

Post-Pubertal Disruption of Medial Prefrontal Cortical Dopamine–Glutamate Interactions in a Developmental Animal Model of Schizophrenia

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Background: A neonatal ventral hippocampal lesion (NVHL) induces behavioral and physiological anomalies mimicking pathophysiological changes of schizophrenia. Because prefrontal cortical (PFC) pyramidal neurons recorded from adult NVHL rats exhibit abnormal responses to activation of the mesocortical dopaminergic (DA) system, we explored whether these changes are due to an altered DA modulation of pyramidal neurons.

Methods: Whole-cell recordings were used to examine the effects of DA and glutamate agonists on cell excitability in brain slices obtained from pre- (postnatal day [PD] 28–35) and post-pubertal (PD > 61) sham and NVHL animals.

Results: N-methyl d-aspartate (NMDA), α -amino-3-hydroxy-5-methylisoxazole propionate (AMPA), and the D₁ agonist SKF38393 increased excitability of deep layer pyramidal neurons in a concentration-dependent manner. The opposite effect was observed with the D₂ agonist quinpirole. The effects of NMDA (but not AMPA) and SKF38393 on cell excitability were significantly higher in slices from NVHL animals, whereas quinpirole decrease of cell excitability was reduced. These differences were not observed in slices from pre-pubertal rats, suggesting that PFC DA and glutamatergic systems become altered after puberty in NVHL rats.

Conclusions: A disruption of PFC dopamine–glutamate interactions might emerge after puberty in brains with an early postnatal deficit in hippocampal inputs, and this disruption could contribute to the manifestation of schizophrenia-like symptoms.

Key Words: Adolescence, dopamine receptors, electrophysiology, GABA transmission, neonatal ventral hippocampal lesion, NMDA, puberty

The neonatal ventral hippocampal lesion (NVHL) has been proposed as a developmental animal model of cortical pathophysiological changes in schizophrenia. The NVHL produces a variety of behavioral and neurochemical abnormalities resembling phenomena observed in schizophrenia (Lipska and Weinberger 1998, 2000). Hyperlocomotion, excessive reactivity to stress, and deficits in sensorimotor gating and social interactions are typically observed in NVHL animals (Becker *et al.* 1999; Le Pen *et al.* 2000; Lipska *et al.* 1993, 2002), and most of these alterations become evident only after puberty (Lipska and Weinberger 1998, 2000). Treatment with classical and atypical antipsychotic drugs has been shown to reverse many of the abnormal behavioral and physiological changes associated with the NVHL (Goto and O'Donnell 2002; Le Pen and Moreau 2002; Lipska and Weinberger 1994), strengthening the notion that neural alterations in this model are relevant to schizophrenia pathophysiology.

Dopaminergic (DA) mesolimbic/mesocortical systems are compromised in these animals. In vivo intracellular recordings of

prefrontal cortical (PFC) pyramidal neurons have revealed an abnormal response to activation of the ventral tegmental area (VTA) in NVHL rats (O'Donnell *et al.* 2002). Ventral tegmental area stimulation typically induces a prolonged plateau depolarization with suppression of action potential firing in the normal PFC (Lewis and O'Donnell 2000). In contrast, an abnormal increase in spike firing was observed in NVHL animals but only after puberty and not with analogous lesions performed during adulthood (O'Donnell *et al.* 2002). Similar abnormal responses to VTA stimulation were observed in the nucleus accumbens (Goto and O'Donnell 2002), and these responses were eliminated with a PFC lesion (Goto and O'Donnell 2004). Thus, a disruption of DA actions in the PFC might be a critical component in NVHL animals. Because PFC DA–glutamate interactions continue to mature after puberty (Tseng and O'Donnell 2005), it is possible that a delayed disruption of these interactions is responsible for the abnormal mesocortical response observed in the PFC of NVHL animals. Here we performed whole-cell patch clamp recordings of PFC pyramidal neurons in brain slices obtained from NVHL and control animals at pre- and post-pubertal ages. We examined the changes in cell excitability induced by DA and glutamate agonists in NVHL animals. Because we have recently reported a strong D₂ attenuation of PFC α -amino-3-hydroxy-5-methylisoxazole propionate (AMPA) and N-methyl d-aspartate (NMDA) responses (Tseng and O'Donnell 2004) that shapes PFC cell firing and in vivo data show that PFC neurons show exaggerated firing in response to mesocortical activation (O'Donnell *et al.* 2002), we explored whether a D₂ downregulation of NMDA responses was affected in NVHL animals.

Methods and Materials

All experimental procedures were performed at Albany Medical College according to the U.S. Public Health Service Guide for Care and Use of Laboratory Animals and were approved by the Albany Medical College Institutional Animal Care and Use Committee.

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NVHL

Pregnant Sprague-Dawley rats were obtained at 18 days of gestation from Taconic Farms (Germantown, New York). At postnatal day (PD) 6, male pups (15–19 g) were randomly separated into two groups either to be lesioned with ibotenic acid or to receive a vehicle injection. Male pups (PD 6–7) were anesthetized with hypothermia by placing them in wet ice for 10–15 min and secured onto a platform on a stereotaxic frame. A cannula was lowered into the ventral hippocampus (anteroposterior [AP]: –3.0 mm; lateral [L]: +3.5 mm; dorso-ventral [DV]: –5.0 mm), and .3 μ L of ibotenic acid (10 μ g/ μ L in mmol: 148 sodium chloride [NaCl], 3 potassium chloride [KCl], .2 sodium phosphate monobasic [NaH₂PO₄], 1.5 sodium phosphate dibasic [Na₂HPO₄], 1.4 calcium chloride dihydrate [CaCl₂], 8 magnesium perchlorate [MgCl₂]; pH 7.4) was delivered with a minipump at a rate of .15 μ L/min. The cannula was left in place for 3 additional minutes before being removed. This procedure was repeated in the contralateral hippocampus. Sham animals received the same volume of vehicle on each side. After surgery, animals were warmed up and returned to their cages, where they remained undisturbed until weaning except for husbandry. All rats were maintained on a 12-hour light/dark cycle with food and tap water available ad libitum until the time of the experiment. The entire rostrocaudal extent of damage (areas with cell loss and cell disorganization) was estimated in all animals by Nissl staining as previously reported. Lesion sizes were estimated roughly as the area at the coronal section in which they were largest, by measuring the diameter of damage extent (O'Donnell *et al.* 2002).

Whole-Cell Patch Clamp Recordings

Rats were anesthetized with chloral hydrate (400 mg/kg, IP) before being decapitated. Brains were rapidly removed into ice-cold artificial cerebrospinal fluid (ACSF) containing (in mmol): 125 NaCl, 25 sodium bicarbonate (NaHCO₃), 10 glucose, 3.5 KCl, 1.25 NaH₂PO₄, .5 CaCl₂, and 3 MgCl₂ (pH 7.45, osmolarity 295 \pm 5 mOsm). Coronal slices (300 μ m) containing only the medial PFC and adjacent white matter were cut on a Vibratome in ice-cold ACSF, transferred and incubated in warm (approximately 35°C) ACSF solution constantly oxygenated with 95% oxygen (O₂)/5% carbon dioxide (CO₂) for at least 90 min before recording. In the recording ACSF, CaCl₂ was increased to 2 mmol and MgCl₂ was decreased to 1 mmol. Patch pipettes (5–8 M Ω) were filled with (in mmol): 115 K-gluconate, 10 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2 MgCl₂, 20 KCl, 2 Mg adenosine triphosphate (MgATP), 2 adenosine triphosphate (bi-sodium salt), .3 5'-guanylate triphosphate (GTP), and .125% Neurobiotin (pH 7.3, 280 \pm 5 mOsm).

All experiments were conducted at 33°–35°C in ACSF (perfusion speed: 2 mL/min) constantly oxygenated with 95% O₂/5% CO₂. The PFC pyramidal neurons were identified under visual guidance with infrared-differential interference contrast (IR-DIC) video microscopy with a 40 \times water-immersion objective (Olympus BX51WI; Olympus America, Melville, New York). The image was detected with an IR-sensitive CCD camera (DAGE-MTI, Michigan City, Indiana) and displayed on a monitor. Whole-cell current-clamp recordings were performed with a computer-controlled amplifier (MultiClamp 700A; Axon Instruments, Sunnyvale, California), and acquired with Axoscope 8.1 (Axon Instrument) at a sampling rate of 10 KHz. The liquid junction potential was not corrected, and electrode potentials were adjusted to zero before acquiring the whole-cell configuration. Input resistance (calculated from the linear portion of the

current-voltage [IV] curve in the hyperpolarized direction), membrane potential, the number of evoked spikes, and the latency to the first spike evoked by a 500-msec-duration depolarizing current pulse were analyzed before and after drug treatment. Changes in cell excitability induced by drugs (DA and glutamate agonists and antagonists) were assessed by repeated delivery of constant-intensity (adjusted during baseline recordings) depolarizing pulses (every 15 sec) while adding the agents to the bath. Typically, current intensities were adjusted to evoke 2 spikes during baseline for drugs with a known increase in cell excitability or 4–5 spikes for drugs with a known attenuation of cell excitability (Tseng and O'Donnell 2004). All drugs (SKF38393, quinpirole, eticlopride, SCH23390, NMDA, and AMPA) were purchased from Sigma (St. Louis, Missouri). They were dissolved into oxygenated ACSF and applied in the recording solution at known concentrations. Both control and drug-containing ACSF were continuously oxygenated throughout the experiments. After 10–15 min of baseline recordings, a solution containing the drug cocktail was perfused for 5–6 min. All comparisons with baseline conditions were conducted 4–6 min after the onset of drug application. The effects observed with NMDA or AMPA were evident after the initial 3 min of drug application and required around 5–10 min to wash out. The effects obtained with D₁ and D₂ agonists were evident after 3 min of drug application and required 15–20 min to partially wash out (Tseng and O'Donnell 2004). All values are expressed as mean \pm SD. Student *t* test was used for two-group comparisons involving a single continuous variable, and drug effects were compared with repeated measures analysis of variance (ANOVA). A two-way ANOVA was preferred to analyze the interactions between drug effects and lesion. The differences between experimental conditions were considered statistically significant when *p* < .05.

Results

Whole-cell current clamp recordings of PFC pyramidal neurons were conducted in brain slices obtained from 38 NVHL and 30 sham-operated post-pubertal (PD 61–85) rats (Figure 1) from 14 litters. Rats from 11 litters were used in both groups, and the remaining 3 litters provided solely NVHL rats. Only one neuron per slice was recorded and typically two to three cells were obtained per animal. All recordings were performed with visual guidance, and Neurobiotin staining revealed that all recorded neurons were pyramidal cells (Figure 2B) located in deep layers of the medial PFC (prelimbic and infralimbic regions). These neurons were silent at rest, showing action potential firing in response to depolarizing current pulses (Figure 2A) and inward rectification with hyperpolarizing current injection (Figure 2C). The PFC pyramidal neurons recorded from NVHL (*n* = 82 cells) and sham (*n* = 71) animals exhibited similar resting membrane potential (NVHL: –70.5 \pm 2.5 mV; sham: –70.6 \pm 2.7 mV, mean \pm SD) and input resistance (NVHL: 140.2 \pm 42.5 M Ω ; sham: 134.8 \pm 34.1 M Ω). No significant differences in action potential threshold or spike kinetics (duration and amplitude) were observed between sham and lesioned groups (data not shown).

Reduced D₂ Suppression and Increased D₁ Enhancement of PFC Pyramidal Neuron Excitability in Adult NVHL Animals

The DA modulation of pyramidal cell excitability was assessed by measuring the number of spikes and the latency to the first spike evoked by constant-amplitude depolarizing current pulses (Tseng and O'Donnell 2004) in slices from NVHL and sham animals. To obtain an accurate measurement of cell

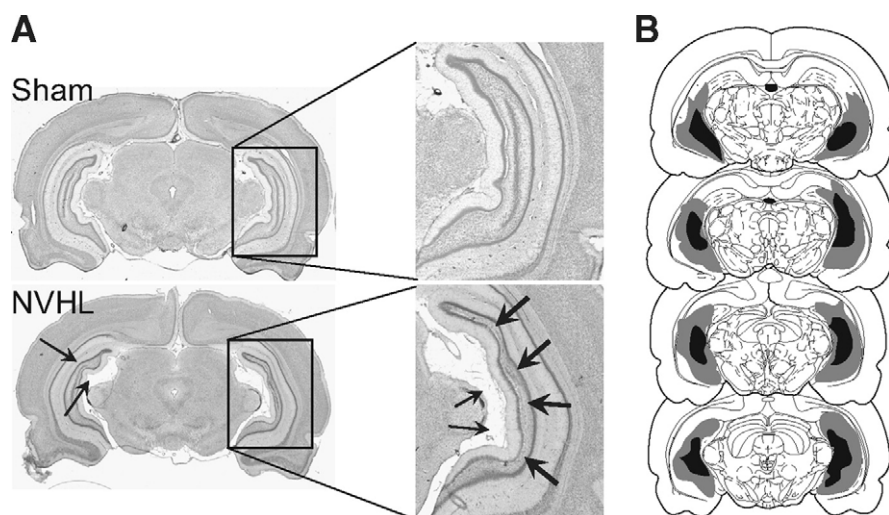


Figure 1. Neonatal ventral hippocampal lesion (NVHL). **(A)** Coronal sections showing the ventral hippocampus of a sham rat (top) and a typical NVHL (bottom), characterized by cell loss (thick arrows), and enlarged ventricles (thin arrows). **(B)** Drawings illustrating the extension of the neonatal lesion observed in adulthood. Gray and dark areas indicate maximal and minimal extents of damage, respectively.

excitability independent of membrane potential changes, continuous direct current was applied through the recording electrode to hold the cell to baseline membrane potential when drugs elicited changes in membrane potential.

Bath application of the D_2 agonist quinpirole induced a concentration-dependent decrease of pyramidal cell excitability in both NVHL and sham animals without apparent changes in the membrane potential, action potential threshold, or kinetics. A two-way ANOVA revealed overall significant effects of “lesion group” and “drug concentration” in both the number of evoked spikes [lesion: $F(1,69) = 59.45$, $p < .0001$; drug: $F(5,69) = 43.49$, $p < .0001$] and the latency to the first spike [lesion: $F(1,69) = 9.58$, $p < .003$; drug: $F(5,69) = 27.34$, $p < .0001$]. However, the inhibitory action of quinpirole was attenuated in animals with an NVHL, with its dose-response curve shifted to the right (Figure 3).

In sham animals, quinpirole ($1 \mu\text{mol/L}$) decreased the number of evoked spikes from $4.1 \pm .2$ to $2.1 \pm .5$ spikes [$p < .001$ compared with NVHL, Tukey post hoc test after significant interaction between drug and lesion, two-way ANOVA: $F(5,69) = 9.1$, $p < .0001$] and increased the latency to the first spike from 44.2 ± 2.4 msec to 78.0 ± 11.6 msec [$p < .001$ compared with NVHL, Tukey post hoc test after significant interaction between drug and lesion, two-way ANOVA: $F(5,69) = 4.7$, $p < .001$]. This concentration of quinpirole failed to modify pyramidal neuron excitability in rats with an NVHL; only higher doses (2 and $4 \mu\text{mol/L}$) yielded the inhibitory effect in NVHL animals comparable to that obtained with $1 \mu\text{mol/L}$ in the sham group (Figure 3). This decrease in neuronal excitability was accompanied by a significant increase in input resistance in all groups (sham: from 132.9 ± 19.8 to $162.7 \pm 16.2 \text{ M}\Omega$ with $1 \mu\text{mol/L}$ quinpirole, $p < .002$, Student t test; NVHL: from 130.2 ± 22.1 to

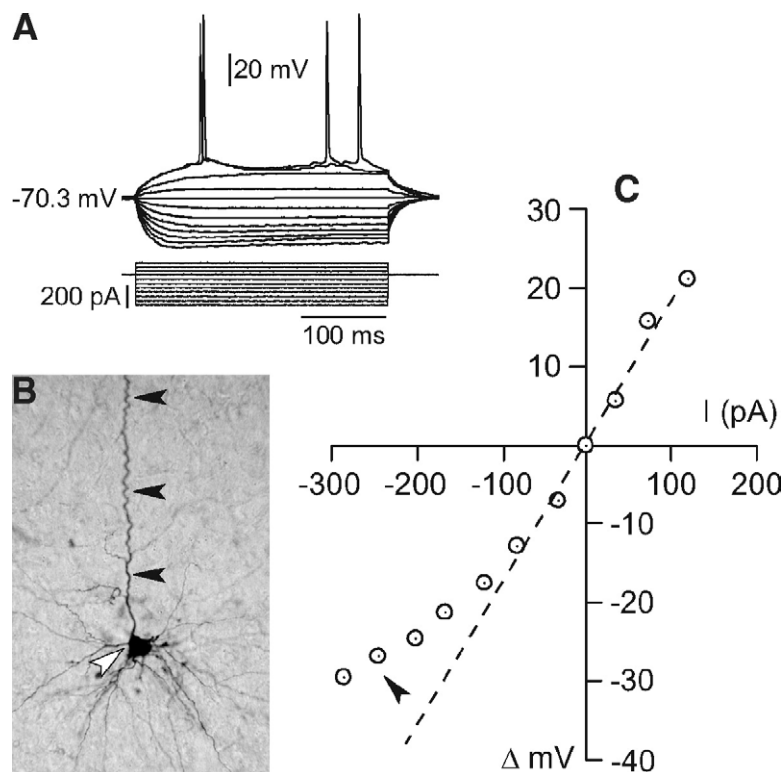


Figure 2. Whole-cell patch clamp recording of medial prefrontal cortical (PFC) pyramidal neurons from adult animals. **(A)** Typical responses to depolarizing and hyperpolarizing somatic current pulses (300 msec, from -300 to $+100 \text{ pA}$) of a deep layer pyramidal neuron recorded from an adult rat. **(B)** Image of a typical deep-layer pyramidal neuron recorded from the medial PFC and labeled with Neurobiotin. Black arrowheads indicate the apical dendrite and the white arrowhead points to the cell body. **(C)** Current-voltage plot obtained from the traces shown in (A). Currents larger than -100 pA yielded an evident inward rectification (arrowhead) in the hyperpolarizing direction.

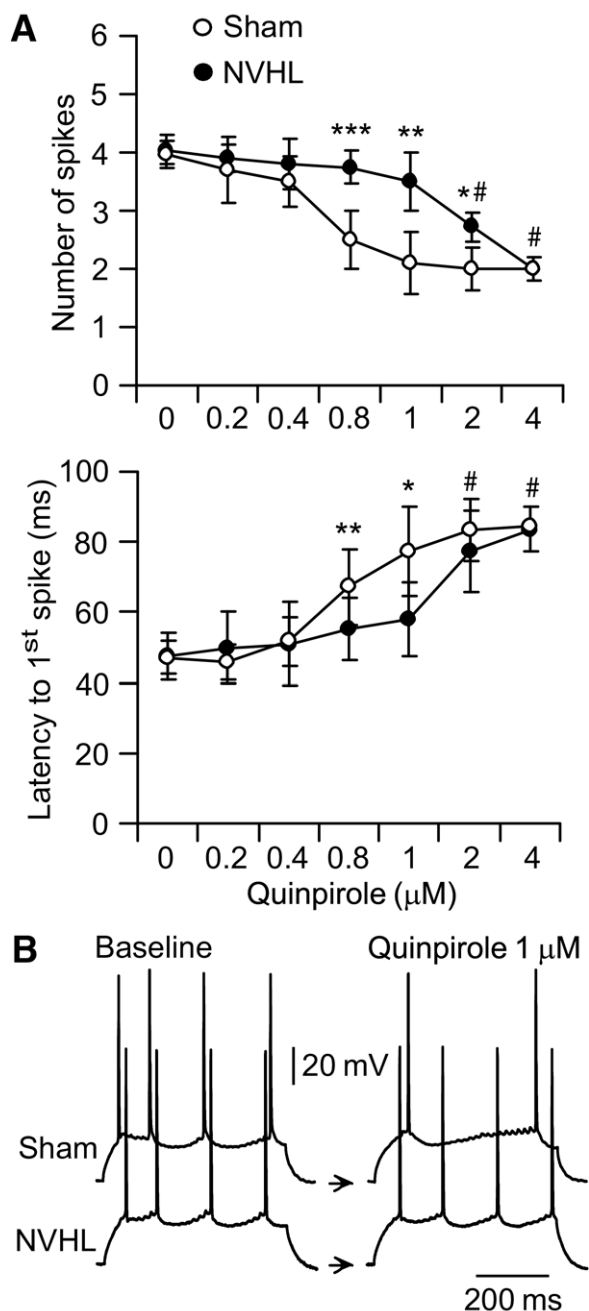


Figure 3. The inhibitory effect of quinpirole on prefrontal cortical (PFC) pyramidal cell excitability is reduced in neonatal ventral hippocampal lesion (NVHL) animals. **(A)** Line graphs summarizing the concentration-dependent effect of quinpirole ($n = 5-6$ cells/dose) on the excitability of PFC pyramidal neurons recorded from adult (postnatal day [PD] 61–78) NVHL and sham-operated animals (all data are mean \pm SD; *** $p < .0005$, ** $p < .005$, * $p < .01$, Tukey post hoc test after significant two-way analysis of variance). Only higher doses (2 and 4 $\mu\text{mol/L}$) yielded the inhibitory effect in NVHL animals (* $p < .0002$ compared with baseline, Tukey post hoc test). **(B)** Representative traces illustrating the effect of 1 $\mu\text{mol/L}$ quinpirole on PFC pyramidal neuron excitability. Quinpirole (1 $\mu\text{mol/L}$) reduced the number of evoked spikes from 4 to 2 spikes in the PFC of sham animals, whereas no apparent effect was observed in the lesioned group.

151.7 \pm 29.1 M Ω with 2 $\mu\text{mol/L}$ quinpirole, $p < .02$, Student t test) and was completely blocked with the D₂ antagonist eticlopride. In presence of 20 $\mu\text{mol/L}$ eticlopride, quinpirole (2 $\mu\text{mol/L}$) failed to

change the number of evoked spikes (from 4 \pm .3 to 3.9 \pm .3 spikes, $n = 5$) or the latency to the first spike (from 48.6 \pm 7.4 to 46.9 \pm 9.7 msec) in cells recorded from NVHL rats. Eticlopride also blocked the inhibitory effect of quinpirole 4 $\mu\text{mol/L}$ in the PFC of both sham and NVHL animals ($n = 3/\text{group}$, data not shown). Thus, the normal D₂ attenuation of pyramidal cell excitability is reduced in animals that developed without a proper hippocampal innervation of the PFC, an effect that could be related to the reduced effect of the agonist on input resistance observed in the lesioned animals.

Bath application of the D₁ agonist SKF38393 resulted in a concentration-dependent excitability increase in pyramidal neurons from both groups of neurons. The two-way ANOVA revealed, similar to what was observed with quinpirole, overall significant effects of “lesion group” and “drug concentration” with SKF38393 in both the number of spikes [lesion: $F(1,54) = 20.73$, $p < .0001$; drug: $F(4,54) = 54.29$, $p < .0001$] and the latency to the first spike [lesion: $F(1,54) = 23.93$, $p < .0001$; drug: $F(4,54) = 65.36$, $p < .0001$] evoked by current pulses. However, the effect of SKF38393 was enhanced in NVHL animals (Figure 4). Bath application of 1 and 2 $\mu\text{mol/L}$ SKF38393 failed to affect pyramidal cell excitability in sham animals but increased the number of evoked action potentials from 1.9 \pm .3 to 2.6 \pm .3 and 3.2 \pm .4 [$p < .001$, Tukey post hoc test after significant interaction between drug and lesion, two-way ANOVA: $F(4,54) = 3.1$, $p = 0.024$], respectively, and reduced the latency to the first evoked spike from 105.8 \pm 9.9 msec to 72.9 \pm 12.6 and 55.7 \pm 15.8 msec [$p < .001$, Tukey post hoc test after significant interaction between drug and lesion, two-way ANOVA: $F(4,54) = 3.8$, $p = 0.009$], respectively, in neurons recorded from NVHL animals (Figure 4). This excitatory effect of SKF38393 was independent of membrane potential depolarization and was not accompanied by changes in input resistance or action potential threshold or duration (data not shown). Furthermore, the D₁ antagonist SCH23390 (10 $\mu\text{mol/L}$, $n = 5$ cells recorded from NVHL animals) blocked the effect of 8 $\mu\text{mol/L}$ SKF38393. The number of evoked spikes and the latency to the first spike remained unchanged when the agonist was administered in presence of the antagonist. Evoked action potentials were 1.9 \pm .4 before drug application and 2.1 \pm .6 after SKF38393 + SCH23390. Similarly, the first spike latency was 107.5 \pm 12.4 msec before SKF38393 + SCH23390 and 103.3 \pm 14.5 msec after drug administration. Altogether, these results suggest that both D₁ and D₂ modulation of PFC neuronal activity are disrupted in animals with an NVHL, in a manner that would cause hyperexcitability in response to mesocortical activation.

Increased Excitatory Effect of NMDA But Not AMPA in the PFC of Adult NVHL Animals

Bath application of NMDA and AMPA depolarize pyramidal neurons, particularly at higher doses (Tseng and O'Donnell 2004). Therefore, pyramidal neuron excitability was measured in all cases, holding the cells to baseline membrane potential with a continuous DC injection when necessary.

Bath application of NMDA induced a dose-dependent increase in excitability in PFC pyramidal neurons recorded from both lesioned and sham animals. However, the response to NMDA was higher in NVHL animals when compared with the sham-operated group (Figure 5). A two-way ANOVA showed overall significant effects of “lesion group” and “drug concentration” with NMDA in both the number of evoked spikes [lesion: $F(1,60) = 47.12$, $p < .0001$; drug: $F(4,60) = 218.5$, $p < .0001$] and the latency to the first spike [lesion: $F(1,60) = 31.96$, $p < .0001$; drug: $F(4,60) = 139.5$, $p < .0001$]. Bath application of 1 $\mu\text{mol/L}$

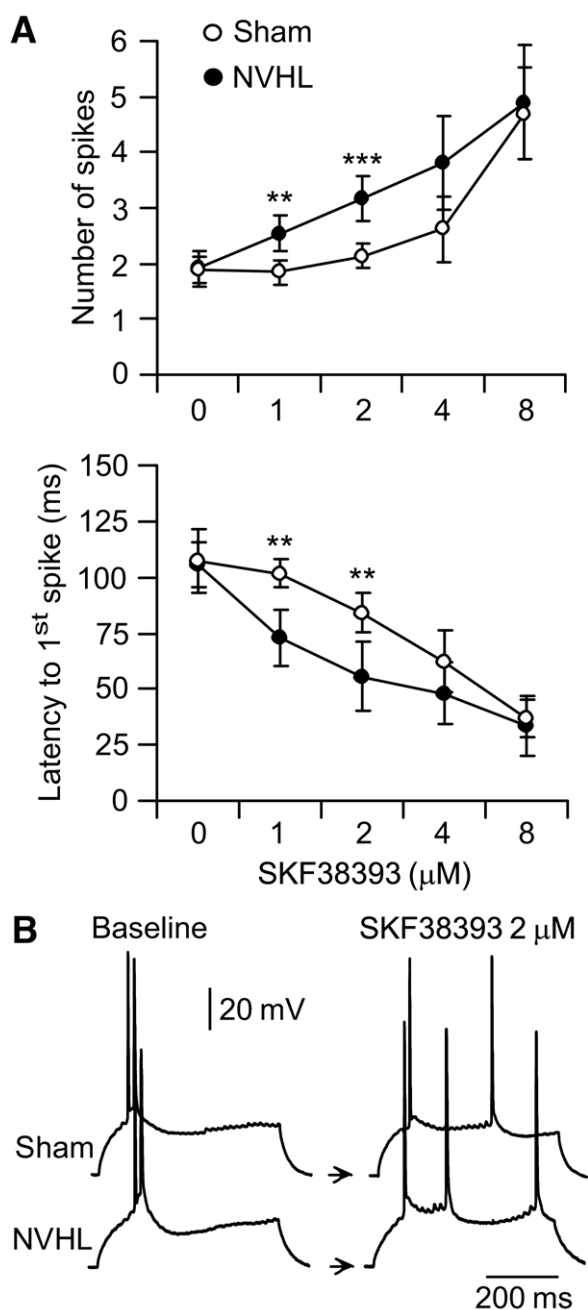


Figure 4. The excitatory effect of SKF38393 on prefrontal cortical (PFC) pyramidal cell excitability is enhanced in neonatal ventral hippocampal lesion (NVHL) animals. **(A)** Line graphs summarizing the concentration-dependent effect of SKF38393 ($n = 5-6$ cells/dose) on the excitability of PFC pyramidal neurons recorded from adult (postnatal day [PD] 61–78) sham-operated and NVHL animals ($***p < .0005$, $**p < .005$, Tukey post-hoc test after significant two-way analysis of variance). **(B)** Traces of responses of two representative pyramidal neurons recorded from a sham and an NVHL rat during baseline and after bath application of 2 μmol/L SKF38393. The number of evoked spikes was not affected by SKF38393 in the neuron recorded from the sham rat. In contrast, bath application of 2 μmol/L SKF38393 increased pyramidal cell excitability from 2 to 3 spikes in the lesioned rat.

NMDA, a dose that failed to affect excitability in sham animals, significantly increased the number of evoked spikes [from $1.9 \pm .2$ to $3.6 \pm .7$ spikes, $p < .001$, Tukey post hoc test after significant interaction between drug and lesion, two-way ANOVA:

$F(4,60) = 7.1$, $p < .0002$] and decreased the latency to the first evoked spike [from 101.1 ± 18.8 to 41.4 ± 9.5 msec, $p < .001$, Tukey post hoc test after significant interaction between drug and lesion, two-way ANOVA: $F(4,60) = 10.7$, $p < .00001$] of PFC pyramidal neurons in lesioned animals (Figure 5). Moreover, the

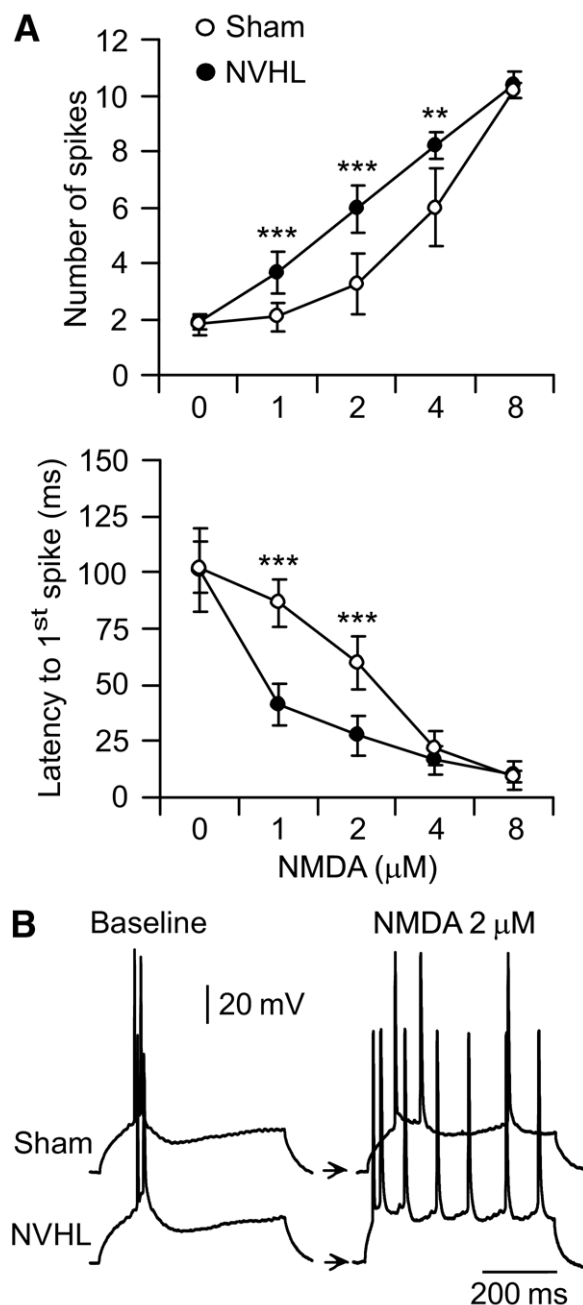


Figure 5. The excitatory effect of N-methyl d-aspartate (NMDA) on PFC pyramidal cell excitability is enhanced in NVHL animals. **(A)** Line graphs summarizing the concentration-dependent effect of NMDA ($n = 5-7$ cells/dose) on the excitability of pyramidal neurons recorded in the PFC of adult (PD 61–78) sham-operated and NVHL animals ($***p < .0002$, $**p < .001$, Tukey post hoc test after significant two-way analysis of variance). **(B)** Representative traces of two PFC pyramidal neurons recorded from a sham and a lesioned animal illustrating their response to 2 μmol/L NMDA. After 5 min of NMDA, the number of evoked spikes increased from 2 to 3 spikes in the sham group, whereas a more pronounced increase (from 2 to 7 spikes) was observed in the lesioned animal. Abbreviations as in Figure 4.

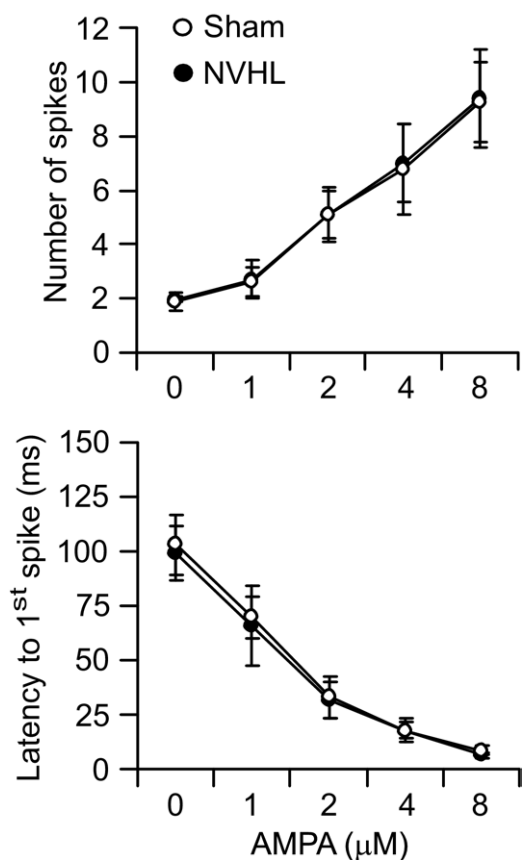


Figure 6. The effect of α -amino-3-hydroxy-5-methylisoxazole propionate (AMPA) on PFC pyramidal cell excitability is unchanged in NVHL animals. The PFC pyramidal neuron excitability increases in a concentration-dependent manner after bath application of AMPA (top: number of evoked spikes; bottom: first spike latency, $n = 5-6$ cells/dose). The PFC pyramidal neurons recorded from sham and NVHL animals (PD 61–78) exhibited similar increase in the number of evoked spikes and decrease of first spike latency. Abbreviations as in Figure 4.

excitatory responses to 2 and 4 $\mu\text{mol/L}$ NMDA were also enhanced in NVHL animals when compared with the sham group (Figure 5). In contrast, the increase in pyramidal cell excitability induced by bath application of AMPA was not different between NVHL and sham animals (Figure 6). These results suggest that alterations in ventral hippocampal inputs

during early postnatal development enhance NMDA-mediated excitation without affecting the responses to AMPA in the adult PFC.

Altered Responses to D_2 , D_1 , and NMDA Are Not Observed in NVHL Animals Before Puberty

Because it has been repeatedly observed that NVHL-induced behavioral (Lipska and Weinberger 1998, 2000) and electrophysiological (O'Donnell *et al.* 2002) changes are evident only after puberty, additional recordings were performed to examine these responses in the PFC of pre-pubertal (PD 28–36) animals. Pyramidal neurons recorded from pre-pubertal sham ($n = 23$) and NVHL ($n = 22$) animals were silent at rest and exhibited similar resting membrane potential (NVHL: -68.5 ± 2.6 mV; sham: -68.4 ± 2.8 mV) and input resistance (NVHL: 140.6 ± 55.3 M Ω ; sham: 134.1 ± 41.2 M Ω). Bath application of quinpirole resulted in similar concentration-dependent decrease of pyramidal cell excitability in both NVHL and sham animals, as revealed by the number evoked of spikes (Figure 7A) and latency to the first evoked spike (data not shown). Similarly, excitatory responses to increasing doses of SKF38393 and NMDA recorded in PFC pyramidal neurons from pre-pubertal lesioned and sham animals were undistinguishable (Figures 7B and 7C). These results indicate that the altered DA and glutamatergic control of pyramidal cell excitability observed in the adult NVHL animals are not present before puberty.

Disruption of D_2 -Glutamate Interactions in the Adult PFC of NVHL Animals

We have recently shown that activation of D_2 receptors attenuates NMDA and AMPA-mediated excitation through different mechanisms in the PFC of developmentally mature animals (Tseng and O'Donnell 2004). To investigate whether these interactions are affected by an NVHL, we examined the effects of quinpirole on NMDA- and AMPA-induced excitability increase in PFC pyramidal neurons recorded from post-pubertal lesioned and sham animals. As reported previously (Tseng and O'Donnell 2004), bath application of .4 $\mu\text{mol/L}$ ($n = 4$, data not shown) or 1 $\mu\text{mol/L}$ quinpirole ($n = 5$) significantly reduced the excitatory effect of 4 $\mu\text{mol/L}$ NMDA in sham animals (Figure 8). However, this D_2 attenuation of NMDA effects was not observed in the lesioned group. Quinpirole (1 $\mu\text{mol/L}$, $n = 6$) failed to attenuate the changes in the number of evoked spikes or the latency to the first evoked spike observed with 4 $\mu\text{mol/L}$ NMDA in the PFC of NVHL rats (Figure 8). The D_2 modulation of AMPA-induced increase in excitability was also disrupted in the PFC of NVHL

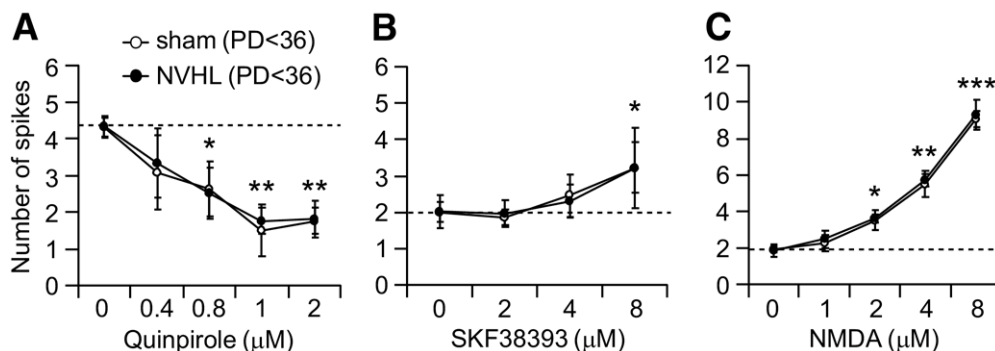


Figure 7. Concentration-dependent effect of quinpirole (A), SKF38393 (B), and NMDA (C) on PFC pyramidal cell excitability (number of spikes evoked by somatic current pulses) recorded from pre-pubertal (PD 28–36) animals. No differences were observed between sham-operated animals and those with an NVHL (4–5 cells/dose). Abbreviations as in Figure 5. (* $P < .05$, ** $P < .01$, *** $P < .001$.)

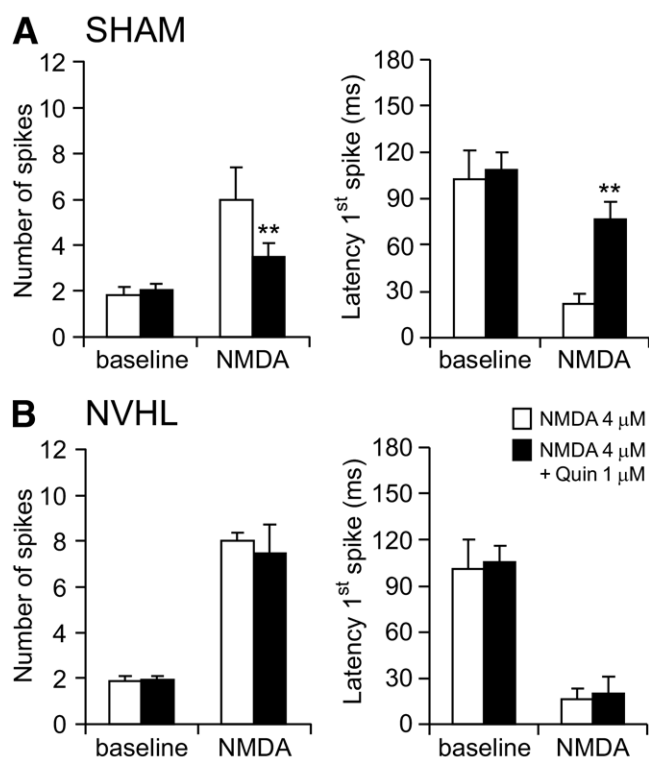


Figure 8. Disruption of D_2 -NMDA interactions in the PFC of NVHL animals. Bar graphs summarize the effect of quinpirole on NMDA responses observed in the PFC of (A) sham and (B) NVHL animals (PD > 61). Bath application of quinpirole (1 μ mol/L) significantly decreased the excitatory effect of NMDA (4 μ mol/L) on PFC pyramidal neuron excitability (** p < .001, Tukey post hoc test after significant two-way analysis of variance). In contrast, the increase in the number of evoked spikes and decrease in first spike latency induced by NMDA were not affected by quinpirole in NVHL animals. Abbreviations as in Figure 5.

rats. Similar to what was observed with the D_2 -NMDA interaction, an attenuation of AMPA responses by quinpirole was only observed in pyramidal neurons recorded from sham animals ($n = 5$; Figure 9). In the PFC of NVHL rats, bath application of 1 μ mol/L quinpirole failed to attenuate the increased number of evoked spikes or to reduce the latency to the first evoked spike induced with .2 μ mol/L AMPA (Figure 9). These results indicate that an early postnatal disruption of the hippocampal inputs also alters the normal DA modulation of glutamatergic function in the adult PFC.

Discussion

We investigated the effects of DA and glutamate on PFC pyramidal neuron excitability in brain slices obtained from NVHL and sham animals. As recently reported (Tseng and O'Donnell 2004), bath application of NMDA, AMPA, or the D_1 agonist SKF38393 induced a concentration-dependent increase in pyramidal neuron excitability, whereas the D_2 agonist quinpirole resulted in a dose-dependent decrease in cell excitability. The excitatory effects of SKF38393 and NMDA but not those mediated by AMPA were significantly enhanced in animals with NVHL. The D_2 -mediated response was also altered in the PFC of lesioned animals, as revealed by a reduced attenuation of cell excitability by quinpirole. These changes were not evident in pre-pubertal animals, suggesting that PFC DA and glutamatergic systems become altered in neonatally lesioned animals only after

puberty. In addition, the typical D_2 attenuation of NMDA- and AMPA-mediated responses (Tseng and O'Donnell 2004) was only observed in the PFC of sham animals. These data indicate that PFC DA-glutamate interactions are also compromised in NVHL animals. Thus, all these changes might underlie the hyper-excitable PFC response to VTA stimulation (O'Donnell *et al.* 2002) and could be responsible for some of the behavioral deficits observed in these animals, which also emerge after puberty (Lipska and Weinberger 1998, 2000).

The PFC pyramidal neurons recorded from NVHL animals are more responsive to bath application of NMDA and the D_1 agonist SKF38393 but not to AMPA. This selective excitability increase induced by NMDA or D_1 activation was observed only in the PFC of post-pubertal (not pre-pubertal) NVHL animals, suggesting that an early disruption of the hippocampal-PFC pathway might alter the normal postnatal development of PFC D_1 and NMDA function. Several mechanisms could account for these changes. It is well known that D_1 receptors increase cyclic adenosine monophosphate (cAMP) levels and protein kinase A (PKA) activity (Nicola *et al.* 2000; Surmeier *et al.* 1995) and can potentiate NMDA-mediated excitation through a postsynaptic PFC-calcium-dependent mechanism (Tseng and O'Donnell 2004; Wang and O'Donnell 2001). It remains to be determined whether an abnormal upregulation of these postsynaptic second messenger cascade events could result in exaggerated pyramidal

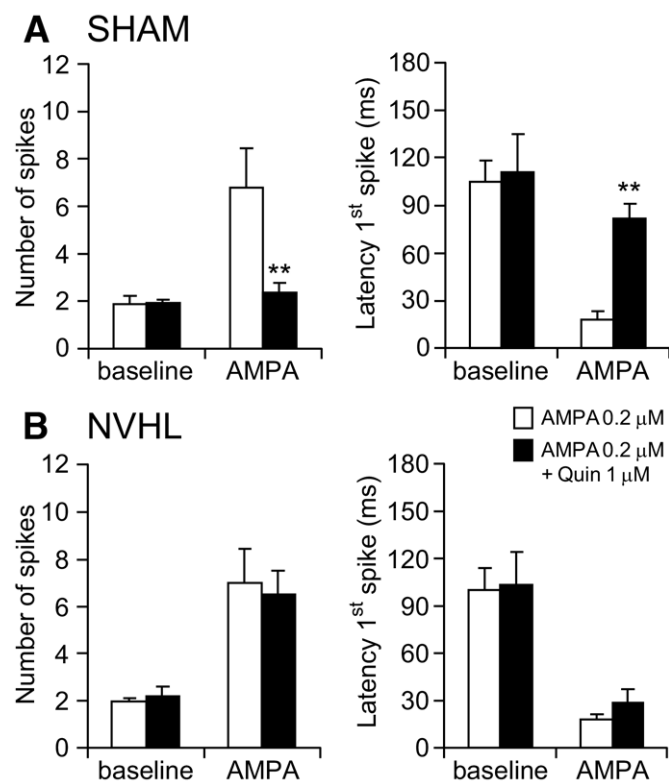


Figure 9. Disruption of D_2 -AMPA interactions in the PFC of NVHL animals. Bar graphs summarize the effect of quinpirole on AMPA responses recorded in the PFC of (A) sham and (B) NVHL animals (PD > 61). The excitatory effects of .2 μ mol/L AMPA on the number of evoked spikes and first spike latency were significantly attenuated with 1 μ mol/L quinpirole in the PFC of sham animals (** p < .001, Tukey post hoc test after significant two-way analysis of variance). In contrast, these inhibitory actions of quinpirole on AMPA responses were not observed in PFC pyramidal neurons from NVHL rats. Abbreviations as in Figure 6.

cell excitability in response to D₁ and NMDA, as observed in the PFC of NVHL animals. A D₁ receptor activation also induces NMDA receptor trafficking to the postsynaptic membrane (Dunah and Standaert 2001), increasing surface expression of NMDA receptors. This interaction could selectively enhance pyramidal neurons' response to NMDA and not to AMPA in the adult PFC. In contrast, the reduced D₂ inhibition and the lack of D₂-mediated attenuation of AMPA and NMDA responses in the PFC of NVHL animals could reflect a disruption of D₂ receptor function. We have shown that the attenuation of PFC pyramidal neuron excitability by D₂ receptors depends on several mechanisms, including a direct postsynaptic modulation of cAMP-PKA and phospholipase C (PLC)–inositol 1,4-5 trisphosphate (IP3)–Ca⁺⁺ second messenger cascades, and indirectly by enhancing firing of local γ -aminobutyric acid (GABA)ergic interneurons (Tseng and O'Donnell 2004). More precisely, the D₂ attenuation of AMPA-mediated excitation involves postsynaptic inhibition of PKA and activation of PLC-IP3-Ca⁺⁺ pathways, whereas the D₂ inhibition of NMDA effects is mediated by activation of local GABAergic transmission (Tseng and O'Donnell 2004). Interestingly, the D₂ activation of PFC GABAergic interneurons emerges after puberty (Tseng and O'Donnell 2006); thus, it is possible that PFC interneurons in NVHL animals acquire an abnormal response to DA during their peri-adolescent maturation. A deficit in PFC interneurons has indeed been suggested by the selective downregulation of glutamate decarboxylase-67 messenger RNA in the PFC of NVHL animals (Lipska *et al.* 2003a, 2003b). This, compounded by the abnormal potentiation of D₁ and NMDA responses and the attenuation of D₂ inhibition of AMPA responses, would yield a hyper-reactive PFC in NVHL animals, especially in response to mesocortical stimulation (O'Donnell *et al.* 2002; Tseng *et al.* 2006a). Thus, PFC DA–GABA–glutamate interactions are compromised in animals that received an early inactivation of the hippocampal formation.

To extrapolate the information obtained in brain slices to an intact brain and, even more, to a disease condition might require considering the limitations inherent in this preparation. The slices used here contained only the medial PFC, and all long-distance afferent projections had been sectioned by the coronal plane of slicing. However, a 300- μ m-thick brain slice does contain a functional local network that allows the exploration of local interactions and actions of specific transmitters within that network. Thus, although these experiments were conducted in a reduced preparation, the cellular changes observed are likely to underscore the manner the entire system responds to mesocortical activation.

Post-pubertal alterations in the PFC of animals with a bilateral NVHL have been proposed to model the cortical deficits observed in schizophrenia (Lipska and Weinberger 1998), a disorder characterized by hypofrontality. Our findings of overall enhancement of PFC pyramidal neuron excitability in NVHL animals are consistent with increased firing observed *in vivo* with VTA stimulation in this model (O'Donnell *et al.* 2002). However, these observations might seem at odds with the common concept of hypofrontality. Hypofrontality typically refers to the lack of PFC activation during working-memory tasks (for review, see Manoach 2003). But it remains unknown whether “hypofrontality” is the result of a hypoactive PFC or a PFC with a hyperactive baseline that has limited room for further increase in activity when needed. For example, some studies have shown absence of changes or even increased activation of the frontal cortices of schizophrenia patients (Callicott *et al.* 1999, 2000, 2003a, 2003b; Honey *et al.* 2002; Manoach *et al.* 1999, 2000). The PFC dysfunction and working memory deficits in schizophrenia are variable

and complex, probably depending on the loads required to conduct the working memory task. In imaging studies, PFC responses typically increase until the task load exceeds the PFC functional capacity, during which the activation decreases (for review, see Manoach 2003). A similar non-linear relationship was observed in schizophrenia but with increases at low working memory loads and a decrease in metabolic activity with loads that would engage the PFC of control subjects (Manoach 2003). The PFC metabolic responses to different intensities of VTA stimulation were tested in both sham and NVHL animals with cytochrome oxidase I staining, revealing a leftward shift only in post-pubertal animals that had received a bilateral NVHL (Tseng *et al.* 2006a). A functional magnetic resonance imaging study has also revealed increased blood flow in several brain regions, including the PFC, in NVHL animals (Risterucci *et al.* 2005). Therefore, the heightened excitability of pyramidal neurons reported here might represent a hyperactive state at low loads, potentially leading to a hypo-frontal state with higher demands.

What could be responsible for such dramatic changes at a late developmental stage? Without doubt, this question is essential to understanding how symptoms in schizophrenia, a disorder with a clear genetic predisposition (Harrison and Weinberger 2005), emerge late in adolescence. Several studies have highlighted a protracted PFC maturation. In human subjects, imaging studies revealed late changes in PFC morphology (Pantelis *et al.* 2005). Local PFC circuits do mature during adolescence in rats (Tseng and O'Donnell 2005 2006) and monkeys (Woo *et al.* 1997). Thus, it is possible that the DA modulation of PFC circuits, including pyramidal cells and interneurons, matures during adolescence. It can be speculated that, in a brain in which hippocampal inputs to the PFC were disrupted during early development, PFC circuits become improperly assembled but do not result in behavioral anomalies until adolescence, because that is the time in which they would acquire an adult profile, particularly in their DA modulation. This possibility and the potential cellular mechanisms involved remain to be explored.

The mesocortical system is activated by salient stimuli (Horvitz 2000) and contributes to cognitive functions, including working memory, attention, and reward (Goldman-Rakic *et al.* 2000; Jay 2003; Schultz 2002). Ventral tegmental area stimulation with trains of pulses mimicking DA cell burst firing, typically observed with salient or reward-predicting stimuli, yields plateau depolarization along with reduced action potential firing (Lewis and O'Donnell 2000). This has been interpreted as a mechanism by which the mesocortical system reduces the impact of weak inputs, enhancing the saliency of stronger activated afferents to the PFC (O'Donnell 2003). This context-dependent DA increase will activate both D₁ and D₂ receptors, with D₁ activation facilitating ongoing NMDA activity (Tseng and O'Donnell 2005; Wang and O'Donnell 2001) and both D₁ and D₂ activation reducing irrelevant excitatory inputs by increasing local inhibitory tone (Goldman-Rakic *et al.* 2000; Gorelova *et al.* 2002; Tseng and O'Donnell 2004, 2006; Tseng *et al.* 2006b) and reducing pyramidal neuron excitability in response to mesocortical stimulation (Lewis and O'Donnell 2000; Tseng *et al.* 2006b). The abnormal excitation by D₁ and NMDA along with the lack of D₂-mediated inhibition of AMPA and NMDA effects in the PFC of NVHL animals might contribute to establish the altered PFC electrophysiological (O'Donnell *et al.* 2002) and metabolic (Tseng *et al.* 2006a) responses observed in these animals. A disruption of DA-mediated inhibition might result in abnormal and inappropriate engagement of pyramidal neuron firing in response to excitatory inputs, which ultimately might lead to

altered cognitive performance in NVHL animals (Chambers *et al.* 1999), a model proposed to mimic some of the cortical deficits observed in schizophrenia (Lipska and Weinberger 1998). Our observation, that this pattern of altered DA–glutamate–GABA interactions within PFC is a late emerging physiology, also might have implications for our understanding of the clinical onset in early adult life of the schizophrenia phenotype.

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