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Early Prefrontal Functional Blockade in Rats Results in Schizophrenia-Related Anomalies in Behavior and Dopamine

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Growing evidence suggests schizophrenia may arise from abnormalities in early brain development. The prefrontal cortex (PFC) stands out as one of the main regions affected in schizophrenia. Latent inhibition, an interesting cognitive marker for schizophrenia, has been found in some studies to be reduced in acute patients. It is generally widely accepted that there is a dopaminergic dysfunctioning in schizophrenia. Moreover, several authors have reported that the psychostimulant, D-amphetamine (D-AMP), exacerbates symptoms in patients with schizophrenia. We explored in rats the effects in adulthood of neonatal transient inactivation of the PFC on behavioral and neurochemical anomalies associated with schizophrenia. Following tetrodotoxin (TTX) inactivation of the left PFC at postnatal day 8, latent inhibition-related dopaminergic responses and dopaminergic reactivity to D-AMP were monitored using *in vivo* voltammetry in the left core part of the nucleus accumbens in adult freely moving rats. Dopaminergic responses and behavioral responses were followed in parallel. Prefrontal neonatal inactivation resulted in disrupted behavioral responses of latent inhibition and latent inhibition-related dopaminergic responses in the core subregion. After D-AMP challenge, the highest dose (1.5 mg/kg i.p.) induced a greater dopamine increase in the core in rats microinjected with TTX, and a parallel increase in locomotor activity, suggesting that following prefrontal neonatal TTX inactivation animals display a greater behavioral and dopaminergic reactivity to D-AMP. Transitory inactivation of the PFC early in the postnatal developmental period leads to behavioral and neurochemical changes in adulthood that are meaningful for schizophrenia modeling. The data obtained may help our understanding of the pathophysiology of this disabling disorder.
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INTRODUCTION

The prefrontal cortex (PFC) stands out as one of the main regions affected in schizophrenia. In the past two decades, several studies have described cytoarchitectural and neuronal morphometric abnormalities consistent with defects in early neurodevelopment at the level of the PFC, particularly in the left hemisphere (Kalus *et al*, 2000; Garey, 2010; Yang *et al*, 2011). In schizophrenia, miswiring of neuronal connections due at least in some cases to neurodevelopmental failures would result in disconnectivity between different integrative brain regions, especially the PFC, temporal lobe, and striatal regions (Lawrie *et al*, 2002; Stephan *et al*, 2009). This disconnectivity may cause the psychic disintegration characteristic of the disease.

It is generally widely accepted that there is striatal dopaminergic dysfunctioning in schizophrenia (eg, Carlsson *et al*, 2001). Consistent with this proposal, a larger striatal dopamine release following administration of D-amphetamine (D-AMP) associated with the emergence or increase of positive symptomatology was observed in unmedicated patients with schizophrenia, in comparison with healthy controls (see Lyon *et al*, 2011 for review). It is also interesting to note that an improvement in cognitive function has been reported in medicated patients with schizophrenia after D-AMP administration (Barch and Carter, 2005). Latent inhibition, an interesting cognitive marker for schizophrenia, has also been found to be reduced in acute patients with schizophrenia (Baruch *et al*, 1988; Gray *et al*, 1995; Rascle *et al*, 2001; but see Swerdlow *et al*, 1996), whereas in chronic patients it has been found to be either enhanced (Rascle *et al*, 2001) or normal (see Kumari and Ettinger, 2010; Swerdlow, 2010 for

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reviews). Latent inhibition, as first described by Lubow and Moore (1959), is a behavioral phenomenon observed in many animal species. It was originally defined as retarded acquisition of the conditioned response (CR) when the conditional stimulus (CS) is first pre-exposed on its own. However, more recent theoretical explanations also lend support to the view that latent inhibition is a performance deficit characterized by a reduction or even disappearance of the CR, when the CS is presented without reinforcement, before conditioning (see De la Casa and Pineño, 2010 for review). Recent evidence from *in vivo* animal studies backs the existence of a differential involvement of dopaminergic neurons innervating the core and dorsomedial shell subregions of the nucleus accumbens in this phenomenon (Jeanblanc *et al*, 2002; Louilot *et al*, 2010). Moreover, as shown recently, unilateral neonatal functional blockade in the left hemisphere of two temporal regions linked to the PFC, the entorhinal cortex, or the ventral subiculum of the hippocampus (Jay and Witter, 1991; Hoover and Vertes, 2007) have been found to be sufficient to induce a disruption of the behavioral manifestation of latent inhibition and the latent inhibition-related dopaminergic variations in adult animals, with a differential reversal of these responses in the left core part of the nucleus accumbens (Peterschmitt *et al*, 2007; Meyer and Louilot, 2011).

The present study was designed to investigate, in adult rats, the consequences of neonatal functional inactivation of the left PFC for two schizophrenia-related markers: latent inhibition expression and reactivity to D-AMP. To that end, and given the aforementioned data, we performed a functional neonatal blockade of the left PFC by locally microinjecting tetrodotoxin (TTX), a well-known $Na+$ channel blocker, at postnatal day 8 (PND8). This point in time in the postnatal neurodevelopmental period in rats is comparable to the middle of the second trimester of gestation in humans (Clancy *et al*, 2001), considered a time window of high vulnerability for developing schizophrenia (Weinberger and Lipska, 1995; Fatemi and Folsom, 2009). TTX interrupts neuronal activity whereas, during development, impulse electrical activity is crucial for shaping connections, once developing fibers reach the target structure (Stryker and Harris, 1986; Katz and Shatz, 1996; Drakew *et al*, 1999; Hutchins and Kalil, 2008; see also Spitzer, 2006). D-AMP behavioral reactivity was assessed by measuring locomotor activity, which is considered as a good marker for animal modeling for schizophrenia (Lipska and Weinberger, 2000). The core part of the nucleus accumbens was chosen for dopamine measurements as it has been reported that it sends direct efferent connections to the motor outputs (eg, Zahm, 1999). Latent inhibition-related dopaminergic responses and D-AMP-induced dopamine release were monitored in the left core alongside behavioral responses using *in vivo* voltammetry in freely moving adult animals (11 weeks).

MATERIALS AND METHODS

Animals and Experimental Design

Animals were housed at $22 \pm 2\,^{\circ}C$ and fed *ad libitum*. Sprague–Dawley female rats (Janvier, Le Genest, France), obtained at 14 days gestation, were housed individually on a 12-h light/dark cycle (lights on at 0800 hours). The size of the litters was limited at birth (PND0) in 12 animals. The supernumerary neonates were given a lethal injection of pentobarbital. On PND8, half of the litter, chosen at random received a PBS microinjection (control groups) and the other half received a TTX microinjection (experimental groups). On PND56 male animals were individually housed on a 12-h reversed light/dark cycle (lights off at 1100 hours). On PND70, the grown-up male rats were surgically implanted with a microsystem designed for monitoring behavior and voltammetric responses in parallel. All the behavioral procedures commenced at least 1 week after implantation of the microsystem in the adult animals. All the experiments were performed during the dark phase of the light/dark cycle. A total of 128 male rats were used in the present study. All the experimental procedures were conducted in accordance with the European Community guidelines for the care and use of experimental animals (Council Directive 86/609/EEC) and authorized by the French Ministry of Agriculture (Authorization 67-244).

Surgery

Neonate surgery. On PND8, rat pups (weight $18.8 \pm 0.2\,g$) were anaesthetized by gas anesthesia by vaporizing isoflurane (Forene, ABBOTT, Rungis, France; for further details see Peterschmitt *et al*, 2007; Meyer *et al*, 2009; Meyer and Louilot, 2011). A stainless steel guide cannula (30 gauge, 12.5 mm length, Small Parts, Miami) was lowered into the PFC at coordinates 1.5 mm relative to bregma (AP), 0.4 mm lateral to the midline (L), and 3.9 mm below the cortical surface (H). PBS (NaCl 8 g/l, KCl 0.2 g/l, $MgCl_2$, $6H_2O$ 0.1 g/l, KH_2PO_4 0.2 g/l, Na_2HPO_4, $2H_2O$ 1.15 g/l, pH: 7.4) or TTX (Sigma, St Quentin-Fallavier, France) dissolved in PBS were infused in a volume of 0.3 µl for a period of 135 s using an infusion pump (Razel, Stamford, CT). The cannula was left in the PFC for 4 min after the end of the microinjection to allow PBS and TTX to diffuse in the targeted structure. The amount of TTX microinjected in the PFC ($100\,\mu M \times 0.3\,\mu l$), approximately 10 ng, is similar to that reported previously in the literature (Zhuravin and Bures, 1991; Peterschmitt *et al*, 2007; Meyer *et al*, 2009; Meyer and Louilot, 2011). It has been reported that the effects resulting from the TTX blockade last 24–48 h (Rothfeld *et al*, 1986). Zhuravin and Bures (1991) reported that microinjection of 10 ng TTX in a total volume of 1 µl at a rate of 1 µl/min has no functional consequences beyond 1 mm/3 h after the microinfusion. Pilot experiments we performed showed that TTX microinjected in neonates in the cortex 1 mm above the infralimbic/prelimbic region of the PFC does not change latent inhibition expression in adult animals. Moreover, according to Malpeli (1999), if the radius of spread varies as the cube root of the volume, the efficient spread for a volume of 0.3 µl TTX is only 0.67 mm, corresponding to an efficient spread of 1 mm for a volume of 1 µl TTX, according to Zhuravin and Bures (1991). Further more, in the Zhuravin and Bures experiment the microinjection speed was 1 µl/min, whereas in our study the infusion speed was 0.3 µl/135 s. This slow injection speed could result in a more limited functional effective spread as has been observed with a slow infusion speed (1 µl/3 min) of lidocaïne, which is more diffusible than TTX (Albert and Madryga, 1980; Zhuravin and Bures, 1991).

The microinjection site in the PFC was identified by postmortem using Evans blue (Sigma), a vital dye added to the solutions of PBS and TTX, and reported as remaining visible in the cerebral tissue several weeks after injection (Martin and Ghez, 1999; Peterschmitt *et al*, 2007; Meyer *et al*, 2009; Brooks *et al*, 2011; Meyer and Louilot, 2011). Each rat pup was identified by means of small three-digit ear tags (Ref 52-4716, Harvard Apparatus, Les Ulis, France). After surgery all of the pups microinjected with either PBS or TTX were placed under a warming lamp and then returned to their mother.

Adult surgery. On PND70, following anesthesia by chloral hydrate (400 mg/kg i.p.) and after being placed in a stereotaxic apparatus (incisor bar set at 3.3 mm below the interaural line; Unimécamique, Epinay/Seine, France), the fully grown male rats (weight 400 ± 25 *g*) were implanted in the left core part of the nucleus accumbens at coordinates 10.2 mm (AP), 1.8 mm (L), and 6.9 mm (H; Paxinos and Watson, 2009), with a specially designed microsystem (Unimécanique; Louilot *et al*, 1987) allowing to monitor behavioral and dopaminergic responses in parallel. After surgery animals were given 7–8 days to recover.

Behavior Analysis

Latent inhibition

Procedure: A total of 70 animals were used for the latent inhibition experiment. Latent inhibition was measured in a three-stage latent inhibition paradigm involving a conditioned olfactory aversion procedure, with banana odor (amyl acetate, Prolabo, Strasbourg, France) as the conditional olfactory stimulus (CS), and LiCl-induced aversion as the unconditional stimulus, as previously reported (Jeanblanc *et al*, 2002; Peterschmitt *et al*, 2007; Louilot *et al*, 2010; Meyer and Louilot, 2011). All pre-exposed and non-pre-exposed animals were microinjected at PND8 with either PBS or TTX in the PFC as indicated before (see neonate surgery). After being implanted with the microsystem, the adult rats were randomly split up into two pre-exposed control groups (pre-exposed PBS-NaCl; pre-exposed TTX-NaCl), two pre-exposed-conditioned groups (pre-exposed PBS-LiCl; pre-exposed TTX-LiCl), two non-pre-exposed control groups (non-pre-exposed PBS-NaCl; non-pre-exposed TTX-NaCl), and two non-pre-exposed-conditioned groups (non-pre-exposed PBS-LiCl; non-pre-exposed TTX-LiCl).

Data analysis: The animals' position in the experimental cage (24 cm wide × 27 cm long × 44 cm high) was followed using a small infrared camera (Ref. 51.8050, CA-H34C, Selectronic, Lille, France) inserted into the ceiling of the cage and linked up to a video monitor and videotape. The banana odor was fed into the cage through a hole in the wall adjacent to the cage door. More precisely, the olfactory stimulus (amyl acetate) was applied to a ball of cotton wool placed in the bottom of a vial, which could be quickly inserted in the hole. Attraction or aversion to the olfactory stimulus was evaluated by the amount of time spent near the olfactive source. The cage floor was divided empirically into two virtual zones. One, containing the hole, was a semicircle accounting for 35% of the total surface area. The rest of the floor made up the second zone. The behavior was analyzed over 10-min periods. It was assumed that an animal moving about the cage at random should spend 35% (210 s) of the 10-min period in the zone containing the hole. Results are expressed as mean ± SEM of the time spent near the hole.

D-AMP-induced locomotor activity.

Procedure: A total of 58 animals were used for the D-AMP experiment. D-AMP-sulfate (Sigma) was dissolved in 0.9% saline, with D-AMP solutions freshly prepared before administration. Adult rats from the same litter were spread across the two controls groups (PBS-NaCl; TTX-NaCl), the two groups injected with D-AMP 0.75 mg/kg (PBS-D-AMP 0.75 mg/kg; TTX-D-AMP 0.75 mg/kg) and the two groups injected with D-AMP 1.5 mg/kg (PBS-D-AMP 1.5 mg/kg; TTX-D-AMP 1.5 mg/kg). Animals were habituated to the experimental cage (24 cm wide × 27 cm long × 44 cm high) for 1 h, after which they received, randomly, either an i.p. injection of NaCl (0.9%) or an i.p. injection of one of the two doses (0.75 mg/kg or 1.5 mg/kg) of D-AMP chosen from the studies that showed that D-AMP administered in this range induces a marked increase in locomotor activity (Sharp *et al*, 1987; Louilot and Choulli, 1997), a good index for animal modeling for schizophrenia (Lipska and Weinberger, 2000). The animals then remained for 90 min in the experimental cage during which their locomotor activity was assessed.

Data Analysis: The behavior of the animals in the experimental cage was monitored using a small infrared camera placed in the top of the cage and connected to a video monitor and videotape. The floor of the cage was divided into four equal virtual quadrants, so that locomotor activity could be measured by directly observing each animal via a video recording and counting the number of times it crossed from one quadrant to another in each 10-min period. Data are expressed as mean ± SEM.

Voltammetric Analysis of the Dopaminergic Signal

The electrochemical procedures were those previously described (Gonzalez-Mora *et al*, 1991; Peterschmitt *et al*, 2007; Meyer *et al*, 2009; Meyer and Louilot, 2011). Differential normal pulse voltammetry (see O'Neill *et al*, 1998 for review), combined with the computerized waveform analysis of the catechol peak, was used to obtain the selective detection of the extracellular dopamine levels in the core part of the nucleus accumbens. It is unlikely that any of the change we observed in the voltammetric dopaminergic signal is due to the oxidation of noradrenaline, particularly because noradrenergic innervation has been found to be largely absent from the core part of the nucleus accumbens (Berridge *et al*, 1997). DNP voltammograms were recorded every minute. The average amplitude of the last 10 peaks (last 10 min) of dopamine signals obtained during the control period (variation of voltammetric signal $<10\%$) was calculated for each animal and set at 100%. Voltammetric variations in the dopamine signal in the core subregion of the nucleus accumbens, recorded min by min, are expressed as

percentages (mean ± SEM) of the mean values before exposure to the olfactory stimulus or to the i.p. injection (NaCl 0.9% or D-AMP). Only variations obtained every 2 min are shown on the graphs.

Statistics

Statistical analysis was performed using a multifactorial ANOVA analysis with repeated measurements on the time factor. Only between-subject ANOVAs are shown unless otherwise indicated. For both the latent inhibition and D-AMP experiments, possible litter effects were first tested. For the latent inhibition experiment, between-subject grouping factors were the conditioning factor with two levels (NaCl, LiCl), microinjection factor with two levels (PBS, TTX), and pre-exposure factor with two levels (pre-exposed, non-pre-exposed). The dependent variables were the time spent near the hole for the behavioral study and the dopaminergic variations for the voltammetric study. For the D-AMP experiment, between-subject grouping factors were D-AMP doses with three levels (NaCl; 0.75 mg/kg; 1.5 mg/kg) and microinjection with two levels (PBS, TTX). The dependent variables were the number of crossings for the behavioral study and the dopaminergic variations for the voltammetric study. For both experiments (latent inhibition, D-AMP response), contrast analysis of the ANOVA was used to test specific hypotheses (see Rosenthal and Rosnow, 1985; Rosenthal *et al*, 2000). Statistical significance was set at $P<0.05$ for all analyses.

Histology

At the end of each experiment, the rats underwent electrocoagulation (see Meyer *et al*, 2009 for details), before receiving a lethal i.p. injection of pentobarbital and then being intracardially perfused, first with NaCl 0.9% and then with a 4% paraformaldehyde solution. Their brains were quickly removed from their skull. The postnatal microinjection site in the left PFC was visualized with the vital dye Evans blue and neutral red staining of the PFC sections, whereas the voltammetric recording site in the left core part of the nucleus accumbens was checked by staining the brain sections with thionin blue. Paxinos and Watson's atlas (Paxinos and Watson, 1998; 2009) was used as a reference.

RESULTS

Histology

The qualitative macroscopic observations of brain sections at the level of the left PFC and left core part of the nucleus accumbens displayed no gliosis or anatomical changes between the animals microinjected with PBS or TTX in the PFC at PND8 (Figure 1).

Latent Inhibition

Behavioral study (retention session). To summarize, pre-exposed PBS animals microinjected at PND8 in the left PFC displayed no significant conditioning effect, unlike other groups. The conditioning effects obtained for pre-exposed TTX animals and non-pre-exposed TTX animals were similar. Time spent near the hole differed statistically for pre-exposed PBS-conditioned animals and pre-exposed TTX-conditioned animals (Figure 2).

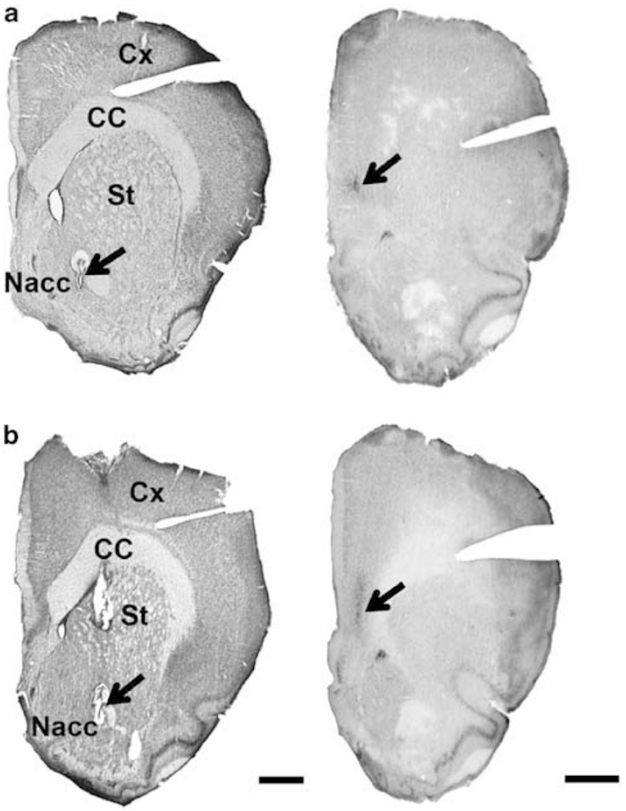

Figure 1 Microphotographs illustrating typical recording sites in the core part of the left nucleus accumbens (left panels) and typical injection sites in the left prefrontal cortex (right panels) of animals microinjected at postnatal day 8 with PBS (a) or tetrodotoxin (TTX) (b). Small arrows (left panels) indicate the location of tracks made by the recording electrodes following electrocoagulation at the end of the experiment. The microinjection sites in the left prefrontal cortex were identified by means of the vital dye Evans blue (arrows, right panels). The locations of both, recording sites and microinjection sites, were checked histologically by thionin blue and neutral red staining of sections, respectively. Scale bar = 1 mm. CC, corpus callosum; Cx, cortex; Nacc, nucleus accumbens; St, striatum.

The ANOVAs for the full hour following animals' exposure to the banana odor showed no significant litter effects ($F[10,59]=1.53$, NS) but revealed significant effects of conditioning (NaCl/LiCl; $F[1,62]=18.07$; $P<0.0001$), and microinjection (PBS/TTX; $F[1,62]=6.96$; $P<0.05$), as well as a significant interaction of microinjection × conditioning ($F[1,62]=6.71$; $P<0.05$), whereas no significant effects were found for pre-exposure or the other interactions (microinjection × pre-exposure, pre-exposure × conditioning, microinjection × pre-exposure × conditioning). Furthermore, contrast analysis of ANOVA for the time spent near the olfactory source during the full hour following stimulus presentation showed a significant conditioning effect for the non-pre-exposed-PBS groups (NPE-PBS-NaCl/NPE-PBS-LiCl; $F[1,62]=7.09$; $P<0.01$), non-pre-exposed-TTX groups (NPE-TTX-NaCl/NPE-TTX-LiCl; $F[1,62]=9.46$; $P<0.005$), and pre-exposed-TTX animals (PE-TTX-NaCl/PE-TTX-LiCl;

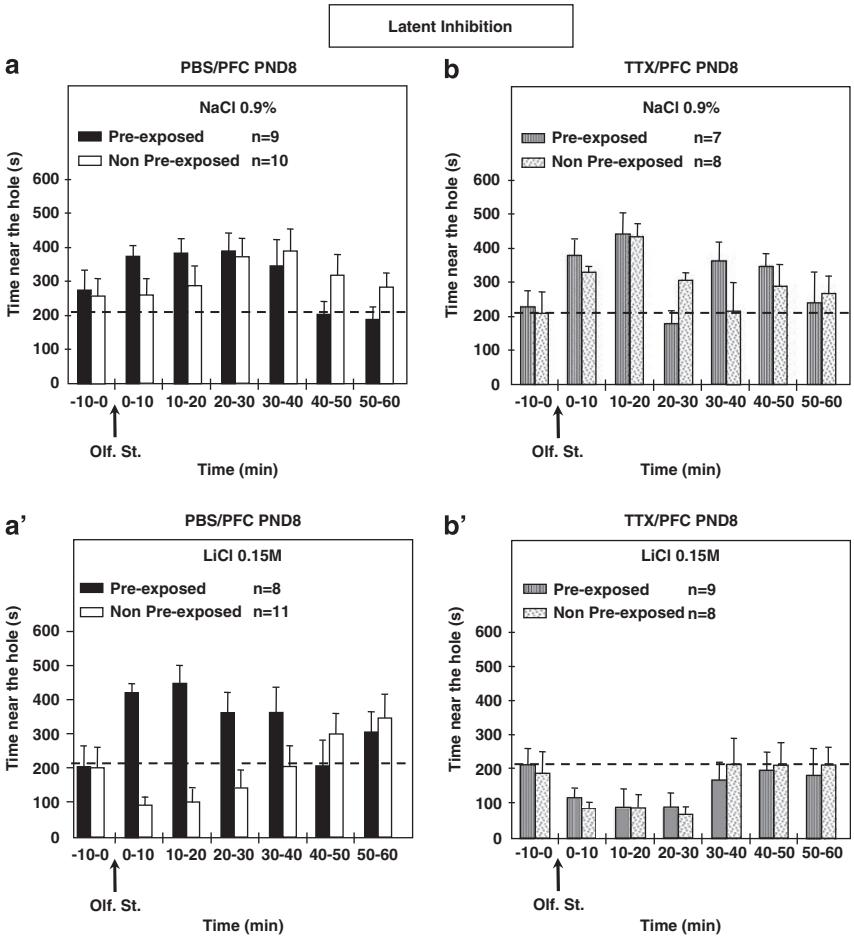

Figure 2 Time spent near the hole during the test session in pre-exposed and non-pre-exposed adult animals microinjected in the left prefrontal cortex with either PBS (a and a′) or tetrodotoxin (TTX) (b and b′) at postnatal day 8. The arrows indicate presentation of the olfactory stimulus (banana odor). During the conditioning session, banana odor was associated in control animals with an i.p. injection of NaCl (0.9%) and in conditioned animals with an i.p. injection of the same volume of LiCl (0.15 M). The dashed line represents 35% of one 10-min period (210 s), corresponding to neutral distribution of the rats in the cage. *n* Represents the number of animals per group. Results were analyzed with factorial ANOVA.

$F[1,62] = 12.24$; $P < 0.001$), whereas in pre-exposed PBS groups (PE-PBS-NaCl/PE-PBS-LiCl) no significant differences were obtained. A clear significant effect of the neonatal microinjection was obtained for the pre-exposed-conditioned animals (PE-PBS-LiCl/PE-TTX-LiCl; $F[1,62] = 17.48$; $P < 0.0001$).

Dopaminergic variations recorded in the core part of the nucleus accumbens (retention session). Only animals with implantation sites clearly located in the core part of the nucleus accumbens (Figure 1, left; Supplementary Figure S1) were considered for the voltammetric analysis (Figure 3).

In summary, concerning the dopaminergic variations recorded in the core subregion of the nucleus accumbens no significant conditioning effect was observed for the pre-exposed PBS animals (conditioned *vs* controls), unlike the other groups. Moreover, increases in the dopamine signal in the pre-exposed PBS-conditioned animals were significantly different from the dopaminergic variations recorded in the non-pre-exposed PBS-conditioned animals, whereas in the case of the pre-exposed TTX-conditioned animals, the dopaminergic responses were moderate and not statistically different from those observed in the non-pre-exposed TTX-conditioned animals.

The overall ANOVA carried out for the dopaminergic variations in the core part of the nucleus accumbens with respect to the full hour following exposure to the banana smell showed significant differences for the conditioning factor (NaCl/LiCl; $F[1,41] = 20.88$; $P < 0.00005$), as well as the pre-exposure factor (NPE/PE; $F[1,41] = 8.09$; $P < 0,01$), but not the microinjection factor (PBS/TTX). The various interactions (conditioning × microinjection, microinjection × pre-exposure, pre-exposure × conditioning or conditioning × microinjection × pre-exposure) were not found to be statistically different. Furthermore, contrast analysis of ANOVA performed on the dopaminergic variations for the whole hour following exposure to the olfactory stimulus revealed a significant effect of conditioning (NaCl/LiCl) for the non-pre-exposed-PBS groups (NPE-PBS-NaCl/NPE-PBS-LiCl; $F[1,41] = 7.36$; $P < 0.01$), non-pre-exposed TTX groups (NPE-TTX-NaCl/NPE-TTX-LiCl; $F[1,41] = 11.46$; $P < 0.005$), and pre-exposed TTX groups (PE-TTX-NaCl/PE-TTX-LiCl; $F[1,41] = 6.67$; $P < 0.05$), whereas no significant difference was observed for the

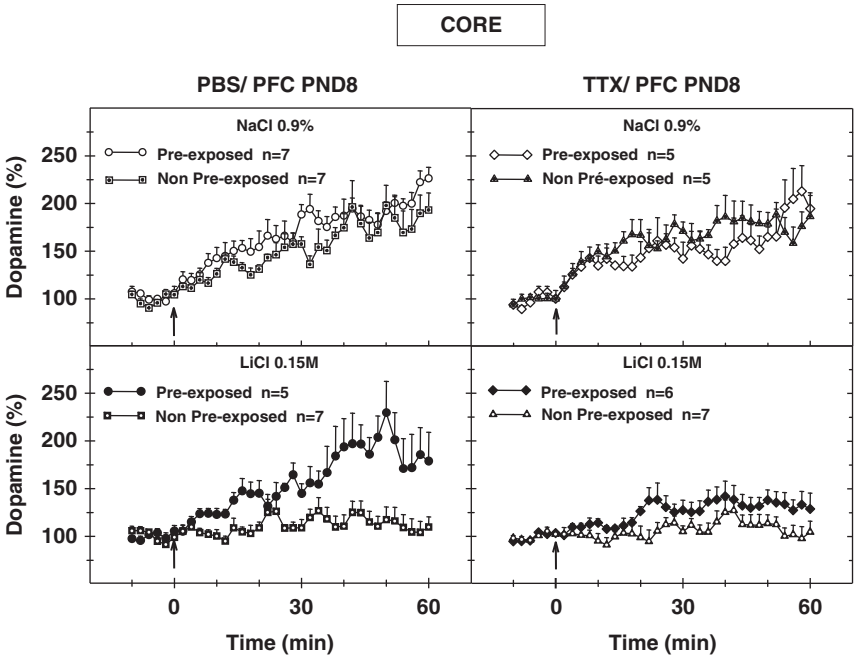

Figure 3 Dopaminergic variations recorded in the core part of the left nucleus accumbens during the test session in adult pre-exposed and non-pre-exposed animals after transient neonatal inactivation of the left prefrontal cortex by tetrodotoxin (TTX) at postnatal day 8 (PND8). Extracellular dopaminergic variations were assessed using differential normal pulse voltammetry and computer-assisted numerical analysis in freely moving adult rats. Voltammograms were recorded every minute. Only mean values and SEM corresponding to two scans are presented. Where no SEM is given, the size is less than the radius of the symbol. The arrows indicate presentation of the olfactory stimulus (banana odor). *n* Is the number of rats per group. Results were analyzed with factorial ANOVA.

conditioning factor for the pre-exposed PBS animals (PE-PBS-NaCl/PE-PBS-LiCl). This contrast analysis also revealed a significant effect of pre-exposure for the PBS-conditioned animals (PE-PBS-LiCl/NPE-PBS-LiCl; $F[1,41] = 9.08$; $P < 0.005$), but no statistical differences for the TTX-conditioned animals (PE-TTX-LiCl/NPE-TTTX-LiCl; $F[1,41] = 1.45$; $P = 0.24$, NS).

D-AMP Challenge

D-AMP-induced locomotor activity. To summarize, after D-AMP challenge a difference in locomotor activity was observed depending on the dose (NaCl 0.9%, D-AMP 0.75 mg/kg, D-AMP 1.5 mg/kg; Figure 4). Moreover, a significant effect of the neonatal microinjection (PBS or TTX) on locomotor activity was obtained for the highest D-AMP dose (1.5 mg/kg; Figure 4c).

The overall ANOVAs carried out for the locomotor activity of the 58 animals during the 90 min postinjection showed no significant litter effects ($F[8,49] = 1.17$, NS) but revealed significant effects of the dose (NaCl 0.9%/D-AMP 0.75 mg/kg, D-AMP 1.5 mg/kg; $F[1,52] = 64.55$; $P < 10^{-6}$) and microinjection (PBS/TTX; $F[1,52] = 7.42$; $P < 0.01$), but not the dose × microinjection interaction. Contrast analysis of ANOVA for locomotor activity during the 90 min following the D-AMP challenge showed a significant microinjection effect only for the highest D-AMP dose 1.5 mg/kg ($F[1,52] = 10.76$; $P < 0.01$), but not for the lowest D-AMP dose 0.75 mg/kg.

Within-subjects analysis revealed a significant time effect ($F[8,416] = 8.12$; $P < 10^{-5}$) and a significant time × dose effect ($F[16,416] = 5.78$; $P < 10^{-5}$), but no significant effects for the time × microinjection or time × dose × microinjection interactions.

D-amp-induced dopaminergic changes recorded in the core part of the nucleus accumbens. Only animals with implantation sites clearly located in the core part of the nucleus accumbens (Figure 1, left; Supplementary Figure S1) were considered for the voltammetric analysis (Figure 5). To summarize, after D-AMP challenge extracellular dopamine levels depend on the injected dose (NaCl 0.9%, D-AMP 0.75 mg/kg, D-AMP 1.5 mg/kg). A significant effect of the neonatal microinjection (PBS or TTX) on the dopaminergic levels was observed only for the highest D-AMP dose (1.5 mg/kg; Figure 5c).

The overall ANOVA for the dopaminergic variations recorded in the core part of the nucleus accumbens in the 90 min postinjection revealed a significant effect of the dose (NaCl, 0.9%/D-AMP 0.75 mg/kg/D-AMP 1.5 mg/kg; $F[2,30] = 21.32$; $P < 10^{-5}$) or neonatal microinjection (PBS/TTX; $F[1,30] = 5.28$; $P < 0.05$) but no significant effect of the interaction (dose × microinjection), although a trend was observed ($F[2,30] = 3.28$; $P = 0.052$). Contrast analysis of ANOVA revealed a significant effect of the neonatal microinjection ($F[1,30] = 11.67$; $P < 0.05$) for the highest dose of D-AMP (1.5 mg/kg), but no effect was obtained for the lowest dose (0.75 mg/kg).

Within-subjects analysis revealed a significant time effect ($F[44,1320] = 9.71$; $P < 10^{-5}$) and significant effects for the time × dose interaction ($F[88,1320] = 5.12$; $P < 10^{-5}$), the time × microinjection interaction ($F[44,1320] = 1.59$; $P < 0.01$), and the time × dose × microinjection interaction ($F[88,1320] = 1.71$; $P < 10^{-4}$).

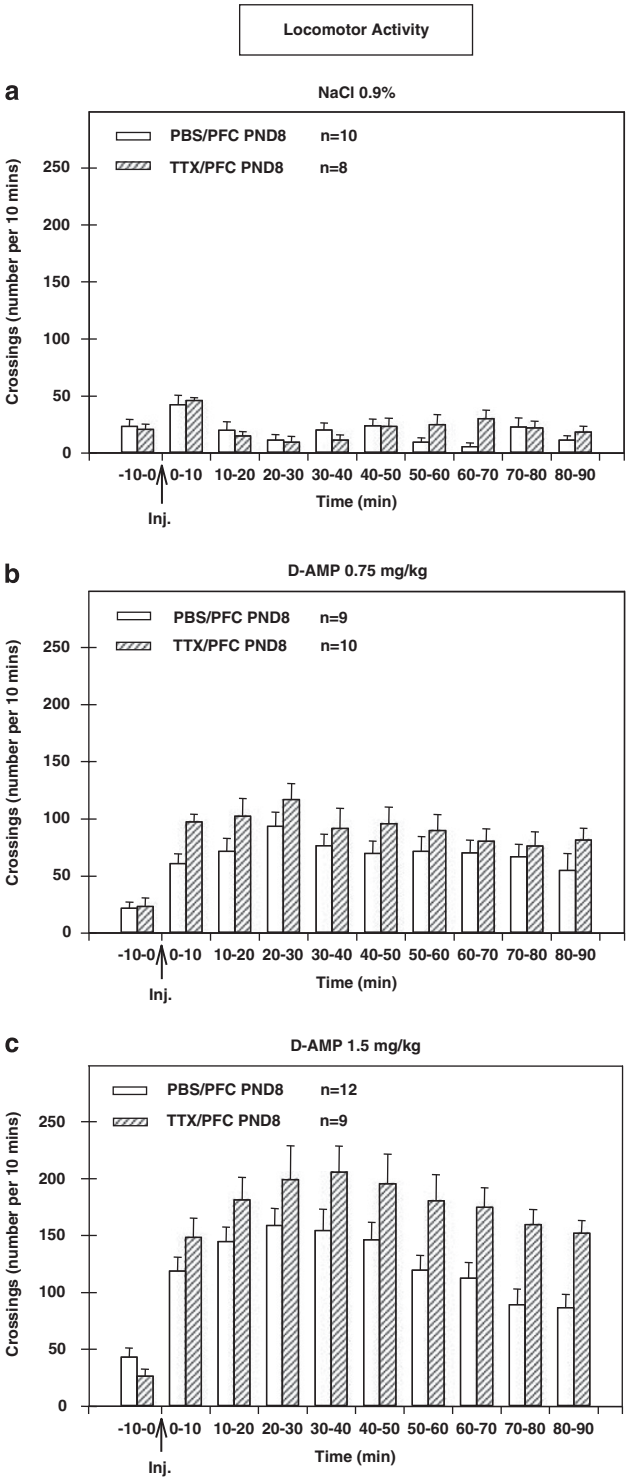

Figure 4 Locomotor activity of animals microinjected at postnatal day 8 (PND8) with PBS (white bars) or tetrodotoxin (TTX; hatched bars) following a NaCl (0.9%) injection (a) or D-AMP challenge (0.75 mg/kg; 1.5 mg/kg, respectively b, c). The arrows indicate the moment of injection (NaCl, D-AMP 0.75 mg/kg, or D-AMP 1.5 mg/kg). *n* Is the number of animals per group. Results were analyzed with factorial ANOVA.

DISCUSSION

To the best of our knowledge, the present study is the first to report the consequences, in adult animals, of a neonatal transitory inactivation of the left PFC for two schizophrenia-related markers; latent inhibition expression and reactivity to D-AMP challenge. The results suggest that neonatal TTX inactivation of the left PFC performed in rats at PND8 leads to disruptions in adulthood of both latent inhibition-related behavioral and dopaminergic responses in the core part of the nucleus accumbens, as well as heightened behavioral and dopaminergic reactivity to D-AMP at the highest dose.

No macroscopic anatomical changes were observed in adulthood in either the PFC or nucleus accumbens of TTX animals. It is conceivable, therefore, that transitory blockade of the PFC early in development involves cellular/molecular mechanisms resulting in impaired communication between the PFC and structures that either receive direct projections from the PFC, such as the nucleus accumbens (Wright and Groenewegen, 1995; Heidbreder and Groenewegen, 2003), or which innervate the PFC, such as the ventral subiculum (Jay and Witter, 1991) or those presenting reciprocal connections with the PFC, such as the entorhinal cortex (Heidbreder and Groenewegen, 2003; Hoover and Vertes, 2007). The first 2 weeks after birth are a critical time window for the development of the PFC insofar as intrinsic and extrinsic connections of the PFC are growing significantly (van Eden *et al*, 1990). Electrical activity has an essential role in the early development of the nervous system (for review see Spitzer, 2006) and is involved in a number of cellular processes, such as axons' myelination (Demerens *et al*, 1996), rearrangement of synaptic connections in target structures (Stryker and Harris, 1986; Katz and Shatz, 1996; Hutchins and Kalil, 2008), and maturation of dendritic spines (Drakew *et al*, 1999). Thus, failure in one or more of these mechanisms, following TTX neonatal inactivation, could result in malfunctioning of the PFC and consequently in changes in control in the dopamine release in the core part of the nucleus accumbens. This suggestion is consistent with recent data showing that a neonatal PFC lesion enhances the sensitivity of the mesoaccumbal dopamine neurons (Bennay *et al*, 2004) and leads to deficient myelination in some projection areas of the PFC, including the nucleus accumbens, hippocampus, and amygdala (Schneider and Koch, 2005; Klein *et al*, 2008).

In the latent inhibition paradigm, neonatal TTX functional blockade of the left PFC at PND8 induced no significant behavioral and dopaminergic differences between non-pre-exposed PBS and non-pre-exposed TTX adult animals. For both postnatal microinjections, attraction or aversion toward the olfactory stimulus (banana odor) was observed for control and conditioned animals. These behavioral results were compatible with those previously obtained with the same aversive conditioning protocol in microinjection-naïve non-pre-exposed animals (Jeanblanc *et al*, 2002; Louilot *et al*, 2010), suggesting that early TTX inactivation of the PFC leaves the olfactory perception intact in microinjected animals. With respect to the pre-exposed PBS animals, latent inhibition-related behavioral and dopaminergic responses for pre-exposed PBS-control and pre-exposed PBS-conditioned rats are comparable to those reported previously in adult rats having had no postnatal microinjection in the PFC (Jeanblanc *et al*, 2002; Louilot *et al*, 2010). Thus, the

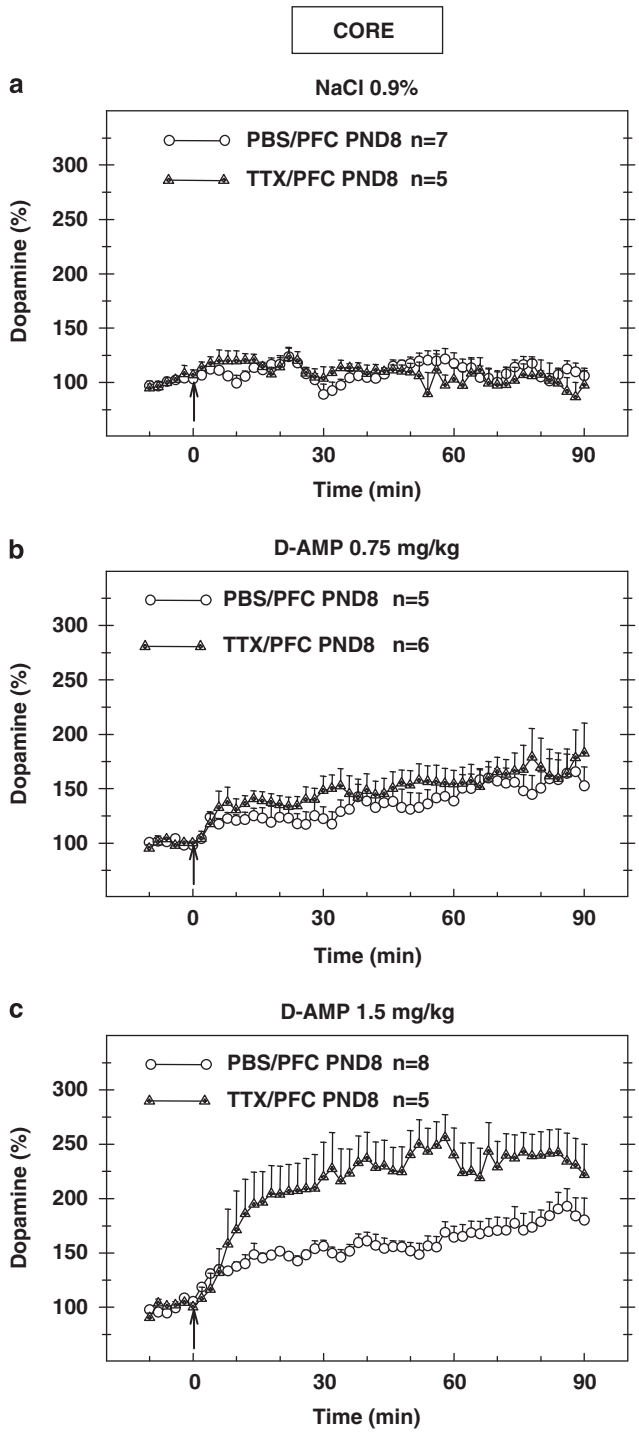

Figure 5 Dopaminergic levels recorded in the core part of the left nucleus accumbens following a NaCl (0.9%) injection (a) a D-AMP challenge (0.75 mg/kg, 1.5 mg/kg, respectively b, c) after neonatal inactivation of the left prefrontal cortex by tetrodotoxin (TTX) at postnatal day 8. Extracellular dopaminergic variations were assessed using differential normal pulse voltammetry and computer-assisted numerical analysis in freely moving adult rats. Voltammograms were recorded every minute. Only mean values and SEM corresponding to two scans are presented. Where no SEM is given, the size is less than the radius of the symbol. The arrows indicate the time of injection of either NaCl (0.9%) or D-AMP (0.75 mg/kg, 1.5 mg/kg). *n* is the number of rats per group. Results were analyzed with factorial ANOVA.

neonatal microinjection and vital dye Evans blue do not appear to have any major effect.

Pre-exposed TTX-conditioned animals expressed no latent inhibition responses, but on the contrary displayed typical conditioned aversive responses toward the banana odor. These data could be interpreted to mean the prelimbic/infralimbic subregion of the PFC is involved in the latent inhibition phenomenon, which would be consistent with recent studies carried out with focal lesions in adults (George *et al*, 2010; Nelson *et al*, 2010), rather than previous works performed with more dorsal lesioning sites in the anteromedian PFC (Lacroix *et al*, 2000; Schiller and Weiner, 2004). However, lesions in the prelimbic/infralimbic subregion of the PFC in adults resulted in enhanced latent inhibition (George *et al*, 2010; Nelson *et al*, 2010), whereas what we observed was that latent inhibition disappeared after postnatal TTX microinjection in the same PFC subregion, suggesting that different mechanisms are involved after intervention in adults and during the postnatal developmental period. The disappearance of the behavioral expression of latent inhibition obtained after neonatal TTX blockade of the PFC is similar to that observed after an identical blockade of the entorhinal cortex or ventral subiculum (Peterschmitt *et al*, 2007; Meyer *et al*, 2009; Meyer and Louilot, 2011). The explanation given for former results was a malfunctioning of a recognition memory system that prevented proper learning and memorization of the characteristics related to CS (banana odor) during pre-exposure to the CS (see Meyer *et al*, 2009; Louilot *et al*, 2010). The PFC could be part of the recognition memory system thought to be involved in the latent inhibition phenomenon and defective after early neonatal inactivation. However, another interpretation is that the results obtained in pre-exposed TTX-conditioned animals are related to neurodevelopmental disturbances in PFC targets, secondary to the neonatal inactivation, such as myelination defects in PFC projection structures, particularly the hippocampal regions, and not to a functional impairment of the PFC *per se*. Such myelination defects have been observed after neonatal ibotenic lesion of the PFC (Schneider and Koch, 2005; Klein *et al*, 2008) and warrant investigation following TTX inactivation.

Dopaminergic variations recorded in pre-exposed TTX-conditioned animals are similar to those obtained in non-pre-exposed TTX-conditioned rats and are characteristic of aversion. A first explanation could be that the disrupted latent inhibition-related dopaminergic responses result mainly from impaired control exerted by the PFC over the extracellular dopamine release in the nucleus accumbens, in interaction with regulating influences from the basolateral nucleus of amygdala. Indeed, dopaminergic transmission in the core part of the nucleus accumbens depends on the functional integrity of the PFC, as shown in anesthetized animals (Louilot *et al*, 1989), and projections from the infralimbic/prelimbic PFC subregion toward the core subregion have been described (Wright and Groenewegen, 1995; Heidbreder and Groenewegen, 2003), as well as a close apposition between the PFC afferents and dopaminergic endings in the nucleus accumbens (Sesack and Pickel, 1992). In other respects, a convergence of afferents from the PFC and basolateral nucleus of amygdala has also been reported at the level of the nucleus accumbens (Wright and

Groenewegen, 1995). Moreover, the basolateral nucleus has been shown to be involved in controlling aversively conditioned dopaminergic responses in the core subregion (Louilot and Besson, 2000). The specific mechanisms involved in the accumbal dopaminergic changes in latent inhibition following the TTX neonatal PFC blockade have still to be clarified, however, given that PFC also appears to be able to regulate the activity of dopaminergic neurons innervating the nucleus accumbens by PFC efferents reaching the ventral mesencephalon (Sesack and Pickel, 1992; Taber *et al*, 1995; Karreman and Moghaddam, 1996; Pennartz *et al*, 1994). The involvement of more complex or indirect regulating pathways can also not be ruled out (see Pennartz *et al*, 1994). Whatever the exact regulatory pathways involved in the observed core dopaminergic variations might be, the relationships between these variations and the behavioral responses could be interpreted with reference to Weiner's switching model of latent inhibition (2003; 2010), according to which the core subregion of the nucleus accumbens is involved in a switching mechanism between responding according to the CS-reinforcement associations acquired during conditioning and responding to the CS-no event associations acquired during pre-exposure, with the switching mechanism inhibited by the shell subregion of the nucleus accumbens. In the switching model, the latent inhibition response is coupled to a rapid increase in dopamine released in the core part of the nucleus accumbens, whereas the CR is associated with a lack of initial increase in dopamine levels (Weiner, 2003, 2010). The present dopaminergic variations recorded in the core in pre-exposed PBS-conditioned animals showing a preference for the CS and in the non-pre-exposed PBS-conditioned, non-pre-exposed TTX-conditioned, and pre-exposed TTX-conditioned animals displaying an aversion toward the CS appear to be consistent with this model.

Regarding the D-AMP challenge, our data show first and foremost that spontaneous locomotor activity, measured before injection of D-AMP, is not altered in TTX-microinjected animals compared with PBS animals. With respect to the PBS and TTX animals, the D-AMP i.p. injection induced a dose-dependent increase in locomotor activity. The highest D-AMP dose (1.5 mg/kg) triggered a larger increase in the locomotor response and longer-lasting effect compared with what was observed with the lowest dose (0.75 mg/kg). Data obtained for PBS-microinjected animals are consistent with previous findings showing that D-AMP induced an increase in locomotor activity in a dose-dependent manner, with a gradual return to baseline values from 60 min after the injection (Sharp *et al*, 1987; Louilot and Choulli, 1997). Our data showing more locomotor activity with the highest D-AMP dose (1.5 mg/kg) in animals subjected to postnatal TTX inactivation, compared with PBS animals, appear to be original. To the best of our knowledge, the behavioral response to D-AMP in animals undergoing TTX neonatal functional inactivation of the PFC has never before been investigated. Our data are consistent with those obtained by Flores *et al* (1996) after excitotoxic PFC postnatal lesion, which showed greater D-AMP-induced locomotor activity in postpubertal animals, but not with those obtained by Lipska *et al* (1998) in the same conditions. The reasons for the discrepancies were not obvious but may be related to the lesion's site or size.

Concerning dopaminergic responses recorded in the core, for PBS and TTX animals we observed a dose-dependent dopaminergic increase following the D-AMP challenge. As with the TTX-animals, a larger increase in dopamine release compared with PBS animals was obtained with the highest D-AMP dose (1.5 mg/kg). With the lower D-AMP dose (0.75 mg/kg) or saline injection, no statistical differences were obtained for the PBS- and TTX-microinjected rats. D-AMP dose-dependent dopaminergic increases observed for PBS-microinjected animals are consistent with results obtained with *in vivo* microdialysis measurements in the nucleus accumbens (Sharp *et al*, 1987). Furthermore, the extent of the present D-AMP-induced dopaminergic responses is consistent with that observed with the voltammetric approach (Ramsson *et al*, 2011). It is also interesting to observe that for the 1.5 mg/kg D-AMP dose, the difference in dopamine levels appears more marked during the 50–60 min postinjection period, whereas the difference in locomotor responses appears at its greatest during the 70–80 min period. At very least, this temporal disconnection can be interpreted as a long-lasting impact of released dopamine on postsynaptic sites in TTX-microinjected animals. In other respects, it is generally accepted that D-AMP increases dopamine levels in the brain by blockade and reversal of the plasmalemmal dopamine transporter (see Sulzer *et al*, 2005). It has also been reported that at a high dose D-AMP can induce a dopaminergic release from storage pools and thus redistribute dopamine from vesicles to the cytoplasm (Sulzer *et al*, 2005). With respect to the TTX animals, it is tempting to attribute the heightened behavioral and dopaminergic hyperactivity to the highest dose of D-AMP (1.5 mg/kg) in the core part of the nucleus accumbens to a much higher release from presynaptic vesicles pools. The greater storage of dopamine in dopaminergic terminals in the nucleus accumbens following early neonatal inactivation of the PFC, as well as the putative mechanisms involved, warrant further investigation. However, it is interesting to note that an increased vesicular storage of dopamine has been proposed as an explanation for the greater reactivity to D-AMP observed in patients with schizophrenia (Lyon *et al*, 2011).

To conclude, the present data suggest that subtle and transient functional inactivation of the infralimbic/prelimbic subregion of the PFC during the postnatal developmental period is sufficient to induce schizophrenia-related behavioral and dopaminergic abnormalities in adulthood. This further suggests that early functional impairment of PFC induced by TTX is a valid approach for modeling the pathophysiology of schizophrenia in animals. To refine this modeling, it would be important to identify the putative disruptions of latent inhibition and D-AMP responses before and after puberty, which is thought to be a second period of vulnerability to developing schizophrenia (Keshavan, 1999; Keshavan and Hogarty, 1999; Paus *et al*, 2008).

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DISCLOSURE

The authors declare no conflict of interest.

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