This is a repository copy of *Partial lesion of dopamine neurons of rat substantia nigra impairs conditioned place aversion but spares conditioned place preference.*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/115082/

Version: Accepted Version

Accepted Manuscript

Partial lesion of dopamine neurons of rat substantia nigra impairs conditioned place aversion but spares conditioned place preference

Bernardo F.C. Lima, Daniele C. Ramos, Janaína K. Barbiero, Laura Pulido, Peter Redgrave, Donita L. Robinson, Alexander Gómez-A, Claudio Da Cunha

www.sciencedirect.com/science/journal/aip/03064522

ASUS E-Service | Imported From IE | SD Science Direct | P Carlos Chagas | Web of Science | periodicos CAPES | r | S Plataforma Sucupira | Grupo de Pesquisa CI

Neuroscience

Supports Open Access | About this Journal | Sample Issue Online | Submit your Article

Get new article feed

Get new Open Access article feed

Subscribe to new volume alerts

Add to Favorites

< Previous vol/iss | No next vol/iss

Neuroscience
Articles in Press - Note to users

Articles

Download PDFs | Export ▾ | All access type

Articles in Press are accepted, peer reviewed articles that are not yet assigned to volumes/issues, but are citable using DOI - find out more.

☐ Partial lesion of dopamine neurons of rat substantia nigra impairs conditioned place aversion but spares conditioned place preference Original Research Article
In Press, Accepted Manuscript, Available online 7 March 2017
Bernardo F.C. Lima, Daniele C. Ramos, Janaína K. Barbiero, Laura Pulido, Peter Redgrave, Donita L. Robinson, Alexander Gómez-A, Claudio Da Cunha
▸ Abstract | ▾ Close graphical abstract | ▸ Research highlights | 📄 PDF (1159 K)
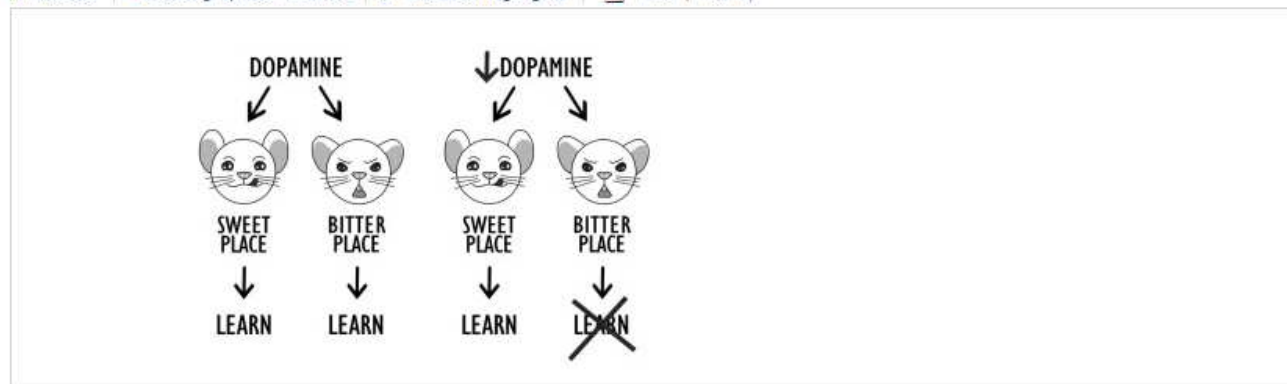
☐ Estrogen receptors ERα and ERβ participation in hypothalamus-pituitary-adrenal axis activation by her

Partial lesion of dopamine neurons of rat substantia nigra impairs conditioned place aversion but spares conditioned place preference

Abbreviated title: Place conditioning and partial SNc dopamine lesion

Bernardo F. C. Lima[a*], Daniele C. Ramos[a*], Janaína K. Barbiero[a], Laura Pulido[a], Peter Redgrave[b], Donita L. Robinson[c], Alexander Gómez-A[a+], & Claudio Da Cunha[a+]

[a]Departamento de Farmacologia, Universidade Federal do Paraná, Curitiba 81.530-980, PR, Brazil.
[b]Department of Psychology, University of Sheffield, UK.
[c]Department of Psychiatry and Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599-7178, USA.

[*]The first 2 authors made equally important contributions to this study.
[+]Corresponding authors: Claudio Da Cunha and Alexander Gómez-A. Departamento de Farmacologia da Universidade Federal do Paraná, 81531-980 Curitiba, Paraná, Brazil.
Email: dacunha.claudio@gmail.com

Abstract

Midbrain dopamine neurons play critical roles in reward- and aversion-driven associative learning. However, it is not clear whether they do this by a common mechanism or by separate mechanisms that can be dissociated. In the present study we addressed this question by testing whether a partial lesion of the dopamine neurons of the rat SNc has comparable effects on conditioned place preference (CPP) learning and conditioned place aversion (CPA) learning. Partial lesions of dopamine neurons in the rat substantia nigra pars compacta (SNc) induced by bilateral intranigral infusion of 6-hydroxydopamine (6-OHDA, 3 µg/side) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 200 µg/side) impaired learning of conditioned place aversion (CPA) without affecting conditioned place preference (CPP) learning. Control experiments demonstrated that these lesions did not impair motor performance and did not alter the hedonic value of the sucrose and quinine. The number of dopamine neurons in the caudal part of the SNc positively correlated with the CPP scores of the 6-OHDA rats and negatively correlated with CPA scores of the SHAM rats. In addition, the CPA scores of the 6-OHDA rats positively correlated with the tissue content of striatal dopamine. Insomuch as reward-driven learning depends on an increase in dopamine release by nigral neurons, these findings show that this mechanism is functional even in rats with a partial lesion of the SNc. On the other hand, if aversion-driven learning depends on a reduction of extracellular dopamine in the striatum, the present study suggests that this mechanism is no longer functional after the partial SNc lesion.

Introduction

Strong evidence exists that dopamine release in the nucleus accumbens (NAc) by neurons of the ventral tegmental area (VTA) is critical for reward-driven associative learning (Salamone, 1994, Phillips et al., 2003b, Bassareo et al., 2007, Kim et al., 2012, Chaudhri et al., 2013, Ilango et al., 2014a, Ilango et al., 2014b, Sciascia et al., 2014) and that the dopamine neurons of the substantia nigra pars compacta (SNc) play a key role in aversion-driven associative learning (Bracs et al., 1984, Da Cunha et al., 2001, Ferro et al., 2005, Manago et al., 2009, Boschen et al., 2011, Kravitz et al., 2012, Dombrowski et al., 2013, Ilango et al., 2014b, Boschen et al., 2015, Pauli et al., 2015). Less, but relevant, evidence also exists that VTA -to-NAc dopamine plays a role in aversion-driven associative learning (Wadenberg et al., 1990, Salamone, 1994, Setlow and Mcgaugh, 1998, 2000, Lalumiere et al., 2005) and that the dorsal striatal dopamine is important for some kinds of reward-driven associative learning (Zaghloul et al., 2009, Kravitz et al., 2012, Rossi et al., 2013, Ilango et al., 2014b, Ramayya et al., 2014, Pauli et al., 2015).

A widely accepted model of reward-driven associative learning proposes that the dopamine released in the striatum in response to "better-than-expected" rewards (positive prediction error) reinforces synapses between: neurons encoding a conditioned stimulus (CS) and neurons encoding a conditioned response (CR); neurons encoding a discriminative stimulus (S) and neurons encoding the response that caused the reward (R); and between neurons encoding the expectation of a rewarding outcome (O) and neurons encoding the instrumental action (A) that caused the reward (Schultz and Dickinson, 2000, Waelti et al., 2001, Yin et al., 2008, Da Cunha et al., 2009, Schultz, 2016). In the same way, it is possible that the pause in the firing of midbrain dopamine neurons caused by omission of an expected reward (negative prediction error) (Schultz, 1998) weakens the above mentioned associations. A solid body of evidence supports the hypothesis that most midbrain dopamine neurons fire as if they encode both positive and negative prediction errors (Schultz, 2010, Hart et al., 2015, Schultz, 2016). Voltammetry evidence further supports this hypothesis (Brown et al., 2011, Oleson et al., 2012, Sunsay and Rebec, 2008, Saddoris et al., 2015, Hart et al., 2014). For example, even the observation of unpredicted reward delivery to a conspecific can cause dopamine release in the ventral striatum (Kashtelyan et al., 2014). There is also substantial evidence supporting the proposal that dopamine release in dorsal and ventral striatum reinforces different types of associative learning including conditioned stimulus-conditioned response (Parkinson et al., 2002, Phillips et al., 2003a, Dalley et al., 2005, Sunsay and Rebec, 2008, Lex and Hauber,

2010, Darvas et al., 2014), stimulus-response and action-outcomeassociations (Tombaugh et al., 1979, Nakajima, 1986, Kim et al., 2012).

The role of midbrain dopamine neurons in aversion-driven associative learning is less understood. Aversive stimuli cause a pause in the firing of most midbrain dopamine neurons (Mirenowicz and Schultz, 1996, Brischoux et al., 2009, Matsumoto and Hikosaka, 2009, Ilango et al., 2012, Schultz, 2016), but also cause an increase of tonic dopamine in the striatum as measured by microdialysis (Dunn and File, 1983, Sorg and Kalivas, 1991, Imperato et al., 1992, Salamone, 1994, Bassareo et al., 2002, Ventura et al., 2007, Dombrowski et al., 2013). A few electrophysiological studies reported cases in which some dopamine neurons were activated by both aversive and rewarding stimuli (Brischoux et al., 2009, Matsumoto and Hikosaka, 2009, Berridge and Kringelbach, 2015). Voltammetry studies reported that aversive stimuli elicit both phasic decrease (Roitman et al., 2008, Badrinarayan et al., 2012, Budygin et al., 2012, Oleson et al., 2012) and phasic increase (Anstrom et al., 2009, Badrinarayan et al., 2012, Budygin et al., 2012, Oleson et al., 2012) in dopamine release in different areas of the striatum. However, it is unclear whether dopamine neurons are encoding an aversion response (Ilango et al., 2014a) or nonselective response driven by the physical salience of the stimulus (Schultz, 2016). It is also under debate whether the dopamine neurons that are activated by aversive stimuli belongs to different categories (Bromberg-Martin et al., 2010) or whether they simply differ in the intensity to which they respond to salient stimuli (Schultz, 2016). An important property of the midbrain dopamine neurons is that even those inhibited by aversive stimuli present rebound activation when the stimulus ends (Brischoux et al., 2009, Matsumoto and Hikosaka, 2009, Schultz, 2016). This may serve as a negative reinforcement signal to drive inhibitory avoidance learning: the released dopamine could reinforce the association between the predictive stimulus and the inhibitory avoidance response. However, it is not clear whether the above mentioned evidence of phasic dopamine changes observed in studies using CSs with clear phasic onset/offset also applies to contextual avoidance learning tasks where the association is with static environmental stimuli.

Conditioned active avoidance learning is more complex than inhibitory avoidance insofar as it is thought to depend on two processes: the learning that a phasic or contextual CS predicts the onset of an aversive stimulus, followed by the learning that this aversive stimulus can be avoided by an instrumental response. Initially, the subject does not know that a particular response causes avoidance of the aversive stimulus. Such an outcome is therefore "better than expected" – a positive prediction error. There is evidence that termination of an aversive event triggers phasic dopamine response (Brischoux et al., 2009; Oleson et al., 2012), and that the

consequent release of dopamine reinforces the association between the CS (i.e. a tone) and the instrumental avoidance response (Dombrowski et al., 2013). Therefore, in addition to reward-driven learning, avoidance learning may also depend on prediction-error triggered dopamine release.

In summary, there is evidence that midbrain dopamine neurons play critical roles in reward- and aversion-driven associative learning but it is not clear whether they do this by a common mechanism or by mechanisms that can be dissociated. While the role of dopamine neurons of the VTA in associative learning is comparatively well understood (Salamone, 1994, Phillips et al., 2003b, Bassareo et al., 2007, Chaudhri et al., 2010, Kim et al., 2012, Chaudhri et al., 2013, Ilango et al., 2014a, Ilango et al., 2014b, Sciascia et al., 2014), that of the laterally located dopamine neurons in SNc is less clear. Therefore, in the present study we addressed this issue by testing whether a partial lesion of the dopamine neurons of the rat SNc affects conditioned place preference (CPP) learning and conditioned place aversion (CPA) learning to the same extent.

Experimental procedures

Animals

Ninety male Wistar rats from Universidade Federal do Parana (UFPR) vivarium were used, weighing 280-310 g at the time of surgery. Rats were maintained in a temperature-controlled room (22±2°C) on a 12/12 light/dark cycle (lights on at 7 a.m.) with food and water *ad libitum*, except for some groups of animals that underwent food restriction as described below. Body weight and water intake were monitored every 3 days. All possible efforts were made to minimize the number of animals used and their discomfort during the experimental procedures. After the end of the experiments, rats were humanely killed by decapitation under deep ketamine/xylazine (140/10 mg/kg) anesthesia. All procedures were approved by the Animal Care and Use Committee of the UFPR (protocol numbers 664 and 846) and conducted in accordance with the Brazilian law (11.794/8 October 2008) and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Neurotoxic lesion of the SNc with 6-OHDA or MPTP

Animals were anesthetized with 3 ml/kg equithesin (1% sodium thiopental, 4.25% chloral hydrate, 2.13% magnesium sulfate, 42.8% propylene glycol, and 3.7% ethanol in water) and secured in a stereotaxic frame. Next, 2 μL of saline (0.9% NaCl), 3 μg 6-OHDA (Sigma,

dissolved in 2 μL of the following solution: 8.66 g NaCl, 0.205 g KCl, 0.176 g CaCl$_2$.2H$_2$O, 0.173 g MgCl$_2$.6H$_2$O and Milli-Q purified water sufficient to complete a final volume of 50 ml) or 200 μg MPTP (Sigma, dissolved in 2 μL of saline) were infused bilaterally into the SNc in the following coordinates: AP -5.0 mm from bregma, ML ± 2.1 mm from midline, DV -7.7 mm from skull, with incisor bar 3.3 mm below the interaural line (Paxinos and Watson, 2005, Dombrowski et al., 2013). We used 6-OHDA and MPTP doses known to cause cognitive deficits without motor impairments (Da Cunha et al., 2001, Gevaerd et al., 2001a, Gevaerd et al., 2001b, Perry et al., 2004, Ferro et al., 2005, Bortolanza et al., 2010). Rats of the SHAM group received saline or the 6-OHDA solution vehicle instead of MPTP or 6-OHDA, respectively. Because there were no significant difference between the measures made in the animals that received saline or vehicle, data were pulled and the animals of these two control subgroups are referred to as SHAM.

Open field test

The open field test was carried out 22-27 days after surgery. Each animal was allowed to freely explore an open field for 5 min. The field was a circular arena (100 cm diameter x 45 cm height) with lines dividing the arena in 18 areas of the same size. The number of times the animal crossed the lines, the number of rears, and the number of grooming episodes were scored (Frussa-Filho et al., 1999).

Place preference testing box

We used a wooden box with a design adapted from White and Carr (1985). The box had two lateral compartments (40x22x30 cm) and a central compartment (10x22x30 cm). A rat could be confined in one of the compartments by inserting guillotine-like dividers. The right lateral compartment had a textured glass floor and vertical stripes painted on the walls. The left lateral compartment had a smooth wooden floor and horizontal stripes painted on the walls.

Conditioned place preference (CPP)

The same rats used in the open field test were later used to test CPP learning. Before surgery these animals were tested for sucrose consumption. In this test each rat was placed in a Plexiglas cage (similar to the home cage) for 3 consecutive days with 10 sucrose pellets each day. The rat had 15 min to eat the pellets on the first day, 10 min on the second day, and 5 min on the last day. All animals ate the sucrose pellets within the designated times.

The CPP protocol used was adapted from White and Carr (1985) and started 3 weeks after surgery. From this day to the end of the experiment, these rats were food restricted to

maintain 85-90% of their free-feeding body weight. In order to check for *a priori* preference for one of the 2 test compartments, after 3 days of food restriction each rat was given 10 min to explore the CPP box freely with the sliding doors opened. Excluding the time spent in the central compartment, the time that each rat spent exploring the right lateral compartment plus the time spent in the left lateral compartment was taken as 100%. Four out of the 6 rats of the SHAM group, 5 out of the 7 rats of the 6-OHDA group and 3 out of the 7 rats of the MPTP group spent between 41% and 48% of the time exploring the left compartment and spent between 51% and 59% of the time exploring the right compartment. The other rats presented the opposite pattern of time distribution between these compartments. These differences did not meet the criterion used for *a priori* place-preference: spend more than 60% in one of the compartments. The compartment in which the animal spent more time was assigned to be the neutral (empty) compartment and the other compartment was assigned to be the appetitive compartment (paired with sucrose pellets). Following this rule resulted in counterbalanced numbers of animals in which a specific compartment was assigned as appetitive (N = 12) and the number of animals in which the same specific compartment was assigned as neutral (N = 8): this 12 versus 8 distribution is not significantly different from a 10 versus 10 distribution (p = 0.52, Chi-square test).

Starting the next day, rats were given 6 sessions of CPP training (1 session per day) and 1 session of place preference testing. Each training session consisted in confining the rat for 20 min in the appetitive compartment and, immediately after, confining it for 20 min in the neutral compartment. The appetitive compartment had 10 sucrose pellets (70 mg/pellet) and the neutral compartment was empty (no pellets). In the test session, the rat was placed in the center compartment with the doors opened. This session was videotaped and the time spent in each compartment was scored by using the ANY-maze software (Stoelting, IL).

In the following 6 days, rats were given one extinction session per day. These sessions were similar to the training sessions, except that both the right and left compartments were empty. On the next day, place preference was tested again.

Conditioned place aversion (CPA)

Three CPA protocols were deployed: (i) naive rats were exposed to a place paired with quinine pellets; (ii) rats were first exposed to a place paired with sucrose pellets, then submitted to extinction sessions in the same location now paired with quinine pellets; and (iii) a quinine solution was delivered orally to naive rats who were then exposed them to a place. In all cases rats were given an alternative choice of a neutral place – not paired either with quinine or

sucrose. Except for the use of quinine pellets or quinine solutions, all CPA protocols were similar to that used for CPP.

For protocols (i) and (ii) the aversive pellets were made of white painted epoxy spheres, immersed in 1.5 mg/ml quinine HCl (Sigma-Aldrich) for 24 h and allowed to dry. They looked identical to the sucrose pellets. Rats trained under protocol (iii) were gently restrained and received 200 μL of a 1 mM quinine solution into the mouth with a syringe. Immediately afterwards, the rat was confined in the aversive compartment for 20 min. During this period the rat received additional doses of quinine every 5 minutes. Next, no solution was given and the rat was confined in the neutral compartment for 20 min. As in the protocol used for CPP, rats were given 1 training session per day for 6 training days and 1 testing day.

Unconditioned responses to sucrose and quinine

Additional groups of rats were used in the two experiments described next. First, SHAM, 6-OHDA and MPTP lesioned rats were anesthetized with ketamine/xylazine (140/10 mg/kg) and two bilateral oral cannulae (heat-flared polyethylene tubing) were implanted by according to the protocol published previously by Roitman et al., (2008). Each cannula entered the mouth just lateral to the first maxillary molar with an ethyl vinyl acetate washer flush against the molar. The other end of each tubing was exteriorized and held at the top of the head with a second washer. After recovery, the external end of each tubing was connected to a syringe. The rat was placed in a transparent box and videotaped from the bottom. Every minute the rat received 200 μL of a 10% sucrose solution through intraoral cannula for 4 times. Three minutes later, the rat received 200 μL of a 1 mM quinine solution through the contralateral cannula for 4 times (once per minute). Immediately after the infusion of the sucrose or quinine solutions the orofacial expressions elicited were recorded for 1 min. The behavior of fast diagonal tongue protrusions was scored and classified as an appetitive response, while gaping responses were scored as an aversive reaction (Berridge and Robinson, 1998).

Behavioral responses to self-administered quinine and sucrose pellets were also recorded. Each rat was placed in a glass cylinder (20 cm diameter, 19 cm high) within a (50 cm long, 23.5 cm wide, and 35 cm high) wooden box with mirrors on the internal walls; this allowed the animal's interaction with the pellets to be videotaped independent of the animal's position (adapted from Grill and Norgren, 1978). On the first day, each rat was given 5 min habituation inside the empty cylinder. The next day the animal was returned to the cylinder with 10 sucrose pellets on the floor. The animal was videotaped from the front and the

recording time started from the moment the animal stood with 4 paws on the floor and ended 1 min after all pellets had been eaten. The following behaviors were scored: (i) time to pick up the first pellet; (ii) time interacting with all pellets (elapsed time from picking up the first pellet to finish eating the last pellet) (iii) inter-pellet intervals (elapsed time from picking up one pellet to picking up the next pellet); (iv) total time spent eating (the sum of the time spent only eating the 10 pellets). On the 3rd day, the animals were returned to the same cylinder but with 10 quinine pellets. Because the quinine pellets could not be consumed, the following differences were made in testing and scoring: (i) the session lasted for 20 min (the mean length of the CPA training sessions); (ii) chewing behavior was scored instead of eating behavior; (iii), the number of times the animal picked up the pellets was scored instead of the number of pellets eaten. The number of rats that presented gaping behavior in response to the quinine pellets was recorded. The typical appetitive orofacial expression that was observed in response to the sucrose solution was not observed when the rats tasted the sucrose pellets.

Evaluation of the nigrostriatal lesion

The brains of 5 rats of each group were processed for tyrosine hydroxylase (TH) immunohistochemistry. The midbrains were dissected and stored for at least 24 h in paraformaldehyde 4% w/v at 4°C, and saturated in a sucrose solution 30% w/v in PBS 0.1 M, frozen on dry ice, sliced in cryostat (Leica Biosystems). One of every 6 coronal slices of 40 μm was collected and incubated in primary anti-TH antibody (1:500; cat # AB152 Chemicon), then transferred to a biotin conjugated secondary antibody solution (1:200, cat # S-1000 Vector Laboratories); next it was transferred to an ABC system solution (cat # PK6101, Vectastain ABC Elite kit, Vector Laboratories), and finally incubated with a 25 mg/ml 3,3'-diaminobenzidine solution. The slices were mounted on glass slides and scanned in a motorized Axio Imager Z2 microscope (Carl Zeiss) equipped with automated scanning VSlide (MetaSystems). TH-immunoreactive neurons were counted in all collected slices of the rostral (-4.6 to -5.3 mm from bregma) and caudal (-5.4 to -5.9 mm from bregma) SNc and VTA as determined by the rat brain atlas of Paxinos and Watson (2005).

The brains of 12 SHAM, 7 6-OHDA, and 9 MPTP rats were quickly removed on ice and the whole striata were dissected and stored at -80ºC. Concentrations of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum were separated and quantified by HPLC with electrochemical detection. The striatal tissue samples were homogenized with an ultrasonic cell disrupter (Sonics, Newton, USA) in 0.1 M perchloric acid containing 0.02% sodium metabisulfite (Sigma), and 50 ng/ml of the internal standard 3,4-dihydroxybenzylamine

hydrobromide (Sigma). After centrifugation at 10,000 g at 4ºC for 30 min, 20 µl of the supernatant was injected in a HPLC system (Shimadzu) with a Synergi Fusion-RP C-18 reverse-phase column (150 x 4.6 mm, 4 µm particle size; Phenomenex) and integrated with a coulometric 197 electrochemical detector (ESA Coulochem III) equipped with a working electrode set at +450 mV vs. a palladium reference electrode (for details, see Dombrowski et al., 2013).

Statistical analyses

Numerical data were tested for normality (D'Agostino-Pearson omnibus' test) and equal variance (standard unpaired t test for unequal variance). Apart from grooming, and the unconditioned responses to sucrose, and quinine pellets, which were analyzed by Kruskal-Wallis ANOVA followed by Dunn's multiple comparison post-hoc contrasts, all other data met these criteria. Open field test data were analyzed by one-way ANOVA. Percent of time spent in the neutral versus appetitive compartments and percent of time spent in the neutral versus aversive compartments data were analyzed by two-way ANOVA followed by Bonferroni-corrected, post-hoc contrasts. Number of neurons, dopamine and DOPAC levels were analyzed by one-way ANOVA followed by the Dunnett's post-hoc contrasts. Sphericity for data of body weight, food intake, water intake, and time spent in neutral and appetitive compartments before CPP training, after CPP training and after extinction were confirmed; these data were analyzed by repeated-measures ANOVA followed by Bonferroni-corrected post-hoc contrasts. Correlations between two variables were analyzed by the Pearson's test. Parametric data were expressed as mean ± SEM and non-parametric data as median (25% percentile / 75% percentile). Differences were considered significant if $p < 0.05$. Calculations were made with the GraphPad Prism for Windows software (version 6.01).

Results

Post-mortem evaluation of midbrain dopamine neuron loss and striatal dopamine depletion

Compared with the SHAM control group, significantly fewer TH immunostained neurons were found in SNc of the MPTP and 6-OHDA lesioned rats (Fig. 1). Statistical analysis confirmed this result in all parts of the SNc; for rostral and caudal counts (group factor $F(2,21) = 14.70$, $p < 0.001$) and for medial and lateral counts (group factor, $F(2,19) = 17.10$, $p < 0.001$). No significant differences were found between number of TH-immunoreactive neurons in the

rostral and caudal parts of the SNc (location factor, $F(1,21) = 3.11$, $p = 0.09$; interaction group X location factor ($F(2,21) = 0.84$, $p = 0.44$) or between the medial and lateral parts of the SNc (location factor, $F(1,19) = 1.77$, $p = 0.20$; interaction group X location factor, $F(2,19) = 0.42$, $p = 0.66$). The lesions were confined to the SNc, as no significant differences were found between the groups in the number of TH-immunostained neurons found in the rostral or caudal parts of the VTA (group factor, $F(2,17) = 0.54$, $p = 0.59$; location factor, $F(1,17) = 2.79$, $p = 0.11$; interaction group X location, $F(2,17) = 1.32$, $p = 0.29$).

Compared to the SHAM group, lesions of the SNc with MPTP or 6-OHDA caused equivalent and significant reductions in tissue levels of dopamine ($F(2,25) = 10.47$, $p < 0.5$) and DOPAC ($F(2,25) = 7.78$, $p = 0.02$) in the striatum.

Body weight, food and water intake

After surgery, animals of all groups exhibited reduced levels of body weight, eating and water drinking over the first few days. SNc-lesioned rats lost more weight (Fig. 2A) and ate less (Fig. 2B) than SHAM-lesioned rats. No significant difference in water intake was observed among groups and food restriction did not alter water drinking (Fig. 2C). No significant difference among groups was observed in body weight, food intake and water drinking when training in the CPP and CPA tasks started.

Repeated-measures ANOVA of weight (Fig. 2A) yielded significant main effects of lesion ($F (2, 22) = 172.4$ $p < 0.001$) and time ($F(7,154) = 190.2$, $p < 0.001$), and a significant interaction between these factors ($F (14,154) = 19.64$, $p < 0.001$). Repeated-measures ANOVA of food intake (Fig. 2B) yielded significant main effects of lesion ($F (2, 22) = 172.4$ $p < 0.001$) and time ($F (7,154) = 190.2$, $p < 0.001$), and a significant interaction between these factors ($F (14,154) = 19.64$, $p < 0.001$). Repeated-measures ANOVA of drinking (Fig. 2C) revealed a significant main effect of time ($F(7,154) = 48.03$, $p < 0.001$), but no significant effect of the lesion ($F(2,22) = 2.37$, $p = 0.11$) or interaction between these factors ($F(14,154) = 0.94$, $p = 0.94$).

Exploratory behavior in an open field

In the open field test the behavior of MPTP, 6-OHDA rats and SHAM control animals was indistinguishable (Table 1). ANOVA showed no significant differences among groups in the number of crossings in both lateral ($F (2, 25) = 0.49$, $p = 0.55$) and central ($F (2, 25) = 0.34$, $p = 0.69$) areas of open field or in the number of rears ($F (2, 25) = 1.32$, $p = 0.28$). Kruskal-Wallis ANOVA also showed no significant differences among groups in the number of grooming episodes ($H(2, 25) = 1.27$, $p = 0.55$), although these were infrequent. These results suggest that

the partial lesion of the rat SNc with MPTP or 6-OHDA did not cause any alterations in motor performance (locomotion) or anxiety (time spent in center of open field) that could affect CPP and CPA scores.

Unconditioned responses to sucrose and quinine

Typical unconditioned orofacial expressions were observed in animals from all groups when they tasted a sucrose solution (licking and tongue protrusions) or a quinine solution (gaping) (Fig. 3). The incidence of fast lateral tongue protrusions in response to infusions of sucrose did not vary among groups ($F_{(2,10)} = 0.28$, $p = 0.75$). Similarly, the incidence of gapes in response to infusions of quinine also did not vary significantly between the groups ($F_{(2,11)} = 0.62$, $p = 0.55$. In addition, when given access to 10 sucrose pellets, rats of all groups consumed all sucrose pellets. These results suggest that the lesion of the SNc did not affect fundamental hedonic responses to these appetitive and aversive stimuli.

Behavioral scores for eating sucrose pellets are shown in Table 2. The only significant difference between sham and lesioned groups was that 6-OHDA rats took significantly longer than SHAM rats to start eating ($H_{(2,20)} = 7.70$, $p < 0.05$, Kruskal-Wallis ANOVA; $p < 0.05$ Dunn test). A significant difference between the time spent eating by MPTP and SHAM rats was significantly different ($H_{(2.10)} = 7,94$, $p = 0.01$; $p < 0.05$ Dunn test). No significant group effect was found for the time interacting with the pellets ($H_{(2,10)} = 1.66$, $p = 0.43$), inter-pellet interval ($H_{(2, 10)} = 1.94$, $p = 0.37$), and number of pellets picked up ($H_{(2,10)} = 1.66$, $p = 0.43$). Moreover, rats of the 6-OHDA and MPTP groups behaved as the rats of the SHAM group when they were previously habituated to eat sucrose pellets and later given access to 10 quinine pellets. Initially they approached and picked up the quinine pellets in the same way as they did for the sucrose pellets. However, immediately afterward they dropped the pellet and opened the mouth (gaping) many times, even though after they were no longer manipulating the pellets. No significant difference among groups was observed for the behavioral scores of responses to quinine pellets (Table 3). Kruskal-Wallis ANOVA yield non-significant group effects for latency to try the first pellet ($H_{(2,10)} = 1.01$, $p = 0.60$), time interacting with the pellets ($H_{(2,10)} = 1.40$, $p = 0.49$), inter-pellet interval ($H_{(2,10)} = 3.44$, $p = 0.17$), time spent chewing ($H_{(2,10)} = 2.40$, $p = 0.30$), and number of pellets picked up ($H_{(2,10)} = 2.29$, $p = 0.31$).

Testing different protocols for CPA learning

The pilot experiments carried out with naive rats showed that protocol (i) failed to cause CPA in naive rats (Fig.4A). Note, this protocol was the same as that used for place conditioning, except

that quinine pellets instead of sucrose pellets. Failure of this protocol to cause CPA in naive rats probably happened because, in contrast to sucrose pellets, rats did not eat the quinine pellets – after they tasted a pellet they usually did not try it again. This problem was solved by using protocol (ii), in which rats that were previously trained in the CPP protocol followed by the extinction sessions underwent 6 CPA training sessions. When these rats were given free choice to explore the chamber, they spent significantly less time in the compartment previously paired with quinine, compared to the neutral compartment (Fig. 4A). Protocol (iii), in which naive rats received a quinine solution into the mouth and where left in the place assigned as aversive was also effective to cause CPA in the test session. Consequently. a two-way ANOVA showed a significant main effect of compartment ($F(1,32) = 33.3$, $p < 0.001$), a non-significant effect of protocol ($F(2,32) < 0.001$, $p > 0.99$), and a significant interaction between compartment and protocol ($F(2,32) = 23.0$, $p < 0.001$). Bonferroni post hoc test showed that the time spent in aversive compartment was significantly less than time spent in the neutral compartment for rats trained under protocol (ii) and (iii) ($p < 0.05$), but not for rats trained under protocol (i).

As described above, before the training and test CPA session, rats trained under protocol (ii) were submitted to CPP training followed by CPP extinction sessions. The data illustrated in Fig. 4B shows that these training and extinction sessions were effective. Thus, a repeated-measures ANOVA that compared scores of animals trained under protocol (ii) before training, after training and after extinction showed a significant compartment factor effect ($F(1,24) = 10.9$, $p < 0.01$), a non-significant time effect ($F(2,24) = 0.0$, $p > 0.99$), and a significant interaction compartment X time ($F(2,24) = 17.4$, $p < 0.001$). In this case, a post hoc Bonferroni's test showed that the time spend in the appetitive compartment was significantly higher than the time spent in the neutral compartment ($p < 0.001$). It also showed that the time spent in the appetitive compartment after training was significantly greater than the time spent in this compartment before training ($p < 0.01$). After extinction, there was no significant difference between the time spent in the appetitive compartment before training and the time spent in this compartment after extinction ($p > 0.2$).

SNc lesion did not impair CPP learning

Partial lesions of the SNc with 6-OHDA or MPTP did not affect CPP learning (Fig 5A). Thus, time spent in the appetitive compartment was significantly higher than the time spent in the neutral compartment for rats of all groups (compartment factor, ($F(1,34) = 63.7$, $p < 0.001$; group factor ($F(2,34) = 0.00$, $p > 0.99$); group X compartment interaction ($F(2,34) = 1.30$, $p =$

0.28) (compartment factor, (F(1,34) = 63.7, p < 0.001; group factor (F(2,34) = 0.00, p > 0.99); group X compartment interaction (F(2,34) = 1.30, p = 0.28).

SNc lesion impaired CPA learning

While SHAM rats learned the CPA task, partial lesion of the SNc prevented CPA learning when they were trained under protocol (ii). As shown in Fig. 5B, on the test day, SHAM rats spent significantly less time in the aversive compartment (previously paired with quinine). On the other hand, the 6-OHDA rats spent significantly more time in the aversive compartment. Time spent by the MPTP rats in the two compartments was not significantly different. A two-way ANOVA of Fig. 5B data showed no significant main effects of compartment (F(1,34) = 2.85, p = 0.10) or group (F(2,34) = 0.00, p > 0.99), but revealed a significant interaction between these factors (F(2,34) = 14.90, p < 0.001). It is possible that the preference of the 6-OHDA lesioned rats for the compartment paired with quinine was biased by the previous pairing of this compartment with sucrose. A positive correlation was found between the time the 6-ODHA rats spent in the appetitive compartment during the CPP test and the time they spent in the aversive compartment in the CPA test (r = 0.71, p < 0.05, Pearson's test). However, no significant correlation between these scores was found for the SHAM (r = - 0.36, p = 0.25) and MPTP rats (r = 0.04, p = 0.93).

To further test whether naive SNc-lesioned rats could learn a CPA task, additional SHAM and 6-OHDA lesioned rats were trained under protocol (iii). This protocol forced the rats to taste a quinine solution which was infused in the mouth immediately before they were confined in the aversive compartment. On the test day, SHAM rats spent significantly less time in the aversive compartment than in the neutral compartment (compartment effect, (F(1,44) = 8.87, p < 0.01) (Fig. 5C). In contrast, the 6-OHDA-lesioned rats spent similar time in the aversive compartment as in the neutral compartment (group effect, F(1,44) < 0.01, p > 0.99; group X compartment interaction (F(2,34) = 5.85, p < 0.05).

Correlations between CPP, CPA and the extent of dopamine neurons loss and dopamine depletion

In the 6-OHDA lesioned rats there was a significant positive correlation between the number of neurons remaining in the caudal SNc and CPP scores (r = 0.93, p < 0.05), while in SHAM lesioned rats there was a significant negative correlation between the number of neurons present in the caudal SNc and CPA scores (r = -0.99, p < 0.05). Except for these results, no other significant correlations were found between number of neurons in any part of the SNc and

the CPP or CPA scores. In the 6-OHDA lesioned rats there was a significant correlation between striatal dopamine levels and CPA scores ($r = 0.70$, $p < 0.05$). No significant correlations were found between dopamine levels and either the CPP or CPA scores in the SHAM lesioned rats

Discussion

The most important aspect of the present study is to show for the first time that a partial lesion of dopamine neurons in the SNc affected aversion-driven, but not reward-driven, associative learning. This effect was independent of the toxin used to lesion the SNc (6-OHDA or MPTP). In addition, impairment of aversive conditioning was observed regardless of the rat history (naive or previously trained in CPP) and of the mode of quinine delivery (self-directed interactions with pellets or experimenter-delivered solution). Moreover, when 6-OHDA, but not MPTP, rats were first trained to prefer a place paired with sucrose pellets, then underwent extinction, and next were trained with quinine paired to the same place previously paired with sucrose, instead of avoiding the quinine-paired place, they preferred this place over the neutral place. The difference in this CPA score between MPTP and 6-OHDA groups suggest that although, we found no significant difference in the number of DA neurons lost in the substantia nigra of 6-OHDA and MPTP rats, the lesion caused by 6-OHDA was a bit higher. The fact that 6-OHDA is a less selective neurotoxin compared to MPTP (Harik et al., 1987) might also have contributed to this difference.

Previous studies demonstrated that SNc dopamine neurons play a role in both appetitive and aversive conditioning. Rossi et al. (2013) reported that optogenetic self-stimulation of the dopamine neurons of the mouse SNc is sufficient to reinforce instrumental learning. Another study (Kravitz et al., 2012) showed that optogenetic activation of medium spiny neurons of the dorsomedial striatum that express D1 dopamine receptors supports CPP while activation of medium spiny neurons expressing D2 dopamine receptors induces CPA. Moreover, Ilango et al. (2014b) found that mice spent more time in a compartment where they received optogenetic stimulation of dopamine neurons in the SNc (or VTA) and avoided the compartment where they received optogenetic inhibition of dopamine neurons in these areas. Many other studies have demonstrated that the dopamine neurons of the SNc also play a key role in several kinds of aversion-driven learning. They include evidence of impaired conditioned avoidance learning in rats with SNc dopamine lesions induced by 6-OHDA (Cooper et al., 1973) or MPTP (Da Cunha et al., 2001, Gevaerd et al., 2001a, Gevaerd et al., 2001b, Perry et al., 2004, Bortolanza et al.,

2010, Dombrowski et al., 2013). The striatal tissue content of dopamine in the MPTP- and 6-OHDA-partially lesioned rats was about 20 to 30% of that in the control rats. A previous microdialysis study from our laboratory found that rats submitted to the same treatment with MPTP had basal tonic levels of dopamine release within the normal range, but released much less dopamine when challenged with amphetamine (Dombrowski et al., 2010). Similar results were reported for rats treated with partial lesion of the SNc induced by intranigral infusion of 6-OHDA (Robinson et al., 1994). In another study, we reported that tonic dopamine release peaked in the striatum while rats were trained in a conditioned avoidance task. However, the tonic levels of dopamine in the striatum of MPTP-lesioned rats did not alter when they were submitted to the same training sessions, and they did not learn the this task (Dombrowski et al., 2013). Together these results suggest that the rats with partial lesion of the SNc probably failed to release the amount of dopamine needed to support aversively-motivated learning. Consistent with this hypothesis, deficits to learn conditioned avoidance tasks were also reported in rats with dorsal striatal lesions (Wendler et al., 2014), dorsal striatal dopamine depletion (Rane and King, 2011), and intra-dorsolateral striatal infusion of D1 (Wietzikoski et al., 2012) or D2 (Boschen et al., 2011) dopamine receptor antagonists. Moreover, SNc lesion impaired learning that depends on negative reinforcement, such as some versions of the Morris water maze task (Miyoshi et al., 2002, Ferro et al., 2005, Da Cunha et al., 2006, Da Cunha et al., 2007), or on punishment, such as inhibitory avoidance (Kumar et al., 2009, Castro et al., 2012, Das et al., 2014). Therefore, it is well established that the dopamine neurons of the SNc are needed for both reward- and aversion-driven learning. However, to the best of our knowledge, this is the first study showing that it is possible to partially lesion dopamine neurons in the SNc in a manner that impairs aversion-driven learning but spares reward-driven learning.

It is unlikely that the CPA impairment observed in the SNc lesioned rats was caused by motor deficits because exploratory behavior of the MPTP and 6-OHDA rats in the open field test was not altered. This agrees with previous studies showing that 2-3 weeks after surgery no motor impairment was observed in rats with partial loss of nigral dopamine neurons induced by similar doses of MPTP (Da Cunha et al., 2001, Da Cunha et al., 2002, Miyoshi et al., 2002, Ho et al., 2011) or 6-OHDA, (Ferro et al., 2005, Tadaiesky et al., 2008, Santiago et al., 2014). Nor is it likely that the CPA impairment was due to differences in motivation for food or water, as intake was similar among groups by the time testing began, in agreement with previous studies (Ferro et al., 2005, Tadaiesky et al., 2008).

It is also unlikely that the CPA impairment was caused by reduced perception of the quinine pellets as aversive. A control experiment showed equal taste "disgust" responses to

quinine in both the control and SNc lesioned (MPTP or 6-OHDA) rats. In addition, like the SHAM rats, 6-OHDA- and MPTP-lesioned rats ate all offered sucrose pellets. The 6-OHDA rats, but not the MPTP rats, presented a longer delay to start eating the sucrose pellets. A similar finding was reported previously by Schwarting and Huston (1996) and is likely due to impairment in approach behavior (Ikemoto et al., 2015) rather than to reduction in hedonic "liking" (Berridge and Kringelbach, 2015). Therefore, we interpret the finding that rats partial SNc lesions did not show conditioned avoidance as a learning, but not a motor or hedonic, impairment.

Therefore, this and previous studies support the hypothesis that nigrostriatal dopamine lesion affects the learning of avoiding aversive stimuli, but does not affect the hedonic response to rewarding and aversive stimuli. In addition, the present study shows that the roles played by dopamine neurons of the SNc on reward- and aversion-driven learning can be separated. The important question is: why can 6-OHDA and MPTP rats learn one but not the other kind of task?

A possible explanation is the hypothesis that there are different clusters of dopamine neurons in the SNc, one supporting CPA learning and the other supporting CPP learning. As early as 1980, Chiodo et al. (1980) reported two populations of dopamine neurons in the rat SNc, one that is activated and another that is inhibited by potentially aversive stimuli. More recently, anatomical differences in the responses of midbrain dopamine neurons to rewarding and aversive stimuli have been reported. For example, Nomoto et al. (2010) found dopamine neurons in the monkey ventromedial SNc with higher sensitivity to reward. Brischoux et al., (2009) reported that the dopamine neurons in the rat dorsal VTA were inhibited by foot shocks while the dopamine neurons in the ventral VTA were phasically excited by foot shocks. Matsumoto and Hikosaka (2009) observed that neurons in the dorsolateral part of the monkey SNc were excited by both aversive and reward stimuli, while neurons in the ventromedial SNc and VTA were excited by a reward stimulus and inhibited by an aversive stimulus. However, other authors claim that instead of different clusters, the distribution of the dopamine neurons in the midbrain is graded in terms of sensitivity to rewarding and salient stimuli. They also claim that the activation observed in some dopamine neurons was not associated with the aversiveness, but the salience of the stimulus (Fiorillo et al., 2013, Schultz, 2016). Fiorillo and coworkers (2013) found that neurons in the "ventral tier" of the monkey SNc present greater suppression in response to aversive stimuli and a subset of these neurons present a rebound activation after suppression. In addition, they observed that neurons in the further rostral part of the SNc were more strongly suppressed by aversive stimuli.

Could the results of the present study reflect clustering of dopamine neurons which are relevant for reward- and aversion-driven learning in different areas of the midbrain? The

dopamine neurons of the VTA were spared in the MPTP and 6-OHDA groups, suggesting that VTA neurons are not sufficient to support CPA. However, as discussed above, other studies have shown that the VTA plays a key role in both kinds of learning (Wadenberg et al., 1990, Salamone, 1994, Setlow and Mcgaugh, 1998, 2000, Phillips et al., 2003b, Lalumiere et al., 2005, Bassareo et al., 2007, Chaudhri et al., 2010, Kim et al., 2012, Chaudhri et al., 2013, Ilango et al., 2014a, Ilango et al., 2014b, Sciascia et al., 2014). In the present study, the most caudal and the most lateral parts of the SNc were partially preserved in the animals treated with MPTP. However, the 6-OHDA rats presented no significant difference among these areas and, like the MPTP rats, they were impaired to learn the CPA but not the CPP task. Nevertheless, in the 6-OHDA rats a positive correlation was found between CPP scores (time spent in the appetitive compartment) and the number of dopamine neurons in the caudal, but not rostral, part of the SNc. One interpretation is that, although 6-OHDA rats were not impaired to learn CPP, this kind of learning is positively modulated by the dopamine neurons of the caudal SNc. In addition, a negative correlation was found between CPA scores (time spent in the aversive compartment) and the number of dopamine neurons in the caudal SNc of SHAM rats, but this correlation was not found in SNc-lesioned rats. This finding is consistent with the hypothesis that CPA depends on reduction of dopamine release by the neurons of the caudal part of the SNc and therefore aversive learning is more sensitive to loss of dopamine neurons. However, the present results suggest that if there are different clusters of dopamine neurons supporting reward- and aversion-driven learning, they are not segregated in different regions of the rat SNc.

Another possible explanation for the finding that the MPTP and 6-OHDA rats could learn the CPP task but not the CPA task is that, compared to reward-driven learning, aversion-driven learning may depend on larger changes in dopamine concentration. Evidence exists that reward-driven learning is reinforced by increased release of dopamine in the striatum in response to "better-than-expected" rewards (Schultz, 2016). Although this evidence refers to studies in which there was a clear phasic onset/offset of the CS, it is possible that in the present study the remaining dopamine neurons in the 6-OHDA and MPTP rats were sufficient to increase extracellular dopamine levels above a threshold critical to support reward-driven learning. In a previous study, we showed that the MPTP lesion did not alter basal tonic levels of extracellular dopamine in the striatum. Moreover, as mentioned above, when these MPTP rats were challenged with amphetamine they exhibited increased dopamine release, though at a lower level compared to SHAM rats (Dombrowski et al., 2010). This might explain why a positive correlation between dopamine neurons spared in the caudal SNc and CPP learning was found

in rats treated with 6-OHDA, but not in SHAM and MPTP rats: although the 6-ODHA rats could learn this task, those rats with more dopamine to release learned the task better. On the other hand, the MPTP lesions were possibly below a limit that could affect CPP scores. Aversion-driven learning may depend on phasic reduction in the extracellular concentration of dopamine in response to aversive stimuli (Roitman et al., 2008, Schultz, 2010, Badrinarayan et al., 2012, Budygin et al., 2012, Hart et al., 2015, Schultz, 2016). This may also be true for tonic levels of dopamine. However, compensatory mechanisms in the dopamine terminals which made possible for lesioned rats to maintain the tonic levels of dopamine (Perry et al., 2005, Da Cunha et al., 2008, Dombrowski et al., 2010) may make it more difficult to decrease extracellular dopamine concentration below baseline. Consistent with this hypothesis, we found a positive correlation between CPA scores and striatal content of dopamine in 6-OHDA rats, but not in SHAM rats. In a future study this hypothesis can be further tested by measuring dopamine release during CPA.

In conclusion, the present study shows that the role of SNc dopamine neurons in reward- and aversion-driven associative learning can be dissociated. The MPTP and 6-OHDA partially lesioned rats can be used to study treatments for learning deficits specific for aversive tasks or in electrophysiological and electrochemical experiments to identify differences in dopamine neuronal activity and dopamine release when they are trained in appetitive- and aversive-driven learning tasks. Histological studies can also take advantage of these animals to investigate putative heterogeneity of dopamine neurons.

Acknowledgements

References

Anstrom KK, Miczek KA, Budygin EA (2009), Increased phasic dopamine signaling in the mesolimbic pathway during social defeat in rats. Neurosci 161:3-12.

Badrinarayan A, Wescott SA, Vander Weele CM, Saunders BT, Couturier BE, Maren S, Aragona BJ (2012), Aversive stimuli differentially modulate real-time dopamine transmission dynamics within the nucleus accumbens core and shell. J Neurosci 32:15779-15790.

Bassareo V, De Luca MA, Di Chiar G (2002), Differential expression of motivational stimulus properties by dopamine in nucleus accumbens shell versus core and prefrontal cortex. J Neurosci 22:4709-4719.

Bassareo V, De Luca MA, Di Chiara G (2007), Differential impact of pavlovian drug conditioned stimuli on in vivo dopamine transmission in the rat accumbens shell and core and in the prefrontal cortex. Psychopharmacol 191:689-703.

Berridge KC, Kringelbach ML (2015), Pleasure systems in the brain. Neuron 86:646-664.

Berridge KC, Robinson TE (1998), What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? Brain Res Rev 28:309-369.

Brown HD, McCutcheon JE, Cone JJ, Ragozzino ME, Roitman MF (2011), Primary food reward and reward-predictive stimuli evoke different patterns of phasic dopamine signaling throughout the striatum. European Journal of Neuroscience 34:1997-2006.

Bortolanza M, Wietzikoski EC, Boschen SL, Dombrowski PA, Latimer M, MacLaren DAA, Winn P, Da Cunha C (2010), Functional disconnection of the substantia nigra pars compacta from the pedunculopontine nucleus impairs learning of a conditioned avoidance task. Neurobiol Learn Mem 94:229-239.

Boschen SL, Andreatini R, Da Cunha C (2015), Activation of postsynaptic D2 dopamine receptors in the rat dorsolateral striatum prevents the amnestic effect of systemically administered neuroleptics. Behav Brain Res 281:283-289.

Boschen SL, Wietzikoski EC, Winn P, Da Cunha C (2011), The role of nucleus accumbens and dorsolateral striatal D2 receptors in active avoidance conditioning. Neurobiol Learn Mem 96:254-262.

Bracs PU, Gregory P, Jackson DM (1984), Passive-avoidance in rats - disruption by dopamine applied to the nucleus accumbens. Psychopharmacol 83:70-75.

Brischoux F, Chakraborty S, Brierley DI, Ungless MA (2009), Phasic excitation of dopamine neurons in ventral vta by noxious stimuli. Proc Natl Acad Sci U S A 106:4893-4899.

Bromberg-Martin ES, Matsumoto M, Hikosaka O (2010), Dopamine in motivational control: Rewarding, aversive, and alerting. Neuron 68:815-834.

Budygin EA, Park J, Bass CE, Grinevich VP, Bonin KD, Wightman RM (2012), Aversive stimulus differentially triggers subsecond dopamine release in reward regions. Neurosci 201:331-337.

Castro AA, Ghisoni K, Latini A, Quevedo J, Tasca CI, Prediger RDS (2012), Lithium and valproate prevent olfactory discrimination and short-term memory impairments in the intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rat model of Parkinson's disease. Behav Brain Res 229:208-215.

Chaudhri N, Sahuque LL, Schairer WW, Janak PH (2010), Separable roles of the nucleus accumbens core and shell in context- and cue-induced alcohol-seeking. Neuropsychopharmacol 35:783-791.

Chaudhri N, Sparks L, Sciascia J, Ayorech Z (2013), Contribution of dopamine receptors to pavlovian-conditioned ethanol-seeking. Alcoholism 37:305A-305A.

Chiodo LA, Antelman SM, Caggiula AR, Lineberry CG (1980), Sensory stimuli alter the discharge rate of dopamine (da) neurons - evidence for 2 functional types of da-cells in the

substantia nigra. Brain Res 189:544-549.

Cooper BR, Breese GR, Grant LD, Howard JL (1973), Effects of 6-hydroxydopamine treatments on active avoidance responding - evidence for involvement of brain dopamine. J Pharmacol Exp Therap 185:358-370.

Da Cunha C, Angelucci MEM, Canteras NS, Wonnacott S, Takahashi RN (2002), The lesion of the rat substantia nigra pars compacta dopaminergic neurons as a model for Parkinson's disease memory disabilities. Cel Mol Neurobiol 22:227-237.

Da Cunha C, Gevaerd MS, Vital M, Miyoshi E, Andreatini R, Silveira R, Takahashi RN, Canteras NS (2001), Memory disruption in rats with nigral lesions induced by MPTP: A model for early Parkinson's disease amnesia. Behav Brain Res 124:9-18.

Da Cunha C, Silva MHC, Wietzikoski S, Wietzikoski EC, Ferro MM, Kouzmine I, Canteras NS (2006), Place learning strategy of substantia nigra pars compacta-lesioned rats. Behavioral Neurosci 120:1279-1284.

Da Cunha C, Wietzikoski EC, Dombrowski P, Santos LM, Bortolanza M, Boschen SL, Miyoshi E (2009), Learning processing in the basal ganglia: A mosaic of broken mirrors. Behav Brain Res 199:156-169.

Da Cunha C, Wietzikoski EC, Ferro MM, Martinez GR, Vital M, Hipolide D, Tufik S, Canteras NS (2008), Hemiparkinsonian rats rotate toward the side with the weaker dopaminergic neurotransmission. Behav Brain Res 189:364-372.

Da Cunha C, Wietzikoski S, Wietzikoski EC, Silva MHC, Chandler J, Jr., Ferro MM, Andreatini R, Canteras NS (2007), Pre-training to find a hidden platform in the morris water maze can compensate for a deficit to find a cued platform in a rat model of Parkinson's disease. Neurobiol Learn Mem 87:451-463.

Dalley JW, Laane K, Theobald DEH, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW (2005), Time-limited modulation of appetitive pavlovian memory by D1 and nmda receptors in the nucleus accumbens. Proc Natl Acad Sci U S A 102:6189-6194.

Darvas M, Wunsch AM, Gibbs JT, Palmiter RD (2014), Dopamine dependency for acquisition and performance of pavlovian conditioned response. Proc Natl Acad Sci U S A 111:2764-2769.

Das NR, Gangwal RP, Damre MV, Sangamwar AT, Sharma SS (2014), A PPAR-beta/delta agonist is neuroprotective and decreases cognitive impairment in a rodent model of Parkinson's disease. Curr Neurovasc Res 11:114-124.

Dombrowski P, Maia TV, Boschen SL, Bortolanza M, Wendler E, Schwarting RKW, Brandao ML, Winn P, Blaha CD, Da Cunha C (2013), Evidence that conditioned avoidance responses are reinforced by positive prediction errors signaled by tonic striatal dopamine. Behav Brain Res 241:112-119.

Dombrowski PA, Carvalho MC, Miyoshi E, Correia D, Bortolanza M, dos Santos LM, Wietzikoski EC, Eckart MT, Schwarting RKW, Brandao ML, Da Cunha C (2010), Microdialysis study of striatal dopamine in MPTP-hemilesioned rats challenged with apomorphine and amphetamine. Behav Brain Res 215:63-70.

Dunn AJ, File SE (1983), Cold restraint alters dopamine metabolism in frontal cortex, nucleus accumbens and neostriatum. Physiology and Behavior 31:511-513.

Ferro MM, Bellissimo MI, Anselmo-Franci JA, Angellucci MEM, Canteras NS, Da Cunha C (2005), Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early phase of Parkinson's disease: Histological, neurochemical, motor and memory alterations. J Neurosci Meth 148:78-87.

Fiorillo CD, Yun SR, Song MR (2013), Diversity and homogeneity in responses of midbrain dopamine neurons. J Neurosci 33:4693-4709.

Frussa-Filho R, Barbosa-Junior H, Silva RH, Da Cunha C, Mello CF (1999), Naltrexone potentiates the anxiolytic effects of chlordiazepoxide in rats exposed to novel environments. Psychopharmacol 147:168-173.

Gevaerd MS, Miyoshi E, Silveira R, Canteras NS, Takahashi RN, Da Cunha C (2001a), L-dopa restores striatal dopamine level but fails m to reverse MPTP-induced memory deficits in rats. International J Neuropsychopharmacol 4:361-370.

Gevaerd MS, Takahashi RN, Silveira R, Da Cunha C (2001b), Caffeine reverses the memory disruption induced by intra-nigral MPTP-injection in rats. Brain Res Bul 55:101-106.

Grill HJ, Norgren R (1978), Taste reactivity test .1. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res 143:263-279.

Harik, S. I., Schmidley, J. W., Iacofano, L. A., Blue, P., Arora, P. K. and Sayre, L. M. (1987) On the mechanisms underlying 1-methyl-4phenyl-1,2,3,6-tetrahydropiridine neurotoxicity: the effect of perinigral infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine, its metabolite and their analogs in rat. J Pharmacol Exp Ther 241:669–676.

Hart AS, Clark JJ, Phillips PEM (2015), Dynamic shaping of dopamine signals during probabilistic pavlovian conditioning. Neurobiol Learn Mem 117:84-92.

Hart AS, Rutledge RB, Glimcher PW, Phillips PEM (2014), Phasic dopamine release in the rat nucleus accumbens symmetrically encodes a reward prediction error term. J Neurosci 34:698-704.

Ho YJ, Ho SC, Pawlak CR, Yeh KY (2011), Effects of d-cycloserine on MPTP-induced behavioral and neurological changes: Potential for treatment of Parkinson's disease dementia. Behav Brain Res 219:280-290.

Ikemoto S, Yang C, Tan A (2015), Basal ganglia circuit loops, dopamine and motivation: A review and enquiry. Behav Brain Res 290:17-31.

Ilango A, Kesner AJ, Broker CJ, Wang DV, Ikemoto S (2014a), Phasic excitation of ventral tegmental dopamine neurons potentiates the initiation of conditioned approach behavior: Parametric and reinforcement-schedule analyses. Front Behavioral Neurosci 8.

Ilango A, Kesner AJ, Keller KL, Stuber GD, Bonci A, Ikemoto S (2014b), Similar roles of substantia nigra and ventral tegmental dopamine neurons in reward and aversion. J Neurosci 34:817-822.

Ilango A, Shumake J, Wetzel W, Scheich H, Ohl FW (2012), The role of dopamine in the context of aversive stimuli with particular reference to acoustically signaled avoidance learning. Front Neurosci 6:9.

Imperato A, Angelucci L, Casolini P, Zocchi A, Puglisi-Allegra S (1992), Repeated stressful experiences differently affect limbic dopamine during and following stress. Brain Res 577:194-199.

Kashtelyan V, Lichtenberg NT, Chen ML, Cheer JF, Roesch MR (2014), observation of reward delivery to a conspecific modulates dopamine release in ventral striatum. Curr Biol 24:2564-2568.

Kim KM, Baratta MV, Yang AM, Lee D, Boyden ES, Fiorillo CD (2012), Optogenetic mimicry of the transient activation of dopamine neurons by natural reward is sufficient for operant reinforcement. PLoS One 7.

Kravitz AV, Tye LD, Kreitzer AC (2012), Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci 15:816-U823.

Kumar P, Kaundal RK, More S, Sharma SS (2009), Beneficial effects of pioglitazone on cognitive impairment in MPTP model of Parkinson's disease. Behav Brain Res 197:398-403.

LaLumiere RT, Nawar EM, McGaugh JL (2005), Modulation of memory consolidation by the basolateral amygdala or nucleus accumbens shell requires concurrent dopamine receptor activation in both brain regions. Learn Mem 12:296-301.

Lex B, Hauber W (2010), The role of nucleus accumbens dopamine in outcome encoding in instrumental and pavlovian conditioning. Neurobiol Learn Mem 93:283-290.

Manago F, Castellano C, Oliverio A, Mele A, De Leonibus E (2009), Role of dopamine receptors subtypes, D1-like and D2-like, within the nucleus accumbens subregions, core and shell, on

memory consolidation in the one-trial inhibitory avoidance task. Learn Mem 16:46-52.

Matsumoto M, Hikosaka O (2009), Two types of dopamine neuron distinctly convey positive and negative motivational signals. Nature 459:838-842.

Mirenowicz J, Schultz W (1996), Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. Nature 379:449-451.

Miyoshi E, Wietzikoski S, Camplessei M, Silveira R, Takahashi RN, Da Cunha C (2002), Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP-induced mesencephalic dopaminergic lesions. Brain Res Bul 58:41-47.

Nakajima S (1986), Suppression of operant responding in the rat by dopamine D1-receptor blockade with SCH-23390. Physiol Psychol 14:111-114.

Nomoto K, Schultz W, Watanabe T, Sakagami M (2010), Temporally extended dopamine responses to perceptually demanding reward-predictive stimuli. J Neurosci 30:10692-10702.

Oleson EB, Gentry RN, Chioma VC, Cheer JF (2012), subsecond dopamine release in the nucleus accumbens predicts conditioned punishment and its successful avoidance. J Neurosci 32:14804-14808.

Parkinson JA, Dalley JW, Cardinal RN, Bamford A, Fehnert B, Lachenal G, Rudarakanchana N, Halkerston KM, Robbins TW, Everitt BJ (2002), Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive pavlovian approach behaviour: Implications for mesoaccumbens dopamine function. Behav Brain Res 137:149-163.

Pauli WM, Larsen T, Collette S, Tyszka JM, Seymour B, O'Doherty JP (2015), Distinct contributions of ventromedial and dorsolateral subregions of the human substantia nigra to appetitive and aversive learning. J Neurosci 35:14220-14233.

Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates. San Diego: Academic Press.

Perry JC, Da Cunha C, Anselmo-Franci J, Andreatini R, Miyoshi E, Tufik S, Vital M (2004), Behavioural and neurochemical effects of phosphatidylserine in MPTP lesion of the substantia nigra of rats. Europ J Pharmacol 484:225-233.

Perry JC, Hipolide DC, Tufik S, Martins RD, Da Cunha C, Andreatini R, Vital M (2005), Intra-nigral MPTP lesion in rats: Behavioral and autoradiography studies. Exp Neurol 195:322-329.

Phillips GD, Setzu E, Hitchcott PK (2003a), Facilitation of appetitive pavlovian conditioning by D-amphetamine in the shell, but not the core, of the nucleus accumbens. Behav Neurosci 117:675-684.

Phillips GD, Setzu E, Vugler A, Hitchcott PK (2003b), Immunohistochemical assessment of mesotelencephalic dopamine activity during the acquisition and expression of pavlovian versus instrumental behaviours. Neurosci 117:755-767.

Ramayya AG, Misra A, Baltuch GH, Kahana MJ (2014), Microstimulation of the human substantia nigra alters reinforcement learning. J Neurosci 34:6887-6895.

Rane P, King JA (2011), Exploring aversion in an animal model of pre-motor stage Parkinson's disease. Neurosci 181:189-195.

Robinson TE, Noordhoorn M, Chan EM, Mocsary Z, Camp DM, Whishaw IQ (1994), Relationship between asymmetries in striatal dopamine release and the direction of amphetamine-induced rotation during the first week following a unilateral 6-OHDA lesion of the substantia-nigra. Synapse 17:16-25.

Roitman MF, Wheeler RA, Wightman RM, Carelli RM (2008), Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. Nat Neurosci 11:1376-1377.

Rossi MA, Sukharnikova T, Hayrapetyan VY, Yang LC, Yin HH (2013), Operant self-stimulation

of dopamine neurons in the substantia nigra. PLoS One 8:7.

Saddoris MP, Cacciapaglia F, Wightman RM, Carelli RM (2015), Differential Dopamine Release Dynamics in the Nucleus Accumbens Core and Shell Reveal Complementary Signals for Error Prediction and Incentive Motivation. J Neurosci 35:11572-11582.

Salamone JD (1994), The involvement of the nucleus accumbens dopamine in appetitive and aversive motivation. Behav Brain Res 61:117-133.

Santiago RM, Barbiero J, Martynhak BJ, Boschen SL, da Silva LM, Werner MFP, Da Cunha C, Andreatini R, Lima MMS, Vital M (2014), Antidepressant-like effect of celecoxib piroxicam in rat models of depression. J Neural Transmission 121:671-682.

Schultz W (1998), Predictive reward signal of dopamine neurons. J Neurophysiol 80:U32-U32.

Schultz W (2010), Dopamine signals for reward value and risk: Basic and recent data. Behav Brain Funct 6:9.

Schultz W (2016), Dopamine reward prediction-error signalling: A two-component response. Nat Rev Neurosci 17:183-195.

Schultz W, Dickinson A (2000), Neuronal coding of prediction errors. Annu Rev Neurosci 23:473-500.

Schwarting RKW, Huston JP (1996), The unilateral 6-hydroxydopamine lesion model in behavioral Brain Res. Analysis of functional deficits, recovery and treatments. Prog Neurobiol 50:275-331.

Sciascia JM, Mendoza J, Chaudhri N (2014), Blocking dopamine D1-like receptors attenuates context-induced renewal of pavlovian-conditioned alcohol-seeking in rats. Alcoholism 38:418-427.

Setlow B, McGaugh JL (1998), Sulpiride infused into the nucleus accumbens posttraining impairs memory of spatial water maze training. Behav Neurosci 112:603-610.

Setlow B, McGaugh JL (2000), D2 dopamine receptor blockade immediately post-training enhances retention in hidden and visible platform versions of the water maze. Learn Mem 7:187-191.

Sorg BA, Kalivas PW (1991), Effects of cocaine and foot shock stress on extracellular dopamine levels in the ventral striatum. Brain Res 559:29-36.

Sunsay C, Rebec GV (2008), Real-time dopamine efflux in the nucleus accumbens core during pavlovian conditioning. Behav Neurosci 122:358-367.

Tadaiesky MT, Dombrowski PA, Figueiredo CP, Cargnin-Ferreira E, Da Cunha C, Takahashi RN (2008), Emotional, cognitive and neurochemical alterations in a premotor stage model of Parkinson's disease. Neurosci 156:830-840.

Tombaugh TN, Tombaugh J, Anisman H (1979), Effects of dopamine receptor blockade on alimentary behaviors - home cage food-consumption, magazine training, operant acquisition, and performance. Psychopharmacol 66:219-225.

Ventura R, Morrone C, Puglisi-Allegra S (2007), Prefrontal/accumbal catecholamine system determines motivational salience attribution to both reward- and aversion-related stimuli. Proc Natl Acad Sci U S A 204:5181-5186.

Wadenberg ML, Ericson E, Magnusson O, Ahlenius S (1990), Suppression of conditioned avoidance-behavior by the local application of (-)sulpiride into the ventral, but not the dorsal, striatum of the rat. Biol Psych 28:297-307.

Waelti P, Dickinson A, Schultz W (2001), Dopamine responses comply with basic assumptions of formal learning theory. Nature 412:43-48.

Wendler E, Gaspar JCC, Ferreira TL, Barbiero JK, Andreatini R, Vital M, Blaha CD, Winn P, Da Cunha C (2014), The roles of the nucleus accumbens core, dorsomedial striatum, and dorsolateral striatum in learning: Performance and extinction of pavlovian fear-conditioned responses and instrumental avoidance responses. Neurobiol Learn Mem 109:27-36.

White NM, Carr GD (1985), The conditioned place preference is affected by 2 independent reinforcement processes. Pharmacol Biochem Behav 23:37-42.

Wietzikoski EC, Boschen SL, Miyoshi E, Bortolanza M, Santos LM, Frank M, Brandão ML, Winn P, Da Cunha C (2012), Roles of D1-like dopamine receptors in the nucleus accumbens and dorsolateral striatum in conditioned avoidance response. Psychopharmacol 219:159-169.

Yin HH, Ostlund SB, Balleine BW (2008), Reward-guided learning beyond dopamine in the nucleus accumbens: The integrative functions of cortico-basal ganglia networks. Eur J Neurosci 28:1437-1448.

Zaghloul KA, Blanco JA, Weidemann CT, McGill K, Jaggi JL, Baltuch GH, Kahana MJ (2009), Human substantia nigra neurons encode unexpected financial rewards. Science 323:1496-1499.

Figure Legends

Fig. 1. Histological and neurochemical evaluation of the lesions induced by infusion of 6-OHDA or MPTP into the SNc. (A) Examples of midbrain slices immunostained for TH. Neuron number was significantly reduced by both toxins in the SNc (B, C) but not VTA (D). Tissue content of dopamine (DA) and DOPAC were significantly reduced in the striatum (E). The mean numbers of counted neurons in the SHAM group were: (B) 166 in the SNc rostral and 303 in the SNc caudal; (C) 198 in the SNc medial and 405 in the SNc lateral; (D) 439 in the VTA rostral and 338 in the VTA caudal. The mean number of counted neurons in the 6-OHDA groups were: (B) 19 in the SNc rostral and 107 in the SNc caudal; (C) 64 in the SNc medial and 28 in the SNc lateral; (D) 386 in the VTA rostral and 355 in the VTA caudal. The mean number of counted neurons in the MPTP groups were: (B) 43 in the SNc rostral and 194 in the SNc caudal; (C) 82 in the SNc medial and 77 in the SNc lateral; (D) 254 in the VTA rostral and 372 in the VTA caudal. (E) The mean contents of striatal DA in the were 9601 ng/g of wet tissue in the SHAM group, 2978 ng/g of wet tissue in the 6-OHDA group, and 1775 ng/g of wet tissue in the MPTP group; the mean contents of striatal DOPAC in the were 957 ng/g of wet tissue in the SHAM group, 677 ng/g of wet tissue in the 6-OHDA group, and 659 ng/g of wet tissue in the MPTP group. The numbers for rats per group in (B), (C), and (D) were: SHAM (n=4), 6-OHDA (n=5), MPTP (n= 5). The numbers of rats per group in (E) were: SHAM (n=12), 6-OHDA (n=7), MPTP (n= 9). Data are expressed as mean ± SEM. * $p < 0.05$ compared to the SHAM group, Dunnett's test after ANOVA.

Fig. 2. Effects of SNc lesion on rat body weight (A), food intake (B), and water intake (C). SHAM (n=9), MPTP (n=7), 6-OHDA (n=9). Data are expressed as mean ± SEM. * $p < 0.05$ compared to SHAM (Bonferroni's test after ANOVA).

Fig. 3. Partial lesion of dopaminergic neurons of the SNc by 6-OHDA or MPTP did not affect orofacial expressions exhibited by rats after tasting quinine or sucrose. (A) Number of lateral tongue protrusions in response to sucrose SHAM (n=5), 6-OHDA (n=4) MPTP (n=4). (B) Number of gapes in response to quinine (right) were unaffected by lesions SHAM (n=5), 6-OHDA (n=5) MPTP (n=4). Bars express mean ± SEM counts.

Fig. 4. Pilot experiments were carried out to set up the best conditioned place aversion (CPA) protocol. Three protocols were tested: (i) exposing naive rats (n = 9) to a place paired with quinine pellets; (ii) exposing rats (n = 5) that were previously exposed to a place paired with sucrose pellets and later submitted to extinction sessions to the same place paired with quinine pellets; and (iii) orally giving quinine solution to naive rats (n = 5) and exposing them to a place. In all cases rats were also exposed to a neutral place that was not paired with quinine. The places paired with sucrose or quinine and the unpaired place are referred to as "appetitive", "aversive", and "neutral", respectively. (A) Only protocols (ii) and (iii) were effective to cause CPA. (B) The rats submitted to protocol (ii) showed no place preference before training, preferred to stay in the appetitive compartment after CPA training and this preference was lost after extinction. Data are expressed as mean ± SEM. * $p < 0.05$ compared to the neutral compartment (Bonferroni's test after ANOVA).

Fig. 5. Effect of SNc lesion with 6-OHDA or MPTP on conditioned place learning (CPP) and conditioned place aversion (CPA) learning. (A) CPA task in which naive rats were paired with sucrose pellets in the appetitive compartment. SHAM (n=6), 6-OHDA (n=7), MPTP (n= 7). (B) CPA task in which the same rats which were first trained for CPP (in A) underwent extinction sessions, and then the same compartment was paired with quinine pellets. (C) CPA task in

which quinine solution was delivered in the mouth of separate naive rats immediately before confinement in the compartment assigned as aversive. No solution or pellet was given to the rats when they were confined in the compartment assigned as neutral. Bars express mean ± SEM time spent in each compartments. SHAM (n=11), 6-OHDA (n=13). * $p < 0.05$ compared to the neutral compartment (two-way ANOVA followed by Bonferroni's test).

DOPAMINE

↓DOPAMINE

SWEET PLACE → LEARN

BITTER PLACE → LEARN

SWEET PLACE → LEARN

BITTER PLACE → ~~LEARN~~

A

B

C

Legend:
- Neutral (white bar)
- Appetitive (gray bar)
- Aversive (black bar)

X-axis labels: Sham, 6-OHDA, MPTP

Y-axis: Time (%), scale 0 to 80
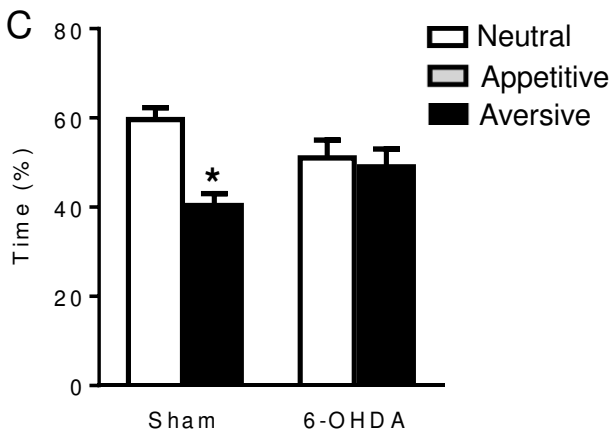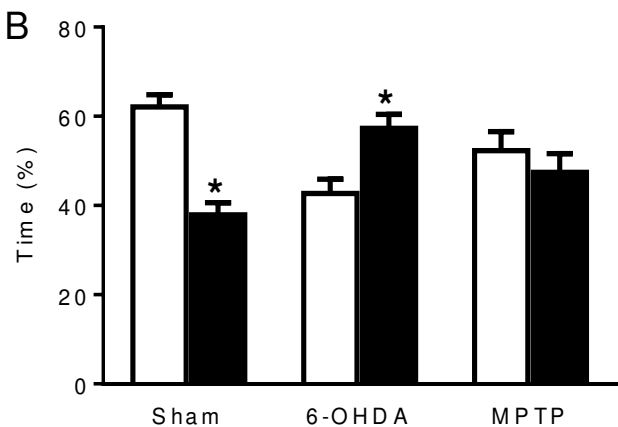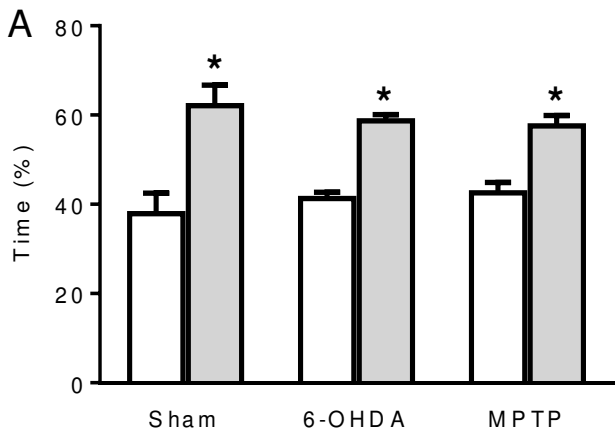
Neutral ☐ Appetitive ▨ Aversive ■

A

Time (%)

(i)   (ii)   (iii)

B

Time (%)

Before training   After training   After extinction

A

	MPTP	6-OHDA	Sham	
Sucrose				
Quinine				

B

Tongue protrusions / min

Gapes / min

Sham ☐
6-OHDA ■
MPTP ▨

A

Body weight (g)

Days after surgery

SHAM
MPTP
6-OHDA

B

Food intake (g)

Days after surgery

C

Water intake (g)

Behavioral tests started

Food restriction started

Days after surgery

A

Sham   MPTP   6-OHDA

B

SNc

Number of neurons (%)

Rostral   Caudal

C

SNc

☐ SHAM
■ 6-OHDA
▤ MPTP

Number of neurons (%)

Medial   Lateral

D

VTA

Number of neurons (%)

Rostral   Caudal

E

Striatum

Tissue content (%)

DA   DOPAC

Table 1. Partial SNc lesions do not significantly affect exploratory behavior in an open field.

	SHAM n=12	MPTP n=7	6-OHDA n=9
Crossings lateral area	79.4 ± 4.8	76.7 ± 6.0	74.0 ± 6.2
Crossings central area	21.4 ± 2.2	20.4 ± 2.9	19.6 ± 1.9
Rearing	16.7 ± 2.89	11.5 ± 2.85	15.2 ± 0.9
Grooming	0.0 (0.0/2.25)	0.0 (0.0/1.0)	0.0 (0.0/0.5)

Parametric data are expressed as mean ± SEM and non-parametric data are expressed as median (25% percentile / 75% percentile)

Table 2. Effects of partial SNc lesions on behavior directed toward sucrose pellets.

	SHAM n = 5	6-OHDA n = 4	MPTP n = 4
Time to start eating 1st pellet (s)	47 (30/71)	275 (90/541)[a]	121 (86/148)
Time interacting with the pellets (s)	77 (70/88)	96.5 (79/236)	105 (62/189)
Inter-pellet interval (s)	8.0 (7.3/9.5)	10.4 (8.4/25.2)	11.2 (6.5/20.6)[b]
Total time spent eating (s)	52 (41/56)	67 (53/98)	35 (27/43)
Number of pellets picked up	10	10	10

Data are expressed as median (25% percentile / 75% percentile). [a] $p < 0.05$, compared to SHAM
[b] $p < 0.05$, compared to MPTP

Table 3. Partial SNc lesions do not significantly alter behavior directed toward quinine pellets.

	SHAM n = 5	6-OHDA n = 4	MPTP n = 4
Time to start eating 1st pellet (s)	29 (24/48)	71 (29/91)	23 (20/80)
Time interacting with the pellets (s)	287 (186/948)	883 (342/1086)	761 (445/1077)
Inter-pellet interval (s)	45 (12/69)	69 (51/79)	42 (20/48)
Total time spent eating (s)	44 (31/144)	40 (12/76)	168 (48/201)
Number of pellets picked up	17 (8/22)	14 (6/17)	23 (11/44)

Data are expressed as median (25% percentile / 75% percentile).

Highlights

Rats with partial lesion of nigrostriatal dopamine failed to learn to avoid a place in which they experienced a bitter taste

Rats with this lesion learned as well as control rats to prefer a place in which they experienced a sweet taste

In both control and lesioned rats unconditioned responses to sweet and bitter tastes were intact and comparable

Therefore, a sub-set of nigrostriatal dopamine neurons play a role in aversion-, but not reward-driven associative learning