Behavioural Brain Research 223 (2011) 241–254

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

# Impairments of exploration and memory after systemic or prelimbic D1-receptor antagonism in rats

Bettina Clausen[a], Todd R. Schachtman[b,*], Louise T. Mark[a], Mette Reinholdt[a], Gert R.J. Christoffersen[a]

[a] Department of Biology, University of Copenhagen, Copenhagen, Denmark
[b] Department of Psychological Sciences, McAlester Hall, University of Missouri, Columbia, MO 65211, USA

ARTICLE INFO

*Article history:*
Received 24 August 2010
Received in revised form 28 March 2011
Accepted 30 March 2011
Available online 7 April 2011

*Keywords:*
D1-receptors
mGlu5
SCH23390
MPEP
Rat
Spatial memory
Object recognition
Exploration

ABSTRACT

D1-receptor antagonism is known to impair rodent memory but also inhibits spontaneous exploration of stimuli to be remembered. Hypo-exploration could contribute to impaired memory by influencing event processing. In order to explore this effect, the D1 receptor antagonist, SCH23390, was administered to rats via routes that either did or did not affect spontaneous exploration: systemic or prelimbic administration, respectively. Effects were tested in spatial and non-spatial memory tasks selected for their requirements for self-initiated exploration of stimuli to be remembered in order to examine the effects on memory: cross-maze and object recognition task. Systemic administration reduced spatial exploration in cross-maze as well as in an open field test, and also reduced object exploration. Spatial (hippocampus-dependent) short-term memory was inhibited in the cross-maze and non-spatial short-term object retention was also impaired. In contrast to these systemic effects, bilateral injections of SCH23390 into the prelimbic cortices altered neither spatial nor object exploration, but did inhibit short-term memory in both cross-maze and object recognition task. Therefore, the inhibiting effects of SCH23390 on both spatial and non-spatial memory were not mediated indirectly via reduced exploration of stimuli to be remembered, but through antagonism of a prelimbic D1-R function that is directly involved in memory formation. Finally, a cooperative regulation of spatial exploration between D1-R and mGlu5 was indicated by a synergistic effect of the antagonists SCH23390 and MPEP.



## 1. Introduction

The dopaminergic D1-receptor (D1-R) antagonist (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH23390) which penetrates the blood–brain barrier [54] has been used since 1983 as a selective D1-R antagonist [48,49] to study the effects of D1-R on behavior. Early reports found that systemically administered SCH23390 affected spontaneous levels of activity as expressed by reduced locomotion in rats [42,60,62,84] and mice [1,8]. In contrast to these findings, hyper-activity produced by 10 μg/kg of SCH23390 has been reported [11,60].

This study sought to further examine the role of D1 receptors on spatial and non-spatial learning using systemic and intra-cerebral injections, while evaluating the relation between activity measures and learning. In order to achieve this goal, several specific aims were pursued. The first aim was to investigate dose-dependent effects of systemically administered SCH23390 on spontaneous exploratory activity of rats exposed to new stimuli. This aim was pursued in three different experimental environments: a cross-maze, an open field and a field with objects positioned in it.

In addition to effects on spontaneous activity, systemic applications of SCH23390 have been found to dose-dependently impair working memory of rats [28,64,91]. As discussed below, reduced working memory function could have had a contributing causal role in the reported hypo-active exploration effects of SCH23390. Therefore the second goal for the present work was to test whether or not exploration in the cross-maze was correlated with a reduced working memory of recently visited maze-arms.

In contrast to these effects of systemically applied antagonist, local infusions into the rat prelimbic cortex of SCH23390 have failed to affect exploratory activity in mice [75] and rats [81] as well as working memory in the T-maze [77] and in the 8-arm maze [81]. These results exist despite the fact that the prelimbic cortex has been implicated in working memory performance [25,26,53,55]. By "working memory" we refer to the relatively small amount of information that is activated in memory at a particular time, in contrast to the huge amount of information stored in long-term memory. The third goal of the present study was therefore to elucidate the contrast between effects of systemic and prelimbic SCH23390 by observing exploration and working memory after bilateral prelimbic injections.

* Corresponding author. Tel.: +1 573 882 3154; fax: +1 573 882 7710.
*E-mail address:* SchachtmanT@missouri.edu (T.R. Schachtman).

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doi:10.1016/j.bbr.2011.03.069

Systemic D1-R antagonism has also been reported to have an inhibitory effect on long term memories using fear potentiated startle [36] and shock avoidance (both using 100–1000 μg/kg), and novel-object place learning (10–30 μg/kg) [7], but not for non-spatial object recognition memory [6]. Medial prefrontal injections of SCH23390 have also impaired long-term food-reinforced lever pressing (0.15–3 nmol) [4], and prelimbic applications of SCH23390 have reduced the learning of a shift between two discrimination tasks – but not the acquisition of either task [73]. It has also produced poor spatial memory of object position but not non-spatial object recognition [75]. Injections of SCH23390 (100 ng) into nucleus accumbens have also decreased spatial response accuracy in the five-choice serial reaction task [70] and intra-amygdalar injections (4 μg) have reduced conditioned fear potentiated startle [36]. Hippocampal infusion (5 μg) has blocked 6-h retention in a delayed match-to-place water maze task [67]. These effects of systemic and intracranial injections therefore indicate a greater D1-R involvement in spatial than in non-spatial memory; and the fourth objective for the present work was to re-examine this possibility. This goal was pursued by observing effects of SCH23390 on spatial conditioning (assessed in a cross-maze and a 3-hole task) versus non-spatial object memory in the object recognition task. This was done after either systemic- or prelimbic administrations.

The hypo-active exploration reported in the presence of systemic SCH23390 is similar to hypo-active exploration induced by an antagonist for subtype 5 metabotropic glutamate receptors (mGlu5): 2-methyl-6-(phenylethynyl)-pyridine (MPEP) [35]. Dose–response curves for this effect were analysed recently during exploration in the cross-maze and open field and during object exploration [20]. The possibility exists that antagonism of the two metabotropic receptors (D1-R and mGlu5) could regulate exploration in a synergistic manner. In addition to their common involvement in the regulation of exploration, the two receptors also activate the same two signalling cascades. MGlu5 couple to $G_{q/II}$-protein and stimulate phosphoinositide hydrolysis [12,21] and also activates adenylate cyclase [12,23,41]. The D1-R activates adenylate cyclase via coupling to heterotrimeric $G_s$-proteins as well as activates phospholipase C [57,86,90,92]. Evidence for group 1 mGlu and D1-R co-operation in phosphorylation of cAMP-response element binding protein (CREB) and the extracellular signal-regulated kinase 2 (ERK2) in rat striatal cells has also been found [87]. Therefore, the fifth aim for the present work was to test this potential synergy by comparing the hypo-activity effects of co-injected doses of SCH23390 plus MPEP with those of either antagonist injected alone.

## 2. Materials and methods

### 2.1. Subjects

Male Sprague-Dawley rats were housed in a vivarium with a 12 h light/12 h dark cycle (lights on at 07:00). For cross-maze experiments, the animals weighed between 200 g and 250 g on the first experimental day. The weight of the rats performing the object recognition task was 250–300 g. The rats had *ad libitum* access to food and water. However, rats trained in the cross-maze and in the 3-hole task (which were reinforced by liquid) were deprived of water 24 h before training and were weighed during the experimental period to ensure that no weight loss greater than 5% of pre-experimental body weight occurred. Rats used for i.p. injections were housed two per cage (distinguished by tail marks), but rats with implanted cannula were housed individually after implantation. The work was carried out with permission from the Animal Experiments Inspectorate of the Danish Government Justice Department.

### 2.2. Cross maze

#### 2.2.1. Apparatus

Experiments were performed in a cross-maze made of black acrylic and divided into 5 zones: four arms (North, East, South, West) and a centre zone. The distance from the end of one arm to the end of the opposite was 100 cm and the maze was enclosed by walls 45 cm high. All corners of the walls were rounded to prevent gnawing and to promote continuous locomotion. The maze was open at the top allowing sight of visual extra-maze cues which provided landmarks for the formation of a spatial map. At the end of all four arms, a 3 cm diameter hole placed 5 cm above the floor allowed access to an outside dispenser which could be moved in or out of reach for the rat. All four dispensers were filled with juice (tomato juice with 10% sugar) in order to equate the odour in each arm. (During place conditioning only one of the filled dispensers could deliver juice to the rat). The maze was positioned on a black PVC floor and was hoisted after experimentation with each rat for cleaning with antiseptic soap, rinsing and drying in order to remove odour traces. The maze was placed inside an aluminium enclosure with dim interior lighting, and external sounds were masked by a constant "white noise" inside the enclosure. The position of the subjects in the maze was monitored with a digitized video-tracking system (Ethovision, Noldus Information Technology, The Netherlands). Frequency and duration of contact with each of the four dispensers was recorded using IR-beam reflections from the nose of the rat.

#### 2.2.2. Procedures

*2.2.2.1. Experiment 1: Exploration and alternation behavior in the cross-maze after i.p. injections.* In experiment 1, 35 rats were divided into five groups given i.p. injections of either vehicle or 7.5-, 15-, 30-, or 50-μg/kg SCH23390 dissolved in 0.9% saline ($n = 7$ in each group). In this as well as all subsequent experiments, only naïve rats were used and were randomly assigned to groups. Injections were given 20 min before a rat was placed in the centre zone with the nose facing the wall between the North and East arms. Spontaneous locomotion was observed for a period of 30 min after the rat had been introduced into the maze for the first time.

*2.2.2.1.1. Data-analysis.* The distance of lateral locomotion was analysed in 6 periods of 5 min. Additionally, the number and identity of arm visits were recorded. The 30 min exploration session was also used to investigate alternation behavior between choices of arms. A spontaneous tendency to avoid revisits into recently visited arms was recorded as a "4/5-percentage" [43] calculated in the following way: First a list of the sequence of entries into identified arms from the centre zone was made. Next, the sequence was divided into cascading quintuplets of entries and the number of quintuplets in which all four arms had been visited was expressed in percent of the total number of quintuplets (the 4/5-percentage). For instance, if entries in the first quintuplet (nos. 1–5) included all four arms, this would count as a score of 1 and if the second quintuplet (nos. 2–6) involved fewer than 4 different arm entries a score of 0 would be recorded, etc. A high 4/5 measure is based on memory of recently visited arms combined with an efficiency-enhancing reluctance to revisit arms [27] and the parameter has therefore been used as a measure of working memory [43]. However, a high 4/5 score can also be achieved through repetitive behavioral procedures; for instance a score of 100% would result from a continuous series of repeated right turns. In order to assess the presence of such perseverant behavior, a supplementary measure of variability of arm-choices was introduced. This behavioral parameter contained a measure for "Transitions" between different movement patterns in the centre zone between arm visits [20]. Transitions were counted from the sequential list of arm entries giving a new list of centre zone movements identified as either: (1) right turn, (2) left turn, (3) diagonal forward passage through the centre zone or (4) re-entry into the just visited arm. Two identical sequential movements (for instance: right followed by right) were scored as no transition, while a change was counted as one transition (for instance: right followed by re-entry). The accumulated number of transitions was expressed in percent of the total number of centre zone passages (minus the first passage of the session): the "*T*-percentage". This percentage was used to clarify whether or not a high 4/5-percentage was achieved by a repetitive behavior.

*2.2.2.2. Experiment 2: Exploration and alternation behavior in the cross-maze after prelimbic injections.* In experiment 2, the behavioral procedures were the same as in experiment 1 except that 35 rats received bilateral infusions into the prelimbic cortices of 1 μl containing either vehicle, 0.01-, 0.05-, 0.5- or 5-nmol SCH23390 in 0.9% saline 20 min before being placed for the first time in the cross-maze ($n = 7$ in each group). Injections were given through micro-cannula implanted at least 10 days earlier.

*2.2.2.2.1. Implantation.* Rats were anaesthetized with a mixture of Hypnorm (VetaPharma, UK) and Midazolam (Hameln Pharmaceuticals, Germany) and placed in stereotaxic frame (David Koppf, USA). Local anaesthetics were applied to the skin over the scalp (lidocaine plus bupivacaine, University Hospital Pharmacy, Copenhagen). Two guide cannula (o.d. 0.6 mm) with internal obdurators and a dust cap were inserted through 0.7 mm scalp holes at coordinates: *Br* +3.7 mm, *L* ±0.8 mm and DV *sub dura* 3 mm [69]. The plastic assembly holding the cannula was mounted with dental cement (Simplex Rapid, Kemdent, Denmark) affixed to 2 bone screws. Immediately after the surgery, analgesium (Temgesic, Schering-Plough, Belgium; 0.12 mg/kg) was given s.c. and lidocaine was applied to the wound. This analgesic procedure was repeated 6 times at 6 h intervals. Daily observation took place during 10 days of recovery which included three occasions of handling before the first day of training.

*2.2.2.2.2. Implant positions.* After the behavioral part of the experiment, the positions of cannula tips within the prelimbic cortex were verified. Brains were placed in 4% formaldehyde and stored frozen at −80 °C. Later, they were placed in 85% n-hexane and sectioned in 30 μm coronal sections in a freeze microtome. The positions of tips were derived from digital photos of cannula tracks in the partially