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Research article

Chronic fluoxetine dissociates contextual from auditory fear memory

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HIGHLIGHTS

- Fluoxetine treatment of adult mice impaired their contextual fear memory but spared auditory fear memory.
- These data suggest that a blunting of hippocampal-mediated aversive memory may be a therapeutic action for this medication.
- Hippocampal perineuronal net (PNN) density was not affected by fluoxetine.

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ABSTRACT

Fluoxetine is a medication used to treat Major Depressive Disorder and other psychiatric conditions. These experiments studied the effects of chronic fluoxetine treatment on the contextual versus auditory fear memory of mice. We found that chronic fluoxetine treatment of adult mice impaired their contextual fear memory, but spared auditory fear memory. Hippocampal perineuronal nets, which are involved in contextual fear memory plasticity, were unaltered by fluoxetine treatment. These data point to a selective inability to form contextual fear memory as a result of fluoxetine treatment, and they suggest that a blunting of hippocampal-mediated aversive memory may be a therapeutic action for this medication.

1. Introduction

Over 350 million people struggle with Major Depressive Disorder (MDD), a condition characterized by excessive guilt and decreased energy, mood and appetite. Severe MDD may also result in suicidal thoughts and psychotic symptoms [1]. Added to these symptoms, MDD is characterized by a bias towards negative memories [2–5] and to negative autobiographical narratives that are sub-served by the hippocampus [6–8]. Selective serotonin reuptake inhibitor (SSRI) treatment has therapeutic effects on this memory bias [9].

Based upon these clinical findings we sought to study the effects of the SSRI, fluoxetine, on negative memories mediated by the hippocampus. To study this we examined the effects of chronic fluoxetine on contextual fear memory, a form of aversive learning where the hippocampus is considered to play a central role [10]. During contextual fear memory rodents form memories of foot shock (unconditioned stimulus; US) associated with multisensory cues (conditioned stimulus; CS).

To determine whether chronic fluoxetine has effects that are specific to contextual fear memory we also studied its effects on auditory fear memory. In auditory fear memory an association is formed between the US and an auditory cue (CS). This form or learning is thought to be primarily mediated by the amygdala [11].

Our studies further examined the effects of chronic fluoxetine on perineuronal nets (PNNs). PNNs are structures consisting of chondroitin sulfate proteoglycans that organize around neurons during brain development [12]. The disruption of PNNs in the mature hippocampus alters contextual fear memory [13]. In addition, fluoxetine alters PNN density in brain circuitry [14]. Therefore, these studies also included an examination of PNNs in the CA1 and DG areas of the hippocampal region.

Our studies found that chronic fluoxetine treatment of adult mice impaired their contextual fear memory, but spared auditory fear memory. These data point to a selective inability to form contextual fear memory as a result of fluoxetine treatment, and they

Abbreviations: MDD, Major Depressive Disorder; SSRI, selective-serotonin reuptake inhibitor; CS, conditioned stimulus; US, unconditioned stimulus; PNN, perineuronal net; DG, dentate gyrus; WFA, wisteria floribunda agglutinin; PBS, phosphate buffered saline; PFA, paraformaldehyde.

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suggest that a blunting of hippocampal-mediated aversive memory processes may be a therapeutic action for this medication. At a histological level, hippocampal PNNs were unaltered by fluoxetine treatment. Although not reflected in PNN density our behavioral findings parallel other studies demonstrating fluoxetine's ability to alter hippocampal-related plasticity [15,16].

2. Methods

2.1. Animals

C57BL/6J mice were bred in our colony maintained at The Scripps Research Institute. These experiments were conducted with male mice and drug treatments were started at ~2 months of age. This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by The Scripps Research Institute guidelines for the humane care and use of laboratory animals and all efforts were made to minimize suffering.

2.2. Drug treatment

Mice were treated with fluoxetine (Eli Lilly), at 0.16 mg/ml in their drinking water. Our measurements of daily water intake estimated this dose to result in fluoxetine treatment of 19 mg/kg per day. Based upon prior studies of plasma fluoxetine this administration would approximate levels in the upper end of fluoxetine's therapeutic range of 20–80 mg daily [17,18]. We chose this dose since it represents fluoxetine levels that are used to target severe and oftentimes treatment resistant depressive symptoms [19,20].The dose is also similar to that used in other studies that have examined fluoxetine's effects on brain plasticity [15,21]

Chronic fluoxetine treatment has been defined as between 24 and 30 days according to prior studies [16,21,22]. In Experiment 1 mice received fluoxetine for 30 days. In Experiment 2 mice received fluoxetine for 24 days. Since fluoxetine is photosensitive, bottles were wrapped in aluminum foil and medication was replaced every 7–10 days. Intake was monitored weekly to ensure regular consumption and comparable intake between experimental groups. Control animals received drinking water alone.

2.3. Behavior

The first experiment used a between-subject design to study the contextual fear memory of one fluoxetine treated group, compared to the auditory fear memory of a second fluoxetine treated group. Fluoxetine-treated mice received the medication in their drinking water for 30 days prior to fear conditioning and continuously up until memory retrieval.

To examine contextual fear memory, fluoxetine-treated mice (n = 6) and drinking water controls (n = 8), underwent contextual fear conditioning in Context A. Context A consisted of a wintergreen scented square chamber with black and white checkerboard pattern and aluminum walls (30-cm length × 24 cm width) and a grid floor that delivered footshock (FreezeFrame). Contextual fear conditioning consisted of 109 s of free exploration followed by four non-signaled footshocks (duration 1 s, intensity 1 mA) with an inter-stimulus interval of 70s. The total duration of training in Context A was 380 s. Twenty fours later contextually fear conditioned animals were re-exposed to Context A for 120 s and freezing levels measured.

To examine auditory fear memory, a separate group of fluoxetine-treated mice (n = 7) or drinking water controls (n = 6) underwent auditory fear conditioning in Context A. Animals were fear conditioned in Context A, as described above, but with white noise (10 s, 85 dB 2800 Hz) preceding and co-terminating with each shock. Twenty four hours later, auditory fear memory was retrieved within Context B. Context B consisted of an opaque plastic container whose floor was covered with sani-chips (Allentown caging, base: 20-cm length × 12-cm width, top: 22-cm length × 14-cm width). This container sat within a larger, lemon-scented fear conditioning chamber (30-cm length × 24-cm width). Mice were placed in the opaque plastic container and their freezing was measured during 12, 10 s exposures to white noise.

The second experiment used a within-subjects design to study the contextual and auditory fear memory of a single fluoxetine treated group. Fluoxetine-treated mice received the medication in their drinking water for 24 days prior to fear conditioning and continuously up until retrieval. Fluoxetine-treated mice (n = 8) or drinking water controls (n = 8) were subject to auditory fear conditioning in Context A. Twenty four hours later, either contextual or auditory fear memory were tested in Context A or B, respectively, using a counterbalanced design. 48 h after the original fear conditioning, animals tested for contextual fear memory were then tested for auditory fear memory and vice versa.

2.4. Perineuronal nets (PNNs)

PNNs were measured in mice exposed to fluoxetine for 30 days (n = 4) or in mice who received drinking water (n = 4). PNNs were visualized according to published methods [23,24]. Briefly, animals were perfused with 4% paraformaldehyde (PFA) in PBS. Brains were postfixed in 4% PFA and then placed in 30% sucrose. Brain sections were collected with a vibratome in ice cold PBS. Sections were incubated in a blocking solution of 3% BSA and 0.2% Triton-X-100 in PBS, pH 7.4, and then incubated in a solution of biotin-conjugated lectin wisteria floribunda agglutinin (WFA) (10 μg/ml). WFA was detected using FITC conjugated streptavidin (10 μg/ml in PBS). Images were collected with a fluorescence microscope and PNNs were counted in the CA1 region and dentate gyrus (DG) by two raters blind to experimental treatment.

2.5. Statistics

Freezing during white noise exposures was totaled for each animal. The effects of fluoxetine on contextual versus auditory freezing were then analyzed with a 2-Way ANOVA or repeated measures ANOVA. A Tukey method for multiple comparisons post-hoc test was used to examine data points with significant differences. PNN data was analyzed with a Student's *t*-test.

3. Results

In the first experiment fluoxetine-treated mice underwent either auditory or contextual fear conditioning. Twenty four hours later, fear memory was retrieved with either auditory cues or contextual cues, respectively. Fluoxetine-treated mice showed no difference in their freezing to auditory cues compared to controls. In contrast, fluoxetine-treated animals showed dramatic reductions in their contextual fear memory compared to controls (Fig. 1). An ANOVA found a main effect for retrieval cue ($F(1,23) = 70.33$, $p < 0.0001$) and fluoxetine ($F(1,23) = 22.63$, $p < 0.0001$), and a significant interaction (retrieval cue x fluoxetine $F(1,23) = 15.93$, $p < 0.001$). Post-hoc testing found a significant decrease in contextual freezing for the fluoxetine-treated group compared to the contextual freezing of the control group ($p < 0.001$). (Fig. 1).

In the second experiment the contextual and auditory fear memory of a single fluoxetine-treated group of mice was tested. Fluoxetine-treated mice underwent auditory fear conditioning. Since this involves exposure to a novel context, the training box, both auditory and contextual associations are formed. Twenty- four