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Intraperitoneal sertraline and fluvoxamine increase contextual fear conditioning but are without effect on overshadowing between cues

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ABSTRACT

Treatment with selective serotonin reuptake inhibitors (SSRIs) can reduce contextual conditioning. Since contexts comprise a variety of potentially competing cues, impaired overshadowing may provide an account of such effects. The present study therefore compared the effects of two SSRIs on overshadowing and contextual conditioning, testing suppression of an ongoing behavioral response (licking) by cues previously paired with foot shock. Conditioning to a 5 s light stimulus was reduced when it was presented in compound with a 5 s noise, thus overshadowing was demonstrated. In two experiments, this overshadowing was unaffected by treatment with either sertraline or fluvoxamine. However, unconditioned suppression to the noise (tested in a control group previously conditioned to the light alone) was reduced after sertraline (10 mg/kg, i.p.). The successful demonstration of overshadowing required the use of a second conditioning session or an additional conditioning trial within the same conditioning session. Neither weak nor strong overshadowing (of the light by the tone) was affected by any drug treatment. Moreover, counter to prediction, conditioning to contextual cues was increased rather than impaired by treatment with sertraline (10 mg/kg, i.p.) and fluvoxamine (30 mg/kg, i.p.).

1. Introduction

Contextual fear conditioning is typically reduced by both acute and chronic treatment with selective serotonergic reuptake inhibitors (SSRIs; Hashimoto et al., 1996, Hashimito et al., 2009; Inoue et al., 2011; Li et al., 2006b; Montezinho et al., 2010; Nishikawa et al., 2007). Contexts comprise a configuration of potential conditioning cues which could compete with each other. Thus the above findings may reflect the role of serotonin (5-hydroxytryptamine, 5-HT) in fear conditioning per se; in other words, fear responses to competing cues might also be reduced by SSRIs.

In an overshadowing procedure, the relative intensity of competing discrete CSs modulates their capacity to become associated with an outcome (unconditioned stimulus, UCS). Normally a more intense CS acquires associative strength at the expense of a less intense CS (Pavlov, 1927). The neural substrates of overshadowing have been little investigated (Horsley et al., 2008; Nelson et al., 2011). If overshadowing is reduced, one possible outcome is learning failure, because no single cue acquires sufficient associative strength. Thus, if salience modulation is impaired, this could reduce overshadowing, and potentially reduce conditioning to contextual cues. The substrates of overshadowing are therefore of interest in relation to contextual conditioning.

In the present study, overshadowing was examined using a fear conditioning procedure that we have previously used to test the effects of amphetamine (Nelson et al., 2011). After pairing of a CS (or two CSs in compound) with footshock UCS, fear was later measured as the suppression of licking in water deprived rats, upon presentation of the CS(s). The same conditioned suppression procedure adopted to assess overshadowing was also used to determine the suppression of licking produced by the contextual cues provided by the conditioning box (Cassaday et al., 2001; Horsley and Cassaday, 2007; Norman and Cassaday, 2003). The effects of two SSRIs were examined in this fear conditioning procedure: sertraline (at 10 and 20 mg/kg i.p.; experiment 1) and fluvoxamine (at 15 and 30 mg/kg i.p.; experiment 2). Based on the fact that contextual conditioning impairment has been widely reported after treatment with SSRIs (Inoue et al., 2011), it was predicted that the overshadowing effect should also be impaired by these treatments.

2. Experimental procedures

2.1. Animals

For each experiment, 72 naïve adult male Wistar rats (Charles River, UK) were caged in pairs on a 12:12 h light/dark cycle with food and water *ad libitum*. They were handled for approximately 5 min per day for 1 week and then placed on water deprivation 24 h prior to the start of each experiment. The mean start weight was 215 g (range 192–237 g in experiment 1 and 200–238 g in experiment 2). The data

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from one rat were lost due to a procedural error (in experiment 1). The work was conducted in accordance with the UK Animals Scientific Procedures Act 1986, Project Licence: PPL 40/3163.

2.2. Drug treatments

Sertraline HCl (Tocris, UK) was dissolved in 2% Tween80 saline for administration of doses of 10 or 20 mg/kg (calculated as the free base) at 2 ml/kg injection volume. Fluvoxamine maleate (Tocris, UK) was dissolved in saline for administration of doses of 15 or 30 mg/kg (calculated as the free base) at 1 ml/kg injection volume (i.p.; warmed to dissolve at 30 mg/ml). Sertraline injections were made 30 min, and fluvoxamine injections were made 60 min prior to the conditioning stage of the procedure. Control rats were injected with the equivalent volume of vehicle.

2.3. Behavioral apparatus

Six identical fully automated conditioning boxes were housed within sound-attenuating cases containing ventilation fans (Cambridge Cognition, Cambridge, UK). The conditioning boxes were steel (25 cm × 25 cm × 22 cm high) with a Plexiglas door (27 cm × 21 cm high), inset at the front. A waterspout was mounted on one wall, 5 cm above the floor and connected to a lickometer supplied by a pump. Licks were registered by a break in the photo beam within the spout, which also triggered water delivery of 0.05 ml per lick. The waterspout was illuminated when water was available. Three wall-mounted stimulus lights and the house light were set to flash on (0.5 s) and off (0.5 s) for a total 5 s duration. In both experiments, these flashing lights served as the CS for the control rats. In the overshadowing group, the 5 s flashing lights CS was presented in compound with a 5 s mixed frequency noise set at 85 dB, delivered by a loudspeaker set in the roof. Footshock of 1 s duration and 1 mA intensity provided the UCS. This was delivered via the grid floor (steel bars 1 cm apart) by a constant current shock generator (pulsed voltage: output square wave 10 ms on, 80 ms off, 370 V peak under no load conditions; MISAC Systems, Newbury, UK). Stimulus control and data collection were by an Acorn Archimedes RISC computer programmed in Basic with additional interfacing using an Arachnid extension (Cambridge Cognition).

2.4. Behavioral conditioning procedures

Water deprivation was introduced 1 day prior to shaping. The rats then had 1 hr and 15 min of *ad libitum* access to water in their home cage after each of the procedural stages described below. This home cage access was in addition to any water drunk in the conditioning boxes (available from the apparatus waterspout on all days of the procedure apart from conditioning). Thus animals were trained, conditioned and tested, after 22 hours of water deprivation, on consecutive days.

2.4.1. Pre-conditioning to establish baseline lick responses

In order to initiate licking behavior, rats were first placed in the conditioning boxes in pairs (with their cage mates) and were shaped for 1 day until all drank from the waterspout. No data were recorded. Thereafter, animals were individually assigned to a conditioning box for the duration of the experiment (counterbalanced by experimental group). There then followed 5 days of pre-training, in which rats drank in their conditioning boxes for 15 min each day (timed from first lick). The licking spout was illuminated throughout, but no other stimuli were presented. Latency to first lick was recorded to assess any pre-existing differences in readiness to drink.

2.4.2. Conditioning with footshock

No water was available within the box, and the waterspout was not illuminated. In experiment 1, the UCS footshock was delivered following termination of the CS in each of 2 conditioning trials per conditioning session (of which there were 2). The first pairing of CS and UCS was presented after 5 min had elapsed, and the second pairing was 5 min after the first, followed by a further 5 min left in the apparatus. In the absence of licking, there were no behavioral measures to record. In experiment 2, three conditioning trials were delivered, as before 5 min apart within a 20 min single conditioning session. In both experiments, the CS was provided by the flashing lights, compounded with the noise stimulus in the overshadowing groups.

2.4.3. Reshaping after footshock

On the day following conditioning, animals were reshaped, following the same procedure as in the pre-conditioning sessions. This both re-established licking after conditioning and provided a measure of contextual conditioning, reflected in the extent to which licking was suppressed in the conditioning boxes.

2.4.4. Overshadowing tests

On the day following reshaping, the animals were placed in the conditioning boxes and presented with the CS. Water was available throughout the test, and the waterspout was illuminated. Once the animals had made 50 licks, the CS was presented for 15 min. The latency to make 50 licks in the absence of the CS (the A period, timed from the first lick made in each box) provided a measure of any individual variation in baseline licking. This was compared with the time taken to complete 50 licks following CS onset (B period) in a suppression ratio (A/(A + B)) to assess the level of conditioning to the CS, adjusted for any individual variation in drink rate. In experiment 1 only, rats underwent a second conditioning session. Following completion of the above procedure, an additional baseline day as per pre-conditioning, was used to re-establish licking. There then followed the same behavioral procedure as before.

2.5. Experimental design and analysis

In both experiments, there were 6 experimental groups run in a 2 × 3 independent factorial design (n = 11–12/cell): conditioning group at levels control or overshadowing; drug at levels saline, 10 or 20 mg/kg sertraline, and saline, 15 or 30 mg/kg fluvoxamine, in experiments 1 and 2 respectively. The same design was applied to analyses of variance (ANOVAs) for the pre-conditioning baselines (to check for pre-existing differences by experimental condition-to-be), the reshaping latencies and number of licks made within the first 5 min (to measure differences in contextual conditioning), suppression to the CS and suppression to the competing tone stimulus (to measure discrete cue conditioning). Post hocs to further examine significant effects of drug were by Tukey test. In each case alpha was set at $p < 0.05$ for the rejection of the null hypothesis. The dependent variables were lick latencies and number of licks within the first 5 min at pre-conditioning and reshaping, and the A period and suppression ratio for the conditioning tests. Where necessary, raw latency data (time to first lick at reshape) were log transformed so that their distribution was suitable for parametric analysis. Non-significant effects on baseline lick responding are not reported.

3. Results

3.1. Experiment 1: effects of sertraline in an overshadowing procedure

3.1.1. Reshaping: contextual conditioning

Fig. 1A shows that following the first conditioning session, there were differences in latency to drink. Statistically, ANOVA showed both a main effect of conditioning group [$F(1,66) = 4.784$, $p = 0.032$] and drug [$F(2,66) = 9.696$, $p < 0.001$]. The main effect of drug arose because the 10 mg/kg sertraline group took overall longer to recommence licking than the saline group [$p < 0.001$; this difference in latency was not