Postnatal prefrontal inactivation results in ketamine-induced increases in dopaminergic responses in the nucleus accumbens and dorsal striatum in adult rats

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Introduction
Numerous studies suggested there are functional disconnections between several cerebral integrative brain regions in schizophrenia, namely the prefrontal cortex and striatum. The origin of these functional disconnections may be neurodevelopmental [1-3]. Abnormalities reminiscent of perinatal brain development disturbances have been observed in the patients’ left prefrontal cortex (PFC) [4-5]. Functional dysregulation of striatal dopaminergic transmission is also generally acknowledged in schizophrenia [6-7].

As well as dopamine, glutamatergic dysfunctions have also been hypothesized in schizophrenia [8]. Consistent with this proposal, non-competitive antagonists of the NMDA (N-Methyl-D-Aspartate) glutamate receptor, such as ketamine, have been shown to cause psychotic states in healthy volunteers [9] and to exacerbate these symptoms in patients with schizophrenia [10-11]. Recently, it has been suggested that striatal dopaminergic dysregulation could be the result of a prefronto-striatal disconnectivity involving glutamatergic NMDA receptors [12-13].

The present study set out to test the hypothesis that early functional disconnection of the left PFC (intralimbic/prelimbic region) in rats during the developmental perinatal period results in increased dorsal and ventral striatal dopaminergic responses and increased locomotor activity after treatment with the NMDA antagonist, ketamine, at adulthood. Several studies have shown electrical activity of neurons during cerebral development to be necessary for establishing the synaptic connections in the target structures [14-15]. Tetrodotoxin (TTX) is a potent and specific blocking agent of sodium channels [16] which interrupts electrical activity. Therefore, reversible functional inactivation of the left PFC was carried out by local TTX microinjection in 8-day-old rats, i.e a critical time of the neurodevelopmental period [17]. Dopamine variations were recorded in the dorsal striatum and ventral striatum (core part of nucleus accumbens) using in vivo voltammetry in freely moving adult rats (11 weeks). Dopaminergic and behavioural responses were monitored in parallel.

Methods
Neonatal reversible inactivation of the anteromedian PFC was performed on postnatal day 8 (PND8). Male pup rats (Sprague-Dawley) were anesthetized with isoflurane (Forene®, ABBOTT, Rungis, France). On PND8, half of the litter,
chosen at random underwent a microinjection of phosphate-buffered saline (PBS), the solvent control group, while the other half, received a TTX microinjection in the left PFC. PBS and TTX were infused locally in a total volume of 0.3 μl over a period of 2 min 15 s. To identify the microinjection site in the left PFC, PBS and TTX solutions were both tinted with Evans Blue, a vital dye remaining visible in the cerebral tissue several weeks after the injection [18-19]. On PND70 male adult rats were implanted with a specially designed microsystem allowing the replacement of the working electrodes before each experiment [20]. Electrochemical procedures were similar to those described previously [19-21]. A conventional three-electrodes potentiostatic setting with reference (Ag/AgCl coated silver wire), auxiliary and working electrodes was used. The working electrodes were pyrolytic carbon-fiber microelectrodes electrochemically pre-treated (12μm diameter, 500μm length). Differential normal pulse voltammetry (DNPV) combined with the computerized waveform analysis of the catechol peak was used to determine selectively the extracellular levels of dopamine [21]. The waveform analysis method takes into account the small but consistent difference (approximately 36-40 mV) between the oxidation potentials of DOPAC and dopamine. The voltammogram is modelled as a sum of normal probability distributions corresponding to the relevant electroactive species; the relative contribution of the two catechols (DOPAC and dopamine) to a mixed peak is resolved by least squares techniques [21]. Differential normal pulse voltammograms (DNPV) were recorded every minute using a Biopulse (Radiometer Analytical, Villeurbanne, France). Control PBS and TTX animals received a subcutaneous (s.c.) injection of NaCl (0.9%); ketamine was administered to PBS and TTX groups at 5 mg/kg s.c. or 20 mg/kg s.c.

Results and Discussion

The obtained results were the following: 1) Clear dose effects were observed for PBS and TTX animals microinjected at PND8; 2) postnatal TTX inactivation of the left PFC performed at PND8 leads to greater increase in dopamine responses in left dorsal striatum and left core part of the nucleus accumbens, and greater increase in locomotor activity, compared to animals microinjected with PBS, only after the highest dose of ketamine (20mg/kg).

This study might provide an anatomo-functional framework to understand the involvement of glutamatergic NMDA receptors in the pathophysiology of schizophrenia and suggests that our approach for the animal modeling of this psychiatric disorder is valid.

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References