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A Neonatal Ventral Hippocampal Lesion Causes Functional Deficits in Adult Prefrontal Cortical Interneurons

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Animals with a neonatal ventral hippocampal lesion (NVHL) develop abnormal behaviors during or after adolescence, suggesting that early insults can have delayed consequences. Many of these behaviors depend on the prefrontal cortex (PFC), and we have reported that PFC pyramidal neurons of adult rats with an NVHL respond to stimulation of the ventral tegmental area with an increase in firing instead of the characteristic decrease. As the dopamine modulation of cortical interneurons matures during adolescence, these findings raise the possibility that maturation of local inhibitory circuits within the PFC may have been altered in NVHL rats. Here, we assessed the state of PFC interneurons in NVHL rats with *in situ* hybridization measures of the mRNAs for the calcium binding protein parvalbumin (PV) and the GABA synthesizing enzyme GAD_{67}, as well as with electrophysiological measures of interneuron function. Although no differences were observed with PV or GAD_{67}, whole-cell recordings in slices revealed abnormal responses to the D_2 agonist quinpirole in interneurons from NVHL rats. The loss of D_2 modulation of local inhibition in slices from NVHL rats was also evident in the absence of a lasting component in the D_2 attenuation of excitatory synaptic responses in pyramidal neurons, which in sham treated rats was picrotoxin sensitive. The results suggest that the neonatal lesion causes improper maturation, but not loss, of PFC interneurons during adolescence, a finding consistent with current interpretations of imaging data in schizophrenia that suggest a hyperactive, "noisy" cortex underlying dysfunction in the PFC and other cortical areas.

Key words: **prefrontal cortex; dopamine; interneurons; GABA; neonatal ventral hippocampal lesion; schizophrenia**

Introduction

Anomalies in cortical interneurons are consistently found in schizophrenia. Postmortem studies have shown reduced markers of interneuron function in the prefrontal cortex (PFC) (Akbarian et al., 1995; Lewis, 1995; Beasley and Reynolds, 1997; Beasley et al., 2002), cingulate cortex (Benes et al., 1991), temporal lobe (Chance et al., 2005), hippocampus (Benes, 1999), amygdala (Benes and Berretta, 2001), and even the motor and visual cortices (Hashimoto et al., 2008a) in brains from schizophrenia patients. The high reproducibility of these findings supports the possibility that interneuron deficits are a central feature in schizophrenia pathophysiology (Benes and Berretta, 2001; Hashimoto et al., 2008b; Lewis and González-Burgos, 2008; O'Donnell, 2008). Many functional deficits are interpreted as arising from interneuron alterations. For example, reduced gamma (i.e., 30–50 Hz) activation in the EEG of schizophrenia patients (Spencer et al., 2003; Cho et al., 2006) is thought to be an expression of altered inhibition in cortical areas because interneurons are critical for this type of activity (Szabadics et al., 2001; Traub et al., 2001; Frankle et al., 2008). The reduced capacity in working memory functions and the inefficient PFC activity suggested by functional imaging studies (Callicott et al., 2000; Manoach, 2003) could also be the consequence of impaired inhibitory circuits. Furthermore, noncompeting NMDA antagonists, a well established pharmacological model of schizophrenia (Javitt and Zukin, 1991), increased glutamate levels (Moghaddam et al., 1997) and pyramidal cell firing (Jackson et al., 2004) in the PFC, likely attributable to blockade of NMDA receptors in interneurons (Homayoun and Moghaddam, 2007). A recent clinical trial with a metabotropic glutamate receptor subunit 2/3 agonist, which would reduce glutamate release, proved almost as effective as olanzapine in treating schizophrenia symptoms (Patil et al., 2007). Thus, preclinical and clinical studies converge remarkably in highlighting interneuron deficits in schizophrenia (Lewis and Moghaddam, 2006).

Because it has become widely accepted that schizophrenia has a strong developmental component (Weinberger, 1987; Harrison and Weinberger, 2005), the need for developmental animal models has become stronger. Many such models have been studied, including the neonatal ventral hippocampal lesion (NVHL) (Lipska et al., 1993; Brake et al., 1999; O'Donnell et al., 2002), prenatal exposure to an antimitotic (Flagstad et al., 2004; Gourevitch et al., 2004; Moore et al., 2006), prenatal stress (Koenig et al.,

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2005), and many genetic models (Miyakawa et al., 2003; Pillai-Nair et al., 2005; Kellendonk et al., 2006; Hikida et al., 2007). The behavioral phenotype in all these models is quite similar: hyperlocomotion, enhanced sensitivity to stress and stimulants, prepulse inhibition deficits, reduced social interactions, and, in some cases, reversal with antipsychotic drugs. Most of these anomalies emerge during adolescence, therefore including a time course similar to the human disease, and in many cases there is also evidence of reduced GABAergic markers (Lipska et al., 2003; Pillai-Nair et al., 2005; Penschuck et al., 2006; Endo et al., 2007). Because we have shown recently that the dopamine (DA) modulation of interneurons matures during adolescence (Tseng and O'Donnell, 2007a,b), it is possible that this maturation is altered in rodent developmental models of schizophrenia. *In vivo* intracellular recordings from adult rats with an NVHL revealed that ventral tegmental area stimulation causes abnormal firing of PFC pyramidal neurons in this model (O'Donnell et al., 2002) instead of the decrease typically observed in naive or sham rats (Lewis and O'Donnell, 2000; O'Donnell et al., 2002). This suggests that inhibitory mechanisms in the PFC may have been altered by the neonatal lesion and the alteration became apparent with the transition to a young adult age. Here, we directly tested that possibility with molecular measures of interneuron-related markers and whole-cell recordings from PFC pyramidal neurons and interneurons in brain slices from NVHL and sham rats.

Materials and Methods

All experimental procedures were approved by the Albany Medical College and the University of Maryland School of Medicine Institutional Animal Care and Use Committees and were conducted according to the United States Public Health Service *Guide for Care and Use of Laboratory Animals*.

Neonatal ventral hippocampal lesion. Pregnant Sprague Dawley rats were obtained at 18 d of gestation from Taconic Farms. At postnatal day 6 (P6), male pups (15–19 g) were randomly separated into two groups to receive vehicle injection (sham) or ibotenic acid injection (lesion). Pups (P6–P7) were anesthetized with hypothermia for 10–15 min and secured on a Styrofoam platform mounted on a stereotaxic frame (David Kopf Instruments). A cannula was lowered into the ventral hippocampus (anteroposterior, −3.0 mm; lateral, +3.5 mm; 5.0 mm below cortical surface) and 0.3 μl of ibotenic acid [10 μg/μl (in mM): 148 NaCl, 3 KCl, 0.2 NaH_2PO_4, 1.5 Na_2HPO_4, 1.4 $CaCl_2$, and 0.8 $MgCl_2$, pH 7.4] was delivered using a minipump at a rate of 0.15 μl/min. The cannula was left in place for 3 additional minutes before being removed. This procedure was repeated in the contralateral hemisphere. Sham animals received the same volume of vehicle on each side. After surgery, pups were warmed up and returned to their cages, where they remained undisturbed until weaning except for husbandry. The extent of damage induced by ibotenic acid (i.e., areas with cell loss and cell disorganization) was estimated in all animals by Nissl staining. All rats were maintained on a 12 h light/dark cycle with food and tap water available *ad libitum* until the time of the experiment.

In situ *hybridization for GAD_{67} and parvalbumin.* Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) 15 min before being decapitated. Brains were immediately frozen after removal from the skull and stored at −80°C. Serial coronal sections (12 μm) were cut from +2.7 to +2.2 bregma (Paxinos and Watson, 1998), and three sections evenly spaced at ~160 μm intervals were selected from each animal and used for *in situ* hybridization studies for each mRNA of interest.

For synthesis of riboprobes, we used a 311 bp fragment for GAD_{67} mRNA and a 339 bp fragment for parvalbumin (PV) mRNA, corresponding to bases 151–461 of the mouse GAD_{67} mRNA (Y12257) and bases 256–594 of the mouse PV mRNA (X59382). Amplified fragments were subcloned into the plasmid pSTBlue-1 (Novagen), and antisense and sense probes were transcribed *in vitro* in the presence of [^{35}S]CTP (GE Healthcare), using T7 or SP6 RNA polymerase.

Hybridization was performed as described previously (Hashimoto et al., 2005). After fixation with 4% paraformaldehyde in PBS, the sections were acetylated, dehydrated through a graded ethanol series, and defatted in chloroform for 10 min. The sections were then hybridized with ^{35}S-labeled riboprobes in hybridization buffer at 56°C for 16 h. The sections were washed in solution containing 0.3 M NaCl, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, and 50% formamide at 63°C, treated with RNase A (20 μg/ml) at 37°C, and washed in 0.1× SSC (150 mM NaCl and 15 mM sodium citrate) at 67°C. Sections were then dehydrated through a graded ethanol series, air dried, and exposed to BioMax MR film (Eastman Kodak).

Quantification was performed without knowledge of subject condition by random coding of the sections. Trans-illuminated autoradiographic film images were captured by a video camera under precisely controlled conditions, digitized, and analyzed using a microcomputer imaging device MCID system (Imaging Research). Images of the sections were also captured and superimposed onto the autoradiographic images to draw contours of the full thickness of the prefrontal cortex, including the cingulate, prelimbic, and infralimbic cortices. Expression levels of each mRNA were determined as optical densities within the contours and expressed as nanocuries per gram of tissue by reference to radioactive standards (Carbon-14 standards; ARC Inc.) exposed on the same film.

Because bilateral lesions were made and the mRNA levels were quantified in the PFC of each hemisphere independently, we used a two-factor design ANOVA with manipulation (lesion vs sham) as a between-subject factor and laterality (left vs right) as repeated measures for each mRNA.

Brain slice preparation. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) 15 min before being decapitated. Brains were quickly removed from the skull into ice-cold artificial CSF (aCSF) oxygenated with 95% O_2–5% CO_2 and containing the following (in mM): 125 NaCl, 25 $NaHCO_3$, 10 glucose, 3.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, and 3 $MgCl_2$, pH 7.45 (295–300 mOsm). Coronal slices (350 μm thick) containing prelimbic and infralimbic regions of the medial PFC were obtained with a Vibratome in ice-cold aCSF and incubated in warm (~35°C) aCSF solution constantly oxygenated with 95% O_2–5% CO_2 for at least 60 min before recording. The recording aCSF solution was delivered to the recording chamber with a minipump at the rate of 2 ml/min, and $CaCl_2$ and $MgCl_2$ were adjusted to 2 and 1 mM, respectively. Patch electrodes (6–9 MΩ) were obtained from 1.5 mm borosilicate glass capillaries (World Precision Instruments) with a Flaming-Brown horizontal puller (P97; Sutter Instruments) and filled with a solution containing 0.125% Neurobiotin and the following (in mM): 115 K-gluconate, 10 HEPES, 2 $MgCl_2$, 20 KCl, 2 MgATP, 2 Na_2-ATP, and 0.3 GTP, pH 7.25–730 (280–285 mOsm). All chemicals and drugs (quinpirole, eticlopride, CNQX, and picrotoxin) were purchased from Sigma, and they were mixed into oxygenated recording aCSF solution in known concentrations.

Whole-cell patch-clamp recordings. All experiments were conducted at 33–35°C. Medial PFC pyramidal cells and interneurons from layers V–VI were identified under visual guidance using infrared (IR) differential interference contrast video microscopy with a 40× water-immersion objective (Olympus BX51-WI). The image was detected with an IR-sensitive CCD camera (Dage-MTI) and displayed on a monitor. Whole-cell current-clamp recordings were performed with a computer-controlled amplifier (MultiClamp 700A; Molecular Devices), digitized (Digidata 1322; Molecular Devices), and acquired with Axoscope 8.1 (Molecular Devices) at a sampling rate of 10 kHz. The liquid junction potential was not corrected, and electrode potentials were adjusted to zero before obtaining the whole-cell configuration.

Interneuron excitability was assessed by counting the number of action potentials evoked by a 500 ms duration constant-amplitude depolarizing current pulse before and after drug treatment. Typically, current intensity was adjusted to elicit between 5 and 15 action potentials during baseline. In each neuron, input resistance and membrane potential were also monitored throughout the entire recording session.

Synaptic responses were tested in pyramidal neurons with electrical stimulation of layers I–II with a bipolar electrode made from a pair of twisted Teflon-coated nichrome wires (tips separated by ~200 μm) and placed ~1 mm lateral to the vertical axis of the recorded neuron. Stimulation pulses (0.4–0.8 mA; 0.3 ms) were delivered every 20 s, and the

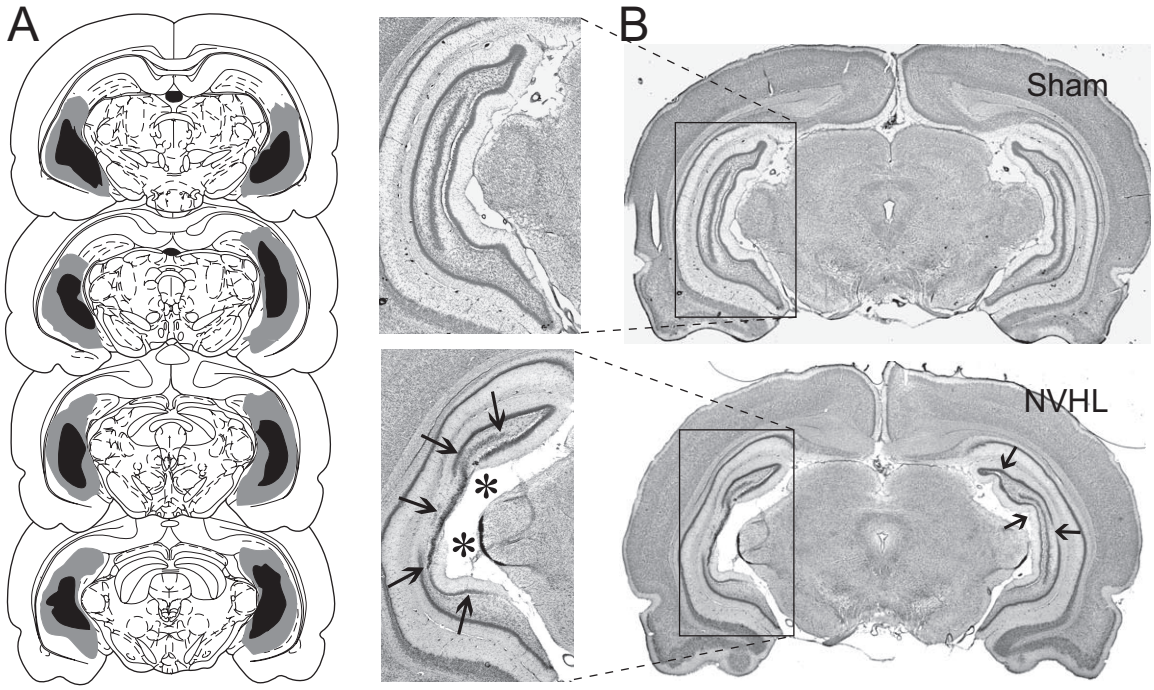

Figure 1. Neonatal ventral hippocampal lesion. ***A***, Drawings showing the extension of the ventral hippocampal damage induced by neonatal ibotenic acid observed in adulthood. Gray and dark areas indicate maximal and minimal extents of damage, respectively. ***B***, Coronal Nissl-stained sections showing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

intensity was adjusted to half the current required to evoke an action potential. If synaptic responses exhibited >15% variation in amplitude during the initial 10 min of recording or the current intensity required was larger than 0.8 mA, the neuron was discarded. Input resistance (measured with hyperpolarizing square pulses), membrane potential, and evoked synaptic responses were analyzed before, during, and after drug application.

Histology. Standard histochemical techniques were used to verify morphology and location of the neurons recorded with Neurobiotin. After completion of the recording session, the entire slices were fixed in 4% paraformaldehyde for 2 h. After a series of rinses in 0.1 M PBS, slices were incubated in 2% Triton X-100 in PBS for 2 h to enhance penetration, followed by 10–12 h in Vectastain Elite ABC reagent (Vector Laboratories) at 4°C. After another series of rinses, slices were reacted with 3,3′ diaminobenzidine and urea-hydrogen peroxide (FAST DAB set; Sigma). Slices were then rinsed, mounted on gelatin-coated slides, air dried for 24 h, cleared in xylene, coverslipped in Permount, and examined on an Olympus CH30 microscope.

Statistics. All measures are expressed as mean ± SD. Drug effects were compared using Student's *t* test or repeated-measures ANOVA, and the differences between experimental conditions were considered statistically significant when $p < 0.05$. In some cases, a two-way ANOVA was used to compare the interactions between experimental groups and the time course of synaptic changes obtained throughout the recording.

Results

Bilateral infusion of ibotenic acid into the ventral hippocampus of neonate rats caused local neuronal loss and cellular disarray that persisted in adulthood. Only rats that showed bilateral anatomical disruption restricted to the ventral hippocampus (Fig. 1), which comprised ~85% of the lesioned animals, were included in the study.

GAD_{67}/PV mRNA expression in the prefrontal cortex of NVHL rats

Ten sham and 10 NVHL rats were used for *in situ* hybridization analyses of GAD_{67} and PV mRNA expression in the PFC. In control animals, the cortical expression patterns of GAD_{67} and PV mRNAs were consistent with previous observations in rodents (de Lecea et al., 1995; Cochran et al., 2003; Lipska et al., 2003; Hashimoto et al., 2005) (Fig. 2*A*,*B*). In all animals, the expression patterns of both mRNAs in the PFC appeared symmetrical between hemispheres. Neither manipulation nor laterality had a significant effect on the expression levels of GAD_{67} ($F_{(1,20)} = 1.649$, $p = 0.214$ for manipulation; $F_{(1,20)} = 0.206$, $p = 0.655$ for laterality) or PV ($F_{(1,20)} = 1.686$, $p = 0.209$ for manipulation; $F_{(1,20)} = 0.005$, $p = 0.942$ for laterality) mRNAs.

Electrophysiological properties of PFC interneurons in NVHL rats

In another set of sham ($n = 22$) and NVHL ($n = 16$) rats, brain slices were prepared for electrophysiological recordings from interneurons and pyramidal neurons. All nonpyramidal neurons ($n = 62$) included in the study were from layers V–VI of the medial PFC (prelimbic and infralimbic regions). Based on their firing pattern, afterhyperpolarization (AHP), and spike-frequency adaptation characteristics (Cauli et al., 1997; Gibson et al., 1999), 27 cells were classified as fast-spiking interneurons (FSIs); those that did not ($n = 35$) were labeled non-fast spiking (NFS) (Fig. 3). FSIs typically responded with constant firing throughout the current pulse (Fig. 3*A*,*B*) and exhibited a larger AHP (19.2 ± 2.9 mV) and faster spike kinetics (0.58 ± 0.08 ms half-width duration) than NFS interneurons (9.4 ± 2.6 mV AHP and 0.9 ± 0.15 ms spike half-width; $p < 0.00001$, unpaired *t* test). FSIs recorded from sham ($n = 14$) and NVHL ($n = 13$) rats exhibited similar resting membrane potential (−62.0 ± 2.0 vs −62.2 ± 1.8 mV, sham vs NVHL) and input resistance (230.5 ± 61.8 vs 231.5 ± 65.5 MΩ, sham vs NVHL). No differences in FSI AHP amplitude (19.2 ± 3.0 vs 19.2 ± 2.9 mV, sham vs NVHL) or half-width duration (5.4 ± 1.7 vs 4.4 ± 1.5 ms, sham vs NVHL) or in action potential kinetics (half-width, 0.58 ± 0.08 vs 0.65 ± 0.09 ms; amplitude, 69.9 ± 5.8 vs 71.3 ± 8.7 mV; spike threshold,

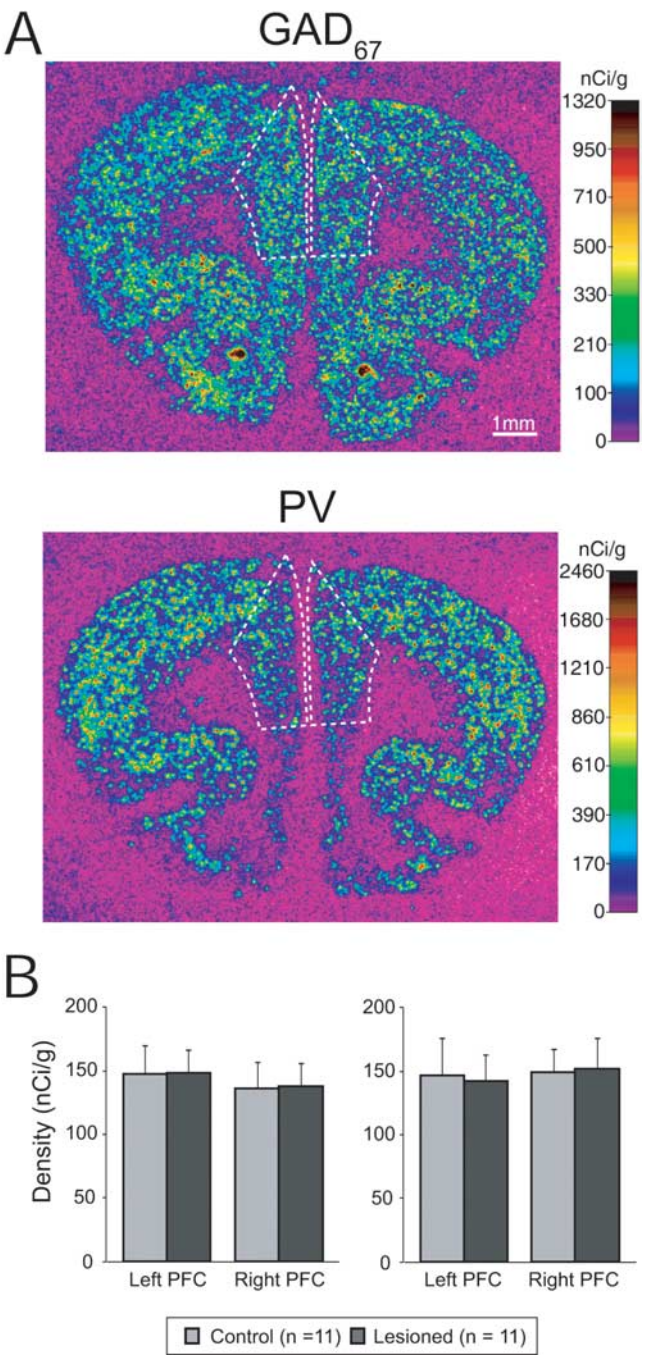

Figure 2. Interneuron markers in the PFC of NVHL rats. ***A***, Representative autoradiograms illustrating the expression of GAD_{67} (top) and PV (bottom) mRNAs in a control rat. The densities of hybridization signals are presented in a pseudocolor manner according to the calibration scales. The densities were quantified in the area indicated by the broken lines. ***B***, Graphs summarizing the mean ± SD for GAD_{67} (left) and PV (right) mRNA levels in right and left PFC of control (gray bars) and NVHL (black bars) rats.

−45.5 ± 1.8 vs −47.0 ± 1.4 mV, sham vs NVHL) were observed between sham and NVHL rats (Fig. 3*C*). Similarly, NFS interneurons recorded from sham ($n = 19$) and NVHL ($n = 16$) rats exhibited similar resting membrane potential (-61.6 ± 4.1 vs. −61.9 ± 3.5 mV, Sham vs. NVHL), input resistance (259.7 ± 55.9 vs 249.7 ± 81.0 MΩ, sham vs NVHL), AHP (half-width duration, 3.2 ± 0.9 vs 3.1 ± 1.0 ms; amplitude, 9.4 ± 2.6 vs 9.9 ± 2.4 mV, sham vs NVHL) and spike kinetics (half-width, 0.91 ± 0.15 vs 0.90 ± 0.17 ms; amplitude, 74.5 ± 6.7 vs 73.4 ± 9.3 mV; spike threshold, −46.1 ± 2.9 vs −46.6 ± 2.6 mV, sham vs NVHL). These results indicate that membrane properties and action potential firing in late adolescent PFC interneurons were not affected by the NVHL.

Postpubertal disruption of PFC interneurons response to quinpirole in NVHL rats

We have shown recently that the D_2 agonist quinpirole enhances FSI excitability in PFC slices from late adolescent and adult (older than P55) but not pre-pubertal (younger than P35) rats (Tseng and O'Donnell, 2007b). In slices from adult rats with a neonatal sham surgery, bath application of quinpirole enhanced interneuron excitability by near 40% ($n = 11$; six FSI and five NFS) (Fig. 4*A*), as observed in naive postpubertal rats (Tseng and O'Donnell, 2007b). The number of spikes evoked by somatic current injection increased from 9.3 ± 2.2 (baseline) to 12.7 ± 2.8 after 10 min of quinpirole incubation ($p < 0.0001$, paired t test) (Fig. 4*A*). This effect was completely blocked by the D_2 antagonist eticlopride (20 μM; $n = 5$), confirming that quinpirole-induced excitability increase was D_2 dependent. Interneuron excitability remained elevated after quinpirole was removed; typically, a washout period of 15–25 min was required to recover to baseline activity. In slices from adult rats with an NVHL, quinpirole failed to increase PFC interneuron excitability ($n = 13$; six FSI and seven NFS). The number of evoked spikes was 10.8 ± 3.1 during baseline and 10.3 ± 3.7 after 10 min of drug application (NS, paired t test) (Fig. 4*B*). Although the majority of interneurons (70%) recorded from NVHL rats did not change excitability after quinpirole, 4 of 13 showed a decrease (Fig. 4*B*). No effects of the D_2 agonist were observed in slices from preadolescent rats with an NVHL or sham operation (Fig. 4*D*,*E*), indicating that the abnormal responses emerge during adolescence (Fig. 4*F*).

NVHL causes the loss of a GABA component contributing to the D_2-mediated inhibition of PFC pyramidal neuron excitatory synaptic response

The attenuation of local excitatory inputs to PFC pyramidal neurons by quinpirole also acquires a mature profile during adolescence attributable to the emergence of a D_2-dependent enhancement of interneuron excitability (Tseng and O'Donnell, 2007a). If the D_2 modulation of PFC interneurons is compromised in NVHL animals, such late component would not be present in late adolescent or adult PFC slices. We examined the effect of quinpirole on synaptic responses evoked in pyramidal neurons by stimulation of layers I–II in slices from sham and NVHL rats. All pyramidal neurons included here were labeled with Neurobiotin to confirm their location and morphology. Stimulation of superficial layers with an electrode placed ~1 mm lateral to the axis of the apical dendrite of the recorded neuron evoked a fast depolarizing AMPA-mediated EPSP. The monosynaptic nature of the evoked response was identified by the short and constant latency (~5 ms) to postsynaptic potential onset. The AMPA antagonist CNQX eliminated the evoked response (data not shown). In similar experiments performed in slices from naive rats, the evoked response remained unchanged after perfusing the slice with the $GABA_A$ antagonists picrotoxin or bicuculline, indicating absence of a detectable fast GABAergic component in this response (Tseng and O'Donnell, 2007a). Bath application of quinpirole (2 μM) reduced EPSP amplitude by ~20%, in both postpubertal sham ($n = 6$) and NVHL ($n = 7$) rats (Fig. 5*B*,*C*). As reported previously (Tseng and O'Donnell, 2007a), this effect was completely blocked with the D_2 receptor antagonist eticlopride ($n =$

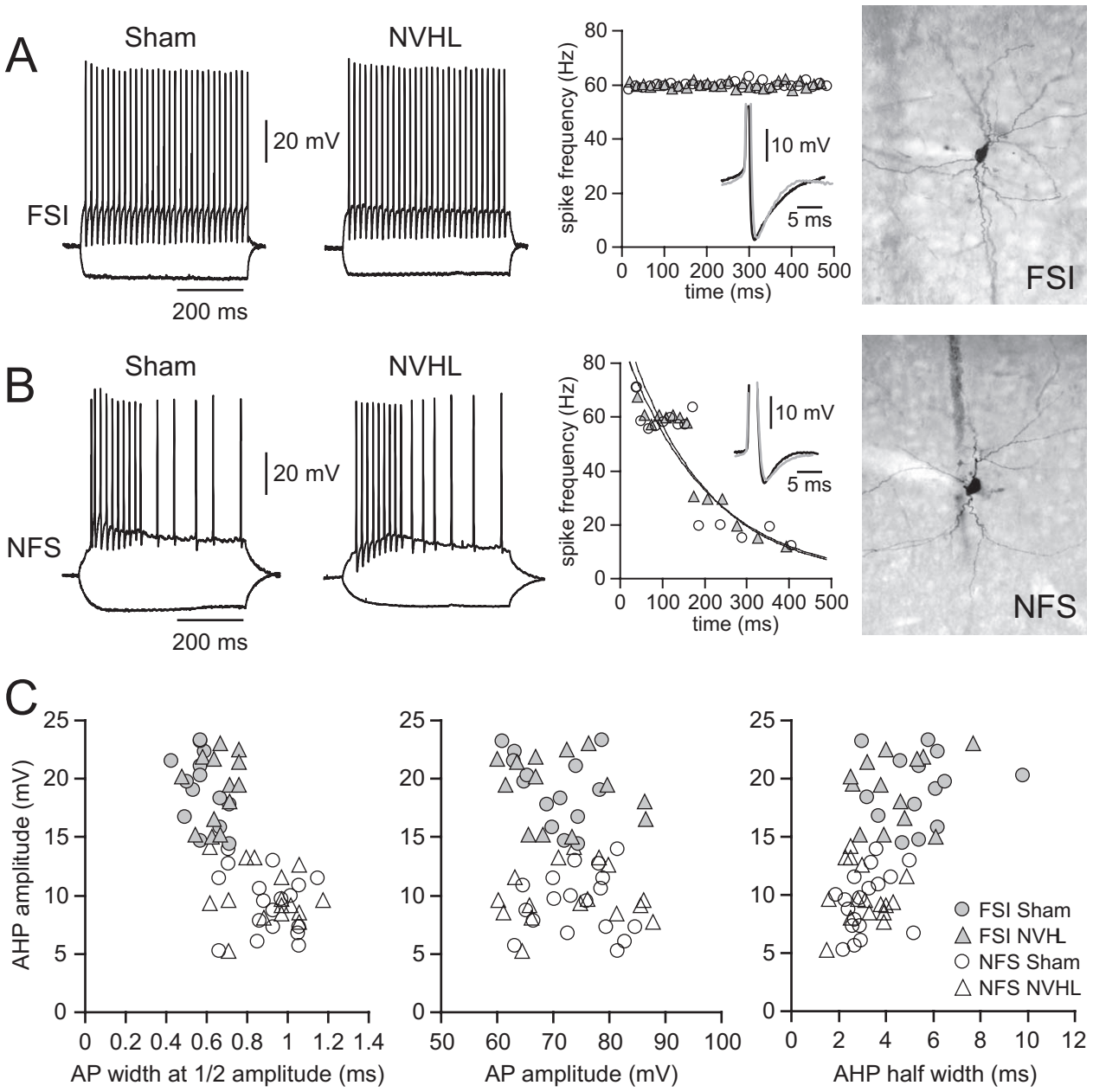

Figure 3. Basic membrane properties of PFC interneurons in NVHL and sham rats. ***A***, Traces illustrating the firing pattern of FSIs in PFC slices from developmentally mature (older than P60) sham and NVHL rats. FSIs exhibited a pronounced fast AHP (middle panel inset) and responded with a constant instantaneous firing rate throughout the current pulse (middle panel: no spike-frequency adaptation). Right, Neurobiotin staining of the FSIs from which the traces were obtained. ***B***, Traces from an NFS interneuron, which instead show increasingly longer interspike intervals when activated with current injection (left and middle panels). Right, Neurobiotin staining of the interneuron recorded. ***C***, Plots of AHP amplitude, half-width, and action potential amplitudes and duration for both cell types.

7), confirming that quinpirole-induced synaptic attenuation is mediated by D_2 receptors (data not shown). However, the duration of this inhibition was significantly longer in sham rats compared with the lesioned group (Fig. 5*D*). Pyramidal neurons recorded from NVHL rats recovered to baseline amplitude by ~10 min after quinpirole was removed, whereas a washout period of at least 25 min was required in the sham group. This persistent post-quinpirole effect in sham rats could be reduced to ~10 min with the $GABA_A$ antagonist picrotoxin (10 μM; $n = 6$). The data suggest that part of the inhibitory action of D_2 receptors on pyramidal neuron excitatory transmission is also compromised in the PFC of late adolescent and adult NVHL rats, particularly the activation of local GABAergic interneurons.

Discussion

Although adolescent and adult rats with an NVHL did not show reduced levels of GAD_{67} or PV, their PFC interneurons had abnormal dopaminergic modulation. The D_2 agonist quinpirole failed to increase cell excitability in FSIs and NFS interneurons from adult rats with an NVHL, and, in many cases, the neurons showed an abnormal decrease in excitability. The lack of activation of GABA interneurons by quinpirole was also reflected in the absence of a late, $GABA_A$-dependent component in the D_2 modulation of synaptic responses within the PFC in slices from NVHL rats. The data suggest that the periadolescent maturation of interneuron function is altered in NVHL rats, even if there is no actual loss of this cell population.

The use of a lesion as a schizophrenia model does require some clarification. If the model has any claim to construct validity, it cannot reside in the lesion as schizophrenia patients to not present a lesion. Because many studies suggest an abnormal PFC in NVHL rats and inactivating the medial PFC reverses behavioral and electrophysiological deficits (Lipska et al., 1998; Goto and O'Donnell, 2004), it is possible that the critical aspect of the NVHL model is an altered PFC function. Our data indicate that the periadolescent maturation of PFC interneurons becomes abnormal as a consequence of the lesion; therefore, we propose that the construct validity in this model arises from the cortical hyperactivity it causes via impaired inhibitory mechanisms. These changes could alter the spatial selectivity of PFC neuronal response to excitatory inputs by setting inappropriate coordination between pyramidal neurons and GABAergic interneurons, which in turn may be associated with the abnormal behavioral performance in NVHL animals.

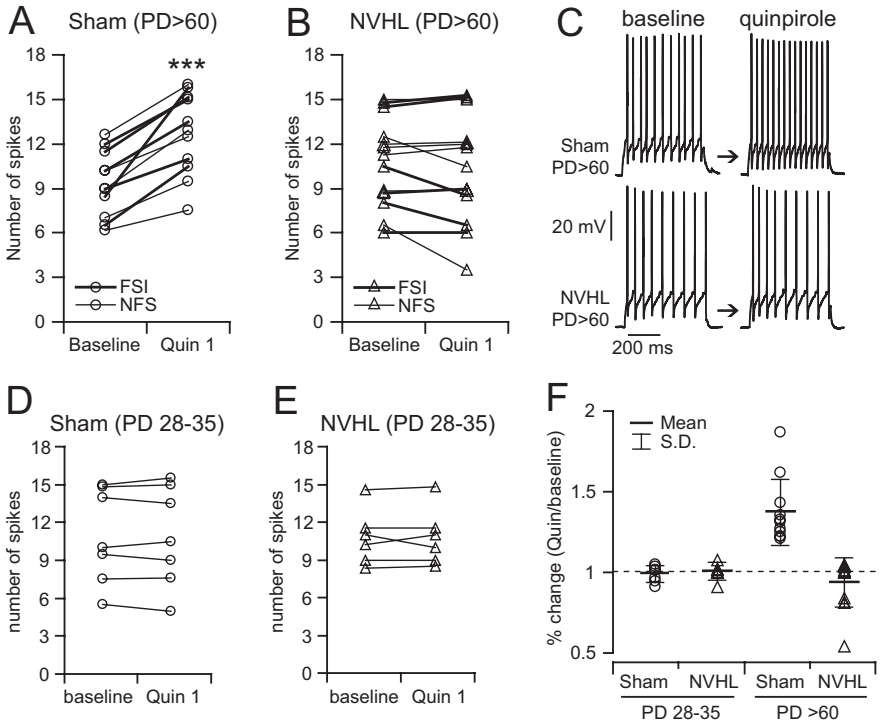

Figure 4. Abnormal maturation of PFC interneurons in NVHL rats. ***A***, ***B***, Plot showing the effect of quinpirole (1 μM) on PFC interneuron excitability in slices from developmentally mature [older than P60 (PD>60)] sham (***A***) and NVHL (***B***) rats. The excitatory action of quinpirole was observed only in the PFC of adult sham rats. In the lesioned animals, quinpirole failed to increase PFC interneuron excitability. Instead, some neurons were inhibited with quinpirole. ***C***, Representative traces illustrating the increase in excitability in a neuron from a sham rat (top) and a neuron from an NVHL rat (bottom). ***D***, ***E***, Plot showing the lack of effect of quinpirole (1 μM) in prepubertal (younger than P35) sham (***D***) and NVHL (***E***) rats. ***F***, Plot of normalized responses in cell excitability changes by quinpirole in all groups. An excitatory action of quinpirole was observed only in the PFC of adult sham rats. In contrast, the majority of adult NVHL interneurons (70%) recorded remained unchanged after quinpirole, resembling the response observed in the PFC of prepubertal rats, whereas others showed a decrease in excitability.

The deficits in PFC interneurons we observed in slices from NVHL rats are functional. There were no differences in PV and GAD_{67} mRNA expression between lesion and sham rats, suggesting that the full complement of GABA neurons, and of PV-containing FSIs in particular, are present in the lesion animals. This finding contrasts with a previous report of reduced GAD_{67} in the PFC of NVHL rats (Lipska et al., 2003). It is possible that the lesion affects PFC interneurons, rendering them into a vulnerable state. Because the expression of both GAD_{67} and PV is activity dependent, it could change with certain conditions (e.g., housing status of the animal and other factors). Many developmental models have reported reductions in PV and/or GAD_{67} without actual interneuron loss (Pillai-Nair et al., 2005; Cabungcal et al., 2006). Although neither the total number of PFC neurons (Thune et al., 2001) nor the densities of PV-immunoreactive (Woo et al., 1997) and PV mRNA-containing (Hashimoto et al., 2003) neurons are reduced in the PFC in schizophrenia, only 55% of PV mRNA-positive neurons expressed detectable levels of GAD_{67} mRNA (Hashimoto et al., 2003). Together, these findings suggest that the interneurons may be present but do not express the normal profile of GABA-related markers. Thus, because these markers are activity dependent, they may be reduced to different extents depending on the condition of the system. The altered modulation by the D_2 agonist without detectable changes in GAD_{67} or PV mRNA expression reported here makes a strong case for abnormal functionality in PFC circuits in this developmental model.

Why does an early hippocampal alteration affect postnatal maturation of PFC interneurons? Although mechanisms can only be speculated on at this time, evidence suggests that altered prefrontal cortical maturation could be attributable to an early downregulation of neurotrophic factors that may depend on hippocampal inputs. First, unpublished observations from our laboratory using DiI injections into the early postnatal ventral hippocampus indicate that the time at which the lesions were done here is when hippocampal fibers are arriving into the PFC, suggesting that both formation of new synapses and the impact of trophic factors released by these fibers may be altered in NVHL. Indeed, the NVHL reduces cortical levels of BDNF (Lipska et al., 2001) and nurr77 (Bhardwaj et al., 2003). Any detrimental effect the impairment of hippocampal inputs may have on PFC circuitry may not be evident until interneurons become fully functional. Our recent work revealed that responses of PFC interneurons to dopamine do not mature until late adolescence (Tseng and O'Donnell, 2007b), which may explain the delayed onset of prefrontal deficits in NVHL rats.

The cellular mechanisms responsible for the abnormal D_2 response in NVHL interneurons are not clear. Although speculative, the abnormal D_2 response could be attributable to altered expression of D_2-like (i.e., D_4) receptors, to recruitment of a different type of G-protein (G_q vs G_i), or to changes in the heterodimerization of receptors in these neurons. DA (in particular D_1 and D_4) receptor expression changes postnatally in rodents (Tarazi and Baldessarini, 2000) and humans (Weickert et al., 2007). Furthermore, the activity of catechol-*O*-methyl transferase, the enzyme responsible for inactivating DA in the PFC, also increased postnatally into the young adult ages (Tunbridge et al., 2007). More research is needed to unveil the mechanisms that may cause abnormal maturation of interneurons in NVHL animals.

The loss of functionality in cortical interneurons seems to be a common finding in schizophrenia research. Individuals with

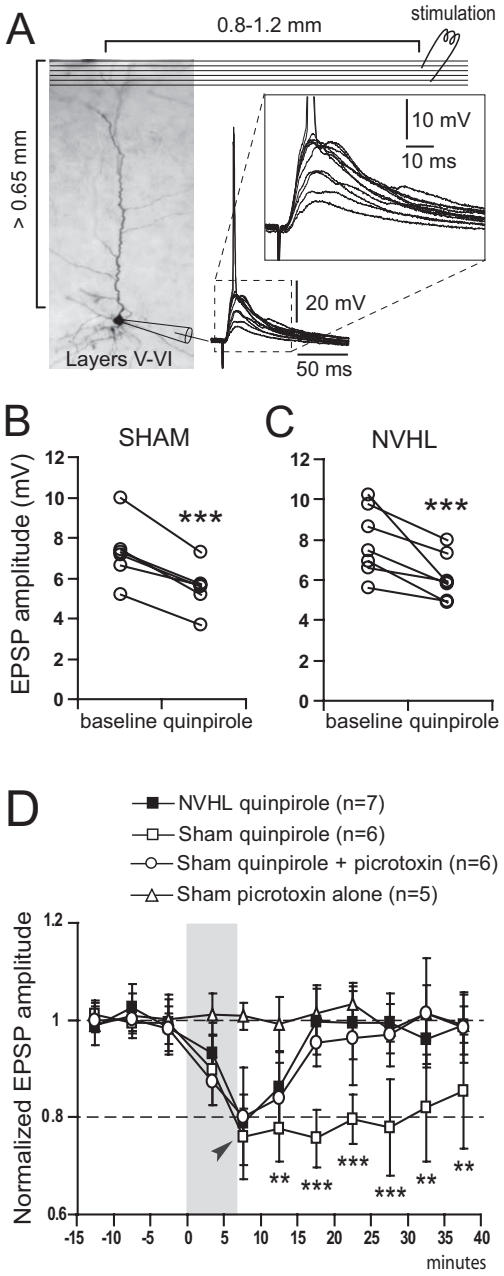

Figure 5. Loss of a D_2-dependent GABA modulation of synaptic responses of pyramidal neurons in NVHL rats. ***A***, Electrical stimulation of layers I–II in the medial PFC of NVHL elicited a primarily AMPA-dependent EPSP in deep layer pyramidal neurons. Diagram illustrating the arrangement of stimulating electrode (top right; layers I–II) and recording sites (bottom left; layers V–VI), along with an overlay of EPSPs recorded in the pyramidal neuron. Inset shows EPSPs at higher magnification. ***B***, Diagram illustrating the decrease in EPSP amplitude by quinpirole (2 μM) in slices from sham rats. ***C***, Plot illustrating the effects of quinpirole in slices from NVHL rats. ***D***, Plot summarizing the time course of these effects. The D_2-dependent synaptic attenuation in pyramidal neurons was persistent in the PFC of sham rats (open squares) but not in NVHL rats (filled squares). The $GABA_A$ antagonist picrotoxin blocked the persistent component of this D_2 effect in slices from sham rats (open circles) but did not affect the response when given alone (open triangles). Thus, the D_2 modulation of corticocortical synaptic responses in adult NVHL rats lacks the recruitment of interneurons. $^*p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.0005$; quinpirole versus quinpirole plus picrotoxin.

schizophrenia do present reduced GABA-related transcripts (Hashimoto et al., 2008b), and clinical neurophysiology studies reveal abnormal patterns associated with poor interneuron performance, such as a loss or reduction in evoked or induced gamma oscillations (Spencer et al., 2003, 2008; Hong et al., 2004; Cho et al., 2006; Ferrarelli et al., 2008). Similar loss of beta/gamma frequency bands is observed with noncompeting NMDA antagonists (Roopun et al., 2008), a model known to target primarily interneuron function (Homayoun and Moghaddam, 2007). Many of those findings revealed absence of high-frequency oscillations that were task dependent. Our finding that abnormal interneuron function is evident when DA agonists are applied implies that, in NVHL rats, when DA cells fire in response to unexpected reward or reward-predicting stimuli (Schultz et al., 1993), the balance between excitation and inhibition in PFC circuits will be altered. Thus, the lack of activation of interneurons during brief high-DA epochs (typically related to the presence of salient stimuli) would contribute to noisy information processing in the PFC and poor assignment of saliency to sensory information associated with the surge in DA as well as impaired working memory functions. Because the symptoms of schizophrenia are broad, it is conceivable that whether cognitive deficits or positive or negative symptoms dominate depends on the cortical region primarily affected by the developmental alteration of interneurons. The cortical functional deficits in NVHL rats do bear some resemblance to current views on schizophrenia pathophysiology, and what makes this model appealing is the emergence of these deficits during adolescence. In summary, the altered periadolescent maturation of PFC interneurons in NVHL rats may reproduce the impact of deleterious combination of predisposing genes and environmental factors that may be present in schizophrenia.

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