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Research report

Evidence that conditioned avoidance responses are reinforced by positive prediction errors signaled by tonic striatal dopamine

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HIGHLIGHTS

- Striatal tonic DA followed prediction errors changes during rat CAR learning.
- Tonic DA was unaffected by unpredictable, unavoidable, and inescapable footshocks.
- Thus, tonic DA does not seem to encode hedonic value or salience of aversive stimuli.
- Lesion of nigrostriatal DAergic neurons impaired CAR learning.
- Thus, CARs seem to be reinforced by prediction errors signaled by tonic dopamine.

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ABSTRACT

We conducted an experiment in which hedonia, salience and prediction error hypotheses predicted different patterns of dopamine (DA) release in the striatum during learning of conditioned avoidance responses (CARs). The data strongly favor the latter hypothesis. It predicts that during learning of the 2-way active avoidance CAR task, positive prediction errors generated when rats do not receive an anticipated footshock (which is better than expected) cause DA release that reinforces the instrumental avoidance action. In vivo microdialysis in the rat striatum showed that extracellular DA concentration increased during early CAR learning and decreased throughout training returning to baseline once the response was well learned. In addition, avoidance learning was proportional to the degree of DA release. Critically, exposure of rats to the same stimuli but in an unpredictable, unavoidable, and inescapable manner, did not produce alterations from baseline DA levels as predicted by the prediction error but not hedonic or salience hypotheses. In addition, rats with a partial lesion of substantia nigra DA neurons, which did not show increased DA levels during learning, failed to learn this task. These data represent clear and unambiguous evidence that it was the factor positive prediction error, and not hedonia or salience, which caused increase in the tonic level of striatal DA and which reinforced learning of the instrumental avoidance response.

1. Introduction

It is well known that learning actions that lead to successful avoidance of negative aversive outcomes depend on release of dopamine (DA) in the striatum [1–3]. However, what causes striatal DA release during aversively motivated learning is controversial. Current hypotheses suggest that DA release is elicited by (i) formation of positive prediction errors (which occur when the outcomes are better than expected) [4], (ii) stimuli with hedonic value [5], or (iii) stimuli that are salient regardless of hedonic valence [6–9]. Phasic and tonic DA release are also proposed to play different roles, the former encoding positive prediction errors [4] and the latter encoding negative prediction errors (outcomes worse than expected) as occurs in response to unexpected aversive stimuli or omission of expected rewards [10]. However, several microdialysis studies, which are believed to measure tonic release of DA

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[11], reported increased extracellular DA in the striatum of rodents submitted to aversive stimuli [12–19]. This debate has been further fuelled by recent reports that aversive stimuli cause phasic activation of a subset of midbrain DA neurons [20–24], a response pattern opposite to what has been typically reported in a number of previous studies [4].

Here we studied alterations of tonic striatal DA during learning of an aversively motivated task – 2-way active avoidance – carried out under conditions in which these hypotheses predict different patterns of DA release. In this task rats learn to move to the opposite side of a shuttle box to avoid a footshock (unconditioned stimulus: US) preceded by a warning cue (conditioned stimulus: CS, auditory tone). A group of sham-operated rats was trained in up to five blocks of 40 trials (with 20-min inter-block intervals), and training stopped when rats achieved an asymptotic level of avoidance. Another group of sham-operated rats underwent a pseudo-training condition in which the total number and durations of CS and US matched those experienced by an avoidance-trained rat in the corresponding training block, but these stimuli were applied in an unavoidable, unpredictable (non-contingent), and inescapable manner (no action was effective to escape from the tone and avoid footshock). Two other groups of rats with partial lesions of substantia nigra pars compacta (SNc) DA neurons underwent the same training procedures. Levels of extracellular DA in the striatum were monitored in microdialysis samples collected at 5 min intervals.

The three hypotheses predict comparable patterns of DA release during active avoidance training: (i) according to the prediction error hypothesis [25] rats learn to expect that the aversive US follows the CS. When the rat accidentally performs an action that results in avoidance of the US, it generates a positive prediction error because this outcome is better than expected. However, after the rat learns how to avoid the US, the outcome will match expectation and prediction errors will tend toward zero. Therefore, the concentration of extracellular DA in the striatum should peak in the first blocks of CS–US pairings and decrease in the subsequent blocks. (ii) The hedonic hypothesis predicts DA release at the end of each footshock, given that relief from footshock is pleasurable. Therefore, DA release would be relatively high initially during training and decrease progressively, as a consequence of the progressive occurrences of the avoidance response. (iii) A similar pattern of DA release is predicted by the salience hypothesis, because the presentation of salient stimuli decreases with learning. In contrast, during pseudo-training, where salient and aversive stimuli are present, the pattern of DA release expected by the prediction error hypothesis is different from the pattern expected by the others. Hedonic and salience hypotheses predict similar patterns of DA release under training and pseudo-training conditions because the same number and duration of aversive and/or salient stimuli are presented under both conditions. However, according to the prediction error hypothesis, no alteration in extracellular DA concentration is expected under pseudo-training because this format offers no possibility for the rat to predict when and for how long the CS or US will occur. In addition, it offers no opportunity for avoiding these stimuli (that is, a complete lack of positive prediction errors).

Although debate continues as to what causes DA release during aversively motivated learning, such release is accepted by many as serving as a teaching signal leading to backward-strengthening associations among CS, US, and selected actions [26–28]. As such, the present study provided unequivocal evidence that striatal DA release is required for 2-way active avoidance learning. This was evidenced by findings showing that such learning did not occur in animals bearing lesions of midbrain DA neurons [29–35], results consistent with previous studies using intra-striatal infusions of D2 and D1 DA receptor agonists and antagonists [36–42].

2. Materials and methods

2.1. Animals

Adult male Wistar rats ($n = 57$; Universidade Federal do Parana (UFPR) breeding stock, Curitiba, Brazil), weighing 250–290 g at the beginning of the experiments, were housed at $22 \pm 2\,^{\circ}C$ on a 12-h light/dark cycle (lights on at 7:00 a.m.) with food and water available ad libitum. All procedures were approved by the Animal Care and Use Committee of the UFPR and consistent with international legislation (EC Council Directive 86/609/EEC).

2.2. Surgeries and drug infusion procedures

Before surgery, rats received atropine sulfate (0.4 mg/kg, i.p.) and penicillin G-procaine (20,000 U/0.1 mL, i.m.) and 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). A group of rats received three injections of acetaldehyde (120 mg/kg, Sigma–Aldrich, i.p., 10 min before and 30 and 60 min after the beginning of surgery). 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyrindine HCl (MPTP, 2 μg, Sigma–Aldrich) in saline (0.9% NaCl) was infused into the SNc via a 30-g stainless-steel needle (0.25 μL/min). SNc coordinates from bregma were (in mm): AP −5.0; ML ±2.1 mm; DV −7.7 from the skull [43]. Sham-operated rats underwent the same procedure, but saline rather than MPTP was infused into the left or right SNc. Sixteen days post-surgery, rats were anesthetized again as described above and a microdialysis guide cannula (15 mm long) was implanted into the striatum-coordinates (in mm) from bregma: AP +1.2; ML ±4.3; DV 2.8 from the skull; 25° angle [43]. It was anchored to the skull and sealed with a stainless-steel wire obturator. Microdialysis experiments were carried out after five days of recovery, when animals were trained in the conditioned avoidance task, as described below.

2.3. Behavioral procedures

The 2-way active avoidance apparatus was an automated 23 cm × 50 cm × 23 cm shuttle-box (Insight Instruments, Ribeirao Preto, Brazil) with a Plexiglas front panel and a floor made of parallel 5 mm o.d. stainless-steel bars 15 mm apart. The box was divided into two equal sized compartments by a 2-cm high Plexiglas bar. Rats were given blocks of 40 trials on the same day, with an interval of 20 min between blocks, until they achieved asymptotic performance (at least seven avoidances in the first 10 of the 40 trials of a block). In each trial, a sound cue (CS: 1.5 kHz, 60 dB, maximum duration of 20 s) was paired with a subsequent 0.5 mA footshock (US: maximum duration of 10 s, starting 10 s after the CS onset) unless the animal crossed to the other compartment of the box after the CS onset but before the US onset (constituting an avoidance response). If the rat crossed to the other compartment of the box after US onset, the US was terminated, but the trial was not coded as an avoidance response. A 10–30 s random interval occurred between trials. The numbers of active avoidances and latencies to respond were recorded automatically by the apparatus. Other groups of sham-operated and MPTP-lesioned rats were pseudo-trained by exposure to the same tone and shock stimuli used to train rats in the conditioned avoidance condition, but with these stimuli presented in a random sequence: they were unpaired, unpredictable, inescapable, and unavoidable. Under pseudo-training, an individual rat received exactly the same number and duration of stimuli in a specific training block that an individual avoidance-trained rat received in the corresponding training block, but the stimuli were presented in a sequence that made either instrumental or Pavlovian learning impossible. As such, the number and duration of the USs and CSs decreased progressively along the blocks.

2.4. In vivo microdialysis procedures

As described previously [44], a concentric microdialysis probe (300 μm o.d.; permeability 6 kDa; Cuprophan; Akzo, Wuppertal, Germany) with active membrane lengths of 4 mm was inserted unilaterally into the striatum via a guide cannula and perfused for 2 h to stabilize. The probe extended from the dorsolateral striatum to the NAc (core and shell). Training or pseudo-training started after four baseline samples had been collected at intervals of 5 min. Each block lasted 20 min, during which three samples were collected; another two samples were collected during the 20 min inter-block intervals. The microdialysis probe was perfused with Ringer's solution (in mM: NaCl, 145.0; KCl, 2.7; CaCl2, 1.2; MgCl2, 1.0, pH 7.4) at a constant flow rate of 1.2 μL/min. All microdialysis samples were collected into polyethylene tubes containing 5 μL of 0.1 M perchloric acid solution (Merck, Darmstadt, Germany), 50 ng/mL dihydroxybenzylamine (DHBA; Sigma–Aldrich) as an internal standard, and 0.06% sodium metabisulfite (Sigma–Aldrich), and stored at $-70\,^{\circ}C$ until high-performance liquid chromatography with electrochemical detection (HPLC-ED) analysis.

2.5. Determination of DA by HPLC-ED

Two protocols were used for sample analysis: one for microdialysis and the other for the postmortem striatal tissues. HPLC-ED analysis of microdialysis samples