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Research report

Ketamine increases striatal dopamine release and hyperlocomotion in adult rats after postnatal functional blockade of the prefrontal cortex

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HIGHLIGHTS

- This study deals with animal modeling of the pathophysiology of schizophrenia
- Consequences of early prefrontal blockade for ketamine challenge were investigated
- TTX blockade increased striatal dopaminergic reactivity to ketamine at adulthood
- TTX blockade enhanced locomotor activity with the highest ketamine dose
- A disruption of glutamate-dopamine relationships in schizophrenia could be considered

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ABSTRACT

Schizophrenia is a complex psychiatric disorder that may result from defective connectivity, of neurodevelopmental origin, between several integrative brain regions. Different anomalies consistent with brain development failures have been observed in patients' left prefrontal cortex (PFC). A striatal dopaminergic functional disturbance is also commonly acknowledged in schizophrenia and could be related to a dysfunctioning of dopamine–glutamate interactions. Non-competitive NMDA antagonists, such as ketamine, can induce psychotic symptoms in healthy individuals and worsen these symptoms in patients with schizophrenia. Our study set out to investigate the consequences of neonatal functional blockade of the PFC for dopaminergic and behavioral reactivity to ketamine in adult rats. Following tetrodotoxin (TTX) inactivation of the left PFC at postnatal day 8, dopaminergic responses induced by ketamine (5 mg/kg, 10 mg/kg, 20 mg/kg sc) were monitored using *in vivo* voltammetry in the left part of the dorsal striatum in freely moving adult rats. Dopaminergic responses and locomotor activity were followed in parallel. Compared to PBS animals, in rats microinjected with TTX, ketamine challenge induced a greater release of dopamine in the dorsal striatum for the highest dose (20 mg/kg sc) and the intermediate dose (10 mg/kg sc). A higher increase in locomotor activity in TTX animals was observed only for the highest dose of ketamine (20 mg/kg sc). These data suggest transient inactivation of the PFC during early development results in greater behavioral and striatal dopaminergic reactivity to ketamine in adulthood. Our study provides an anatomo-functional framework that may contribute toward a better understanding of the involvement of NMDA glutamatergic receptors in schizophrenia.

1. Introduction

Schizophrenia is a complex and devastating neuropsychiatric disorder that may result from defective connectivity between several integrative brain regions [1,2]. The prefrontal cortex (PFC) appears to be one integrative region crucially affected in this disorder. Recently, cytoarchitectural and neuronal morphometric abnormalities have been described in patients with schizophrenia, reminiscent of early neurodevelopment defects at the level of the PFC, particularly in the left hemisphere [3–6]. In addition, for several decades now a striatal dopaminergic imbalance has been acknowledged in schizophrenia [7–9], as supported by several brain imaging studies [8,10–17]. The dopaminergic dysregulation may result from prefronto-striatal disconnectivity [1,18–20]. Moreover, a dysfunction of dopamine–glutamate interactions has also been suggested in schizophrenia [21–23]. Consistent with this suggestion, non-competitive antagonists of the NMDA (N-Methyl-D-Aspartate) glutamatergic receptor administered at subanesthetic doses, such as phencyclidine (PCP) and ketamine, have been shown to induce schizophrenia-like symptoms in healthy individuals

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[24–27] and to exacerbate psychotic symptoms in patients with schizophrenia [28,29]. The striatal dopaminergic dysregulation in schizophrenia could result from a prefronto-striatal disconnectivity involving NMDA receptors [8,30].

Taking the aforementioned data into account, the aim of the present study was to investigate the consequences of early functional inactivation of the left PFC for dopaminergic and locomotor reactivity to ketamine challenge in adult rats. At postnatal day (PND) 8, we carried out a functional blockade of the left PFC (infralimbic/prelimbic region) by local microinjection of tetrodotoxin (TTX), a well-known Na+ channel blocker [31]. PND8 is a critical time for brain development in rats, comparable to the middle of the second trimester of human gestation, which is considered a period of high vulnerability for developing schizophrenia at least in some cases [32,33]. During brain development impulse neuronal activity, which is interrupted by TTX in the present study, is crucial for shaping connections in the target structures [34–38]. Behavioral reactivity to ketamine was assessed by locomotor activity, a good index for animal modeling for schizophrenia [39,40]. Ketamine-induced dopamine release was monitored, parallel to locomotor activity, in the left part of the dorsal striatum using *in vivo* voltammetry in freely moving adult animals (11 weeks).

2. Materials and methods

2.1. Animals

All animals were housed in a temperature-controlled room (22 ± 2 °C) with access to water and food *ad libitum*. A total of 74 male Sprague-Dawley rats born to mothers from the R. Janvier breeding center (Le Genest-Saint-Isle, France) were used. The mothers were kept during gestation in individual cages on a 12 h light/dark cycle (lights on at 7 am). All the experimental procedures were conducted in accordance with 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). Every effort was made to minimize the suffering and number of the animals used.

2.2. Design of the experiment

The day the pups were born was considered postnatal day 0 (PND0). The size of the litters was limited at birth to 12 animals. Supernumerary pups received a lethal intraperitoneal (i.p.) injection of pentobarbital. On PND8, the pups were randomly given a microinjection of either PBS (control group) or TTX (experimental group) in the PFC. On PND56, the male rats were individually housed in plexiglas cages under a reversed light/dark cycle (lights on between 11 pm and 11 am). On PND70, the grown-up male rats were implanted with a specially designed microsystem that allowed behavior and dopaminergic responses to be monitored in parallel. Following surgery, the animals were given 7 days to recover. The voltammetric recordings were performed on PND77. All the experiments were performed during the dark phase of the light/dark cycle.

2.3. Neonatal and adult surgeries

2.3.1. Neonatal surgery: TTX inactivation of the PFC

Neonatal reversible inactivation of the anteromedian PFC was performed at PND8. On PND8, half of the pups in the litter, chosen at random, received a PBS microinjection (control group) and the other half a TTX microinjection (experimental group). The neonates weighed around 20 g at the time of surgery.

The rat pups were anaesthetized by vaporizing isoflurane (Forene®, ABBOTT, Rungis, France) with an anesthesia vaporizer (Univentor 400, Univentor®, Zetjun, Malta) connected to an air pump (Dymax 30, Charles Austen Pumps®, Byfleet, UK) and released through a specially adapted gas anesthesia mask. Induction was obtained with a concentration of 3.9–4.1% isoflurane in air. Once the neonates were anesthetized, a scalp incision was performed according to the anteroposterior axis to expose the skull. Thereafter the pups were immediately placed on a specially adapted stereotaxic apparatus (Unimécanique®, Epinay/Seine, France) (incisor bar set at 2.8 mm below the interaural line). Anesthesia was maintained throughout surgery at a concentration of 1.9–2.2% isoflurane in air.

Neonatal microinjection of either PBS or TTX was performed with a stainless steel guide cannula (30 gauge, 12.5 mm length, Small Parts, Miami, USA), connected by a flexible catheter (tube Tygon® S 54HL, Fisher Bioblock, Illkirch, France) to a microsyringe (10 μl Hamilton, Bonaduz®, Bonaduz, CH). The microinjection cannula was lowered into the PFC at coordinates 1.5 mm anterior to the 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: 7.42) or TTX (Sigma, St Quentin-Fallavier, France) dissolved in PBS were infused in a volume of 0.3 μl for a period of 2 min 15 s using an infusion pump (Razel, Stamford, CT, USA). The cannula was left in the PFC for 4 min after the end of the microinjection to allow for the diffusion of PBS and TTX in the targeted structure. The amount of TTX microinjected in the PFC (100 μM × 0.3 μl), approximately 10 ng, is similar to that reported previously in the literature [41–43]. According to Malpeli [44], 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. Indeed, Zuhravin and Bures [41] reported that microinjection of 10 ng TTX in a total volume of 1 μl (*i.e.* 3 times more than the volume we used) at a rate of 1 μl/min has no functional consequences beyond 1 mm 3 h after the microinfusion. Importantly the inactivating effects of a local microinjection of 10 ng TTX have been reported to last 1 or 2 days [45]. However, a similar amount of TTX microinjected in the ventromedial prefrontal cortex has been reported to affect a more complex behavioral task compared to the one investigated by Rothfeld et al. [45] for only 4 h [46]. Thus, in our conditions the duration of TTX effects should be at least 4 h and at most 48 h. To identify the microinjection site in the PFC, the PBS and TTX solutions were both colored with Evans Blue (Sigma, France), a vital dye described as remaining visible in the cerebral tissue several weeks after injection [42,43,47]. Each rat pup was marked for identification purposes with small three-digit ear tags (Ref 52-4716, Harvard Apparatus, Les Ulis, France). After surgery, the neonates were placed under a heating lamp until they woke up and then returned to their mothers.

2.3.2. Adult surgery: implantation of the microsystem

On PND70, the fully-grown male rats (weight 400 ± 25 g) microinjected in the anteromedian PFC at PND8 with either PBS or TTX were placed in a stereotaxic apparatus (incisor bar set at 3.3 mm below the interaural line; Unimécanique, Epinay/Seine, France) following chloral hydrate anesthesia (400 mg/kg i.p.) and implanted with a specially designed microsystem (Unimécanique; [48]) that allowed behavioral and dopaminergic responses to be monitored in parallel in the left dorsal striatum at coordinates 10.0 mm anterior to the interaural line (AP), 1.65 mm lateral to the midline (L), and −5.8 mm below the cortical surface (H) [49]. After surgery, there is a post-operative recovery period of at least 7 days before the voltammetric recordings and behavioral testing.

2.4. Drug administration

Ketamine (Imalgene®, Merial, Lyon, France) was dissolved in saline at 5 mg/ml, 10 mg/ml and 20 mg/ml for subcutaneous injection (sc) at 0.5 ml/kg. Fresh ketamine solutions were prepared prior to each experiment. Control rats received the equivalent volume of saline (NaCl 0.9%).

2.5. Ketamine-induced locomotor activity

After being left for 1 h to become accustomed to the experimental cage (24 cm wide × 27 cm long × 44 cm high), the adult rats received, randomly, either a sc injection of NaCl (0.9%) (control groups) or a sc injection of one of the three doses (5 mg/kg, 10 mg/kg or 20 mg/kg) of ketamine (experimental groups). The doses were chosen from studies showing that ketamine administered in this range increases, dose-dependently locomotor activity see [50,51]. After the sc injection, the animals were left in the experimental cage for 1 h during which their locomotor activity was assessed. Their behavior was monitored using a small infrared camera in the ceiling of the cage linked up to a video monitor and video tape. The floor of the cage was divided into four equal virtual quadrants. Locomotor activity was 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 ± S.E.M.

2.6. Voltammetric analysis of the dopaminergic signal

The electrochemical procedures were those previously described [42,43,52–54]. The average amplitude of the last 10 peaks (last 10 min) of dopamine signals obtained during the control period (variation of voltammetric signal less than 10%) was calculated for each animal and set at 100%. Voltammetric variations of the dopaminergic signal, recorded min by min in the dorsal striatum, are expressed as percentages (mean ± S.E.M.) of the mean values determined prior to the sc injection (NaCl 0.9% or ketamine). Only variations obtained every 2 min are shown on the graphs.

2.7. Statistics

Statistical analysis was performed using a multifactorial analysis of variance (ANOVA) with repeated measurements on the time factor. Only between-subject ANOVAs are shown unless otherwise indicated. Between-subject grouping factors were ketamine doses with 4 levels (NaCl; ketamine 5 mg/kg; ketamine 10 mg/kg; ketamine 20 mg/kg) and neonatal microinjection with 2 levels (PBS, TTX). The dependent variables were the number of crossings for the behavioral study and the striatal dopaminergic variations for the voltammetric study. *Post hoc* contrast