomotor activity testing, and in number of rearings and open- and closed-arm entries in the elevated plus maze. nVHL rats showed less total percent GABA and glutamate release increase in the PFC and this was significant for glutamate release.

That control, sham and nVHL groups did not differ on (1) time-to-platform for the sample trials in the match-to-sample task or (2) swim speeds in the acquisition and match-to-sample tasks suggests that the cognitive deficits observed in the present study cannot be attributed to non-mnemonic effects of the lesions (e.g., sensory, motor or motivational changes). These results are consistent with the findings of Le Pen et al. [30] showing that rats receiving nVHLs performed similar to controls in a cued platform task in the water maze.

The present experiment included two control groups, one that received sham lesions and one that was anesthetized, had the scalp incised and sutured but received no central injections. This allowed us to examine possible behavioural and neuochemical effects of cannulation and injection of saline into the VH. For the lower activity measure during the amphetamine phase of the locomotor

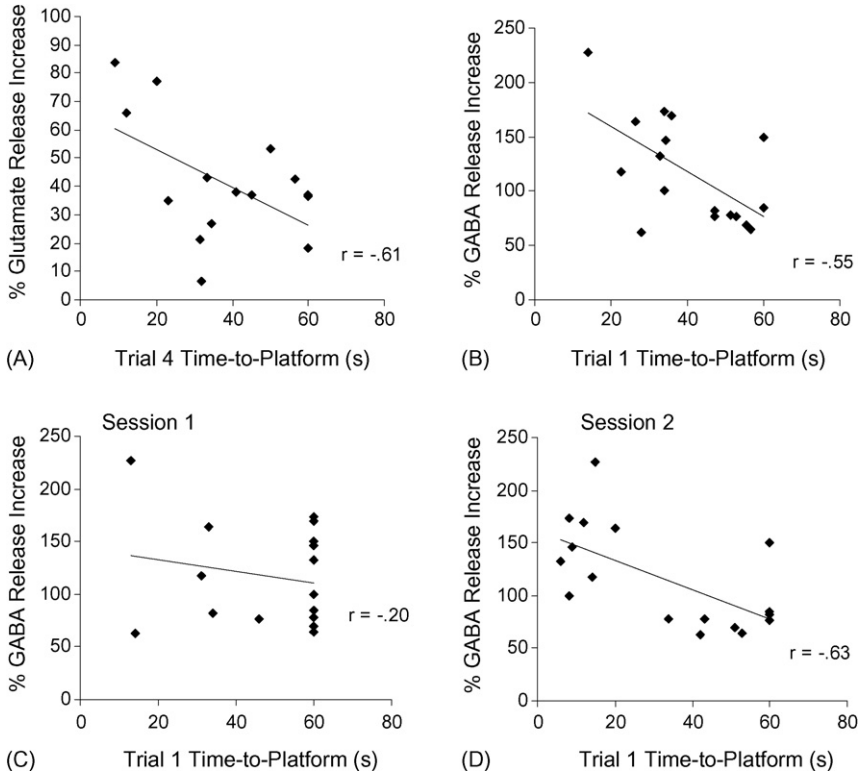

Fig. 9. Top: Significant correlation (<.05) between A) total percent glutamate release increase and average time-to-platform (s) during trial 4 and B) total percent GABA release increase and average time-to-platform (s) during trial 1 of the water maze acquisition task averaged over sessions 1 and 2 for neonatal ventral hippocampal lesion (nVHL) rats. Bottom: Correlation between total percent GABA release increase and time-to-platform (s) during trial 1 of C) session 1 and D) session 2 of the water maze acquisition task for nVHL rats.

activity study the female sham group showed increased activity like the nVHL group that was significantly higher compared to the uninjected control group. With this one exception, no other significant differences were seen between the two control groups. Thus, based on the present findings, there is little evidence that the injection of saline alone into the nVH produces reliable effects in adult rats. However, Lipska et al. [39] have shown that rats given nVH injections of the transient sodium channel blocker tetrodotoxin showed impairments in adulthood that were smaller than those seen in the nVH lesion rats. In unpublished work on water maze experiments we have observed that sham-operated rats performed at a level in between the control and nVH lesion groups. These findings suggest that it is possible that the vehicle injection into the nVH produced small and/or transient damage sufficient to affect behaviour. Thus, in spite of our generally negative findings, further studies are needed to test this possibility.

Cognitive deficits have frequently been observed in nVHL rats. The majority of studies have used males; deficits were seen in the T-maze in continuous alternation tasks at delays of 0 [37,47] to 30 s [47] and discrete paired-trial alternation tasks at delays of 0–40 s [37,48], in the radial maze in win-shift tasks [10,32], and in the water maze in place-learning [30,65,76], match-to-sample [76] and probe tasks [30]. These deficits were evidenced at pre-pubertal [10,32,47,48], pubertal [10,32] and post-pubertal ages [10,30,32,37,65,76]. The current findings that post-pubertal male nVHL rats were impaired in the water maze on the place-learning task and the match-to-sample task at delays of 0 and 30 s are thus consistent with these studies.

Relatively fewer experiments have focused on cognitive deficits in female nVHL rats. Chambers et al. [10] and Levin and Christopher [32] found that female nVHL rats tested at pre- and post-pubertal ages were impaired in the win-shift version of the radial arm maze. Our finding of impairment in nVHL female rats in the water maze is consistent with these findings. Silva-Gomez et al. [65] reported that female nVHL rats were not impaired in a place-learning task in the water maze. The reasons for this negative finding remain unclear. Our results add to previous findings showing impaired cognitive abilities in female nVHL rats.

Previous studies investigating locomotor activity in post-pubertal male rats found that spontaneous [2,14,15,30,42,62,65] and amphetamine-induced locomotor activity was significantly increased in nVHL rats compared to controls [9,42,73,76]. Locomotor activity was not significantly enhanced following saline injections [9,76] with the exception of one study where male nVHL rats demonstrated increased locomotor activity relative to sham lesioned rats in the first 10 min of a 60-min testing phase [42]. Overall, our finding of increased lower and upper spontaneous and amphetamine-induced locomotor activity in male nVHL rats is consistent with these reports.

Two studies investigated locomotor activity in female nVHL rats; one found significantly increased spontaneous locomotor activity on P56 [65], while the other found significantly increased spontaneous locomotor activity on P100, but not on P56 [9]. The difference in locomotor activity results on P56 may have been due to estrous stage at the time of testing, as this has been shown to influence spontaneous locomotor activity [13]. Locomotor activity following amphetamine was enhanced in female nVHL rats on P56 and P100 [9]. Similar to these reports, the current study showed that female nVHL rats demonstrated significantly increased upper and lower spontaneous and amphetamine-induced activity relative to control rats. To our knowledge, this is the first study to investigate upper activity (e.g., rearing and jumping) in nVHL rats. Upper activity was affected in a similar manner to lower activity in the nVHL group. Overall these studies suggest that similar to male nVHL rats, female nVHL rats demonstrate increase spontaneous and amphetamine-induced locomotor activity in adulthood.

Wood et al. [76] showed that nVHL rats, particularly those raised by high arched back nursing dams, spent more time on the open arms of the elevated plus maze, but the gender of their rats was not clear. Our current finding that male and female nVHL rats demonstrated greater percent open-arm time on the elevated plus maze agrees with Wood et al. [76] and shows for the first time that this effect occurs in males and females. These findings with nVHL rats are consistent with Schwabe et al. [64] who reported that rats that received neonatal lesions to the medial prefrontal cortex (mPFC), a target area for functional and cellular developmental changes following nVHLs [36], spent more time on the open arms of the elevated plus maze relative to controls. In contrast, Becker et al. [4] found no difference between control and nVHL rats in time spent on open arms; however, rats were tested at a later age of P91 and were housed singly after weaning rather than in groups. Considering that increased age and individual housing increase anxiety behaviours [61], and that control and nVHL rats both spent minimal time on the open arms, these factors may have obscured the effects of nVHLs and make comparisons with the current study difficult.

Behavioural abnormalities in nVHL rats have been linked to prefrontal-temporolimbic circuits [15,47,68,72]. Rat VH afferents primarily target the PFC [70]; the absence of appropriate hippocampal innervation during a critical period of development in the nVHL model is thought to have long-term consequences for PFC neural circuits and accordingly for a variety of behaviours modulated by these circuits [36,53]. Previous studies of male rats have supported the role of abnormal excitatory neurotransmission in the PFC of nVHL rats. Thus, nVHL rats demonstrated excessive firing of PFC pyramidal neurons in response to ventral tegmental area stimulation [54], reduced spine density, dendritic length and arborization of PFC pyramidal neurons [15], reduced mRNA expression of the flop isoform of the AMPA receptor subunit GluR3 in the PFC [67], enhanced glutamate binding and reduced potassium-stimulated L-aspartate release in the frontal cortex [63]. The current study showing for the first time significantly lower K^+-evoked glutamate release in the PFC of nVHL rats agrees with these findings and additionally extends them to female nVHL rats.

Abnormal PFC GABAergic transmission has also been found in the nVHL model. Thus, male nVHL rats demonstrated decreased mRNA expression for the GABA synthesizing enzyme GAD_{67} [40], increased number of $GABA_A$ receptors [14], and increased mRNA expression for the $GABA_A$ receptor subunits $\alpha 1$ [49] and $\beta 2$ [14] in the PFC. These findings suggest that there may be reduced GABA release in this model. In the current study, nVHL rats indeed demonstrated a lower GABA release relative to control rats, however this difference was not statistically significant. Considering the above documented changes in PFC GABAergic function, the question arises why was K^+-evoked GABA release not significantly reduced? It may be that the reduction in GABA release only occurs in a small select population of neurons and this may not be detectable in the overall release response. Although no studies to date have investigated how subtypes of GABA neurons are differentially affected by nVHLs, numerous studies of schizophrenic patients suggest that the PV-containing subtype is disproportionately affected [35,44]. Similarly, studies using the subchronic NMDA receptor antagonist animal model of schizophrenia have demonstrated changes in GABA markers specific to the PV-containing subtype of neurons [1,11,28]. In the rat PFC, PV+ neurons represent approximately 40 percent of GABA neurons [16]. Thus, our finding that GABA release was non-significantly reduced in nVHL rats may reflect an effect on a relatively small number of neurons. Interestingly, the potassium-evoked GABA and glutamate release showed a significant negative correlation with water maze acquisition scores in nVHL rats. Although these correlations were only found on one of four trials for each neurotransmitter and must be interpreted cautiously, this observation might suggest that changes in GABA and glutamate release predict cognitive impairment.

In conclusion, the current study demonstrates that both male and female nVHL rats show abnormalities in water maze acquisition and match-to-sample tasks, spontaneous and amphetamine-induced locomotor activity, and elevated plus maze behaviour when tested in adulthood. This, the first study to investigate K^+-evoked GABA and glutamate release in the PFC of such rats also demonstrates a significant reduction in glutamate release, thus supporting the hypothesis that nVHL is associated with reduced glutameteric function in the PFC. Future studies will determine if changes in markers of PFC GABA function associated with the nVHL model are dependant on abnormalities in specific subtypes of GABA neurons.

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