Behavioural Brain Research 293 (2015) 81–88

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Brain regions associated with inverse incentive learning: c-Fos immunohistochemistry after haloperidol sensitization on the bar test in rats

Lexy N. Pezarro Schimmel[a], Tomek J. Banasikowski[a], Emily R. Hawken[a,b], Eric C. Dumont[a,b,c], Richard J. Beninger[a,c,d,*]

[a] Centre for Neuroscience Studies, Kingston, ON K7L 3N6, Canada
[b] Biomedical and Molecular Science, Kingston, ON K7L 3N6, Canada
[c] Psychiatry Queen's University, Kingston, ON K7L 3N6, Canada
[d] Psychology Queen's University, Kingston, ON K7L 3N6, Canada

HIGHLIGHTS

- We pair low-dose of haloperidol with the environment of a bar test.
- Pairing daily haloperidol with the bar test produces sensitized descent latencies.
- Unpaired rats received haloperidol in their home cages; they did not sensitize.
- Paired rats have less c-Fos in the nucleus accumbens core and ventral pallidum.
- Paired and unpaired have less c-Fos in the striatum and nucleus accumbens shell.

ARTICLE INFO

Article history:
Received 6 April 2015
Received in revised form 23 June 2015
Accepted 27 June 2015
Available online 17 July 2015

Keywords:
Dopamine
Basal ganglia
Ventral pallidum
Nucleus accumbens core

ABSTRACT

Inverse incentive learning is the loss by stimuli of their ability to elicit approach and other responses. We used c-Fos immunohistochemistry to identify brain regions associated with inverse incentive learning. Rats that had daily treatments with haloperidol (0.25 mg/kg) paired with placing their forepaws on a horizontal bar elevated 10 cm above the floor initially descended almost immediately but over days descent latencies grew longer, revealing inverse incentive learning. Control rats that were tested daily and received haloperidol (Unpaired group) or saline later in their home cage showed no evidence of increased descent latencies. On the final test day, all groups were tested after haloperidol and only the Paired group showed increased descent latencies. c-Fos levels in the nucleus accumbens core and ventral pallidum were lower in the Paired group than in the Unpaired and Saline groups even though all groups received haloperidol on the test day. Compared to the Saline group both the Paired and Unpaired groups showed evidence of lower c-Fos levels in the dorsal striatum and nucleus accumbens shell, possibly a result of daily haloperidol injections. No group differences in c-Fos were found in the piriform cortex, ventral hippocampus, ventral tegmental area or lateral habenula. Results reveal, by means of different patterns of c-Fos expression, brain region-specific changes in neuronal activity associated with inverse incentive learning. Results support possible underlying neuroplastic changes for learned decreases in responsivity to environmental stimuli.

Abbreviations: ANOVA, analysis of variance; BSA, bovine serum albumin; DA, dopamine; DAB, diaminobenzidine; dd H_20, double distilled water; DLS, dorsolateral striatum; DMS, dorsomedial striatum; GABA, γ-aminobutyric acid; lHab, lateral habenula; NAc, nucleus accumbens; NAcC, nucleus accumbens core; NaCl, sodium chloride; NAcS, nucleus accumbens shell; PBS, phosphate buffered saline; TB, trizmabase; TBS, trizmabase sodium chloride; TBS-T, trizmabase sodium chloride and Triton X-100; vHipp, ventral hippocampus; VP, ventral pallidum; VTA, ventral tegmental area.

* Corresponding author at: Dept. Psychology, Queen's University, 62 Arch Street, Kingston, ON K7L 3N6, Canada. Fax: +1 613 533 2499.
E-mail address: Beninger@queensu.ca (R.J. Beninger).

http://dx.doi.org/10.1016/j.bbr.2015.06.041
0166-4328/

1. Introduction

Rewarding stimuli produce increases in dopaminergic neurotransmission that lead to incentive learning, i.e. the acquisition by previously neutral stimuli of an increased ability to elicit approach and other responses [5,8]. A simple experimental example of incentive learning is conditioned activity. If an animal repeatedly receives injections of a dopamine (DA)-enhancing stimulant drug such as amphetamine or cocaine paired with placement into a distinctive environment, the animal will show significantly elevated locomotor activity in that environment when it receives a saline injection there [6,7]. Control unpaired animals with a similar history of exposure to the test environment and a similar history of amphetamine or cocaine injections but outside of the test environment, do not show elevated activity during the saline test confirming the conditioned nature of the response [6,7].

DA antagonists can produce a similar but inverted conditioning effect in the horizontal bar test that measures the length of time an animal spends standing on its hind legs with its forepaws resting on a horizontal bar elevated above the floor [31]. High doses of many DA receptor antagonists (e.g. chlorpromazine, haloperidol) produce unconditioned catalepsy in the bar test, rats remaining motionless for extended periods of time [31]. However, Schmidt and co-workers showed sensitization of descent latencies with low doses of haloperidol. They found that repeated pairing of a distinctive test environment with a low dose of haloperidol initially produced no increase in the descent latency of rats but gradual increases in descent latencies were observed over days with repeated testing [19,33]. Control unpaired rats undergoing bar testing every day but receiving haloperidol outside the testing environment did not show descent latency sensitization. When they were subsequently tested on the bar following an injection of haloperidol, descent latencies were short, confirming the conditioned nature of the sensitization effect. When both the paired and unpaired groups were tested following an injection of saline, increased descent latencies were observed in the paired but not in the unpaired group, demonstrating a conditioned response [3,4,19].

Sensitization in the bar test and conditioned increases in descent latencies can be understood as *inverse incentive learning*; stimuli repeatedly paired with decreases in dopaminergic neurotransmission gradually lose their ability to elicit approach and other responses. According to this framework, increases in descent latency reflect decreases in the ability of stimuli associated with bar testing to elicit responses from the animals. Although the mechanisms of inverse incentive learning are largely unknown, DA receptors may be implicated. Indeed co-treatments with low doses of haloperidol plus the DA D1-like receptor antagonist SCH 23,390 blocked conditioned increases in descent latency normally seen in paired rats on the saline test day [3]. Furthermore D3 receptor antagonists block the expression of conditioned increases in descent latency when given on the saline test day without significantly affecting the acquisition of increased descent latencies in the bar test [4].

Here, we used c-Fos immunohistochemistry to map neuroanatomical regions potentially involved in sensitization to low doses of haloperidol on the bar test [10,11]. Many studies have linked catalepsy to the dorsal and ventromedial striatum, and nucleus accumbens (NAc) [27,43]; these areas may also be involved in inverse incentive learning measured with the bar test. Areas linked to these striatal regions have been implicated in context-dependent incentive learning [2,10,15,23,26,28,40,41]. They may also be involved in inverse incentive learning; thus we evaluated striatal input regions including the cerebral cortex, ventral hippocampus (vHipp), substantia nigra, and ventral tegmental area (VTA), and output regions including the globus pallidus and ventral pallidum (VP) for c-Fos expression.

We trained paired and unpaired rats and a saline group in bar-test sensitization and then carried out a haloperidol test on all groups. Immediately following the test, rats were euthanized and brains prepared for c-Fos immunohistochemistry. Based on the work of Schmidt and co-workers and our previous observations we hypothesized that the paired but not the unpaired or saline group will show sensitization of descent latencies [1,3,4,19,20,29,32,33,38]. We hypothesized that the paired group will show changes in c-Fos immunohistochemistry in striatal regions previously implicated in catalepsy, including the dorsal striatum and NAc [27,43]. Possible changes may also be seen in striatal output regions but group differences were not expected in striatal input regions. We found sensitization of descent latencies in the paired group and lower c-Fos expression in the NAcC and the VP.

2. Methods

2.1. Subjects

Experimentally naïve male albino Wistar rats ($n = 29$) weighing 200–225 g were obtained from Charles River Canada (St. Constant, QC). They weighed approximately 300 g at the start of the experiment. The rats were housed in a colony room on a reverse 12-h light/dark cycle with lights on at 1900 h. The average room temperature was 21 °C with humidity of about 70%. The rats were housed in groups of two or three in clear Plexiglas cages (45.0 × 25.0 × 22.0 cm) on Beta Chip bedding (Northeastern Products Corp., Warrensburg, NY) with continuous access to food (LabDiet 5001, PMI Nutrition International, Brentwood, MO) and water. The rats were treated in accordance with the guidelines of the Animals for Research Act and the Canadian Council on Animal Care. The Queen's University Animal Care Committee approved the project.

2.2. Drugs

Haloperidol, (4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one) (Sigma–Aldrich) was prepared in a 0.3% distilled-water solution of tartaric acid. Injections were administered intraperitoneally in a volume of 1.0 ml/kg.

2.3. Behavioural procedure

Rats were randomly assigned to experimental (Paired, $n = 10$) and control groups (Unpaired, $n = 9$; Saline, $n = 10$). The bar test sensitization procedure consisted of injecting rats with haloperidol or saline outside the testing room and putting them back into their home cages. Sixty minutes later, they were tested on a horizontal bar (see below) by gently placing both forepaws on the bar. Descent latency was measured as the time span from placing the animal on the bar until the first active paw movement (i.e. one forepaw left the bar or the hind legs left the floor to climb onto the bar). A cut-off time of 180 s was used, i.e. the trial was terminated when the animal did not make an active paw movement within that time.

2.4. Experimental design

The experiment was co-designed by Dr. Peter Ossenkopp from the University of Western Ontario, London ON. The experimental design was adapted from the 3-group sensitization procedure for quinpirole sensitization used by [22]. Bar test sessions occurred between 0900 and 1700 h, during the dark phase of the light–dark cycle. The test apparatus consisted of two distinct rectangular Plexiglas horizontal bar chambers (32 × 42 × 30 cm), one with three black sides and one with three white sides; the fourth side was clear