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Involvement of dopaminergic mechanisms in the nucleus accumbens core and shell subregions in the expression of fear conditioning

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ABSTRACT

The involvement of dopamine (DA) mechanisms in the nucleus accumbens (NAC) in fear conditioning has been proposed by many studies that have challenged the view that the NAC is solely involved in the modulation of appetitive processes. However, the role of the core and shell subregions of the NAC in aversive conditioning remains unclear. The present study examined DA release in these NAC subregions using microdialysis during the expression of fear memory. Guide cannulae were implanted in rats in the NAC core and shell. Five days later, the animals received 10 footshocks (0.6 mA, 1 s duration) in a distinctive cage A (same context). On the next day, dialysis probes were inserted through the guide cannulae into the NAC core and shell subregions, and the animals were behaviorally tested for fear behavior either in the same context (cage A) or in a novel context (cage B). Dialysates were collected every 5 min for 90 min and analyzed by high-performance liquid chromatography. The rats exhibited a significant fear response in cage A but not in cage B. Moreover, increased DA levels in both NAC subregions were observed 5–25 min after the beginning of the test when the animals were tested in the same context compared with accumbal DA levels from rats tested in the different context. These findings suggest that DA mechanisms in both the NAC core and shell may play an important role in the expression of contextual fear memory.

The conditioned emotional response procedure is one of the most commonly used paradigms to investigate the neurobiological basis of fear. In aversive Pavlovian conditioning, an emotionally neutral stimulus, such as a context, is paired with an aversive unconditioned stimulus (US), such as footshock. The context acquires aversive conditioned properties and elicits responses similar to those induced by footshock itself. Conditioned freezing behavior can be elicited simply by placing the animal in the chamber in which footshock was previously experienced [4].

Most recent studies on fear conditioning have implicated the amygdala, dorsal hippocampus, and nucleus accumbens (NAC) as key structures for the emergence of fear learning. The amygdala integrates stimuli inputs from the environment and signals to other structures the degree of threat the stimuli represent [4,16]. The hippocampal formation is involved in contextual but not conditioned fear to discrete cues [17,23], and the NAC has been implicated in the acquisition and expression of conditioned fear [12].

Neurochemical experiments have shown that aversive conditioned stimuli (CS) can evoke dopamine (DA) release in the NAC [7,29]. A joint action with the amygdala is envisaged because the basolateral nucleus of the amygdala (BLA) sends a dense glutamatergic projection to the NAC [28] that synapses in close apposition to mesoaccumbens DA varicosities [9], thereby providing a potential site at which this glutamatergic projection could act presynaptically to increase DA efflux in the NAC [5].

The NAC contains two anatomically and functionally distinct subregions: a medioventral shell and a dorsolateral core [10,30]. The NAC, including its core and shell subregions, is a major area of convergence for inputs from the amygdala, hippocampus, and perirhinal and prefrontal regions, forming a complex network mediating dissociable functions in the acquisition, encoding, and retrieval of aversive learning and memory processes [4].

The distinct pattern of NAC core and shell output targets, with the core projecting mainly to motor structures and the shell projecting mainly to more limbic regions, suggests that the two subregions may mediate different behavioral processes [30]. Recently, functional dissociations of the NAC shell and core have been provided

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using excitotoxic and electrolytic lesions [20], as well as excitatory amino acid modulation selective to one or both NAC subregions [13]. The differential involvement of the shell and core in responding to a variety of stimuli suggests potential differences in the functional roles of these subregions in the modulation of conditioned fear. The main goal of the present study was to explore the neuroanatomical and neurochemical specificity of these NAC subregions during the expression of contextual fear conditioning by determining dialysate DA levels in the shell and core subregions of the NAC.

Male Wistar rats weighing 250–300 g were obtained from the breeding facility of the University of São Paulo at Ribeirão Preto. Animals were housed in a temperature-controlled ($22 \pm 1\,^{\circ}C$) room and maintained on a 12 h light/12 h dark cycle with lights on at 7:00 a.m. Rats were housed in pairs and given free access to food and water. The experiments reported in this paper were performed in accordance with the recommendations of the SBNeC (Brazilian Society of Neuroscience and Behavior) and complied with the United States National Institutes of Health Guide for Care and Use of Laboratory Animals.

Animals were anesthetized with tribromoethanol (250 mg/kg, i.p.) and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with the incisor bar set −3.3 mm below interaural zero. Each animal was implanted with two guide cannulae for dialysis probes (CMA/12, CMA/Microdialysis AB, Stockholm, Sweden) aimed at the NAC core (membrane length 1 mm) and shell (membrane length 2 mm), one in the left hemisphere and the other in the right hemisphere. With bregma as the reference point for each plane, the following coordinates were used: NAC core (AP + 1.4, ML 4.0, DV 6.8 mm [21]) and NAC shell (AP + 1.6, ML 0.8, DV 6.0 mm [21]). Thus, the microdialysis probe was introduced through the guide cannula.

Experiments began 5 days after guide cannula implantations. The animals were subjected to the contextual fear conditioning paradigm. This procedure has been used routinely in this laboratory [16,19]. Using this paradigm, freezing is elicited only in animals that are placed in the same context where they previously received footshocks. Briefly, the animals were conditioned to a distinctive context (cage A). This training chamber measured 25 cm × 25 cm × 15 cm, with the lateral walls and ceiling constructed of black and transparent Plexiglas, respectively. The floor consisted of 15 stainless bars (2.0 mm diameter) spaced 1.2 mm apart. The animals were placed in this cage, and 3 min later received 10 US (0.6 mA, 1 s duration) with a variable intertrial interval of 15–45 s. The shocks were delivered through the cage floor by a constant current generator built with a scrambler (Albarsh Instruments, Brazil). Application of the stimulus was controlled by a microprocessor and an input/output board (Insight Equipment, Brazil). Each animal was removed 2 min after the last shock and was returned to its home cage. The training session lasted about 15 min. A smaller cage (15 cm × 15 cm × 15 cm) with the lateral walls and ceiling made of white and opaque Plexiglas, respectively, served as cage B (different context) and was used only during testing sessions.

On the next day, the microdialysis probe was inserted into NAC shell or core and was perfused with Ringer's solution (in mM: NaCl, 147.0; KCl, 4.0; $CaCl_2$, 2.2) at a constant flow rate of 2.0 μL/min (Microinjection pump, BAS, West Lafayette, IN, USA). Following an equilibrium period, dialysate samples were collected every 5 min into vials containing 10 μL perchloric acid solution (0.05N) with dihydroxybenzylamine as the internal standard. Control levels were defined as the average of four baseline samples (four consecutive samples differing by 10%). After the collection of basal samples (considered 100%), animals were either placed in cage A (same context where they received footshocks) or in cage B. This timepoint was considered time 0. Dialysate samples were collected for 90 min. Microdialysis probes were implanted in the NAC in two groups of animals ($n = 6$, $n = 5$ for the same and different context groups, respectively) to examine the effects of the conditioned fear response on extracellular DA levels in both nuclei. Shell and core dialysates were collected simultaneously.

One day after the training sessions, the animals were placed either in cage A or cage B as described above, and the microdialysis probes were inserted into the NAC shell and core, one in the left hemisphere and the other in the right hemisphere. Testing sessions were conducted without presentation of footshocks. The criterion used to assess contextual fear was the time rats spent freezing during a period of 5 min. Freezing was operationally defined as the total absence of movement of the animal with the exception of respiration.

The amount of DA in the collected fractions was analyzed using high-performance liquid chromatography coupled with electrochemical detection (BAS, West Lafayette, IN, USA). The reverse phase ODS column was a UniJet 100 mm × 10 mm C-18 with 3 μm particle size (BAS, West Lafayette, IN, USA). The HPLC system consisted of a BAS Epsilon electrochemical detector (BAS, West Lafayette, IN, USA) with a glass-carbon electrode and a pump (PM-92e, BAS, West Lafayette, IN, USA). The potential was set at 650 mV (compared with the Ag–AgCl reference electrode). The mobile phase was composed of 50 mM NaH_2PO_4, 0.1 mM Na_2-EDTA, 0.5 mM *n*-octyl sodium sulfate, and 15% methanol (pH adjusted to 5.5) and was filtered and pumped through the system at a flow rate of 80 μL/min. The injection volume was 20 μL. This set-up allowed the analysis of DA in the dialysate in a single run, which lasted approximately 6 min. The detection limit of the assay was ∼5 pg/sample.

Upon completion of the experiments, the animals were given a lethal dose of urethane (0.5 mg/kg, i.p.) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7.4). The brains were removed and maintained in PBS solution for 2 h and cryoprotected in 30% sucrose in 0.1 M PBS until soaked. Serial 60 μm coronal sections were cut using a freezing microtome, mounted on gelatin-coated slides, and stained with 5% cresyl violet (Sigma–Aldrich, St. Louis, MO, USA) to localize the positions of the dialysis probes according to the Paxinos and Watson rat brain atlas [21].

Data are presented as mean ± S.E.M. of the percentage of basal values calculated as the mean of the first three consecutive microdialysis samples obtained immediately before the test session in cage A (same context) or cage B (different context). Data for the DA collected from each NAC subregion (shell or core) were subjected to a two-way repeated-measures analysis of variance (ANOVA), with group (same or different context) as the between-subjects factor and time sample of the dialysate as the within-subjects factor. Results showing significant overall changes were subjected to Tukey's *post hoc* test. Dialysate values were not corrected for *in vitro* probe recovery. Time of contextual conditioned freezing was subjected to Student's *t*-test. The level of significance was set at $P < 0.05$.

The representative sites of the dialysis probes inserted into the NAC shell and core are shown in Fig. 1. Fig. 2 shows the time of conditioned freezing exhibited by rats during the test session in cage A (same context) or in cage B (different context) for the groups implanted with a microdialysis probe in NAC shell or core. A robust increase in the time spent freezing was observed when rats were tested in the same context in which they previously received footshocks ($F_{1,9} = 13.35$, $P < 0.05$, Fig. 2). Immobility was the predominant behavior of rats subjected to the same context. Fifteen to 20 min after the beginning of the sessions, rats from both groups (same and different contexts) displayed similar behaviors such as occasional scratching, grooming, or some head movements.