Spontaneous Recovery of a Conditioned Taste Aversion differentially alters extinction-induced changes in c-Fos protein expression in rat amygdala and neocortex.

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Abstract

Conditioned taste aversions (CTAs) may be acquired when an animal consumes a novel taste (conditioned stimulus; CS) and then experiences the symptoms of poisoning (unconditioned stimulus; US). Animals will later avoid the taste that was previously associated with malaise. Extinction of a CTA is observed following repeated, non-reinforced exposures to the CS and represents itself as a resumption of eating/drinking the once-avoided tastant. SR of a CTA (a revival of the taste avoidance) occurs when the CS is offered after a latency period in which the CS was not presented. This study investigated changes in the Amygdala (AMY), Gustatory neocortex (GNC), and Medial Prefrontal Cortex (mPFC) functioning during acquisition, extinction and SR of a CTA. Brain *c-Fos* protein expression was analyzed in fluid-deprived rats that acquired a CTA [3 pairings of 0.3% oral saccharin (SAC) and 81mg/kg i.p. Lithium Chloride (LiCl)] followed by extinction training resulting in 90% reacceptance of SAC. Other animals were extinguished but were tested for SR of the CTA upon exposure to SAC following a 30-day latency period of water drinking. Rats were sacrificed on the final day of SAC exposure and brain *c-Fos* protein expression was evaluated via immunohistochemistry. Animals exhibited a significant SR of the CTA. The numbers of *c-Fos*-labeled neurons in GNC and mPFC was low following CTA acquisition, increased dramatically as rats fully extinguished the aversion, and then declined significantly following SR. Low levels of *c-Fos* expression in the central nucleus of AMY were observed throughout EXT with little change in expression detectable following SR. *C-Fos* expression in basolateral AMY decreased significantly from EXT to SR. These measurements suggest the dynamic nature of brain activity during acquisition, extinction and SR of a CTA and further reinforce an important role for cortical and amygdalar neurons in the reorganization of learned information. *Supported by NIMH grant: 2-R15-MH063720*

Introduction

- Conditioned Taste Aversions (CTAs) may be formed when an animal consumes a novel taste (CS) and then experiences the symptoms of poisoning (US).
- A significant amount of work has been focused on how the brain establishes a CTA (see Yamamoto, 1993, *Neurosci. Res.*, 16, 181-185, for review). However, relatively little has been done on how the brain adjusts during ***extinction*** of this classically conditioned response (Houpt et al., 1994, *Neurosci. Let.*, 172, 1-5; Houpt et al.,1996, *Learn. & Mem.*, 3, 25-30; Berman & Dudai, 2001, *Science*, 291, 2417-2419) or during ***spontaneous recovery*** of a CTA (Rosas & Bouton, 1996, *Anim. Learn. Behav.* 24, 341-348).
- We used *c-Fos* protein immunohistochemical techniques to label neural activity. Evidence suggests that the expression of *c-Fos* (the protein product of the immediate early gene *c-fos*) not only mediates sensory experience but may also be instrumental in the associative aspects of a CTA (Lamprecht & Dudai, 1996, *Learn. & Mem.*, 3, 31-41).
- This study sought to document if/how the number of *c-Fos*-labeled neurons would change during the course of extinction and spontaneous recovery of a CTA.
- We measured behavioral responses during acquisition, extinction and spontaneous recovery of a CTA and analyzed correlated *c-Fos* expression in several brain areas:
  - **Gustatory Neocortex (GNC)** and **Medial Prefrontal Cortex (mPFC)**: GNC is known to mediate CTA acquisition (Bermudez-Rattoni, 1987, *Brain Res.,* 416, 147-152) and CTA extinction (Mickley et al., 2004, *Brain Res.*, 1016, 79-89). Prefrontal cortex has been implicated as mediating the extinction of several other learned responses (Barrett et al., 2003, *J. Neurosci.*, 23, 5740-5749; Herry & Garcia, *J. Neurosci.*, 2002, 22, 577-583; Milad & Quirk, *Nature*, 420, 70-74).
  - **Amygdala:** Both Basolateral (BLA) and Central nuclei (Ce) have known interconnections with cortical structures and play a significant role in extinction (Maren & Quirk, *Nat. Rev. Neurosci.*, 5, 844-852; Bahar et al., 2003, *Eur. J. Neurosci.* 17, 1527-1530).

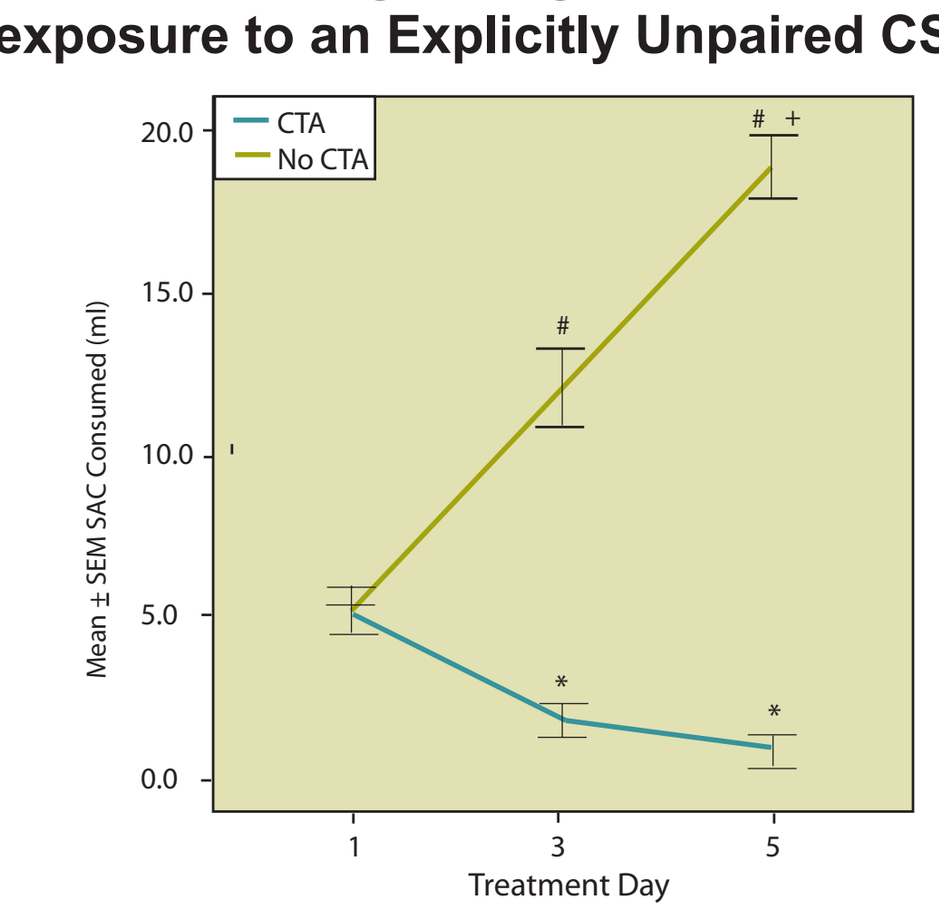
Results

Behavioral Data

CTA Acquisition

SAC consumption in the No-CTA (Explicitly Unpaired; EU) CS, US groups increased over the course of the 3 trials indicating that these rats did not acquire a CTA. Conversely, SAC consumption in all of the CTA groups decreased over the 3 trials indicating that these rats acquired a CTA.

Saccharin drinking during the formation of a CTA or exposure to an Explicitly Unpaired CS and US

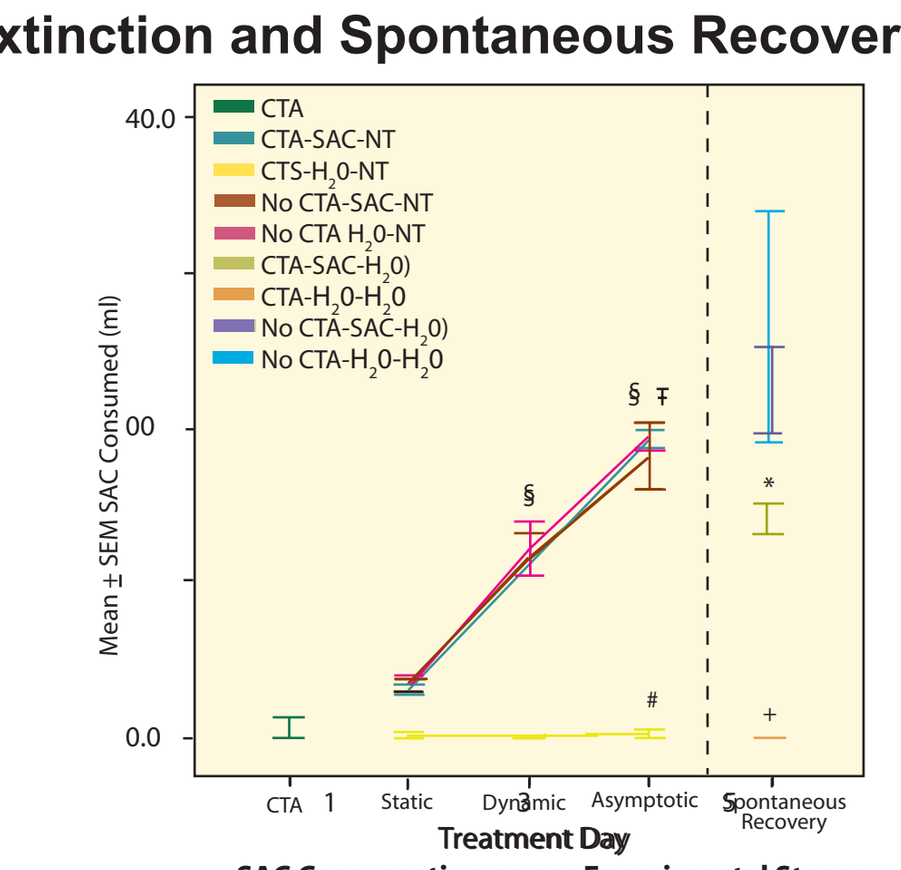


. Significantly less than CTA Treatment Day 1 and No CTA Treatment Day 1
 # Significantly greater than No CTA Treatment Day 1 and CTA Treatment Day 1, 3, & 5
+ Significantly greater than No CTA Treatment Day 3

CTA Extinction and Spontaneous Recovery

Rats avoided SAC when it was previously associated with LiCl. But this aversion extinguished after subsequent, non-reinforced SAC exposures. [See Mickley et al. 2004, *Brain Res.*, 1016, 79-89 and Mickley et al, 2005, *Brain Res.*, 1051, 176-182; for further details on CTA extinction curves]. Spontaneous Recovery of a CTA (i.e., a significant decrease in SAC drinking) follows a latency of 30 days of daily water consumption.

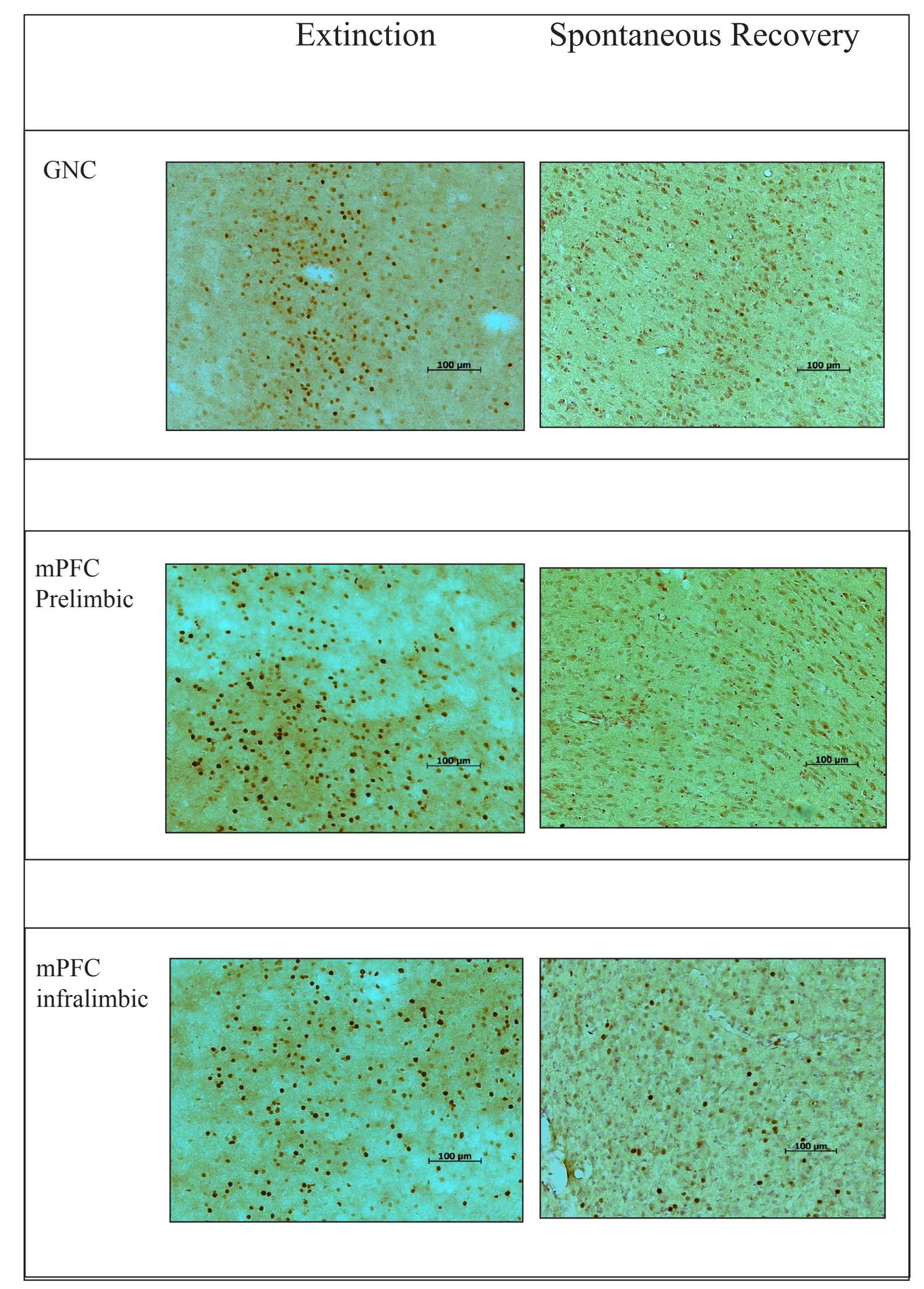
Extinction and Spontaneous Recovery of a CTA



. Significantly less than Asymptotic CTA-SAC-NT
+ Significantly less than all other groups in Spontaneous Recovery stage
 # Significantly less than all other groups in Static, Dynamic, and Asymptotic stages
§ Significantly greater than Static CTA-SAC-NT
Significantly greater than Dynamic CTA-SAC-

Immunohistochemistry
Extinction and Spontaneous Recovery of a CTA

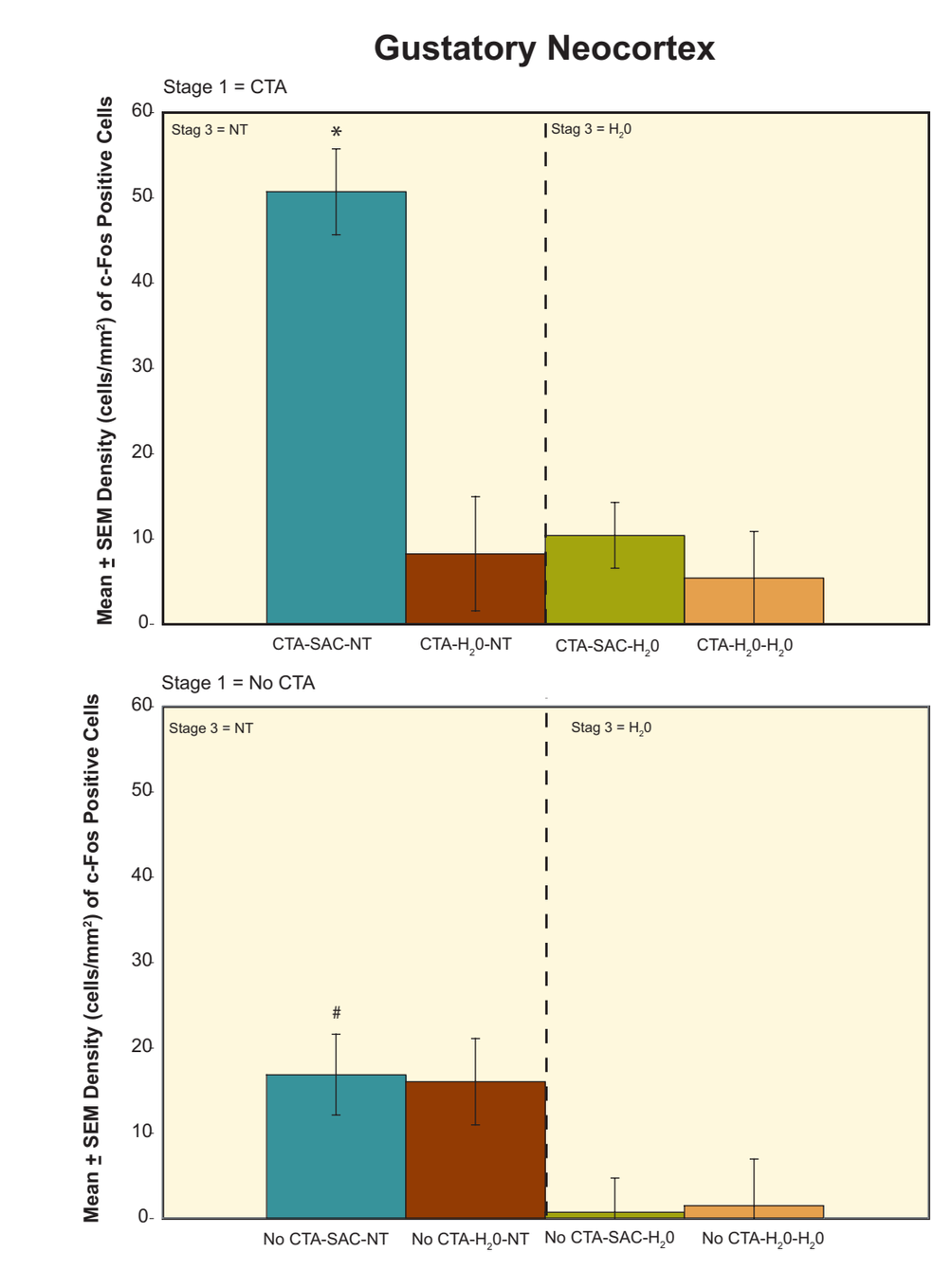
The number of cortical cells expressing *c-Fos* is high in rats that have acquired a CTA and then extinguished the aversion. However, a significant reduction in c-Fos labeled cells accompanies spontaneous recovery of the CTA.



Cerebral Cortex

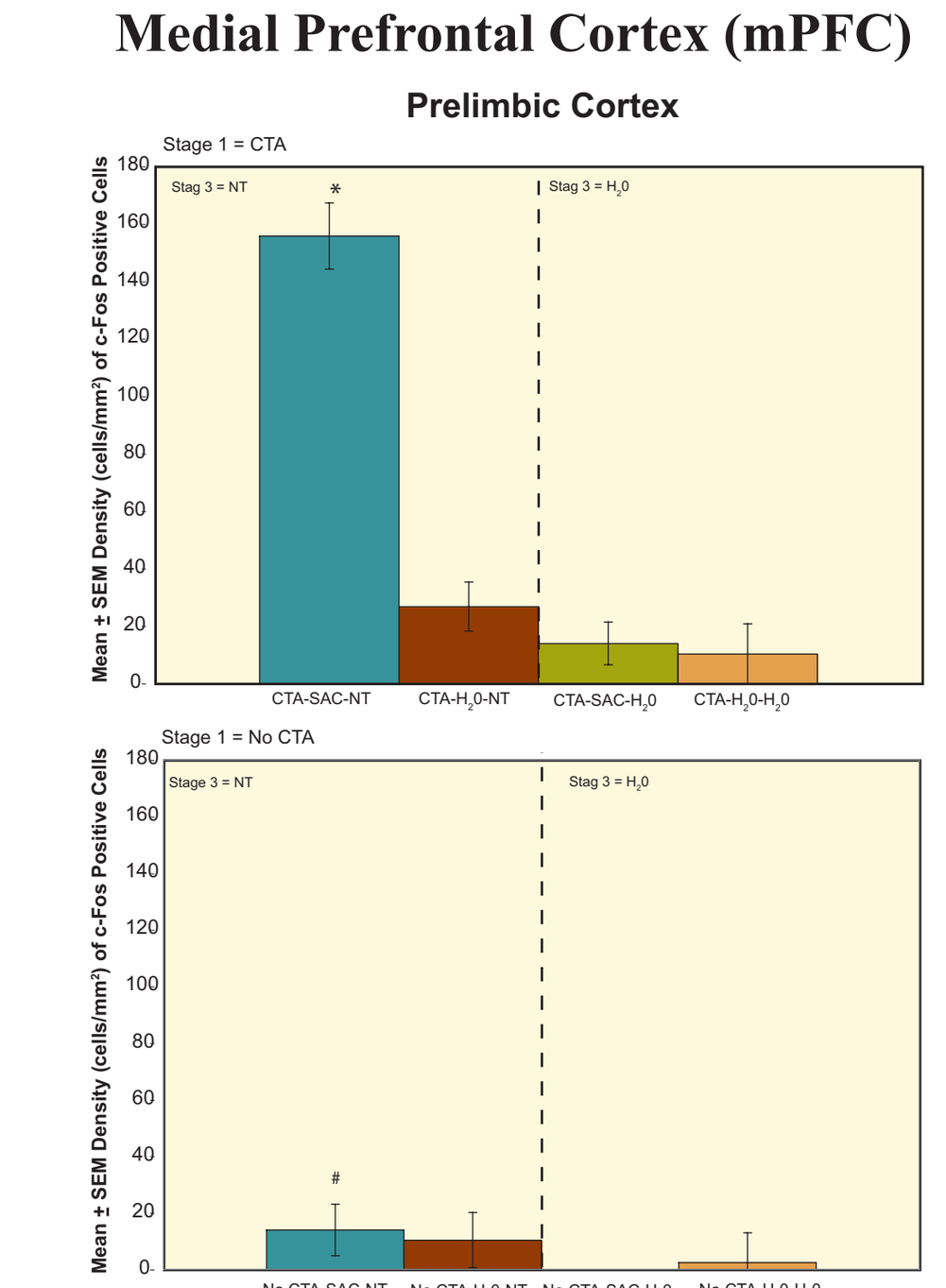
In the cortex, CTA extinction produces a significant increase in cells expressing *c-Fos* protein. Spontaneous recovery of the CTA reverses this increase and produces levels of expression similar to that seen in rats that have a CTA.

Surprisingly, rats that did not acquire a CTA also exhibited relatively low levels of *c-Fos* expression.

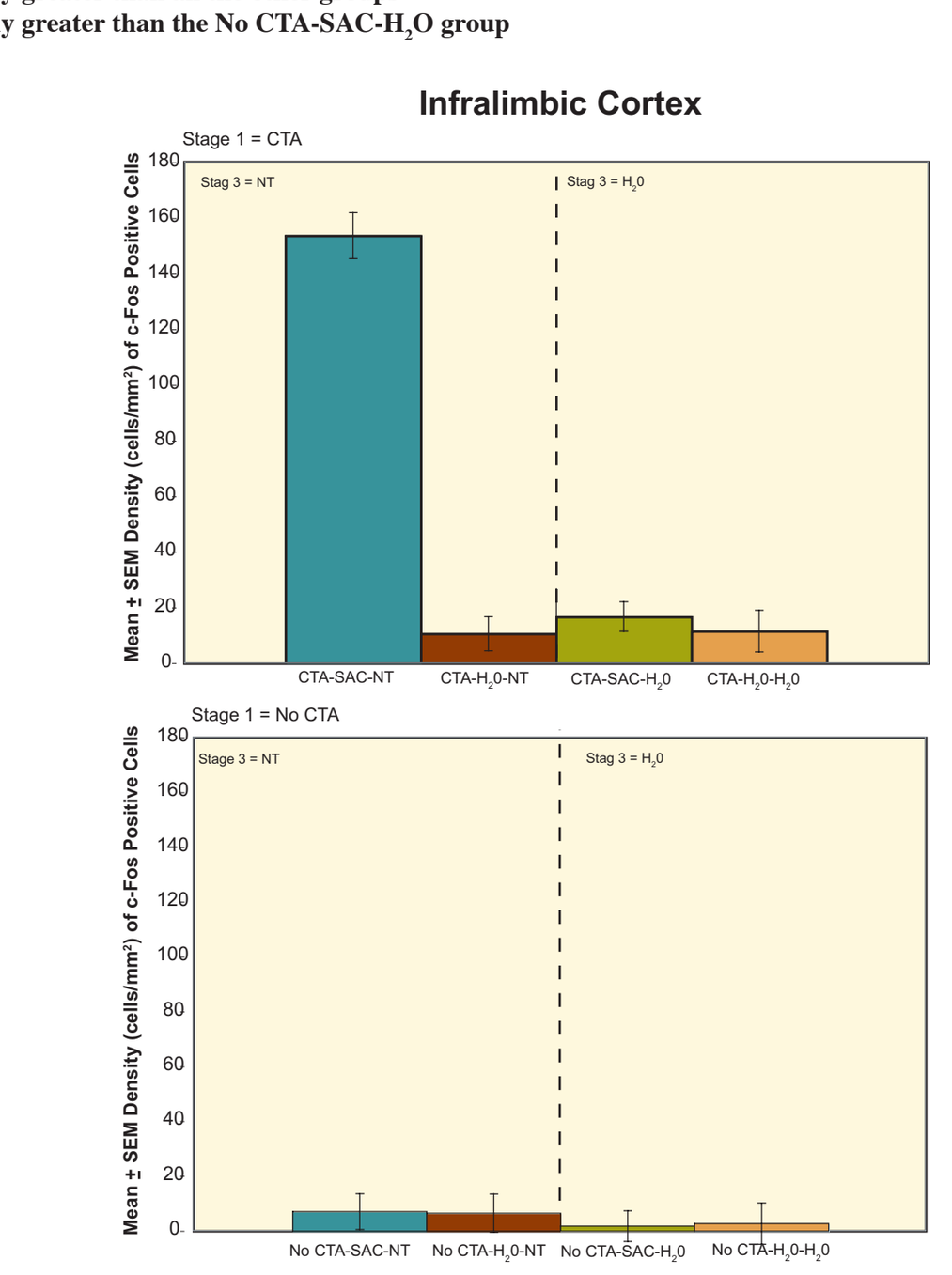


*** Significantly greater than all the other groups**
 # Significantly greater than the No CTA-SAC-H_2O group

Medial Prefrontal Cortex (mPFC)



*** Significantly greater than all the other groups**
 # Significantly greater than the No CTA-SAC-H_2O group



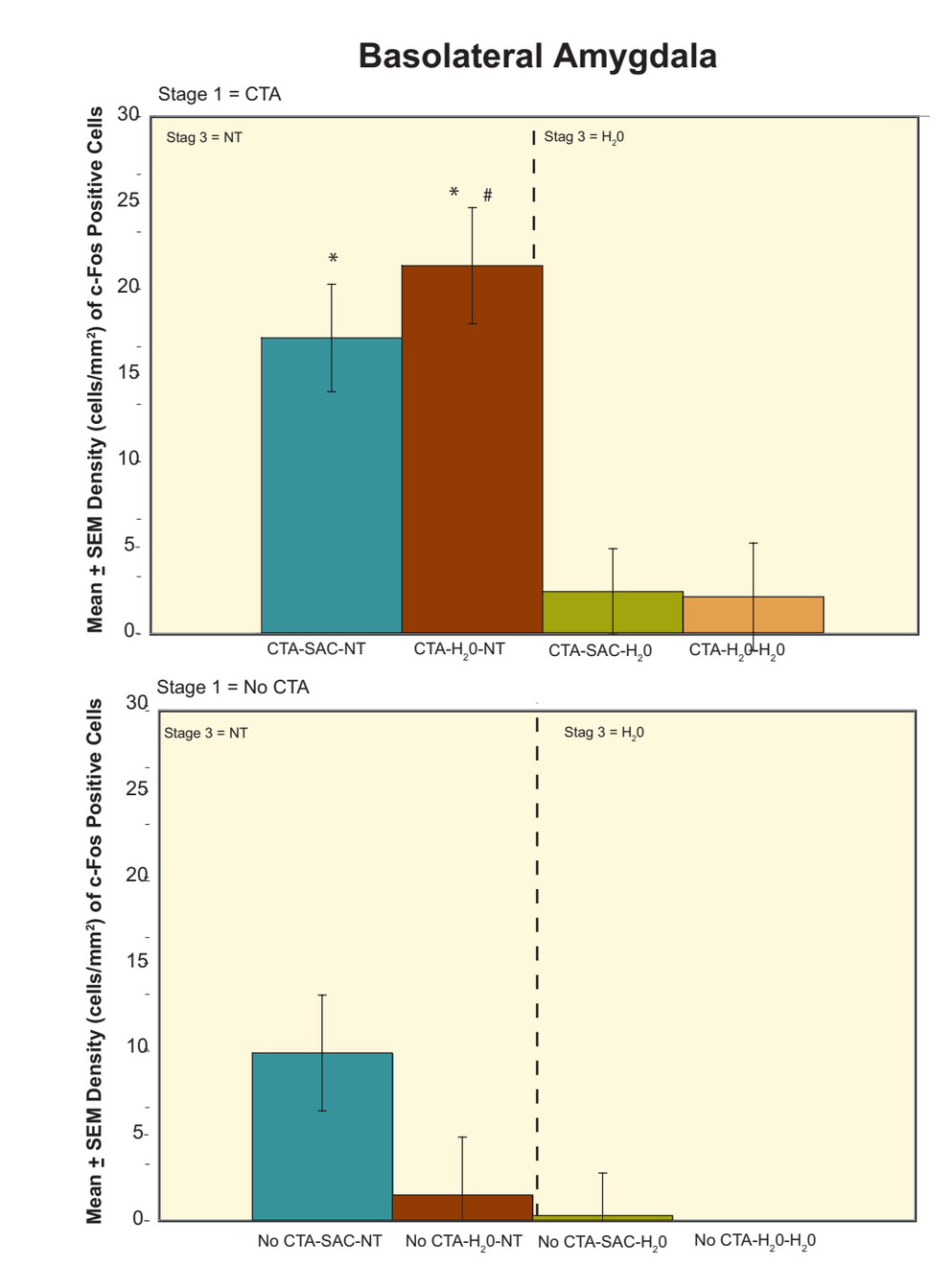
*** Significantly greater than all the other groups**

Amygdala

Basolateral Amygdala (BLA)

Asymptotic CTA extinction does not change the density of c-Fos expression in the BLA. However, spontaneous recovery of a CTA brings with it a significant reduction in the density of c-Fos labeled cells – a pattern similar to that seen in rats with a strong CTA.

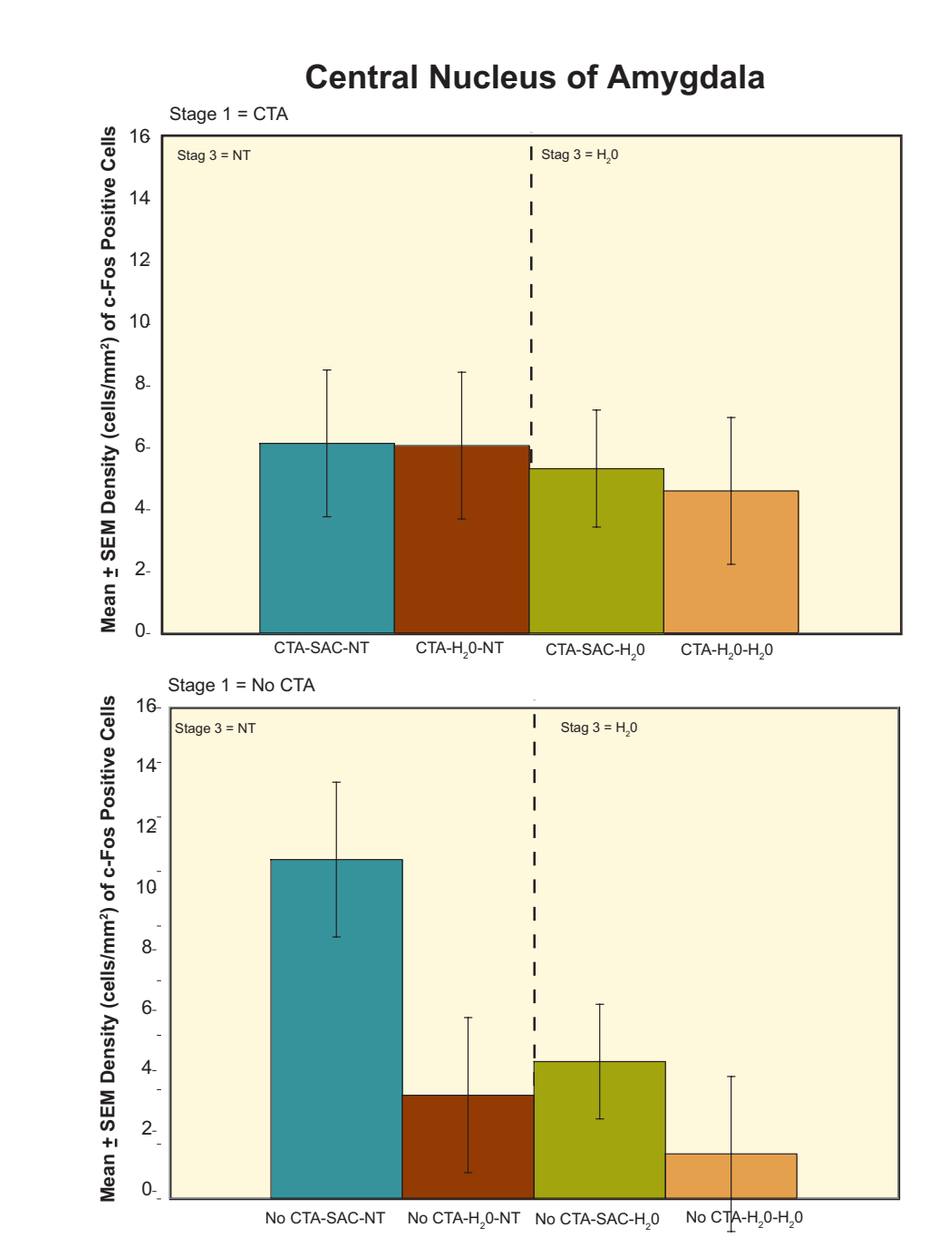
A long latency between CTA acquisition and re-exposure to SAC on the final day of the study significantly reduced the c-Fos expression in BLA.



*** Significantly greater than the CTA-SAC-H_2O group**
 # Significantly greater than the CTA- H_2O -H_2O group

Central Nucleus (Ce)

The density of CN cells expressing c-Fos is low and does not differ significantly among rats that have acquired a CTA, extinguished the CTA or spontaneously recovered the aversion.



Methods

Subjects: Adult, male Sprague-Dawley rats.
Group Designations:

Table 1: Summary of Conditioning Procedures and Extinction/Spontaneous Recovery Timelines

Stage of Experiment	*Stage 1 (Conditioning)* CTA or EU						*Stage 2 (Extinction)* SAC or H_2O	*Stage 3 (Latency)* H_2O or No Treatment (NT)	
Treatment periods	Treatment Day 1	Treatment Day 2	Treatment Day 3	Treatment Day 4	Treatment Day 5	Treatment Day 6	Liquid Consumed from Day 7 until Sacrifice or end of Extinction period	Liquid consumed daily during latency period of 30 days	Liquid Consumed on the Day of Sacrifice
Group Designations									
CTA w/ Extinction (CTA+SAC+NT) *	SAC** + LiCl***	Water	SAC + LiCl	Water	SAC + LiCl	Water	SAC	N/A	SAC
CTA w/o Extinction (Short Test) (CTA +H_2O+NT)	SAC + LiCl	Water	SAC + LiCl	Water	SAC + LiCl	Water	Water	N/A	SAC
CTA w/ Spontaneous Recovery (CTA + SAC + H_2O)	SAC + LiCl	Water	SAC + LiCl	Water	SAC + LiCl	Water	SAC	Water	SAC
CTA w/o Extinction (Long Test) (CTA+H_2O+H_2O)	SAC + LiCl	Water	SAC + LiCl	Water	SAC + LiCl	Water	Water	Water	SAC
No CTA (Yoked to CTA w/ Extinction) (EU+SAC+NT)	SAC	LiCl & Water	SAC	LiCl & Water	SAC	LiCl & Water	SAC	N/A	SAC
No CTA (Yoked to CTA w/o Extinction Short Test) (EU+H_2O+NT)	SAC	LiCl & Water	SAC	LiCl & Water	SAC	LiCl & Water	Water	N/A	SAC
No CTA (Yoked to CTA w/ Spontaneous Recovery group) (EU+SAC+H_2O)	SAC	LiCl & Water	SAC	LiCl & Water	SAC	LiCl & Water	SAC	Water	SAC
No CTA (Yoked to CTA w/o Extinction Long Test) (EU+H_2O+H_2O)	SAC	LiCl & Water	SAC	LiCl & Water	SAC	LiCl & Water	Water	Water	SAC

* Summary of Stage Designation (Stage 1 + Stage 2 + Stage 3)
** SAC = 0.3% Saccharin in Deionized Water
*** LiCl = Lithium Chloride (81.0 mg/kg dose, i.p.)

Table 2: Number of rats in each treatment group, stage of extinction or Spontaneous Recovery*:

Group Designation	Static Extinction	Dynamic Extinction	Asymptotic Extinction	Spontaneous Recovery
CTA w/ Spontaneous Recovery				12 (12) [12] {12} |11| "11"
CTA w/o Extinction (Long Test)				7 (6) [6] {6} |7| "7"
No CTA (Yoked to CTA w/ Spontaneous Recovery group)				6 (11) [11] {11} |11| "11"
No CTA (Yoked to CTA w/o Extinction Long Test)				6 (7) [6] {6} |6| "6"
CTA w/ Extinction	9	8	9 (7) [5] {5} |7| "7"	
CTA w/o Extinction (Short Test)	9	9	9 (4) [9] {9} |6| "7"	
No CTA (Yoked to CTA Extinction)	7	7	6 (8) [8] {8} |6| "6"	
No CTA (Yoked to CTA w/o Extinction Short Test)	5	5	5 (7) [7] {7} |6| "6"	
Conditioned Control	6			

* Key: Behavioral (GNC) [Prelimbic] {Infralimbic} |BLA| "CE". First number represents the number of rats included in the behavioral analyses. The 2nd-5th number represents the number of rats in the immunohistochemical analyses.

Procedures:
- All rats were water deprived for 23 hours per day for the duration of the experiment beginning two days prior to their conditioning trials.

Conditioned Taste Aversion (CTA) Procedure:
- The conditioning procedure included three conditioned stimulus (CS) + unconditioned stimulus (US) trials administered to each rat every other day (see Table 1). The trials were as follows:
  - For rats in the CTA groups (the CS & US were paired to create an aversion to the CS), the CTA was established by oral presentation of 0.3% Saccharin Sodium Salt (SAC) (this is the CS) followed by an 81 mg/kg Lithium Chloride (LiCl) injection, (i.p.) (US).
  - For rats in the "No CTA" (Explicitly Unpaired) groups (the CS & US were unpaired to avoid the formation of a taste aversion), SAC was presented for 30 minutes followed 24 hours later by a LiCl injection.
  - An additional control group (CTA Controls) included rats that received 3 CTA conditioning trials over 6 days, SAC on day 7, and were then sacrificed 90 minutes thereafter.

Extinction Procedure:
- Baseline drinking was computed by taking an average of familiar SAC drinking from similarly sized rats.
- Rats in the extinction groups were presented with SAC for 30 minutes daily (supplemented by water for 30 minutes) until their drinking met one of the predetermined criteria (see nomenclature proposed by Nolan et al., 1997, *Physiol. & Behav.*, 14, 161-170):
  - *Static Phase*: 10% baseline drinking
  - *Dynamic Phase*: 40% baseline drinking
  - *Asymptotic Phase*: 90% baseline drinking
- After rats in the spontaneous recovery groups had extinguished to asymptote (90% of baseline drinking) they received water each day for 30 days before they had a last, single 30-minute re-exposure to SAC.
- On the day that the appropriate behavioral criterion was met for one of the experimental conditions, the rat (along with its yoked control) was sacrificed following its SAC drinking.

Histology:
- Rats were sacrificed 90 minutes following their last SAC exposure. Brain sections were collected and assayed for *c-Fos* protein immunoreactivity (Hsu et.al., 1981, *Am. J. Clin. Pathol.*, 75, 734-738 & Hsu et.al., 1981, *J. Histochem. Cytochem.*, 29, 577-280).
- Brain nuclei were located using standard demarcations from The Rat Brain in Stereotaxic Coordinates (Paxinos & Watson, 1998, 4th ed.). Sub-nuclei were selected based on their role in gustation [see: Paxinos, (Ed.) The Rat Nervous System, 1995].
- *Brain Nuclei counted:* Gustatory Neocortex (GNC); Medial Prefrontal Cortex (mPFC; both Prelimbic and Infralimbic areas); Amygdala [AMY; both Basolateral (BLA) and Central (CE) nuclei].
- Cells staining positive for *c-Fos* protein (only round, dark, uniformly stained cells) were counted per brain nucleus. The observers were blind to the experimental condition of the rats.

Summary and Conclusions

Cortex
- C-Fos expression in GNC and mPFC parallels behavioral changes observed as rats acquire, extinguish and spontaneously recover a CTA.
  - CTA = Low *c-Fos* densities
  - Extinction = High *c-Fos* densities
  - Spontaneous recovery = Low *c-Fos* densities
- Interestingly, non-conditioned control rats also exhibited relatively low levels of *c-Fos* expression in the cortical area sampled.
- These changes in neural activity are apparently not a reflection of volume of SAC consumed since control rats, drinking similar volumes, did not exhibit parallel changes in *c-Fos*-labeling.
- These data reflect a dynamic role for the GNC and mPFC in acquisition, extinction, and spontaneous recovery of a CTA.

Amygdala
- A change in expression of *c-Fos* labeled BLA cells does NOT accompany CTA extinction, but spontaneous recovery of the CTA significantly reduces the expression of *c-Fos* protein in this nucleus.
- The data suggest that BLA may have a role in the spontaneous recovery of a CTA.
- *C-Fos*-labeled cells in the Ce are generally low, independent of the CTA acquisition, extinction or spontaneous recovery history of the animal.
- The relatively low levels of *c-Fos* expression in the Ce of the amygdala, independent of CTA treatment, extinction or spontaneous recovery, suggest a less-important a role for this nucleus in the processing of CTA learning and memory.

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