Table 1- Group Nomenclature, number of subjects, and timeline

<table>
<thead>
<tr>
<th>Group Nomenclature</th>
<th>N</th>
<th>20-day Water deprivation schedule</th>
<th>Conditioning Extinction Extinction Latency Period</th>
<th>5th Test Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAC</td>
<td>5</td>
<td>Water</td>
<td>SAC = L+ SAC = L− SAC = L+ SAC = L−</td>
<td>SAC Extinction</td>
</tr>
<tr>
<td>dPAG Stimulation</td>
<td>10</td>
<td>Water</td>
<td>SAC = L+ SAC = L− SAC = L+ SAC = L−</td>
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</tr>
</tbody>
</table>

Methods

Introduction

As anxiety disorders, like phobia and PTSD, gain clinical attention, there is a demand to understand the behavioral and neurological processes that underlie extinction (Endo and Stafford, 2010; Henry et al., 2010).

Foams can re-emerge following the passing of time (spontaneous recovery; SR) or other forms of relapse (Bouton, 1995; Rescorla and Heth, 1976).

The neural pathways that modulate acquisition and extinction of learned fears include the amygdala, prefrontal cortex, and periaqueductal gray (PAG). The PAG is referred to as the “final common pathway” of affective and defensive behaviors (Graeff, 1990; 324).

Recent works suggest that the neurological processes of fear extinction and those underlying the reoccurrence of once-extinguished fears are related (Costantini et al., 2011) and that the PAG, specifically the dPAG, may be involved in modulation of spontaneous recovery (Mickley et al., 2011).

The inactivation of the PAG impairs fear acquisition, suggesting a role of the PAG in the prediction of negative outcome, risk assessment and rescaling of expectations, all of which influence the processes of extinction and SR (McNally and Westbrook, 2010). CTA is a defensive reaction to a learned fear (Parker, 2003) and may be acquired when an animal consumes a novel taste (C) and then experiences the symptoms of poisoning (the US). Garcia, Kinokforn & Hutt, 1981. Garcia, Kinokforn & Kneiding, 1995.

Chances in neural activity in the amygdala, hippocampus, and PAG correlate with various stages of extinction and SR of a conditioned taste aversion (CTA) (Mickley et al., 2011). Increased neural activity in the PAG, specifically the basolateral PAG (bPAG), indicated by c-fos immunoreactivity, is correlated with a decreased likelihood of SR (Helmstetter et al., 2000).

In the current study, we aimed to determine if SR of a CTA could be modulated through electrical stimulation of the PAG. We predicted that stimulation should increase c-fos activity and produce a decrease in SR of the CTA.

CTA Extinction

• Starting on experimental Day 7, animals began a series of every-other-day CS-only SAC presentations in extinction to the CTA.
• On odd days, animals were presented SAC followed by a 15min latency, preceded by 30min access to water every other day (odd days, see Table 1 throughout EXT).
• On even days, animals were given only water. This procedure continued until animals demonstrated 90% of baseline SAC consumption (referred to as “asymptomatic” extinction; Nolan et al., 1997).

Latency Period and Electrode implantation

• Animals received 60 min ivitaly access to water for 30 days.
• Rats were chronically implanted with intracranial electrodes aimed at the dPAG. Approximately 15 days into the latency phase to ensure proper recovery prior to the SR test.
• Bilateral bipolar electrodes were centered on the following coordinates to deliver electrical stimulation to the dPAG: −7.68mm, posterior to bregma, m 3.0mm to the midline, and 1.5mm ventral.

• Rats had 3 in 1 days of ad libitum water exposure after surgery before going back on the water deprivation schedule.

C-fos immunohistochemistry

• Brains were removed, stored, and sliced at 40µm.
• Staining was for c-fos protein immunoreactivity, and quantitated with neutral red (Hernandez, and Rosenberg, 1966).
• Cells with dark, punctate nuclear staining were counted as c-fos positively stained cells bodies were not counted. A positive control was used to ensure c-fos did not result from faulty staining procedures (Farman et al., 1997).

Results

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• Brain slices were viewed to determine the location of electrode tip and to see if c-fos expression was found in this area (see Fig. 1).
• Electrode placements were characterized as in (N=10) or outside (N=9) the structures (See Fig. 1 for electrode placement diagram; Fig. 4 for SAC consumption).

Table 2- Summary of CTA extinction, and inactivation of the dPAG

<table>
<thead>
<tr>
<th>Extinction Condition</th>
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<th>PAG Stimulation</th>
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CTA Extinction

• The data indicate that the extinction procedure produced reacquisition of the SAC.
• SAC consumption was low on the first day of extinction for both treatment groups (SAC stimulation: MCW = 0.12 ± 0.03 ml; Mann-Whitney), stimulation control: 0.12 ± 0.03 ml). This is similar to SAC consumption on the final conditioning day.
• The number of days to reach asymptomatic extinction (see Fig. 4) and the slopes of extinction curves were comparable between the two groups (PAG stimulation: 27.90 ± 0.11 days), slopes = 0.63 ± 0.11; stimulation control: 24.08 ± 2.40 days, slope = 0.65 ± 0.10).
• The amount of SAC consumed on the day rats achieved asymptomatic extinction was also similar between PAG stimulation rats [12.19 ± 0.48 ml] and stimulation control [17.08 ± 0.88 ml] (See Fig. 4).

CTA Extinction

• Rats that experienced electrical stimulation of the dPAG and/or the cPAG exhibited a stronger SR of the CTA than rats that received stimulation in closely adjacent brain structures (See Fig 1 for electrode placement diagram, Fig. 4 for SAC consumption).
• A 1-way ANOVA with repeated measures compared SAC consumption at asymptomatic extinction between stimulation and stimulation control groups. All animals demonstrated a SR of their CTA (P< 0.01) (>73.06 ± 0.01).
• Rats receiving dPAG stimulation drank significantly less SAC at the test day than did the control rats (F=1.17; p < 0.01). (See Fig. 4)

SAC Consumed at Asymptomatic Extinction and Spontaneous Recovery Tests

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