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Research report

# Glutamatergic neurotransmission in the inferior colliculus influences intrastriatal haloperidol-induced catalepsy

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## HIGHLIGHTS

- A modulation of the IC in the catalepsy induced by haloperidol is proposed.
- The IC plays a role in sensorimotor gating and glutamatergic mechanisms are involved.
- MK-801 injected in the IC reduced ventral striatum haloperidol-induced catalepsy.
- NMDA injected in the IC facilitated dorsal striatum haloperidol-induced catalepsy.
- The IC integrates information from the auditory system and influences motor output.

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## ABSTRACT

The inferior colliculus (IC) is an important midbrain relay station for the integration of descending and ascending auditory information. In addition, it has also been implicated in the processing of acoustic information of aversive nature, as well as in sensory-motor gating. There is evidence that glutamate-mediated mechanisms at the IC level influence haloperidol-induced catalepsy. The present study investigated the influence of glutamate-mediated mechanisms in the IC on catalepsy induced by intrastriatal microinjection of haloperidol (10 μg/0.5 μl). Male Wistar rats received bilateral intracollicular microinjections of the glutamate receptor agonist NMDA (10 or 20 nmol/0.5 μl), the NMDA receptor antagonists MK-801 (15 or 30 nmol/0.5 μl) or physiological saline (0.5 μl), followed by bilateral microinjections of haloperidol (10 μg/0.5 μl) or vehicle (0.5 μl) into the dorso-rostral or ventro-rostral striatum. The catalepsy test was performed positioning both forepaws of the rats on an elevated horizontal wooden bar and recording the time during which the animal remained in this position. The results showed that the administration of physiological saline in the IC followed by the microinjection of haloperidol in the dorso-rostral region of the striatum was not able to induce catalepsy. However, when the bilateral administration of NMDA into the IC was followed by microinjection of haloperidol into the dorso-rostral striatum, catalepsy was observed. The microinjection of haloperidol into the ventro-rostral striatum induced catalepsy, counteracted by previous administration of MK-801 into the IC. These findings suggest that glutamate-mediated mechanisms in the IC can influence the intrastriatal haloperidol-induced catalepsy and that the IC plays an important role as a sensorimotor interface.



## 1. Introduction

The inferior colliculus (IC), a midbrain structure implicated in auditory processing, is distinguished from other auditory centers in the brainstem by its connections with motor systems [1]. The IC projects to the intermediate and deep layers of the superior colliculus [2] that control head, eye and pinnae movements for orientation toward objects in space. There is also evidence that motor systems project to the IC, since projections from the substantia nigra pars reticulata (SNpr) [3] and from the globus pallidus [4] to the IC have been reported in rats.

Studies aimed at the investigation of defensive behavior produced by electrical stimulation of the midbrain tectum have shown that local injections of NMDA into the IC produce behavioral activation interspersed with freezing very similar to the defense reactions elicited by electrical stimulation of this structure [5,6]. Lesions

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of the SNpr increase defensive responses induced by electrical or chemical stimulation of the deep layers of the superios colliculus [7] and the central nucleus of the IC [8–10]. Moreover, microinjection of MK-801 into the IC of rats is reported to disrupt prepulse inhibition (PPI) of the acoustic startle reflex, suggesting that blockade of NMDA receptors of the IC affects the PPI response, an operational measure of sensorimotor gating that reflect the functioning of a pre attention filtering system [11]. Together, these results indicate that glutamate-mediated mechanisms are involved in sensorimotor gating activated by emotional stimuli at this midbrain level [12].

Parkinson's disease is a neurodegenerative disease characterized by cell loss in the dopaminergic nigrostriatal system, which result in decreases in striatal dopamine (DA) concentration [13]. The antipsychotic haloperidol blocks DAergic receptors in the nigrostriatal pathway leading to extrapyramidal motor side effects [14]. In animal models, haloperidol induces a behavioral state known as catalepsy in which the animals are unable to correct externally imposed postures [15]. For this reason, haloperidol-induced catalepsy has long been used as an animal model for screening drugs for parkinsonism [16].

We previously showed that the intracollicular administration of glutamate receptor antagonists (AP7 and MK-801) and agonist (NMDA) alters haloperidol-induced catalepsy in rats, which is reduced by the antagonists and increased by the agonist [17]. Following this line of research, we also showed in a more recent study the involvement of neuronal glutamatergic circuits in the IC in the regulation of the catalepsy induced by L-NOARG, a nitric oxide synthase inhibitor, which can also impair striatal DA function [18]. Altogether these findings suggest participation of the IC in the modulation of motor function.

However, in the studies mentioned above, haloperidol was administered systemically. Furthermore, the possible involvement of the IC in the intrastriatal haloperidol-induced catalepsy has not yet been investigated. Therefore, in the present study, we investigated if catalepsy induced by haloperidol microinjected directly into the striatum can be influenced by glutamatergic neural circuitry in the IC. To this aim microinjections were performed bilaterally into the rostral part of the striatum since this region seems to be most sensitive to the cataleptogenic action of haloperidol [19].

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats weighing 250 and 300 g, from the animal house of CEDEME—Federal University of São Paulo (UNIFESP), were used, which had free access to food and water throughout the experiment. The animals were kept in Plexiglas-walled cages in a 12:12 h dark/light cycle (lights on at 07:00 am) and with a room temperature of 22 ± 1 °C and humidity of 55 ± 5%. All protocols were used according to the recommendations of the Brazilian Society for Neuroscience and Behavior (SBNeC), which are based on the guidelines of the American National Institute of Health for the Care and Use of Laboratory Animals (Publication No.85-23, revised 1985) and were approved by the ethics committee of Federal University of São Paulo (UNIFESP) (1666/11). All efforts were made to minimize the number of animals used and their suffering.

### 2.2. Surgery

The animals were anesthetized with 92 mg/kg ketamine (Ketamine Agener, União Química Farmacêutica Nacional, Brazil) and 9.2 mg/kg xylasine (Calmiun, União Química Farmacêutica Nacional, Brazil) and fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The upper incisor bar was set at 3.3 mm below the interaural line so that the skull was horizontal between bregma and lambda. Each animal was implanted with bilateral guide cannulae aimed at the IC and the ventro-rostral or dorso-rostral striatum. Guide cannulae were introduced vertically into the IC using the following coordinates, with lambda serving as reference: anteroposterior (AP) = −1.2 mm; mediolateral (ML) = ±1.5 mm; and dorsoventral (DV) = 4.5 mm [20]. Cannulae were lowered to the dorso-rostral or ventro-rostral striatum at the following coordinates: AP = 1.4 mm; ML: ±2.5 mm; and DV: 4,5 mm and AP = 1.4 mm; ML: ±2.5 mm; and DV: 6.7 mm, respectively, using bregma as the reference [20]. The guide cannulae were fixed to the skull with acrylic resin and two stainless steel screws. A stylette inside the guide cannulae prevented obstruction. At the end of surgery, each rat was treated with an intramuscular injection of penicillin G-Benzatine (120,000 UI; 0.2 ml) followed by an intramuscular injection of the analgesic and anti-inflammatory flunixin meglumine at 2.5 mg/kg. All animals were allowed a recovery period of 7 days after surgery with *ad libitum* access to food and water.

### 2.3. Drugs and doses

Haloperidol (Sigma-Aldrich, St. Louis, MO, USA) was dissolved with 40% glacial acetic acid and diluted with physiological saline to obtain the required concentration of 10 μg/0.5 μl. The drugs *N*-methyl-D-aspartate (NMDA; Sigma-Aldrich, St. Louis, MO, USA) and (+)-5-methyl-10, 11-dihydro-5H-dibenzo (a,d)-cyclohepten-5,10-imine (MK-801; Research Biochemicals International, Natick, MA, USA) were both dissolved in physiological saline. All microinjections were performed in a volume of 0.5 μl, and the animals from the control groups received an equivalent volume of physiological saline or vehicle.

### 2.4. Microinjection procedure

After removal of the stylettes, microinjections were delivered using thin stainless steel dental needles (Mizzy, São Paulo, Brazil; 30 gauge, outer diameter, 0.3 mm) introduced bilaterally through the guide cannulae until their lower ends were 1 mm below the cannulae tips. This infusion needle was connected to a 10 μl Hamilton syringe by polyethylene tube, and a volume of 0.5 μl of drug solution or vehicle was delivered over 1 min by an infusion pump (Insight Equipment, Brazil). The needle was left in place for an additional 1 min after injection. After that, the stylettes were again inserted.

### 2.5. Induction and evaluation of catalepsy

Five minutes after intracollicular administration of drug or saline, the animals received intrastriatal administration of haloperidol. Catalepsy, a prolonged maintenance of an externally imposed abnormal posture, was assessed by means of the bar test [21]. Briefly, the rat was gently placed with its forepaws on a horizontal wooden bar (1.5 cm in diameter × 25 cm long) positioned 8 cm above the floor. The duration of catalepsy was measured as the time (in seconds, maximum 300 s) during which the animal remained in this imposed posture. This test procedure was performed at 0, 30, 60, 90 and 120 min after intrastriatal administration of haloperidol or vehicle. The experiments were conducted in a quiet room.

The following experiments were performed. *Experiment 1*: in order to investigate the effects of the glutamate receptor agonist NMDA (10 or 20 nmol/0.5 μl; $n = 5$ and 7, respectively; NMDA/Halo groups) or physiological saline solution ($n = 7$; Sal/Halo group),