Time-dependant effects of systemic muscimol on extinction (EXT) of a conditioned taste aversion (CTA)

G. Andrew Mickley, Zana Hoxha, Stephanie Bacik.
Department of Psychology and the Neuroscience Program,
Baldwin-Wallace College, Berea, OH 44017 USA.

Abstract

GABA (γ-aminobutyric acid) receptor manipulation has been proposed as an important mediator of extinction (EXT). The current experiments paired 0.3% saccharin (SAC) with 81 mg/kg Lithium Chloride (LiCl; i.p.) to establish a strong CTA in adult, male Sprague-Dawley rats. During extinction, subsequent opportunities to drink SAC were either preceded, or followed, by an injection of the GABA agonist muscimol (1.0 mg/kg, i.p.). Muscimol given 0.75 hr after SAC exposure significantly impaired EXT learning in rats compared to rats that received muscimol 0.5 hr before SAC exposure. The days required to reach asymptotic EXT did not differ between rats that received muscimol before daily SAC drinking and those that received no muscimol. A second study tested the hypothesis that muscimol given after CS exposure can act as a US and cause a CTA. This experiment paired rat SAC drinking with muscimol (1.0 mg/kg, i.p.), instead of LiCl. Each subsequent exposure to SAC during the acquisition phase resulted in approximately the same consumption of SAC as on the first day of exposure. Thus, these rats neither developed a strong aversion nor did they overcome the initial neophobia (natural avoidance of a novel taste) and readily accept the SAC. These data suggest that the GABA agonist muscimol can impair EXT of a CTA but this phenomenon depends on the timing of the drug administration relative to CS re-exposures. The exact mechanism by which muscimol retards EXT is currently unknown. The drug may be, (a) acting as a weak US, (b) impairing sensory processing of the SAC taste, (c) blocking the taste memory itself, or (d) having direct effects on EXT memory formation. Continuing studies are addressing these possibilities.

Introduction

- Growing evidence suggests that extinction (EXT) is not an unlearning, or forgetting, of a conditioned response (for review, see Leslie, 2004).
- Rather, extinction may be the active *inhibition* of the initial conditioned response (Davis & Myers, 2002).
- Since γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the brain, it is probable that it plays a major role in extinction (Davis & Myers, 2002).
- Extinction has been both attenuated and facilitated by $GABA_A$ manipulation (Harris & Westbrook, 1998a&b; McCabe et al., 2004; Stowell et al., 2000).
- McCabe et al. (2004) reported that later administration of the drug chlordiazepoxide (CDP, a benzodiazepine) facilitated extinction, but when the drug was given at the start of extinction trials, the effect was not immediately seen.
- Conditioned Taste Aversions (CTAs) may be acquired when an animal consumes a novel taste and then experiences the symptoms of poisoning (Garcia et al., 1955).
- The current studies investigate the effects of systemic muscimol (a $GABA_A$ and $GABA_C$ receptor agonist; Akirav et al., 2006) on extinction of a CTA.

Methods

Subjects: Male Sprague-Dawley rats

Drugs and Solutions:

- 0.3% Saccharin (SAC = conditioned stimulus; CS): Solution was made by mixing 3.0g of SAC in 1.0 L of DI water.
- 81 mg/kg (i.p.) Lithium Chloride (LiCl = Unconditioned Stimulus; US) solution was made by mixing 2.0mL of 0.9% physiological saline with 0.162 g LiCl.
- 1 mg/kg (i.p.) Muscimol solution was made by mixing 1mg muscimol in 1mL 0.9% physiological saline (Houston et al., 2002)).

Group Designations and Procedures:

Conditioned Taste Aversion (CTA) Procedure:

CTA Groups:

- Three, alternate-day pairings of SAC+LiCl were followed by 30 minutes access to water (see Table 1).
  - Groups:
    - **CTA + EXT + SR**
    - **CTA + (muse then SAC) EXT + SR**
    - **CTA + EXT (SAC then muse) + SR**

Explicitly unpaired group: **EU**

- SAC & LiCl were unpaired to avoid the formation of a CTA
- Rats received 30 minutes access to SAC followed 24 hours later by a LiCl injection.

Muscimol as US group: **SAC + muse (US)**

- Rats were treated like the CTA groups except that 1 mg/kg muscimol (i.p.) substituted for the US

Extinction (EXT) Procedure:

- **CTA + EXT + SR** rats extinguished the CTA through daily 30-minute presentations of SAC followed by 30 minutes access to water.
  - Extinction trials continued until SAC consumption reached 90% of baseline SAC consumption (asymptotic extinction).
  - Baseline was computed by taking an average of familiar SAC drinking from similarly sized rats.

Table 1. Group Nomenclature and Experimental Design

Group Designation (N)	Treatment Day 1	Treatment Day 2	Treatment Day 3	Treatment Day 4	Treatment Day 5	Treatment Day 6	Testing of muscimol injection i.p.-(mg/kg)	Liquid Consumed from Day 7 Until Asymptotic Extinction	Liquid Consumed Daily After Extinction Trials For Spontaneous Recovery Test	Liquid Consumed For Spontaneous Recovery Test
CTA + Extinction + Spontaneous Recovery (**CTA + EXT + SR**)	SAC + LiCl	60 min. access to water	SAC + LiCl	60 min. access to water	SAC + LiCl	60 min. access to water	N/A	SAC	Water	SAC
N=13										
EU-Unpaired (**EU**)	SAC+H2O	LiCl 48 min. access to water	SAC+H2O	LiCl 48 min. access to water	SAC+H2O	LiCl 48 min. access to water	N/A	Water	Water	SAC
N=20										
CTA + muscimol inj. before EXT (**CTA + SAC(muse) + SR**)	SAC + LiCl	60 min. access to water	SAC + LiCl	60 min. access to water	SAC + LiCl	60 min. access to water	0.75 hr. before SAC exposure during extinction trials	SAC	Water	SAC
N=8										
CTA + muscimol inj. before EXT trials + Spontaneous Recovery (**CTA + muse) SAC + SR**)	SAC + LiCl	60 min. access to water	SAC + LiCl	60 min. access to water	SAC + LiCl	60 min. access to water	0.5 hrs before SAC exposure during extinction trials	SAC	Water	SAC
N=8										
SAC (CS) paired with muscimol (US) (**SAC + muse(US) + SR**)	SAC + muse	60 min. access to water	SAC + muse	60 min. access to water	SAC + muse	60 min. access to water	N/A	N/A	N/A	N/A
N=8										

- **CTA + (muse then SAC) EXT + SR** rats were extinguished as described above but received a daily muscimol (1mg/kg; i.p.) injection 0.5 hr before each SAC exposure
- **CTA + EXT (SAC then muse) + SR** rats were extinguished as described above but received a daily muscimol (1mg/kg; i.p.) injection 0.75 hr after each SAC exposure
- Muscimol injections lasted 18 days, but extinction trials continued until animals reached asymptotic extinction (i.e., 90% baseline SAC consumption)

Spontaneous Recovery (SR) Procedure:

- The following groups received one hour access to water for 30 days after asymptotic extinction.
  - **CTA + EXT +SR,**
  - **CTA + (muse then SAC) EXT + SR**
  - **CTA + EXT (SAC then muse) + SR**
- On the 30th day, SR was assessed by allowing 30 minutes access to SAC and measuring the amount consumed.

Results

Conditioned Taste Aversion (CTA) Acquisition

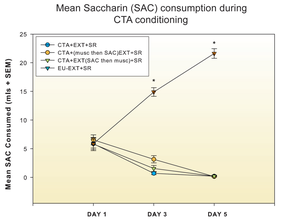

Figure 1. Saccharin drinking decreased significantly in the groups **[CTA+EXT+SR , CTA+(musc then SAC)EXT+SR**, and **CTA+EXT(SAC then musc)+SR]** that had 3, SAC+LiCl pairings -indicating that these rats acquired a CTA. In the Explicitly Unpaired group **(EU-EXT)** saccharin drinking increased over the course of the 3 trials indicating that these animals did not acquire a CTA.

A repeated measure ANOVA [Treatment (CTA or EU) X Trial] revealed a significant interaction between Treatment and Trial [F (3,42) = 7.182, p < 0.05]. At the start of the study, all rats exhibited similar (low) levels of SAC consumption (neophobia). These data represent a decline in SAC drinking in rats that received SAC+LiCl pairings and a reliable rise in SAC drinking in the EU animals.

* = significantly different (p < 0.05) from all CTA treatment groups.

GABA receptor stimulation with muscimol alters the time course of CTA extinction

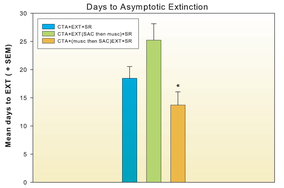

Figure 2. The number of days to achieve asymptotic CTA extinction was significantly reduced in rats that received a muscimol injection *before* daily SAC exposures **[CTA+(musc then SAC)EXT+SR]** as compared to rats that received the muscimol *after* tasting SAC **[CTA+EXT(SAC then musc)+SR]**.

One-Way ANOVA [Treatment x EXT Days] revealed a significant treatment effect F (2,28) = 4.698, p < 0.001.

* = Significantly different from **CTA+EXT (SAC then musc)+SR**.

Extinction times for rats in the **CTA +EXT+SR** group were moderate and not significantly different from either of the muscimol-treated groups.

GABA receptor stimulation with muscimol alters the Saccharin consumed per day during CTA extinction

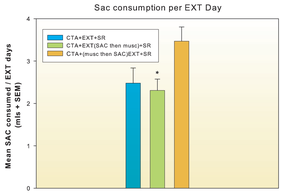

Figure 3. During extinction, daily consumption of SAC was significantly lower in the rats that had SAC before muscimol treatment **[CTA+EXT (SAC then musc)+SR]** as compared to the rats that drank SAC after the muscimol **[CTA+(musc then SAC) EXT+SR]** [t (14) =-2.75, p < 0.05]. Daily SAC drinking of rats in the **CTA +EXT+SR** group were moderate and not significantly different from either of the muscimol-treated groups.

* = Significantly different from **CTA+(musc then SAC)+EXT+SR**.

Results

Rats given muscimol before daily SAC exposure exhibited accelerated extinction of a CTA.

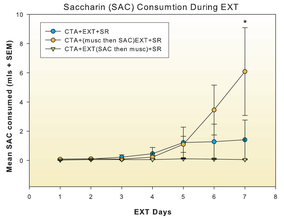

Figure 4. Over the course of the first 7 non-reinforced SAC exposures, rats in **CTA+EXT (musc then SAC)+SR** group re-accept saccharin significantly faster than **CTA+EXT+(SAC then musc)+SR** animals indicating an accelerated EXT [F (2,28) = 3.438, p< .05].

Muscimol (1.0 mg/kg, i.p.) does not act as an Unconditioned Stimulus and produce a CTA

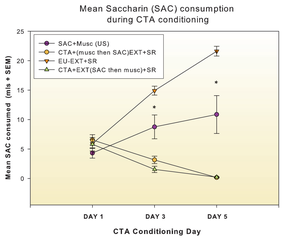

Figure 5. When super-imposed over the CTA acquisition data from Figure 1, it becomes apparent that using 1.0 mg/kg of muscimol (i.p.) **[SAC+Musc(US)** group] is ineffective in producing a CTA.

A repeated measure ANOVA [Treatment (CTA or EU) X Trial] revealed a significant Treatment X Trial interaction [F (4,44) = 6.337, p < 0.05]. The rats that received muscimol as a US (instead of the LiCl) consumed significantly less SAC than EU rats [F (4, 58) = 88.951, p < 0.05] but significantly more SAC than rats treated with LiCl (as a US) [F (4, 58) = 59.501, p < 0.05].

* = significantly different (p < 0.05) from CTA and EU groups.

Muscimol (1.0 mg/kg, i.p.) administered during extinction of a CTA does not significantly change spontaneous recovery of the aversion

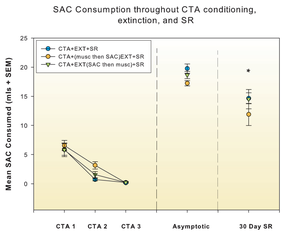

Figure 6. During the spontaneous recovery test, SAC consumption decreased significantly [F(1,24) = 33.45, p < 0.001] (indicating a SR of the CTA). However there were not statistically significant differences between the rats that had received Muscimol during and EXT and those that had not received the drug.

Summary and Conclusions

- The GABA agonist muscimol can impair extinction of a CTA.
- This phenomenon depends on the timing of the drug administration relative to CS re-exposures (see also Akirav, In Press).
- The data are not consistent with the explanation that muscimol is merely acting as a US (also see Houston et al., 2002).
- Muscimol given during extinction does not reliably alter SR of the CTA.
- The exact mechanism by which muscimol retards EXT is currently unknown.
- GABA receptor blockade may:
  - Impair sensory processing of the SAC taste
  - Block the taste memory itself
  - Alter EXT memory formation

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