

Pharmacological activation of the lateral orbitofrontal cortex on regulation of learned fear and extinction

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ARTICLE INFO

Keywords:

Obsessive-compulsive disorder
Post-traumatic stress disorder
Orbitofrontal cortex
Pavlovian fear extinction

ABSTRACT

Obsessive-compulsive disorder (OCD) is usually accompanied with hyperactivity of the orbitofrontal cortex (OFC). OCD patients have anxiety issues, and there is high comorbidity of OCD and post-traumatic stress disorder (PTSD). One of the leading factors of PTSD is the failure of fear extinction. In this study, we examined whether hyperactivity of the OFC interfered with extinction processes. The lateral OFC (lOFC) was pharmacologically activated with *N*-methyl-D-aspartate (NMDA) in behaving rats during encoding, consolidation, and retrieval, of Pavlovian fear extinction. We found that when we brought the lOFC on-line before extinction training or retrieval test, there was a general initial suppression of fear expression regardless behavioral history, which was followed by development of nonspecific fear response. Moreover, pre-extinction activation of the lOFC impaired the encoding of extinction demonstrated by a general up-shift of fear levels during retrieval test compared to controls. We also found that regardless of whether the lOFC was activated or not, immediate post-extinction manipulation interfered extinction consolidation in general. To conclude, activation of the lOFC altered expression of learned fear and negatively impaired extinction outcome. Our results provided a new angle to study the etiology of comorbid OCD and PTSD.

1. Introduction

Fear is an intrinsic emotion that animals express when they are under dangers or threats, and thus prepares the animals to cope with the coming situations and avoid further harms (Campese et al., 2016; LeDoux, 1994). By protecting an individual from life-threatening conditions, fear stands its vital role in evolution (Janak & Tye, 2015; LeDoux, 1996; Mineka & Ohman, 2002). However, when fear goes beyond control and is expressed inappropriately, it interferes with the daily function of the affected individuals and takes a toll on the victims' lives. Post-traumatic stress disorder (PTSD), panic disorder, phobias, and obsessive-compulsive disorder (OCD) are only a few psychiatric disorders that develop with abnormal regulation of fear (Bouton, Mineka, & Barlow, 2001; Cromer, Schmidt, & Murphy, 2007; Kessler et al., 2005; Pare, Quirk, & LeDoux, 2004; Rothbaum & Davis, 2003; Stevens et al., 2013). According to the prediction of World Health Organization, anxiety and depressive disorder combined will become the second most burdensome illness in the world (Graham & Milad, 2011). Thus, understanding the underlying psychological and neurobiological mechanisms of how fear is generated and how it can be extinguished is

of great urgency.

In laboratory settings, Pavlovian fear conditioning and extinction is one of the most widely used behavioral paradigms to study how fear memory is formed and regulated (LeDoux, 2000; Quirk & Mueller, 2008). The conditioning procedure is conducted by pairing a neutral conditioned stimulus (CS; e.g. tone) with an aversive unconditioned stimulus (US; e.g. mild footshock). Fear response to the CSs, such as freezing, develops after a few pairings (Blanchard & Blanchard, 1972; Fanselow, 1980; Fendt & Fanselow, 1999; Giustino & Maren, 2015). After the individual acquires learned fear, the extinction procedure is carried out by exposing the subject to the CSs without the aversive outcomes. Fear response gradually declines, which requires an active inhibitory "CS-no US" learning that undergoes encoding and consolidation processes (Milad, Rauch, Pitman, & Quirk, 2006; Quirk & Mueller, 2008).

As mentioned above, abnormal regulation of fear may lead to certain severe anxiety disorders. For example, some veterans and survivors of physical and sexual assault, abuse, accidents, disasters, and many other serious events, developed PTSD (Peri, Ben-Shakhar, Orr, & Shalev, 2000; Shin, Rauch, & Pitman, 2006). The symptoms of PTSD

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include re-experiencing, avoidance, and hyperarousal (First, Frances, & Pincus, 2004). It is worth noticing, though, that not everyone who lives through a dangerous event gets PTSD. There are several risk factors (Brewin, Andrews, & Valentine, 2000), such as having another mental health problem. A number of reports suggested that OCD is one of the common comorbid disorders with PTSD (Becket, 2002; de Silva & Marks, 1999; Gershuny, Baer, & Jenike, 2002; Shavitt et al., 2010; Simon, Adler, Kaufmann, & Kathmann, 2014). OCD is characterized by intrusive, unwanted thoughts (i.e., obsessions) and ritualized, repetitive behaviors (i.e., compulsions) (First et al., 2004; Simon et al., 2014). Abnormalities in thalamo-cortico-striatal loops have been identified as the general aberrance in OCD patients, especially the hyperactivity of the orbitofrontal cortex (OFC) (Shin & Liberzon, 2010; Simon et al., 2014).

The likely comorbidity of PTSD and OCD provides us with a new perspective of PTSD etiology. It is well documented that input from the medial prefrontal cortex (mPFC) to the amygdala is critical for proper fear regulation after extinction (Milad & Quirk, 2012; Quirk & Mueller, 2008; Sierra-Mercado, Padilla-Coreano, & Quirk, 2011; Sotres-Bayon & Quirk, 2010). Anatomy data suggested that the lateral OFC (lOFC), including lateral orbital (LO) area and the adjacent agranular insular (AI; including the ventral “AIV” and dorsal “AID” subdivisions) area, project heavily to the sensory input area of the amygdala, including the lateral (LA) and basolateral (BLA) nuclei (McDonald, Mascagni, & Guo, 1996; Rempel-Clower, 2007), while electrophysiology data suggested that activation of the lOFC suppressed the information flow on the mPFC-LA/BLA pathway (Chang, 2017; Chang & Grace, 2018). These evidences support the possibility that activation of the lOFC mimicking OCD condition may interfere the extinction processes. In this study, we examined the hypothesis that hyperactivity of the lOFC negatively affects fear extinction, in which failure of the extinction has been suggested as one of the leading causes of PTSD (Milad et al., 2006; Rothbaum & Davis, 2003). In behaving rats, lOFC was pharmacologically activated during the encoding (pre-extinction), consolidation (post-extinction), and retrieval (pre-test) phase of Pavlovian fear extinction (Fig. 1).

2. Material and methods

2.1. Subjects

A total of 127 male Long-Evans rats (250–300 g; National Laboratory Animal Center, Taiwan) were used in this study. Animals were individually housed in a temperature ($22 \pm 1^\circ\text{C}$) and humidity (60–70%) controlled facility on a 12-h light/dark cycle (7:00 a.m. to 7:00 p.m.) with food and water available *ad libitum*. Animals were handled for at least five days (about 10 s per day) before surgery, and all experimental procedures were performed according to the guidelines approved by Institutional Animal Care and Use Committees (IACUC) of both the National Tsing Hua University and National Chiao Tung University.

2.2. Surgery

Animals were anesthetized with ketamine (80–100 mg/kg) and xylazine (8–10 mg/kg) before being placed in a stereotaxic apparatus (Stoelting). Core body temperature was maintained at 37°C by a

temperature-controlled heating pad (CWE). Two 26-gauge stainless steel guide cannulae (Plastics One) were implanted bilaterally aiming the lOFC (relative to bregma, anteroposterior [AP] +3.5 mm, medio-lateral [ML] ± 3.0 mm, dorsoventral [DV] -4.0 mm) (Chang, 2017). Three additional anchor screws were mounted, and then the headstage was fixed in place with dental acrylic. After surgery, carprofen (5 mg/kg) was injected subcutaneously as an analgesic. Animals were placed back to their home cages and monitored until awake. Rats were allowed to recover for at least five days before the behavioral procedures, while the dummy cannulae (Plastics One) were changed daily.

2.3. Behavioral procedures

A 2×2 design with factors of “Drug” (vehicle “VEH” and *N*-methyl-D-aspartate “NMDA”) and “Group” (extinction “EXT” and no-extinction “NoEXT”) were conducted, yielding a total of four groups (V-EXT, V-NoEXT, N-EXT, and N-NoEXT) in each experiment. The chamber position of each animal was counterbalanced across experimental groups and training squads. All behavioral experiments were conducted in four identical observation chambers in sound-attenuating cubicles (Med-Associates) under the same context settings. Specifically, all lights (room ceiling lights, chamber ceiling lights, and chamber stimulus lights) remained on, while ventilation fans in each cubicle supplied background noise at 65 dB. The cubicle doors remained half open during behavioral sessions. Between training squads, the chambers were cleaned with a 1% acetic acid solution. To provide a distinct odor, stainless-steel pans containing a thin layer of this same solution were placed underneath the grid floors before the rats were placed inside. The rats were transported to this context using transparent cuboid boxes with a thin layer of beddings in the boxes.

On Day 1, animals were conditioned with five tone (10 s, 80 dB, 2 k Hz)-footshock (2 s, 1.0 mA) pairings with 60 s inter-stimulus intervals (ISIs). On Day 2, animals underwent the extinction/exposure process. The EXT group was given 45 tone (10 s) presentations with 30 s ISIs, while the NoEXT group was placed in the chamber but received neither tone nor footshock for equivalent amount of time. On Day 3, all animals were tested with 45 tone (10 s) presentations with 30 s ISIs.

2.4. Behavioral pharmacology

33-gauge injectors (Plastics One) extending 1.0 mm beyond the guide cannulae were attached to polyethylene tubes, which were connected to Hamilton syringes (5.0 μl) located on an infusion pump (Harvard Apparatus). NMDA (0.75 $\mu\text{g}/0.5 \mu\text{l}$) or VEH (sterile saline) was injected at the rate of 0.2 $\mu\text{l}/\text{min}$ for 2.5 min, followed by another 1 min for the drug diffusion. The dosage of NMDA was chosen based on previously published reports (Floresco, Todd, & Grace, 2001; Legault, Rompre, & Wise, 2000). Drug infusions were conducted immediately before or after behavioral sessions at three different time points: Pre- (Experiment 1, “encoding”) or Post-Extinction (Experiment 2, “consolidation”) on Day 2, and Pre-Test (Experiment 3, “retrieval”) on Day 3 (Fig. 1).

2.5. Histology

For histological verification of cannula placements, animals were killed with CO_2 followed by decapitation. Their brains were removed, fixed with 8% paraformaldehyde (PFA) in 0.2 M phosphate buffer (PB) for at least 48 h, and then switched to 25% sucrose solution in 0.1 M PB until saturated. Coronal sections (60 μm) were collected with a cryostat (-20°C) and mounted onto subbed slides. Sections then underwent standard Nissl stain to confirm injection sites (Fig. 2A). We targeted the lOFC area; nonetheless, animals with cannula tips located in the dysgranular insular cortex (DI) just dorsal of AID were also included in the data analyses because of the spread of the drug infusion (Fig. 2B–D).

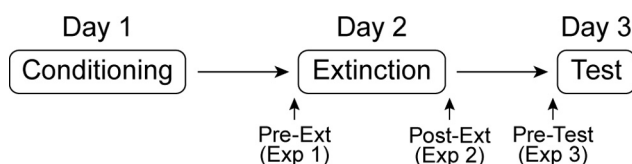


Fig. 1. Experimental design of this study.

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