Muscimol Alters Extinction of a Conditioned Taste Aversion

Anthony DiSorbo, Gina N. Wilson, Stephanie Bacik, Zana Hoxha, Jaclyn M. Biada, and G. Andrew Mickley
Department of Psychology, Neuroscience Program, Baldwin-Wallace College, Berea, OH 44017-2088 USA

Abstract

Gamma-aminobutyric acid (GABA) plays a role in fear extinction. Here we explored how manipulation of $GABA_A$ systems at different times during conditioned taste aversion (CTA) acquisition or extinction would affect extinction. In Experiment 1, rats acquired a CTA to 0.3% saccharin-flavored water (SAC) when it followed an injection of lithium chloride (LiCl; 81.0 mg/kg, i.p.), but not muscimol (1.0 mg/kg, i.p.), confirming that this dose of muscimol does not possess unconditioned stimulus properties. Following conditioning, rats received extinction training in which the $GABA_A$ agonist muscimol (1.0 mg/kg, i.p.) was administered either before or after each extinction trial. Muscimol had no effect on extinction rate when administered before extinction trials, but hindered extinction when administered after extinction trials. Muscimol's inhibitory effects may have impeded extinction learning by disrupting synaptic mechanisms required to consolidate information experienced during extinction trials. In Experiment 2, we studied the effects of muscimol on CTA acquisition and subsequent extinction. Rats received muscimol (1.0 mg/kg, i.p.) either before, or after, CTA conditioning trials. Following CTA acquisition, all rats were given CTA extinction training without any muscimol administration. Although all treatment groups developed CTAs, the group that received muscimol before CTA conditioning trials extinguished rapidly in comparison to either rats that received muscimol after conditioning trials or received no muscimol during conditioning. Muscimol may have weakened CTA formation, thus allowing faster extinction of the fear. Muscimol's time-dependent effects on CTA further confirm a role for GABA in extinction of a defensive reaction to a learned fear. Differences between muscimol's effects on CTA conditioning and CTA extinction indicate that fear conditioning and extinction involve, to some degree,

Introduction

- A Conditioned Taste Aversion (CTA) may be formed when an animal consumes a novel taste (CS) and then experiences the symptoms of poisoning (US) (Garcia et al., 1955).
- A growing literature suggests that the brain neurotransmitter gamma-aminobutyric acid (GABA) and its receptors play a role in extinction of conditioned fears (Davis & Myers, 2002).
- This study was aimed at documenting the time-dependent role of GABA in CTA acquisition and extinction learning. Furthermore, we sought to differentiate between the role GABA plays in the two forms of learning.
- Muscimol is a $GABA_A$ agonist that has been used systemically to produce changes in learning retention. For example, muscimol injected systemically immediately after conditioning disrupts consolidation (Castellano & McGaugh, 1990).
- We tested the effect of muscimol administration on CTA acquisition and extinction (EXT).

Method

Subjects: Adult, male Sprague-Dawley rats

Experiment 1:

Table 1. Experiment 1: Summary of group names, numbers, and treatments.

Group Designation	Number of Subjects	Conditioning						Extinction Phase
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	
CTA + EXT	12	SAC[1] LiCl	Water	SAC LiCl	Water	SAC LiCl	Water	SAC
CTA + EXT(Mus)[2]	9	SAC LiCl	Water	SAC LiCl	Water	SAC LiCl	Water	SAC(Mus)[7]
CTA + (Mus)EXT[3]	7	SAC LiCl	Water	SAC LiCl	Water	SAC LiCl	Water	(Mus)SAC[8]
SAC + Mus	8	SAC Mus	Water	SAC Mus	Water	SAC Mus	Water	N/A[9]
No CTA (EU)[4]	10	SAC	Water	SAC	Water	SAC	Water LiCl	N/A

[1]SAC = 0.3% saccharin salt dissolved in deionized water.
[2]LiCl = lithium chloride solution (81mg/kg; i.p.) prepared in physiological saline.
[3]Mus = muscimol injection (1mg/kg; i.p.)
[4]EXT(Mus) = muscimol was administered 45 min after SAC exposure throughout extinction
[5]Mus(EXT = muscimol was administered 30 min before SAC exposure throughout extinction
[6]No CTA (EU) = "explicitly unpaired" treatment group
[7]SAC(Mus) = muscimol was administered 45 min after SAC exposure
[8](Mus)SAC = muscimol was administered 30 min before SAC exposure
[9]N/A = the extinction phase was not necessary since rats never acquired a true CTA

Procedure: Experiment 1

- Rats were water-deprived for 23 hours per day for the duration of the experiment beginning two days prior to their conditioning trials.
- **Conditioning procedure:**
  - Three conditioned stimulus (CS) + unconditioned stimulus (US) trials were administered every other day for a total of three pairings (see Table 1).
  - ***CTA groups:*** the CTA was established by 30 minute oral presentation of 0.3% saccharin solution (SAC) followed by an 81.0 mg/kg lithium chloride (LiCl) injection (i.p.).
  - ***SAC + Mus:*** same schedule as the CTA groups except a 1.0 mg/kg injection of muscimol was given in lieu of LiCl. The purpose of this treatment group was to determine if muscimol could act as a US
  - ***No CTA (EU):*** SAC was presented for 30 minutes followed by a LiCl injection 24 hours later. This procedure controls for presentations of the CS and US without producing a CTA.
- **Extinction procedure:**
  - We used the CTA extinction nomenclature proposed by Nolan et al. (1997):
    - Static Phase: 10% baseline drinking
    - Dynamic Phase: 40% baseline drinking
    - Asymptotic Phase: 90% baseline drinking
  - Baseline drinking was computed by taking an average of SAC drinking from similarly sized rats familiar with the sweet taste (Mickley et al., 2007).
  - Rats were presented with SAC for 30 minutes (followed by water for 30 minutes) until their drinking reached asymptote (90% baseline consumption).
  - Rats were injected with muscimol (1.0 mg/kg, i.p.) either 30 minutes before [***CTA+(Mus)EXT*** group] or 45 minutes after [***CTA+EXT(Mus)***group] SAC presentation. ***CTA+EXT*** rats received physiological saline injections (i.p.) at 30 minutes before or 45 minutes after SAC.
  - After the nineteenth day of extinction training, muscimol injections were terminated to determine the effects of drug cessation.

Experiment 2

Table 2. Experiment 2: Summary of group names, numbers, and treatments.

Group Designation	Number of Subjects	Conditioning						Extinction Phase
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	
CTA + EXT	4	SAC LiCl	Water	SAC LiCl	Water	SAC LiCl	Water	SAC
(Mus)CTA[1] ***+ EXT***	4	(Mus)SAC LiCl	Water	(Mus)SAC LiCl	Water	(Mus)SAC LiCl	Water	SAC
CTA(Mus) + EXT	5	SAC(Mus) LiCl	Water	SAC(Mus) LiCl	Water	SAC(Mus) LiCl	Water	SAC
(Mus)CTA + (Mus)EXT[2]	6	(Mus)SAC LiCl	Water	(Mus)SAC LiCl	Water	(Mus)SAC LiCl	Water	(Mus)SAC

[1]Mus(CTA = muscimol was administered 30 minutes before SAC exposure throughout conditioning
[2]CTA(Mus) + muscimol was administered 45 minutes after SAC exposure throughout conditioning
[3]Mus(CTA+Mus)EXT = muscimol was administered 30 minutes before SAC exposure throughout conditioning and extinction

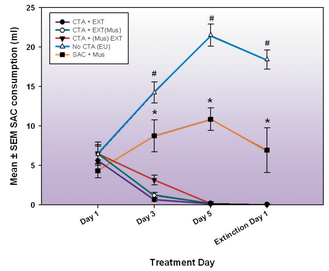
Procedure:

- Rats were water-deprived for 23 hours per day for the duration of the experiment, beginning two days prior to their conditioning trials.
- **Conditioning procedure:**
  - Three conditioned stimulus (CS) + unconditioned stimulus (US) trials were administered every-other day for a total of three pairings (see Table 2).
  - Rats were injected with muscimol (1.0 mg/kg, i.p.) either 30 minutes before [***(Mus)CTA+EXT***] or 45 minutes after [***CTA(Mus)+EXT***] SAC presentation. ***CTA+EXT*** rats received physiological saline injections (i.p.) at 30 minutes before or 45 minutes after SAC.
  - The 30 minute latency between muscimol administration and saccharin presentation is a sufficient period of time to allow any muscimol-induced hypodipsia to subside, consistent with Houston et al., (2002).
- **Extinction procedure:**
  - Rats were presented with SAC for 30 minutes (followed by water for 30 minutes) until their drinking reached asymptote (90% baseline consumption).
  - Baseline drinking was computed by taking an average of SAC drinking from similarly sized rats familiar with the sweet taste.
  - Rats in the ***(Mus)CTA+(Mus)EXT*** treatment group received muscimol (1.0 mg/kg, i.p.) 30 minutes before SAC presentation throughout extinction training to test for any state-dependent effects of muscimol (Nakagawa et al., 1995).

Results

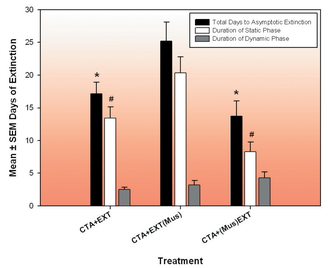
Experiment 1

- **CTA Acquisition**
  - SAC consumption in the ***CTA*** groups decreased throughout conditioning, indicating that these rats developed a CTA.
  - The SAC consumption of ***NO CTA (EU)*** rats increased over the course of this period, indicating that they did not develop a CTA to SAC.
  - ***SAC+Mus*** rats' SAC consumption remained static through conditioning – perhaps suggesting that post-SAC consumption injection of muscimol disrupts the formation/consolidation of a safe taste memory.



***SAC consumption is significantly less than the *No CTA (EU)* group and significantly greater than the three *CTA* groups.**
#SAC consumption is significantly greater than the *SAC+Mus* and three *CTA* groups.

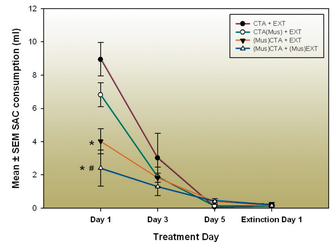
- **CTA Extinction**
  - All rats extinguished the CTA.
  - Rats in the ***CTA+EXT(Mus)*** group required significantly more days to reach asymptote than did the other groups. This difference is reflected exclusively in the Static Phase of extinction.



*** Total days to Asymptotic Extinction is significantly greater than the *CTA+(Mus)EXT* and *CTA+EXT* groups.**
#Duration of Static Phase is significantly greater than the *CTA+(Mus)EXT* and *CTA+EXT* groups.

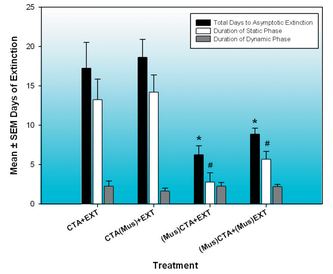
Experiment 2

- **CTA Acquisition**
  - SAC consumption in all groups decreased throughout conditioning, indicating that all groups developed a CTA.
  - Rats that received muscimol treatments before SAC presentation consumed significantly less SAC than animals receiving other treatments on Day 1.



*** SAC consumption is significantly less than the *CTA+EXT* group.**
 # SAC consumption is significantly less than the *CTA(Mus)+EXT* group.

- **CTA Extinction**
  - All rats extinguished the CTA.
  - Rats receiving muscimol before conditioning trials extinguished their CTA significantly faster than rats receiving muscimol after conditioning trials or saline controls.



*** Total Days to Asymptotic Extinction are significantly less than the *CTA+EXT* and *CTA(Mus)+EXT* groups.**
 # Duration of the Static Phase is significantly less than the *CTA+EXT* and *CTA(Mus)+EXT* groups.

Summary and Conclusion

Experiment 1

- Rats that received post-extinction trial muscimol injections exhibited impeded extinction, specifically during the static phase of extinction. Muscimol did not have a notable effect on the dynamic phase of extinction in any of the treatments.
- ***SAC+Mus*** rats did not develop a CTA, nor did they exhibit a waning of neophobia, providing the corroborating evidence that the impeded extinction observed in the current study may be due to retrograde amnesia (Rossato et al., 2003; Salinas & McGaugh, 1995).
- Cessation of post-extinction trial muscimol injections did not correspond with a rapid reacceptance of the once-aversive taste. Such findings suggest that muscimol was not inhibiting expression of extinction behavior, but impeding extinction learning itself.

Experiment 2

- Rats in Experiment 2 that received muscimol after conditioning trials still developed a CTA, which required the same amount of extinction training as the ***CTA+EXT*** group to reach asymptotic consumption.
- ***(Mus)CTA+EXT*** rats developed a CTA, but extinguished quickly, possibly due to weaker CTA formation. Effects were not state dependent because ***(Mus)CTA+(Mus)EXT*** exhibited similar acquisition and extinction behavior.

Conclusions

Conclusions

- The $GABA_A$ agonist muscimol produced different, but profound, effects on CTA acquisition and extinction.
- The timing of muscimol administration before/after presentation of the CS can dramatically alter either CTA acquisition or extinction.
- Data suggest that the neural substrates of CTA acquisition may be different (to some degree) than the ones required for CTA extinction (see Bahar et al., 2003)
- Uncovering the exact role of GABA in extinction could lead to the development of new pharmaceutical treatments for treating phobias, PTSD, and anxiety disorders (Barad, 2005).

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