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Research report

# Dopamine in the prefrontal cortex regulates rats behavioral flexibility to changing reward value

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ABSTRACT

Prefrontocortical dopamine (DA) plays an essential role in the representation of reward value and is implicated in behavioral flexibility. We here tested the effect of systemic and local blockade of DA D1- and D2-receptors in the medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC) by using an operant paradigm, where rats have to adjust their behavior to changing reward value.

Rats were trained in a Skinner box, where different numbers of lever-presses for pellet-rewards were assigned to and switched between two levers. After rats commit to the efficient lever the lever-occupancy reversed and rats had to switch to the now efficient one. Rats were either intraperitoneally injected with the DA D1-receptor antagonist SCH23390 (40 μg/kg), the DA D2-receptor antagonist sulpiride (10 mg/kg), or phosphate buffered saline (PBS). Two other groups received bilateral local mPFC- or OFC-infusions of SCH23390, sulpiride (both 3 μg/0.5 μl), or PBS (0.5 μl) through previously implanted cannulae. After initial detection of reverse of lever-occupancy, systemic and local blockade of D1-receptors increased the number of switches back to the previously efficient lever, thus reducing the total number of reverses completed. D2-receptor blockade deteriorated this measure after local mPFC-infusion. Notably, initial detection of reverse of lever-occupancy was not affected.

Blockade of DA receptors within the prefrontal cortex do not deteriorate the detection of changes in reward value, whereas maintenance of behavioral adaptation is disturbed. Interestingly, blockade of DA receptors in the mPFC and OFC had similar effects, *i.e.*, these regions apparently act in a cooperative manner.



## 1. Introduction

When environmental conditions change, animals must be able to adapt their behavior to the new situation. This behavioral flexibility is a dynamic process that involves detection of the altered situation, inhibition of the previously applied response pattern together with acquisition and maintenance of a new association or strategy. Experimentally, behavioral flexibility is often tested by reversal learning, which uses the reversal of a stimulus response pattern, or reversal of a specific rule. It is also tested by using either an intra-dimensional shift, where the subject that has been previously trained to a particular stimulus or strategy is required to transfer that rule to a novel stimulus of the same dimension, or an extra-dimensional shift, where a subject is required to shift a response set to an alternative, previously irrelevant dimension. Both reversal learning and shifting to a new strategy or stimulus-reward association require the inhibition of responses to a previously reinforced stimulus or strategy [18,43].

The prefrontal cortex (PFC) regulates a number of higher-order cognitive and executive functions that serve to optimize performance in complex tasks, including working memory, decision-making, and behavioral flexibility [16,22,35,45]. Preclinical animal studies have shown that inactivation or local pharmacological manipulation of different subregions of the PFC, particularly alterations of the DA level, lead to deficits in tasks, which require the animals to disregard a previously beneficial behavioral strategy and engage in a novel or previous one [19].

Generally, the medial prefrontal cortex (mPFC) seem to be involved in the selection of higher-order rules (*e.g.*, cross-modal attentional shift), whereby the orbitofrontal cortex (OFC) is thought to be involved in lower order rules, for example reversal learning with no change in perceptual processing [16,44,54]. The different

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outcome of OFC and mPFC lesions is consistent with previous observations that mPFC and OFC subserve different types of inhibitory control or behavioral flexibility [4,17,34]. However, some studies have also reported deficits in reversal learning after lesions of the mPFC [32], while others reported that reversal learning is only impaired when stimuli are difficult to discriminate [8]. Additionally, the OFC has been shown to be important for goal directed appetitive behaviors and neurons within the OFC respond to reward predicted stimuli [46]. Notably, DA neurons have also been implicated in these behavioral function [48]. However, the tasks previously used are often heterogeneous in nature, and different anatomical divisions of the PFC as well as different local ablative and pharmacological manipulations of transmitter system have been used for testing, making these studies difficult to compare. Thus, a greater understanding of the PFC will come from using tasks that load more specific cognitive and executive processes.

In this study we used a novel test for measuring behavioral flexibility in an operant behavioral task, where different demands, *i.e.*, a different number of lever-presses at each lever for a pellet-reward, were assigned to two levers of a Skinner box. After the rat selected the efficient lever, the required number of instrumental responses reversed, so that the other lever became more efficient (adapted from Ref. [23]). The number of lever-presses the rat needed to identify this reversal served as an indication for its behavioral flexibility. We here aimed to compare the effects of systemic, as well as local blockade of DA D1- and D2-receptors in the mPFC and OFC during testing in this paradigm. Notably, our procedure uses (1) within session reversals, which minimizes the likelihood of affecting the results with memory effects, (2) the reversal of demand is indicated by an internal cost-benefit analysis of the reward value, and (3) the reversal does not lead to a complete denial of the reward, but an increase in the number of lever-presses, *i.e.*, the cost, to get the reward.

## 2. Materials and methods

### 2.1. Animals

Experiments were performed on 36 male Wistar rats (Harlan-Winkelmann GmbH, Borchen, Germany) weighing 250–300 g. They were housed in groups of six animals in Makrolon® Type IV cages under controlled ambient conditions (22 °C, 12 h light/dark cycle, lights on at 07:00 a.m.). Rats had free access to water, while food was restricted to 12 g rodent chow/(rat day) to maintain their weight at 250–300 g. All experiments were performed between 12:00 and 5:00 p.m. During testing casein pellets were used as rewards (45 mg casein pellet, Bioserv, Frenchtown, USA). The Principles of Laboratory Animal Care (NIH publication no. 86-23 revised 1985) and German regulations for animal experimentation were followed.

### 2.2. Apparatus

The operant behavioral task was conducted in automated operant chambers (29.5 cm × 28.5 cm × 23.5 cm; operant-lever-box, modular test cage system, Coulbourn Instruments, PA, USA). The chambers were equipped with two levers (2 cm × 3.5 cm, 13 cm distance) with a food dispenser in between the levers. Equipment programming and data recording were computer controlled.

### 2.3. Flexibility test

The rats obtained rewards at both levers, but the effort at one lever was *high* (less-efficient lever, fixed ratio FR 25, *i.e.*, 25 lever-presses for one pellet-reward) and at the other *low* (efficient lever, FR 9, *i.e.*, 9 lever-presses for one pellet-reward). Whenever the rats acquired seven pellets in a row at the efficient side, *i.e.*, without changing to the less-efficient side for even one lever-press, the lever-occupancy reversed (*i.e.*, the formerly efficient lever became the less-efficient lever and vice versa). Whenever the rats changed to the less-efficient side, the counter for the criterion "seven pellets in a row" restarted and the animal had to press a minimum of 63 levers again (7 × 9 lever-presses at efficient side). Ten reverses or 60 min ended the trial.

The following parameters were recorded and analyzed. Overall performance in this paradigm was assessed by the "number of reverses" achieved within 60 min. Failure of the rats to achieve reverses could be primarily due to two reasons. First, rats may not recognize or care about the switch in lever-occupancy and thus stay on the less-efficient side after reverse. This can be measured by counting the number of lever-presses before changing the first time to the efficient lever, *i.e.*, "pre-ratio shift response", which may be an indicative of perseveration on the previous efficient side. These errors are used as an index of how quickly animals are able to shift away from a previously efficient lever, which is no longer appropriate.

The second rationale for not achieving reverses could be that after changing to the efficient side, *i.e.*, after recognizing the change in lever-occupancy, rats repeatedly switch back to the less-efficient side, thus starting the counter for the criterion "seven pellets in a row" over and over again. This behavior can be measured by counting the number of switches back to the previous efficient lever per reverse, which are in the following termed "post-ratio shift response".

We also analyzed the number of lever-presses per second as a measure for motor impairments. Additionally, we measured the total number of pellets per session.

### 2.4. Habituation and training

The animals were first accustomed to the operant chambers, the taste of the casein pellets and the noise of the food dispenser. After this accommodation phase the rats were trained for lever-pressing in sessions of 30 min on a continuous schedule of reinforcement (*i.e.*, FR 1) on both levers. Thereafter, the rats were trained to switch between both levers by using a two-lever progressive ratio-paradigm. Here lever-presses were increased on a progressive scale. Successive reinforcement could be earned according to the following number of lever-presses: from 1 to 10 – one additional lever-press per ratio, from 11 to 20 – two additional lever-presses per ratio and so forth. Whenever the rat changed onto the other lever, this counter was restarted for both sides. Thus, it was most efficient for the rat to repeatedly change between the two levers. One session lasted 60 min or at least 10 switches between levers within 60 min. The day after learning that it is effective to change between the two levers (*i.e.*, three consecutive days with at least 10 switches between levers within 60 min), the training for the flexibility test started. In order to familiarize the rats with the principle of the paradigm, we initially used lever-occupancies that made it very easy for the rat to decide which lever was the efficient and which lever was the less-efficient one. In a previous study [21] we found that a difference of 29 and 5 is very easy for the rats, whereas with 25 and 9 it was more difficult for the rats to get 10 reverses within 1 h. Therefore, one lever was first allocated with FR 5, the other with FR 29. After 2 days most rats had achieved the principle of the task, *i.e.*, performed 10 reverses within 60 min at three consecutive days, the levers were allocated with FR 25 (less-efficient lever) and FR 9 (efficient lever).

### 2.5. Surgery for local injections

Once the rats learned the task, guide cannulae were implanted into the mPFC and OFC under chloral hydrate (360 mg/kg, i.p.) anesthesia. The anesthetized rats were placed in a stereotaxic apparatus and the cannulae bilaterally implanted by using the following coordinates: OFC 3.5 mm anterior, ±2.6 mm lateral and −3 mm ventral to bregma; mPFC 2.7 mm anterior, ±1.1 mm in triangle of 5° and −2.6 mm ventral to bregma. These coordinates were adapted on Ref. [42]. Guide cannulae were prepared from 22-gauge stainless steel tubing and fitted with 28-gauge stainless steel stylets in order to maintain patency. The guide cannulae were anchored with dental acrylic cement to three small anchor screws. The rats were allowed to recover 5 days before training started again and continued until reaching baseline.

### 2.6. Systemic blockade

The criteria for baseline performance were 10 reverses on three consecutive days. During these days the number of "post-shift response" (*i.e.*, the switches back to previous efficient lever) was allowed to vary for ±1, and the number of "pre-shift response" (*i.e.*, lever-presses before first switching) for ±25 lever-presses around the mean value for these measures. As soon as the rats reached this criterion, actual testing started the following day. Rats were intraperitoneally injected with the DA D1-receptor antagonist SCH23390 (40 μg/kg), the DA D2-receptor antagonist sulpiride (10 mg/kg) in a 2 ml solution, or 0.01 M PBS (2 ml/kg). All substances were administered 30 min before testing with SCH23390 and PBS administered pseudo-randomly. Sulpiride was injected last because in our hands it had been shown to have long-lasting effects (unpublished observations). RS(+)-SCH23390 (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) was dissolved in distilled water. (RS)-(±)-sulpiride (Tocris, Biotrend, Cologne, Germany) was first dissolved in a drop of glacial acetic acid, filled up to the required volume, and the pH adjusted to 6.0 with sodium hydroxide. The pH of all solutions was adjusted to 6.0 with sodium hydroxide or hydrochloride acid. The dose of SCH23390 and sulpiride are based on previous studies that used similar doses [29,41]. Between each injections rats were tested until reaching baseline criterion again.