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Research report

Monitoring dopamine transmission in the rat nucleus accumbens shell and core during acquisition of nose-poking for sucrose

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HIGHLIGHTS

- Dialysate dopamine was monitored within subjects during responding for sucrose.
- Accumbens shell dopamine progressively increased with training.
- Core dopamine increased transitorily at the beginning of training.
- Responding under extinction increased shell but not core dopamine.
- Non-contingent sucrose increased dopamine in both shell and core.

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ABSTRACT

On the basis of between subjects monitoring of in vivo dopamine (DA) transmission in the rat nucleus accumbens (NAc) shell and core during response-contingent and non-contingent sucrose feeding we have hypothesized that long term, daily exposure to sucrose feeding results in the acquisition of conditioned/discriminative stimuli capable of activating accumbens shell DA transmission in a non-habituating fashion. In order to verify this hypothesis we have now monitored within the same subject the changes in accumbens shell and core DA during acquisition of fixed ratio 1 (FR1) nose-poking for sucrose pellets. Once full training was obtained, dialysate DA was monitored in the same rat on three different sessions: responding for sucrose, extinction and non-contingent sucrose presentation. Dialysate DA steadily increased in the shell during operant sessions as training progressed but was activated in the core only early and transiently in training (5th session). After full training, reinforced as well as non-reinforced responding for sucrose activated DA selectively in the NAc shell. Non-contingent sucrose feeding activated DA in the shell and in the core. No habituation of shell DA responsiveness was observed under contingent and non-contingent sucrose feeding. These observations are consistent with the hypothesis that learning of FR1 nose-poking for sucrose involves acquisition of conditioned activation of DA transmission in the shell and active suppression in the core and that loss of habituation of shell DA responsiveness is related to change from primary-rewarding to conditioned/discriminative as driving stimuli of DA transmission in this area.

1. Introduction

A large body of evidence indicates that nucleus accumbens (NAc) dopamine (DA) plays a fundamental role in behaviour motivated by conventional (e.g., food) and drug reinforcers [1–3]. NAc, in turn, is not a homogeneous area, being distinguished into a ventro-medial shell and a dorso-lateral core with different input-out connections and different roles in behaviour [4–9].

Brain microdialysis and voltammetry studies have shown that NAc DA transmission differentially responds to palatable food in the two NAc subdivisions. Thus, NAc shell DA, but not NAc core and prefrontal DA undergoes rapid, single-trial habituation upon repeated feeding of a palatable food [10–16]. In contrast, monitoring of dialysate DA during responding for food [17–19] did not result in significant differences in DA responsiveness in the shell as compared to the core.

Abbreviations: CS, conditioned stimulus; CSs, conditioned stimuli; DA, dopamine; FR1, fixed ratio 1; NAc, nucleus accumbens.

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In a recent between subject study we reported that during responding for sucrose, DA transmission increases in the NAc shell but not in the core [20]. The same selective increase of NAc shell DA occurred under extinction in rats trained to eat sucrose in a response-contingent as well as non-contingent (passive) fashion in which only conditioned/discriminative cues were presented. In contrast, DA increased both in the shell and core during response non-contingent sucrose presentation. We explained these observations by hypothesizing that repeated sucrose feeding results in the acquisition of conditioned/discriminative stimuli capable of selectively activating accumbens shell DA transmission in a non-habituating fashion and that during responding for sucrose the stimulatory DA response in the core is actively suppressed. In order to verify this hypothesis we have now monitored within the same subject the changes in accumbens shell and core DA during acquisition of fixed ratio 1 (FR1) responding for sucrose pellets. Once full training was obtained, dialysate DA was monitored in the same rats on three different sessions: responding for sucrose, extinction and non-contingent sucrose presentation.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Harlan Italy, Udine, Italy) weighing 250–275 g were housed in group of six per cage with standard food (MIL topi e ratti, GLP diets, Stefano Morini, S. Polo D'Enza, RE, Italy) and water *ad libitum*, for at least 1 week in the central animal room, under constant temperature (23° C), humidity (60%) and a 12 h light/dark cycle (light from 8.00 a.m. to 8.00 p.m.).

All animal experiments were conducted in accordance with the guidelines for care and use of experimental animals of the European Communities Council (2010/63/UEL 276 20/10/2010) and with the Italian law (DL: 04.03.2014, N° 26) and approved by the Ethical Committee of University of Cagliari (CESA). All efforts have been made to minimize suffering and the numbers of animals used.

2.2. Surgery

Rats were anaesthetized with Equitesin (0.97 g pentobarbital, 4.25 g chloral hydrate, 2.1 g MgSO4, 42.8 ml propylene glycol, 11.5 ml 90% ethanol/100 ml; 5 ml/kg i.p.). A guide cannula (Plasticone, Roanoke, VA, USA) was stereotaxically and unilaterally implanted under the following coordinates: NAc shell (A: 2.0; L: 1 from bregma, V: −3.6 from dura), NAc core (A: 1.6; L: 1.9 from bregma, V: −3.4 from dura) according to the atlas of Paxinos and Watson [21]. Guide cannulae were plugged with a dummy cannula.

After surgery, rats were housed in individual cages (45 × 21 × 24 cm) under the same conditions mentioned above. Rats were left to recover for 10 days and during the first 5 days they were administered with Gentamicin sulphate (40 mg/kg s.c.). Rats were manipulated once a day for 5 min during the whole training period.

After recovery rats were fed with 15 g of standard food each day (MIL topi e ratti, GLP diets, Stefano Morini, S. Polo D'Enza, RE, Italy) in order to keep their weight around 90% of their *ad libitum* weight. Water was available *ad libitum* for the whole duration of the experiments.

2.3. Microdialysis

2.3.1. Probe preparation

Microdialysis probes were prepared according to the method of Lecca et al. [22,23], using AN69 membrane (Hospal Dasco, Italy). The dialyzing portion of the probe was 1.5 mm long. For each experimental session a new probe was utilized.

2.3.2. Microdialysis experiments

At the beginning of each microdialysis session, the microdialysis probes were connected to an infusion pump and perfused with a Ringer's solution (147 mM NaCl, 4 mM KCl, 2.2 mM $CaCl_2$; see Lecca et al. [23] on the use of 2.2 mM Ca^{2+} in the Ringer) at a constant rate of 1 μl/min, the dummy cannula was removed and the microdialysis probe was inserted through the guide cannula. The final coordinates of the microdialysis probe were: NAc shell (A: 2.0; L: 1 from bregma, V: −7.6 from dura), NAc core (A: 1.6; L: 1.9 from bregma, V: −7.4 from dura). Rats were placed in the operant box. After 10 min dialysate samples (5 μl) were started to be taken every 5 min and injected without purification into a high-performance liquid chromatograph (HPLC) equipped with a reverse phase column (LC-18 DB, 15 cm, 5 μm particle size, Supelco) and a coulometric detector (ESA, Coulochem II, Bedford, MA) to quantify DA. The first electrode of the detector was set at +125 mV (oxidation) and the second at −175 mV (reduction). The composition of the mobile phase was: 50 mM NaH_2PO_4, 0.1 mM Na_2–EDTA, 0.5 mM *n*-octyl sodium sulfate, 15% (v/v) methanol, pH 5.5 (obtained adding Na_2HPO_4). With these conditions the sensitivity of the assay for DA was 5 fmol/sample. Basal dialysate DA was calculated as the mean of the last three consecutive samples differing by no more than 10%, collected during the 60-min period preceding each experimental session [20].

At the end of each microdialysis session the probe was removed and the guide cannula was plugged again with a sterilized dummy cannula. Each rat was then returned to the homecage.

2.4. Sucrose

Sucrose pellets of 45 mg each were utilized as food (Test Diet, Richmond, IN).

2.5. Training on FR1 responding for sucrose and dopamine monitoring during training

Ten days after guide cannula implant, rats ($n = 13$) were trained to self-administer sucrose pellets every day for 2 weeks, apart from weekends, for a total of 10 sessions. Sessions lasted 1 h and took place between 9.00 a.m. and 2.00 p.m. in acoustically isolated and ventilated operant cages (Coulbourn Instruments, Allentown, NJ, USA). Two nose-poke holes were placed on one wall, 2 cm from the cage's floor. The active nose-poke was illuminated by a green-yellow light and the inactive one by a red light. The food dispenser was placed between the nose-pokes holes. On the same wall was located a loud speaker emitting a tone of 4500 Hz.

Rats were trained to respond for sucrose under a FR1 schedule [20].

The number of nose-pokes made and rewards earned were recorded by Graphic State 2 software, Coulbourn Instruments, USA.

Each 1 h session was composed of a cyclic alternation of three phases:

Phase 1: lasting 15 s during which the house light and the nose poke lights were turned on and a tone was activated to signal reward availability. Failure to respond correctly for more than 15 s resulted in switch off of visual and auditory cues and start of phase 3 without going into phase 2.

Phase 2: a sucrose pellet was dropped into the food dispenser and after 5 s phase 3 was initiated.

Phase 3: all cues were turned off and reward was not available for 7 s.

In rats ($N = 13$, seven implanted in the shell and six in the core) dialysate DA was monitored within subjects during training of FR1 responding for sucrose. After recovery from surgery, animals started the sucrose self-administration training, under the same conditions described above. Microdialysis was monitored during