Neuropharmacology 62 (2012) 2440–2446

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

# Role of the anterior thalamic nucleus in the motor hyperactivity induced by systemic MK-801 administration in rats

Ximena López Hill, María Cecilia Scorza*

*Laboratory of Cell Biology, Instituto de Investigaciones Biológicas Clemente Estable, Avenida Italia 3318, 11600 Montevideo, Uruguay*

ARTICLE INFO

*Article history:*
Received 13 October 2011
Received in revised form
4 January 2012
Accepted 28 January 2012

*Keywords:*
NMDA receptor antagonists
Anterior thalamus nucleus
Muscimol
Hyperlocomotion

ABSTRACT

Non-competitive N-methyl-D-aspartate receptor (NMDA-R) antagonists have been extensively used in rodents to model psychotic symptoms of schizophrenia. Although the motor syndrome induced by acute and systemic administration of low doses of dizocilpine (MK-801) has been extensively characterized, its neurobiological basis is not fully understood. NMDA-R antagonists can disinhibit excitatory inputs in certain brain areas, but the precise circuitry is not fully known.

We examined the involvement of the anterior thalamic nucleus (ATN) in hyperlocomotion and other related behaviors (stereotypies, ataxia signs) induced after acute systemic administration of MK-801. Since GABAergic neurons of the reticular thalamic nucleus (RTN) exert the main inhibitory control on thalamic projection neurons, we hypothesized that systemically injected MK-801 might block NMDA-R on RTN GABAergic neurons. This effect would subsequently result in disinhibition of GABAergic inputs onto ATN projections to cortical motor areas, thereby inducing behavioral effects. We evaluated the behavioral syndrome induced by the systemic administration MK-801 (0.2 mg/kg) in control rats and in rats subjected to a bilateral stereotaxic infusion of the $GABA_A$ agonist muscimol (0.2 μl of 2.5 and 5.0 mM; 0.5–1 nmol per application, respectively) into the ATN. As previously reported, MK-801-induced hyperlocomotion in parallel with disorganized movements (e.g. not guided by normal exploration) slight ataxia signs and stereotypies. All responses were antagonized by pre-infusion of muscimol but not saline into the ATN. According to our results we suggest that the ATN plays a role on hyperlocomotion evoked by MK-801 and could involve a thalamic GABAergic disinhibition mechanism.



## 1. Introduction

The strongest evidence that links schizophrenia to excitatory neurotransmission is the finding that the non-competitive N-methyl-D-aspartate (NMDA) receptor (NMDA-R) antagonists produce psychotic symptoms in healthy individuals and exacerbate clinical symptoms in schizophrenic patients (Coyle, 1996; Javitt and Zukin, 1991; Kim et al., 1980; Olney and Farber, 1995). This evidence suggests the existence of glutamatergic abnormalities in schizophrenia (Lahti et al., 1995, 2001; Malhotra et al., 1997).

In rodents, the systemic administration of the NMDA-R antagonists such as phencyclidine (PCP), ketamine or dizocilpine maleate (MK-801), elicited a behavioral syndrome characterized by hyperlocomotion, ataxia signs and stereotypies (Andiné et al., 1999; Bradford et al., 2010; Geyer and Ellenbroek, 2003; Javitt, 2004; Moghaddam and Jackson, 2003; Scorza et al., 2008). Despite a large body of literature characterizing the above behavioral syndrome, the brain networks and involved cellular mechanisms are not fully understood.

It has been postulated that NMDA-R antagonists may produce a disruption of the excitatory/inhibitory (E/I) balance via disinhibition of GABAergic inputs to glutamate-containing neurons, enhancing AMPA-mediated neurotransmission in cortical and subcortical regions (DeGiorgio et al., 1999; Kehrer et al., 2008; Krystal et al., 2003; Marek et al., 2010; Moghaddam et al., 1997; Sharp and Tomitaka, 2001; Tomitaka et al., 2000). The thalamus, which provides the main subcortical excitatory input to the cortex, has become an important focus of research in schizophrenia (Andreasen, 1997; Young et al., 2000). Indeed, MK-801 and PCP enhanced *c-fos* expression in various thalamic nuclei that project to motor and association cortical areas (Kargieman et al., 2007; Santana et al., 2011; Väisänen et al., 2004). Likewise, the connectivity between the anterior thalamus nuclei (ATN) and the retrosplenial cortex (RSC) is associated with the neuronal injury induced by MK-801 (Sharp et al., 1991; Tomitaka et al., 2000). The activity of ATN neurons is tightly controlled by GABAergic neurons of the reticular thalamic nucleus (RTN) while ATN neurons send glutamatergic collateral projections to the GABAergic neurons of the RTN

* Corresponding author. Tel.: +598 2487 1616x115; fax: +598 2487 5548.
*E-mail address:* scorza@iibce.edu.uy (M.C. Scorza).

0028-3908/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2012.01.025

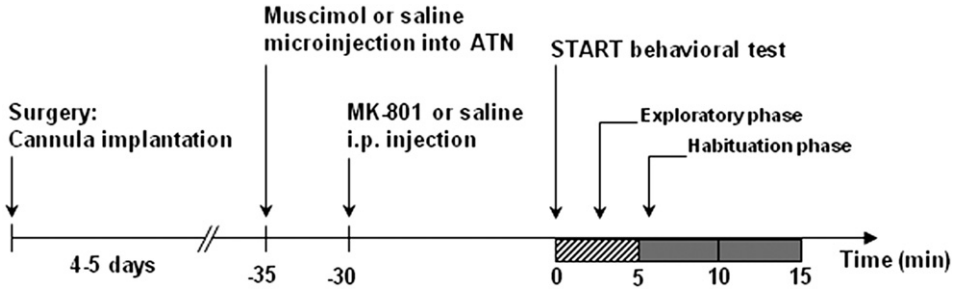


**Fig. 1.** Schematic representation of the experimental protocol.

(Gonzalo-Ruiz et al., 1996, 1997) to modulate the thalamic-cortical outputs. Interestingly, it has been proposed that this thalamic-cortical circuit is involved in psychosis (Tomitaka et al., 2000) in accordance with evidence that shows thalamic abnormalities in schizophrenia (Andreasen, 1997; Buchsbaum et al., 1996; Young et al., 2000). Here, we investigated the role played by the ATN as a possible substrate involved in the induction of the behavior responses by MK-801. The study was based on the working hypothesis that behavioral actions of MK-801 are caused by hyperactivity of the ATN, following blockade of NMDA-R in GABAergic afferents of the RTN to this thalamic nucleus. We examined the behavioral effects of the systemic administration of the NMDA-R antagonist MK-801 in control rats and in rats whose $GABA_A$ tone in the ATN was increased by the local stereotaxic application of the $GABA_A$ agonist muscimol in order to normalize the E/I balance in the ATN.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats (250–300 g) obtained from the IIBCE animal facilities (Montevideo) were used in this study. The animals were housed in groups of 6 in plastic cages (50 × 37.5 × 21 cm) with food and water available *ad libitum* under controlled conditions (temperature 22 ± 2 °C, 12-h light–dark cycle, lights on at 7:00 A.M.). All procedures were carried out in accordance to the IIBCE Bioethics Committee's requirements (followed the Guiding principles in the care and use of animals – DHEW Publications, NIH, 80-23) and under the current ethical regulations of the national law on animal experimentation N°18.611. Adequate measures were taken to minimize discomfort or stress of the animals, and all efforts were made in order to use the minimal number of animals necessary to produce reliable scientific data.

### 2.2. Drugs

(+)-MK-801 [dizocilpine (5R,10S)-(1)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine hydrogen maleate] was obtained from Sigma RBI. Muscimol was kindly provided by Dr. Pablo Torterolo (Faculty of Medicine, Uruguay). Ketamine and xylazine were from Konsol Köning S.A Laboratory. MK-801 and muscimol were dissolved in saline. Aliquots were prepared and stored at −20 °C.

### 2.3. Surgical procedures

The animals were anesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine (90 mg/kg) and xylazine (5 mg/kg) and mounted in a stereotaxic apparatus (David Kopf Instruments, USA). Following a scalp incision, skull landmarks were visualized and coordinates were determined from Paxinos and Watson (2005) atlas. Two small holes were bilaterally drilled in the skull at the ATN coordinates (from duramater and bregma in AP: −1.5 mm, L: ±1.0 mm and DV: −6.0 mm; according to Tomitaka et al., 2000). Thereafter, injection stainless steel bilateral cannula (28 G, 3280PD style-2.0/SPC, Plastics One, USA) were slowly inserted into both holes to reach the ATN. Two small stainless steel screws serving as anchors were cemented to the skull with dental acrylic. After recovering from anesthesia the rats were returned into their home cages to the animal rooms until the day of the experiment (typically 4–5 days after cannula implants). During this period the animals were housed in groups of 3–4 to reduce the risk of injury with the implanted cannula.

### 2.4. Treatments

On the day of experiment, animals were first microinjected bilaterally into the ATN, with either muscimol (2.5 and 5.0 mM) or vehicle (saline). The intra-thalamic microinjections were performed by connecting the bilateral injection cannula to plastic tubing and these to an infusion pump (Harvard Apparatus, Instech USA). Then, the animals were allowed to move freely in the cage while saline or muscimol were perfused at a flow rate of 0.1 μl/min during 2 min (final volume of 0.2 μl), which corresponds to a total amount of 0.5–1.0 nmol of muscimol (2.5 and 5.0 mM, respectively) per injection site. Five min after the muscimol microinjection into the ATN, rats received an intraperitoneal (i.p.) injection of saline or MK-801 (0.2 mg/kg) and 30 min later, animals were individually placed in the centre of the Open Field (OF) and allowed to move freely for 15 min. The volume of injection was set at 1 ml/kg.

### 2.5. Behavioral experiments

Rats were moved in their home cages to the experimental room, identified and weighed one day before the behavioral experiments to allow acclimation to the test

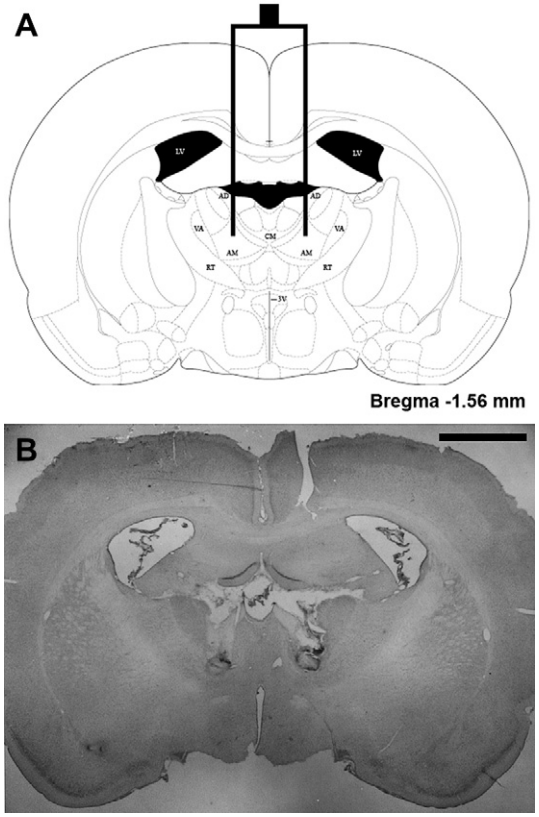


**Fig. 2.** Localization of the cannula in the anterior thalamic nucleus. Panel A shows a representation of the position of the bilateral cannula in the anterior thalamic nucleus (ATN). Diagram was taken from Paxinos and Watson (2005). Panel B shows a coronal section stained with hematoxilin/eosine showing the level of the bilateral cannula position in the brain. Bar scale in B: 2 mm.