

Systemic Baclofen Decreases Gustatory Discrimination and Induces Conditioned Taste Aversion in Adult Rats

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Abstract

Studies that intend to measure drug-induced changes in learning and memory are challenged to parse out the effects of drugs on sensory and motor systems as well as associative systems in the brain. In the context of conditioned taste aversion (CTA), drugs that alter the sensorium of experimental animals and affect their ability to taste and/or feel malaise may inadvertently distort the conclusions drawn from these studies. Therefore, the current experiments evaluated the extent to which the GABA_B agonist, baclofen (BAC), altered the sensory abilities of rats to differentiate between two concentrations of saccharin (SAC). Here we report that 2 or 3mg/kg (i.p.) BAC, but not 1mg/kg BAC, impaired the ability of animals to discriminate between 0.3% and 0.6% SAC in a two bottle preference test. Further, when SAC consumption was preceded by 2 or 3mg/kg (i.p.) BAC rats depressed their subsequent SAC drinking. On the final day of testing, we evaluated the visceral effects of BAC administration on lithium chloride (LiCl)-induced suppression of water drinking. We found that BAC did not disrupt the LiCl-induced hypodipsia.

A second experiment evaluated if the suppression of SAC and water drinking (revealed in Experiment 1) was mediated by putative amnesiac effects of BAC or whether BAC possessed US properties in the context of the CTA paradigm. The results of the second experiment indicated that 2 and 3mg/kg (i.p.) BAC were capable of inducing a CTA, which was extinguishable via repeated presentations of SAC only. The time necessary to reach an asymptotic level of CTA extinction was not significantly different in those animals that received the 3mg/kg dose of BAC compared to more conventionally SAC + lithium chloride (LiCl) conditioned animals. However, rats treated with 2 mg/kg BAC extinguished faster than animals conditioned with either 3 mg/kg BAC or LiCl. Our data indicate that, depending on the dose, BAC can alter SAC taste discrimination and act as a potent US in the context of a CTA paradigm.

Introduction

- Baclofen [BAC; 4-amino-3-(4-chlorophenyl) butanoic acid], a GABA_B receptor agonist, disrupts performance on a variety of memory tasks [1, 2, 8, 9, 15, 16].
- However, tests of GABAergic system manipulations on a conditioned taste aversion (CTA) have produced variable results.
 - Chester and Cunningham [2] showed that BAC does not alter ethanol-induced conditioned taste aversion in mice, nor does BAC, itself, have malaise inducing properties at a dose of 2mg/kg. However, other labs have demonstrated that GABA_B(1a) knockout mice fail to acquire a CTA while GABA_B(1b) knockouts failed to extinguish a CTA [9].

- The challenges of using drugs like BAC to test memory in the context of an appetitive paradigm include the fact that GABAergic system manipulations reportedly cause hypodipsia, sedation, vertigo and hyperphagia [4, 5, 13].

- In order to separate out drug effects on learning/memory vs. drug effects on sensory or appetitive factors this investigation asked the following questions:

- Does BAC alter basal food or liquid consumption?
- Does BAC compromise rats' ability to perceive the conditioned stimulus (CS)?
- Does BAC cause malaise itself, thus acting as a US or interfering with actions of a US?

- Given the previous literature (see above), in our first experiment, we hypothesized that BAC would have no effects on taste discrimination or overall SAC consumption.

- Results of the first experiment led us to a second experiment in which we hypothesized that BAC at 3mg/kg would have the visceral effects of an unconditioned stimulus if used in a CTA paradigm.

Methods

Experiment 1 - Taste Discrimination and Hypodipsia Test

- Subjects: Naïve male and female Sprague-Dawley rats (Mean + SEM = 440.15 + 29.04 grams) were used.
 - Two days prior to the first discrimination test, all animals were introduced to a 23-hr fluid deprivation cycle that consisted of a 1 hr presentation of fluid/day to ensure animals were motivated to drink at the time liquid was present; this first day of fluid deprivation the animals were given 1 hr of water only.
- Following the first fluid deprivation day, animals were subjected to subsequent SAC (0.3% and 0.6%) exposure and discrimination testing as described in Table 1.
- On the fifth day of testing, animals were given an injection of saline or BAC (1, 2, or 3mg/kg, i.p.). Thirty min later animals were injected with LiCl (81mg/kg, i.p.) and immediately presented with one sipper tube of water for 30min.

Group Nomenclature	N	Treatments on SAC Discrimination Tests Days 1 and 3		Treatments on LiCl-induced Hypodipsia Test Day 5	
		Measures on Rest Days Days 2 and 4	Measures on Rest Days Days 2 and 4	Measures on Rest Days Days 2 and 4	Measures on Rest Days Days 2 and 4
SAL	5	SAL + SAC**	Water	SAL+(LiCl+Water) [†]	
BAC 1mg/kg	5	BAC + SAC	Water	BAC+(LiCl+Water)	
BAC 2mg/kg	5	BAC+SAC	Water	BAC+(LiCl+Water)	
BAC 3mg/kg	5	BAC+SAC	Water	BAC+(LiCl+Water)	

*SAL = physiological saline injection (0.9% NaCl dissolved in deionized water, 1ml/kg, i.p.)
**SAC = two-bottle SAC preference test using 0.3% SAC and 0.6% SAC (%w/v; SAC salt dissolved in deionized water to specific concentration).
†BAC = BAC injection (BAC dissolved in physiological saline to either 1, 2 or 3mg/ml; 1ml/kg, i.p.)
‡(LiCl+Water) = LiCl injection (81mg/ml LiCl dissolved in physiological saline; dose = 81 mg/kg, i.p.) which was immediately followed by the presentation of one water bottle.

Experiment 2 - Conditioned Taste Aversion

- Male Sprague-Dawley rats (Mean + SEM = 467.74 + 38.43 grams) were used.
 - Rats underwent 2 days of 23-hr fluid deprivation in order to acclimate them to the drinking schedule.
 - Following the two days of fluid deprivation, animals began the CTA acquisition phase of the experiment, which was subsequently followed by CTA extinction for those groups that acquired a CTA after three CS-US pairings. See Table 2 for the animals' drinking and drug injection schedule throughout this experiment.
- Animals were considered to have reached asymptotic extinction of their CTA after reaching 90% of a premeasured baseline level of SAC consumption for similarly sized rats [10, 12].

Table 2. Experiment 2: Group Nomenclature and Treatments

Group Nomenclature	N	Conditioning			Extinction
		Days 1, 3, 5	Days 2, 4, 6	Extinction	
SAC+BAC 2mg/kg	5	SAC+BAC*	Water	SAC [†]	
SAC+BAC 3mg/kg	5	SAC+BAC	Water	SAC	
SAC+LiCl	5	SAC+LiCl [†]	Water	SAC	
EU(LiCl)	5	SAC	Water+LiCl		
EU(BAC 2mg)	5	SAC	Water+BAC		
EU(BAC 3mg)	5	SAC	Water+BAC		

*BAC = BAC injection (BAC dissolved in physiological saline to either 2 or 3mg/ml; 1ml/kg, i.p.; dosing specified in group nomenclature)
†SAC = 30min presentation of 0.3% SAC (%w/v; SAC salt dissolved in deionized water) occurred everyday throughout extinction until animals reached asymptotic consumption.
‡LiCl = LiCl injection (81mg/ml LiCl dissolved in physiological saline; dose = 81 mg/kg, i.p.)

Experiment 1: Effects of BAC on a SAC taste discrimination task

High doses of Baclofen (2-3mg/kg, i.p.) disrupt performance on a SAC taste discrimination test whereas a lower dose (1mg/kg, i.p.) does not.

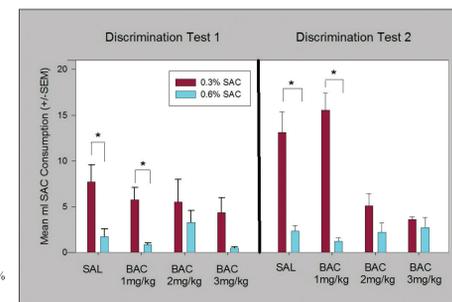


Figure 1. Mean SAC Consumption (ml ± SEM) on SAC Discrimination Test Days. RM-ANOVA showed that on both SAC Discrimination Test Days the SAL and BAC 1mg/kg groups drank significantly more 0.3% SAC than 0.6% SAC. The BAC 2mg/kg and BAC 3mg/kg groups did not drink significantly different amounts of 0.3% SAC and 0.6% SAC on either test day. The SAL and BAC 1mg/kg groups also showed an increased SAC consumption between test 1 and test 2, but the BAC 2mg/kg and BAC 3mg/kg groups did not increase their consumption of 0.3% SAC. Note: On the First Taste Exposure Day (not illustrated in figure), when no drug was given, all groups showed a preference for 0.3% SAC despite the overall low SAC consumption due to neophobia [Mean SAC consumptions ± SEM: 0.3% SAC = 5.2 ± 1.1ml; 0.6% SAC = 2.3 ± 0.7ml]. *Significant within group difference between 0.3% and 0.6% SAC. p<0.05.

Hypodipsia Test

- There was no indication on Day 5, that LiCl-induced hypodipsia was affected by BAC injection. A Repeated Measures ANOVA showed a significant decrease overall in water consumption from the Rest Days (mean water consumption on Days 2 and 4) to water consumption on the LiCl-induced Hypodipsia Test Day (Day 5) [F(1, 13) = 6.256, p<0.027] and Tukey post hoc analysis showed no differences between treatment groups.

Experiment 2: Effects of BAC (2-3mg/kg) on CTA Acquisition and Extinction

High doses of Baclofen (2-3mg/kg) act as an unconditioned stimulus to induce a CTA to 0.3% SAC over three CS+US pairings.

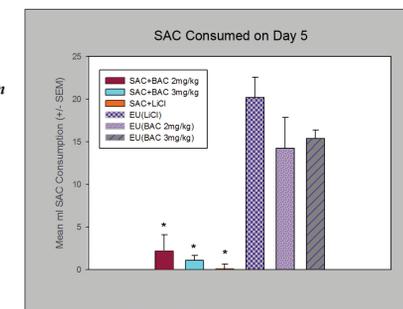
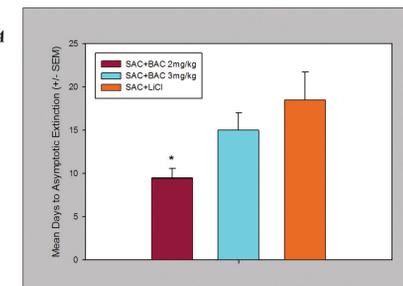


Figure 2. Mean ml SAC Consumption (± SEM). All animals that received either SAC+LiCl or SAC+BAC pairings acquired a strong taste aversion to SAC by the third CTA trial, as illustrated by the significant difference in SAC drinking between animals given EU training and those that had SAC paired with LiCl or BAC. All of the EU groups that did not receive CS+US pairings did not acquire a CTA, as indicated by high SAC drinking on trial 3, which was significantly greater than their drinking on trial 1. The SAC+LiCl, SAC+BAC 2mg/kg and SAC+BAC 3mg/kg groups were all drinking near-zero amounts of SAC on trial 3, which was significantly less than their SAC drinking on trial 1. Drinking on the first conditioning trial was not significantly different between any groups [Mean SAC consumption ± SEM on trial 1 = 4.35 ± 1.22ml]. Note: A BAC 1mg/kg group is not used in Experiment 2 because this group did not indicate a disruption of SAC discrimination capabilities in Experiment 1 nor did they demonstrate any possible US effects of BAC exposure in Experiment 1. *Significant difference between EU groups and the following CS+US groups: SAC+LiCl, BAC 2mg/kg, and BAC 3mg/kg. p<0.05.

Time requirements for extinction of the CTA are dependent on the dose of BAC used as the unconditioned stimulus during CTA acquisition.

Figure 3. Mean Days (± SEM) to Asymptotic EXT.

The LiCl and BAC 3mg/kg groups did not differ significantly in the number of days required to reach asymptotic EXT. However, the BAC 2mg/kg group extinguished significantly faster than the LiCl and BAC 3mg/kg groups. * Significantly less than both LiCl and BAC 3mg/kg groups. p<0.05.



Results

Summary & Conclusions

- Experiment 1 revealed that BAC dose-dependently (2 and 3 mg/kg, i.p.) reduced taste discrimination as well as total fluid consumption in rats. Moreover, within-group comparisons indicated that 1mg/kg BAC did not alter gustatory discrimination or consummatory behaviors.
- BAC at 1, 2, or 3mg/kg did not disrupt LiCl-induced hypodipsia, so the US effects of LiCl remain intact when co-administered with BAC.
- Both doses of BAC tested in experiment 2 (2 and 3mg/kg) did induce a CTA, as SAC consumption decreased significantly over 3, CS+US pairings in the animals receiving SAC+BAC. This suppression was comparable to that observed in rats that received SAC+LiCl, a conventional CTA pairing.
 - A difference in malaise-inducing properties of the US injections are likely the variable that led to differences in extinction times; BAC at 2mg/kg is a weaker US, while BAC at 3mg/kg may evoke malaise comparable to LiCl (81mg/kg).
- Despite some variance in the literature [refer to 2, 3, 5, 11, 12], it is not surprising that BAC possesses US properties, due to the cascade of neurophysiological effects it produces in the CNS [3, 15]. Through direct action as a GABA_B agonist, BAC indirectly reduces levels of a variety of other neurotransmitters (e.g., norepinephrine, dopamine, acetylcholine, serotonin, glutamate, aspartate and GABA) [15].
- Overall, our data indicated that BAC given at 2 or 3mg/kg (i.p.) impairs sensory abilities and decreases gustatory discrimination in rats. But, these effects may be augmented by dose-dependent US properties of BAC that, when paired with the gustatory stimulus, SAC, induced a CTA in Experiment 2.
 - BAC may still be used successfully in future CTA manipulations at a dose of 1mg/kg (i.p.), since that dose did not produce changes in SAC taste discrimination or LiCl-induced hypodipsia. Nor did it produce a CTA.

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