Effects of acute intragastric administration of D-Cycloserine in rats with lesion of orbito-medial prefrontal cortex: possible anxiogenic effects on extinction of fear conditioning

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Introduction

Extinction is a process of cortical inhibition. One region that has received considerable attention as a component of the brain's extinction circuitry is the medial prefrontal cortex (Sotres-Bayon, Cain, & LeDoux, 2006)

Dorsal prefrontal cortex (mPFCd) lesions produce a general increase in fear reactivity to CS fear conditioning and context fear while ventral prefrontal cortex (mPFCv) lesions has no effect during acquisition of fear conditioning but extend the fear response to CS (but not to context) during extinction (Morgan & LeDoux, 1995)

Ventromedial orbital prefrontal cortex (OPFC) lesions result in generalized fear and impaired extinction in a discriminative fear conditioning task (Zelinski, Hong, Tyndall, Halsall, & McDonald, 2010)

D-cycloserine (DCS) - a partial agonist of glycine site of N-methyl-D-aspartate receptor - facilitates the extinction of learned fear in a fear conditioning task, but also seems to reduce fear to a non-extinguished CS (Ledgerwood, Richardson, & Cranney, 2005)

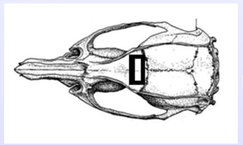
Intercalated cell (ITC) in the amygdala are a likely site of action of DCS and a possible site of potentiation of prefrontal or basolateral inputs (Pare, Quirk, & LeDoux, 2004)

The aim of this study was to asses the function of orbito medial prefrontal cortex (OM) in the extinction of fear conditioning and the possible changes in the effects of DCS associated to OM lesion.

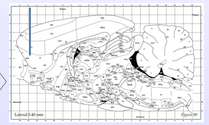
Method

Thirty-two male Wistar rats (300±20 g) were used

Animals were anesthetized with a mixture of Ketamine (70mg/Kg) and Xylacine (5mg/Kg), and fixed in a stereotaxic frame



A flap of skull overlying the frontal cortex was removed

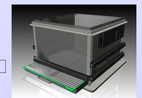


Standard isolated electrodes were directed to the following coordinates relative to Bregma (AP = 3.8 mm; ML = ± 0.4 mm; DV = 4.6 mm)

Rats were randomly assigned to one of four groups: lesion+DCS, lesion-DCS, control+DCS and control-DCS

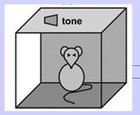
Lesion	omPFCv	Sham
DCS	8	10
Saline	6	8

After 5 days, the animals started the experimental procedure



Habituation (Day 0): Free exploration of the conditioning box during a 10-min period. No explicit stimulus was presented in this phase.

Conditioning (days 1 and 2): Three presentations of the CS (tone; 800Hz; 65dB; 20s) followed by the US (foot shock; 0.5s, 0.2mA alternating current) on each day. The inter-trial time was 42.5±17.5 s.



Extinction (days 3 to 11): The procedure for extinction was exactly the same as for conditioning days except that the US was never presented.

At the end of day three, the intragastric administration of DCS (15mg/kg) or saline solution (0.9 %) was made by means of a standard intragastric administration needle

Freezing time was measured for periods of: (1) 20 s prior the presentation of CS, (2) 20 s during the presentation of CS and (3) 20 s after the termination of CS

After the end of experiments, the animals were intracardiacally perfused with 120ml of saline solution (0.9%) followed by 200ml of paraformaldehyde (4%)

Brains were coronally sectioned (Vibratome, 1500) and 50um slices were obtained and treated for Nissl staining with Cresyl Violet

Results

Differences in freezing time for day four to nine were analyzed using a two way ANOVA (drug treatment x lesion). When necessary the comparison between the averages of the groups was done using the Student Newman–Keuls test as post hoc test. Alpha was set at 0.05 for all instances.

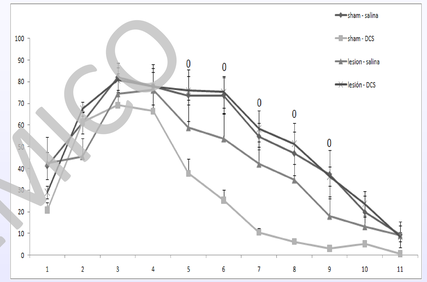


Fig. 1. Extinction Curve.

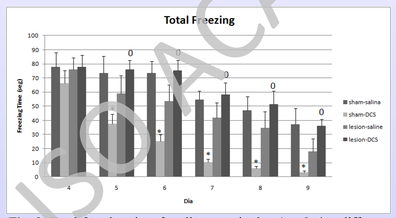


Fig. 2. Total freezing time for all groups in day 4 to 9: * = different from the group with the same lesion but different drug treatment o = different from the group with the same drug treatment but different lesion.

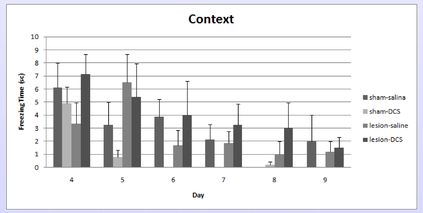


Fig. 3. Context freezing time for all groups in day 4 to 9.

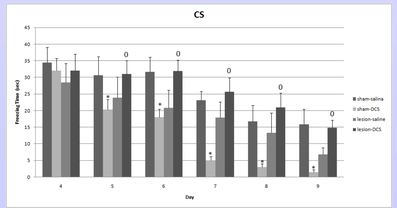


Fig. 4. CS freezing time for all groups in day 4 to 9: * = different from the group with the same lesion but different drug treatment o = different from the group with the same drug treatment but different lesion.

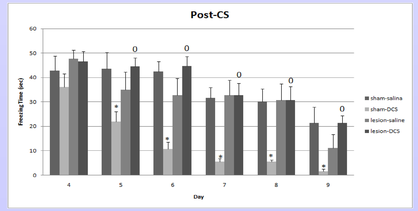


Fig. 5. Post-CS freezing time for all groups in day 4 to 9: * = different from the group with the same lesion but different drug treatment o = different from the group with the same drug treatment but different lesion.

Discussion

Lesion of OM had no effects during acquisition and extinction of fear conditioning

Intragastric acute administration of DCS facilitates the extinction to CS as reported in other studies

Lesion of OM prevent the effects of DCS in the extinction of fear conditioning

DCS had anxiogenic effects in rats selected for low anxiety behaviors in the elevated plus maze (Ho et al., 2005). Medial prefrontal cortex structures are associated to emission of appropriated responses to anxiogenic stimuli (Gonzalez et al., 2000)

Fear extinction as a behavioral flexibility process. Reversal and OM function

Differences between fear and anxiety in fear conditioning and fear extinction research

Conclusion

Complete lesion of mPFCv and DCS vs Infralimbic cortex lesion and DCS

Local administration of DCS in OM

Lesion of OM and intra-amygdala (BLA) administration of DCS

Specific effects of DCS in fear and anxiety tasks

Effects of OM lesion in freezing behavior

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