Stimulation of the dorsal periaqueductal gray enhances spontaneous recovery of a conditioned taste aversion

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Introduction

- As anxiety disorders, like phobias and PTSD, gain clinical attention, there is a demand to understand the behavioral and neurological processes that underlie extinction (Groblewski and Stafford, 2010; Herry et al., 2010).
- Fears can re-emerge following the passing of time (spontaneous recovery; SR) or other forms of relapse (Bouton, 1993; Rescorla and Heth, 1975).
- The neural pathways that mediate 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, p. 324).
- Recent works suggest that the neurological processes of fear extinction and those underlying the reoccurrence of once-extinguished fears are related (Costanzi et al., 2011) and that the PAG, specifically the dlPAG, 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 resetting 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 (CS) and then experiences the symptoms of poisoning (the US) (Garcia, Kimeldorf & Hunt, 1961; Garcia, Kimeldorf, & Knelling, 1995).
- Changes in neural activity in the amygdala, mPFC, 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 dorsolateral PAG (dlPAG) as indicated by *c-fos* immunoreactivity, is correlated with a decreased likelihood of SR occurring (Mickley et al., 2009).
- **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.**

Methods

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

Group Nomenclature	N	23-hour Water deprivation acclimation	Conditioning		Extinction		Latency Period	SR Test Day
		3 Days	Days 1, 3, 5	Days 2, 4, 6	Odd Days	Even Days	30 Days	1 Day
PAG Stimulation	*10*	Water	SAC* + LiCl†	Water	SAC	Water	Water	PAG Stimulation + SAC
Stimulation Controls	*9*	Water	SAC + LiCl	Water	SAC	Water	Water	Non-PAG Stimulation + SAC

[a] 23-hr water deprivation schedule was maintained throughout the study; [b]0.3% sodium saccharin salt dissolved in water (SAC); [c] Lithium Chloride 81 mg/kg. i.p. (LiCl); [d] the latency period provided a sufficient time lapse before evaluating the SR of the CTA. Thirty days is known to produce a robust SR of a CTA (Mickley et al., 2011).

CTA Acquisition

- On days 1, 3, and 5, animals received the CS, SAC, for 30 minutes, followed by a 15-minute latency period where no fluids were consumed and animals were administered the US (LiCl).
- US administration was followed by a 30-minute access to water for hydration purposes.
- Days 2,4 and 6 were rest days on which the animals received 1 hr of water only and no injections

CTA Extinction

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

Latency Period and Electrode Implantation

- Animals received 60 minutes/day access to water for 30 days.
- Rats were chronically implanted with intracranial electrodes aimed at the dlPAG, 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, ± 0.90mm lateral to the midline, and 5.4mm ventral.
- Rats had 3 ± 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 40 µm.
- Slices were assayed for *c-fos* protein immunoreactivity, mounted, and counterstained with neutral red (Herrera and Robertson, 1996).
- Cells with dark, punctate nuclear staining were counted as *c-fos* positive. Diffusely stained cells bodies were not counted. A positive control was used to ensure *c-fos* did not result from faulty staining procedures (Rinaman et al., 1997).

Results

Histology

- 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 dPAG.
- *c-Fos* expression was observed in animals that underwent brain stimulation (see Fig 2).

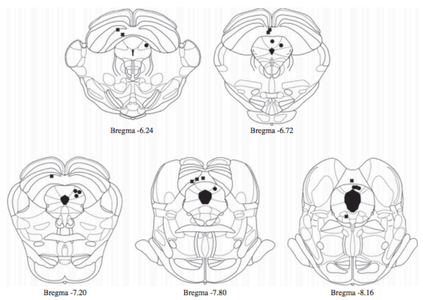

Figure 1 – Drawings of coronal rat brain sections illustrate the electrode tip location for rats that underwent simulation of the dorsomedial PAG or the dorsolateral PAG (indicated by the dot on the right side of these illustrations). Electrode placements that missed either the dmPAG or dlPAG are indicated by an X on the left side. *Note:* Although stimulation was bilateral, electrode placements are illustrated here in one hemisphere only (left showing target "misses" and right indicating target "hits") for simplicity of presentation. Drawings adapted from Paxinos and Watson, (2008).

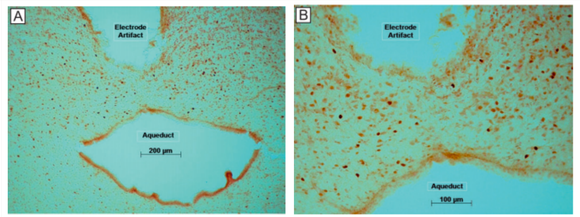

Figure 2 – Low (Panel A) and higher (Panel B) magnification of a representative section of the dPAG with *c-fos* expression. This expression is concentrated adjacent to the tip of the electrode. (Placement coordinates: dPAG, -8.16mm posterior to bregma).

Behavioral Results

CTA Acquisition

- All animals demonstrated a strong CTA. On the third day of conditioning, the volume of SAC was less than 1ml (see Fig. 3), representing a significant decline from the first day of conditioning prior to CS-US pairing.

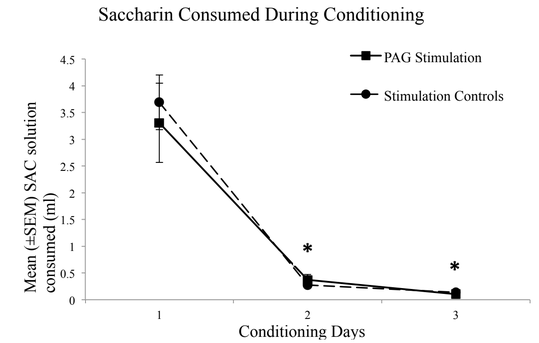

Figure 3- Suppression of 0.3% SAC consumption following multiple pairings with LiCl (81mg/kg i.p.). This is a significant reduction in SAC consumption [t(21)=7.58, p<0.001*] compared to the first conditioning day. No significant difference between the PAG stimulation group and the stimulation control group was found.

CTA Extinction

- The data indicate that the extinction procedure produced reacceptance of the SAC solution.
- SAC consumption was low on the first day of extinction for both treatment groups [PAG stimulation rats: 0.12 ± 0.03ml (Mean±SEM); stimulation control rats: 0.12 ± 0.01ml]. This is similar to SAC consumption on the final conditioning day.
- Both the number of days to reach asymptotic extinction (see Fig. 4 caption) and the slopes of extinction curves were comparable between the two groups [PAG stimulation (27.90 ± 0.11 days), slope = 0.63 ± 0.11; stimulation control (24.08 ± 2.40 days), slope = 0.66 ± 0.17].
- The amount of SAC consumed on the day that rats achieved asymptotic extinction was also similar between PAG stimulation rats [16.21 ± 0.48 mls] and stimulation control [17.08 ± 0.68 mls]. (See fig. 4).

Spontaneous Recovery Test

- Rats that experienced electrical stimulation of the dlPAG and/or the dmPAG 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 measured compared SAC consumption at asymptotic extinction between stimulation and stimulation control groups). All animals demonstrated a SR of their CTA [F(1,17) = (173.96, p<0.001].
- Rats receiving dPAG stimulation drank significantly less SAC at the SR test than did the stimulation controls [F(1,17) = 8.39, p=0.01]. (See Fig. 4)

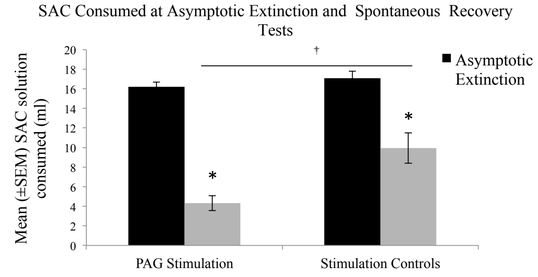

Figure 4 – Volume of 0.3% SAC consumed at the end of CTA extinction and during the SR test 30 days later. All rats achieved asymptotic extinction (operationally defined as 90% of baseline SAC drinking) and the animals in each group exhibited a SR of the CTA. * = significant suppression of SAC consumption compared to that consumed at the end of extinction training. However, stimulation of the dmPAG/dlPAG potentiated the SR as compared to rats experiencing stimulation outside the dorsal PAG († = significantly different, p = 0.01).

Summary and Conclusion

- Rats receiving dPAG stimulation exhibited a significantly *enhanced* SR of their CTA when compared to control animals that received comparable electrical stimulation outside the dPAG.
- Thus the correlation originally observed between enhanced *c-fos* protein expression in the dlPAG and reduced SR (Mickley et al., 2011) did not appear to support a cause-and-effect relationship.
- Our findings that activation of the dPAG can enhance SR of a CTA may prompt future studies that aim to manipulate this structure in a way that reduces the reoccurrence of conditioned defensive reactions to fears.

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