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Short communication

Medial orbitofrontal cortex lesion prevents facilitatory effects of D-cycloserine during fear extinction

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HIGHLIGHTS

- We evaluate the relationship between orbitofrontal cortex and D-cycloserine in fear extinction.
- Orbitofrontal cortex lesion prevents the facilitatory effect of the D-cycloserine.
- Pharmacological treatments could be influenced by prefrontal activity.

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ABSTRACT

Animal models of fear extinction have an important clinical relevance to pharmacological and exposure-based therapies for anxiety disorders. Lesions of prefrontal structures impair fear extinction. On the other hand, D-cycloserine is able to enhance this process. We hypothesize that the integrity of cortical structures involved in inhibitory control of emotional responses is crucial for the facilitatory effects of D-cycloserine. Here, we showed that medial orbitofrontal cortex lesion prevents D-cycloserine enhancement of fear extinction. These preliminary results suggest that effects of pharmacological treatments could be dependent on cortical activity state to promote fear memory reduction.

Anxiety and stress-related disorders such as post-traumatic stress-disorder (PTSD) are largely maintained by pathological fear memories. Pavlovian fear conditioning is a classical animal model to study the neurobiological mechanisms of anxiety disorders. Fear conditioning involves pairing a neutral/conditioned stimulus (CS) with an aversive unconditioned stimulus (US) such as a footshock. Following this, the presentation of CS elicits fear responses like freezing or fear-potentiated startle. When the CS is repeatedly presented alone, animals learn that it no longer predicts the occurrence of the US, and consequently suppress fear expression. This is known as extinction.

Over the past few decades, considerable research has been conducted to understand the neural networks and neurochemical mechanisms underpinning fear extinction [1–3]. A large body of evidence suggests that fear extinction depends on the interaction of the ventromedial prefrontal cortex (vmPFC) – generally defined as the prelimbic and infralimbic subregions and medial orbitofrontal cortex (OFC) – and amygdala [4]. Increased neural activity in the prelimbic cortex (PL) was observed in rats in response to CS presentation during extinction [5]. In addition, fear extinction retrieval was associated with increase of infralimbic cortex (IL) activity. During extinction, excitatory projections from vmPFC, particularly of IL, target GABAergic intercalated cells (ITC) in the amygdala to inhibit fear responses [6,7].

Despite the focus on PL and IL modulation during fear extinction, recently, the role of orbitofrontal cortex (OFC) in fear and appetite extinction has been assessed. Zelinski et al. [8] showed that excitotoxic lesions of the lateral orbitofrontal cortex prevent extinction during discriminative fear conditioning and promote over-generalized fear responses. Learning theories argue that fear extinction is caused by the discrepancy between the expected value of the US that is elicited by the CS, and the lack of experienced

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US [9]. Two functions proposed for the OFC are representation of US expectancy information and discrepancy detection [10,11]. In fact, Panayi and Killcross [12] showed that OFC is required for the representation of CS outcome expectancy values; furthermore, OFC inactivation, immediately prior to extinction session, impairs learning extinction of appetitive conditioning [12]. Rodriguez-Romaguera et al. [13] showed that deep brain stimulation of the dorsal striatum induced the expression of the plasticity marker pERK in PL/IL, OFC, and amygdala (Central Nucleus and ITC), this result is consistent with fear extinction memory enhancement.

Besides the anatomical research, pharmacological studies are crucial for the design of new therapeutic interventions in anxiety disorders [14–16]. D-cycloserine (DCS), an agonist at the glycine-binding site of the NMDA receptor, enhanced fear extinction in rats that received systemic or intra basolateral amygdala administration prior to [17] or immediately after extinction training [18]. Gupta et al. [19] showed that DCS alone or in conjunction with extinction training led to a strong facilitation of extracellular signal-regulated kinase (ERK) in the medial prefrontal cortex. This increase in ERK suggests that DCS may augment local synaptic plasticity in order to facilitate fear extinction.

To date, there is evidence suggesting that OFC represents US expectancy during appetitive extinction [10,11]; however, little is known about the role of this structure in acquisition and extinction of fear memories. In this study, we used electrolytic lesion of the medial orbitofrontal cortex (mOPF) and oral gavage DCS administration in order to investigate the function of mOFC during acquisition and extinction of cued fear memory and its relation with the extinction enhancement of DCS. We hypothesize that the integrity of cortical structures involved in inhibitory control of emotional responses is crucial for the facilitatory effects of D-cycloserine.

All experiments were conducted in accordance to Colombian Guidelines for Laboratory Animal Care (law 84/1989 and 8430/1993) and the US National Institute of Health Guide for Care and Use of Laboratory Animals (No. 86–23, revised 1985).

Thirty-five male Wistar rats (300 ± 20 g) obtained from the animal facilities of the Universidad de los Andes were used. The animals were housed in groups of four per cage, under a 12:12 h dark-light cycle (lights on at 07:00 h), $21 \pm 2\,^{\circ}$C. Free access to food and water was allowed throughout the experiment.

A stereotaxic surgery was performed to cause a bilateral electrolytic lesion in the OFC. For this, the animals were anesthetized with a mixture of Ketamine (Rotexmedica, 75 mg/kg, i.p.) and Xylacine (Bayer, 5 mg/kg), and fixed in a stereotaxic frame (Narishige). Standard isolated electrodes were aimed at the OFC with the following coordinates relative to Bregma: AP = 3.8 mm; ML = ±0.4 mm; DV = 4.6 mm. Half the animals in each group received a 150 uA anodal direct current, during 10 s, for electrolytic lesion (lesion group). The other half had the electrode inserted in the same place but no current was used (sham surgery). After a 1-week recovery period from surgery, animals were submitted to the behavioral procedures.

Fig. 1 shows the extent of the larger and the smaller lesions from the rats included in the mOFC lesion groups. As can be seen, the larger lesion included damage to anterior and posterior regions of the mOFC and some portions of anterior prelimbic (PrL) and infralimbic cortex (IL). In some subjects, little damage to anterior ventral orbitofrontal cortex (VO) was detected. The smaller lesion included damage to mOFC and some portions of PrL, but not VO. No damage was detected in lateral orbitofrontal cortex (LO).

Each group of animals (lesion and sham surgery) was divided in two subgroups to receive 1.0 mL/kg of D-cycloserine (15 mg/kg—VESALIUS-PHARMA) or sterile saline (0.9%, wt/vol) by oral gavage.

All sessions were performed in a conditioning chamber ($60 \times 60 \times 30$ cm). The floor of the box consisted of 4 mm aluminum bars spaced 11 mm apart. The animals were allowed to freely explore the conditioning chamber during a 10-min period (day 0). No explicit stimulus was presented in this phase. Conditioning (day 1 and 2) consisted of three presentations of the CS followed by the US on each day. Each rat was placed in the chamber and after 150 s the CS (tone; 800 Hz; 65 dB; 20 s) was presented for the first time and co-terminated with the US (foot shock; 0.5 s, 0.5 mA alternating current). The inter-trial interval was 42.5 ± 17.5 s. Fifty seconds after the last CS–US presentation, the rat was removed from the conditioning chamber and placed in its home cage. For extinction trials, three CS presentations of 20 s (without US pairing) were performed with an inter-trial interval of 42.5 ± 17.5 s. Nine days of extinction were performed. After the first day of extinction (day 3), oral DCS or saline solution was administered. Freezing time was evaluated during all CS presentations, totaling 60 s of CS measure in each day (conditioning and extinction sessions). Freezing was defined as the absence of all movements, except those related to breathing.

Factorial ANOVA with repeated measures (ANOVA-RM) was performed to compare between or within-group comparisons during conditioning and extinction session. Additionally, two-way ANOVA was used in order to analyze the number of days required for each group to reach the 80% extinction criterion (80% reduction on freezing time to CS). Tukey post-hoc test was used for all instances and significance was set at $p < 0.05$. All data were expressed as mean ± standard error of means (SEM).

Evidence from rodent studies links the vmPFC to fear memory regulation and expression [20,21]. Lesions of vmPFC cortex disrupt fear extinction acquisition, as well as extinction retrieval [22]. However, little is known about the involvement of orbitofrontal structures in fear memory regulation. First, we assessed the effect of mOFC lesion during the acquisition of fear conditioning. ANOVA-RM revealed significant effects of time ($F_{2,66} = 96.174$, $p < 0.001$) but not of lesion ($F_{1,33} = 0.009$, $p = 0.922$) nor significant time × lesion interaction ($F_{2,66} = 0.718$, $p = 0.491$) during conditioning. Tukey post-hoc test showed that both groups (lesion and sham) expressed more freezing levels during conditioning day 2 ($p < 0.05$) compared with conditioning day 1 and pre-shock (Fig. 2A). Consistent with previous reports on prefrontal cortex modulation of fear extinction, lesion of mOFC showed no effect on the acquisition of fear conditioning [23–25].

To establish the role of mOFC on fear extinction and its relation with the pharmacological effect of DCS, two independent analyses were performed: (1) the ability of all groups to extinguish fear conditioning, and (2) the days required to reach the criterion of 80% reduction in the freezing. For this, rats in the sham and lesioned groups were randomly assigned to receive either DCS or vehicle, and after the first extinction session (day 3), each rat received oral DCS or saline solution, as appropriate.

Freezing of early (day 3) and late (day 11) extinction was analyzed to assess the acquisition of fear extinction. ANOVA-RM revealed significant effects of time ($F_{1,31} = 197.500$, $p < 0.001$) but not of lesion ($F_{1,31} = 0.282$, $p = 0.599$), drug ($F_{1,31} = 2.164$, $p = 0.151$), lesion × drug interaction ($F_{1,31} = 1.692$, $p = 0.202$), time × lesion interaction ($F_{1,31} = 0.305$, $p = 0.584$), time × drug interaction ($F_{1,31} = 0.144$, $p = 0.706$) and time × lesion × drug interaction ($F_{1,31} = 0.222$, $p = 0.640$). Tukey post-hoc test showed that all groups expressed more freezing during early extinction when compared with late extinction ($p < 0.001$) (Fig. 2B and C). By the end of extinction training, all groups exhibited comparable, low levels of freezing independently of lesion or drug treatment. Interestingly, this experimental approach showed a lack of difference between lesion and control in the acquisition of fear extinction.