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Do Rats Consume Ethanol to Regulate a Negative Emotion Induced by a Successive Negative Contrast Procedure?

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by
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Abstract

According to the self-medication hypothesis, individuals may consume drugs or alcohol, or engage in other behaviors in order to reduce a negative emotional state (Khantzian, 1985; Gross, 2013; Crum et al., 2013). Rats experiencing a negative state induced by various stressors (Bertholomey et al., 2010), or a decrease or loss in reward value of a sucrose solution (Manzo et al., 2015; Manzo et al., 2014) demonstrate increased consumption of alcohol. I used successive (SNC) and anticipatory negative contrast (ANC) procedures to further examine this hypothesis and the previous findings (Manzo et al., 2015), that rats increase consumption and preference for ethanol in post-shift sessions of a SNC procedure. The results of the present study confirm these findings, and in addition, demonstrate that an ANC procedure does not affect ethanol consumption.

Keywords: Successive negative contrast, anticipatory negative contrast, self-medication hypothesis, ethanol

Introduction

Emotion regulation involves an effort to decrease negative emotional states or increase positive emotional states (Gross, 2013). Individuals, for example, may try to change their negative emotional state by changing their behavior, which could include using drugs and drinking alcohol (Butler, 2011). In anxiety disorders the emotion system is dysregulated and negative emotional states are more extreme than in normally functioning individuals. Anxious individuals that self-medicate by drinking are attempting to reduce their negative emotion, but this emotional regulation is more likely to develop into alcohol dependence and preventing this self-medicating behavior could reduce the potential for dependence (Crum et al., 2013). Also chronic or cyclic alcohol use may go counter to an individual's goal of reducing negative emotional states in the long-term by increasing anxiety in humans. Nevertheless, alcohol consumption is an effective emotion regulator in the short term similar to pharmacological treatments for anxiety like the benzodiazepines (Crum et al., 2013).

Self-Medication Hypothesis

The self-medication hypothesis states some individuals use alcohol and drugs to relieve symptoms of anxiety, depression, and other disorders (Khantzian, 1985). The hypothesis was later revised by Khantzian to state that people self-medicate to improve a dysphoric state instead of in response to their condition, and that the drug of choice corresponds with their current state (Khantzian, 1999). This later revision may not be supported (Lembke, 2012), but nevertheless the self-medication hypothesis is supported by the literature (Markou, Kosten, and Koob, 1998), and Khantzian's own more recent research (Khantzian, 2013).

Stress produces a state of tension which is hypothesized to be reduced by the anxiolytic effect of alcohol consumption (Noori et al., 2014). According to the tension reduction

hypothesis (Becker et al., 2011), individuals consume alcohol to alleviate common symptoms of a psychological disorder such as dysphoria, anxiety and stress. This hypothesis shows similarity to the self-medication hypothesis.

Animal Models

In order to test the self-medication hypothesis in animal models, which are used to generate findings that can be translated to human research, it is first necessary to demonstrate that laboratory animals experience negative emotions such as anxiety and that pharmacological treatments can reduce the negative emotions. There is substantial evidence that this is the case (Andrews et al., 2014; Kumar et al., 2013; Xiaobai et al., 2012). For example, the elevated plus maze is a well validated model of anxiety in rats (Kumar et al., 2013). Anxiety is measured by time spent in and entries into the open arms of the maze (Arabo et al., 2014). Anxious animals spend more time in the closed arms of the maze while anxiolytic substances increase open arm activity (Acevedo et al., 2014). Stressed rats show anxiety-like behavior in this maze while rats given an anxiolytic substance like ethanol or the benzodiazepine chlordiazepoxide (CDP) exhibit less anxious behavior (Acevedo et al., 2014).

To create an animal model of self-medication the next step is for researchers to expose rodents to stressors (or inject them with anxiogenic substances), and provide opportunities for the rodents to self-administer an anxiolytic substance. To be consistent with a self-medication interpretation the measured behavior needs to result from negative reinforcement (Acevedo et al., 2014). Negative reinforcement increases behavior to ameliorate a negative emotion while positive reinforcement increases behavior to increase a pleasurable state (Ameringer et al., 2015). Negative reinforcement has been used to explain self-medicating behavior in animals as early as Conger (1956). Stressors such as foot-shock, the forced swim test, social defeat stress,

and yohimbine injection, for example, induce alcohol seeking and consumption in rodents (Bertholomey et al., 2010; Caldwell & Riccio, 2010; Bertholomey et al., 2013), presumably through negative reinforcement. However, it is also possible that ethanol seeking behavior and increased consumption is due to positive reinforcement, especially when rats selectively bred to prefer ethanol are used as subjects (Knapp et al., 2011).

There is some evidence that in a post-session choice test ethanol can serve as a negative reinforcer, consumed to reduce an aversive emotional state. Manzo and colleagues (2014) tested this hypothesis by exposing rats to a 22% sucrose solution for 10 trials (acquisition of sucrose consumption phase) and then removed the sucrose solution for several days (extinction phase). The rats increased consumption of a 2% ethanol solution in a two hour ethanol choice test following the extinction sessions compared to when sucrose was available during daily sessions. This increased consumption of ethanol during the extinction phase was interpreted as self-medication to relieve the negative emotional reaction caused by the loss of sucrose availability (Manzo et al., 2014).

Contrast Procedures. Decreasing the value of a sucrose solution can also induce an anxious state similar to removal of the solution (Manzo et al., 2015; Kamenetzky et al., 2008). In a *successive negative contrast* (SNC) procedure a group of rats receives access to a highly preferred sucrose solution for several days in succession (pre-shift sessions) and then a less rewarding solution (either a lower sucrose concentration or saccharin) replaces the highly preferred solution for several days (post-shift sessions) (Rowan & Flaherty, 1991). The downshift marks the transition from pre-shift to post-shift solution, which is a reduction in value to the animals (Manzo et al., 2015). The rats experiencing a downshift in the solution show reduced consumption of the less preferred solution compared to a control group that received

only the less preferred solution throughout all sessions without a downshift. This reduced consumption in the downshifted group relative to the control group is called *negative contrast*. This downshift produces an anxious state similar to the total removal of the sucrose solution in the extinction phase as reported by Manzo et al (2014).

What is the evidence that SNC is mediated by a negative emotional reaction to the downshifted solution? The contrast effect produced in a SNC procedure can be prevented by administering alcohol (Kamenetzky et al., 2008) or CDP (Flaherty & Rowan, 1986) before the downshift sessions. Interestingly CDP and alcohol ameliorate the contrast effect on the second post-shift day, but not the first (Flaherty & Rowan, 1991). This is expected since the rats must first experience the downshift in sucrose reward before an emotional response can be elicited (Bentosela et al., 2006). That is, only on the second post-shift day can the animals anticipate the presentation of the less preferred solution. Additionally, ethanol administered during the extinction phase of a sucrose consumption task accelerates extinction of sucrose-seeking in rats (Kamenetzky et al., 2009), indicating ethanol may also reduce a negative reaction induced by reward loss. Since ethanol can change consumption before SNC downshift or extinction sessions, a rat may seek and consume this anxiolytic substance after a session that produces a negative emotional state (Kamenetzky et al., 2008; Manzo et al., 2015). The negative contrast effect produces a well characterized negative emotional state, as compared to the different stressors used to produce alcohol seeking discussed previously (Manzo et al., 2015). Since SNC produces an aversive emotional state, post-session ethanol availability provides an opportunity for negative reinforcement. That is, consumption of ethanol can reduce this negative state.

Additional evidence of a negative emotional reaction caused by reward reduction comes from concomitant hormonal measures. The stress hormone corticosterone is elevated in

extinction and negative contrast post-shift sessions (Bentosela et al., 2006). Corticosterone has been found elevated before and after the second post-shift session, and administration of corticosterone enhances the consummatory SNC effect (Flaherty et al., 1985; Ruetti et al., 2009). This stress hormone is released in response to stressors like reward loss (Bentosela et al., 2006). Though rats do not show increased anxiety on the elevated plus maze after post-shift days the incentive downshift produces an aversive state characterized by frustration as the rats expect a greater reinforcement (Cuenya et al., 2012; Genn et al., 2004). A variety of drugs that activate GABAergic neurons are effective in eliminating the negative contrast on the second post-shift day (Genn et al., 2004). Alcohol and GABA have similar ameliorating effects on negative contrast because alcohol's anxiolytic property is due in part to activation of GABA receptors (Meyer & Quenzer, 2013). The emotional component of consummatory SNC allows anxiolytic substances like ethanol to influence sucrose consumption during post-shift sessions (Ruetti et al., 2009). CDP prevents the negative contrast due to its anxiolytic properties (Genn et al., 2004).

To test the self-medication hypothesis Manzo et al (2015) used SNC to induce an anxious state in rats, again followed by access to a substance with antianxiety properties. These included either a 2% ethanol solution or 1 mg/kg CDP solution. The experimental group of rats had access to a 32% sucrose solution during pre-shift trials and 4% solution in post-shift trials. In post-shift trials the rats exhibited a contrast effect by decreasing consumption of the sucrose solution to levels below unshifted controls. In addition, only the rats experiencing a contrast effect increased consumption of both ethanol and CDP while preference for either anxiolytic substance was not observed during the pre-shift sessions. These findings support the hypothesis that animals regulate an anxious state (i.e., self-medicate) by consuming anxiolytic substances like alcohol or CDP (Manzo et al., 2014; Manzo et al., 2015).

Types of Incentive Contrast Effects

The goal of the present experiment is to build on the work of Manzo et al (2015) to evaluate anti-anxiety self-medication in the initial consumption of ethanol. To better evaluate the self-medication hypothesis using a negative contrast procedure to elicit a negative emotion it is necessary to distinguish between different types of incentive contrast procedures and their effects.

Incentive Contrast. Incentive contrast is defined as a change in behavior in response to a change in the magnitude or incentive of a reinforcer (Zentall, 2007). As mentioned previously the contrast effect occurs when a group of rats experiencing the sudden change in a reward shows significantly different responding than a group exposed to the lesser reward since the beginning of the experiment. For example in the seminal study by Crespi (1942), rats shifted from a larger number of food pellets to a smaller number ran slower in a runway than rats reinforced with the smaller number of pellets from the start. This demonstrates a negative incentive contrast effect. By comparison in a positive incentive contrast effect rats run faster when shifted from a smaller to larger number of pellets than rats reinforced with the larger number the entire time (Crespi, 1942). Crespi used a *discrete trial instrumental procedure* to study contrast effects. Another approach to study contrast effects is the *behavioral contrast* procedures where animals are tested in concurrent schedules of reinforcement in operant chambers. In behavioral contrast procedures an animal will change its rate of responding to one schedule of reinforcement due to a change in reinforcement on a different, but concurrent, schedule of reinforcement (Zentall, 2007). The reduction in reinforcement in one schedule leads to an increase in responding in the other schedule of reinforcement, which has remained unchanged.

Yet another procedure to study incentive contrast effects is the *consummatory contrast procedure* whereby animals do not run to obtain the reward (discrete-trial instrumental procedure) nor respond on schedules of reinforcement (behavioral contrast procedures), but are simply provided the opportunity to consume the reward usually as a solution (Flaherty, 1982). Although there are commonalities among these various procedures, there are also some substantial differences in the contrast effects that emerge from these procedures (Zentall, 2007). This thesis will investigate consummatory incentive contrast procedures rather than discrete-trial instrumental procedures or behavioral contrast procedures. I will now review two types of consummatory incentive contrast procedures: SNC and ANC.

Successive Negative Contrast (SNC). As described previously in a SNC procedure a group of rats has access to a rewarding sucrose solution for a number of sessions. The sucrose solution is then lessened for a number of downshift sessions. The consistent finding is that the rats decrease consumption of this second solution compared to a control group that was exposed to that same sucrose solution for the duration of the experiment (Flaherty & Rowan, 1991). As reviewed previously, pharmacological and behavioral studies support the hypothesis that the contrast effect in this procedure is mediated by the elicitation of a negative emotion.

Anticipatory Negative Contrast. In anticipatory negative contrast (ANC), rats are provided access to two bottles presented in succession during daily sessions (Flaherty & Rowan, 1986). For the contrast group a less rewarding saccharin solution is followed by a more rewarding sucrose solution (Saccharin-Sucrose). The control group, however, is given access to the less rewarding saccharin solution in both bottles (Saccharin-Saccharin). Thus, both groups receive the same saccharin solution in the first bottle, but only the contrast group receives the more preferred sucrose solution in the second bottle. After several days of training the contrast

group consumes less of the saccharin solution in the first bottle compared to the control group. This reduced consumption of saccharin in the first bottle relative to the control group is a negative contrast effect and occurs because only the contrast group reduces intake as a result of anticipating the more preferred sucrose in the upcoming second bottle (Flaherty et al., 1989). Researchers propose this type of contrast effect is due to expectation of the sucrose solution (Flaherty et al., 1995).

ANC can also be investigated as a within-subjects procedure rather than as a between-subjects procedure by giving one group of rats Saccharin-Saccharin sessions and Saccharin-Sucrose sessions on alternate days for the duration of the experiment. ANC research shows that on control days (Saccharin-Saccharin), rats consume more of the saccharin in the first bottle compared to contrast days (Saccharin-Sucrose) when they consume less of the saccharin in the first bottle (Wright, Gilmour, & Dwyer, 2013). Within-subjects ANC (WS-ANC) is a Pavlovian conditioning procedure because discriminative context cues are introduced to signal the second solution for each session, allowing the rats to predict the second of the solutions (Binkley et al., 2014). For example, the presence of white noise and dim light on contrast days signals that sucrose is available in the second bottle and the absence of these cues on alternate days signals the availability of the less preferred saccharin on control days (Wright et al., 2013). In ANC the rats gradually learn which cues are associated with which type of session and their consumption behavior changes as they learn to anticipate which sessions will be followed by sucrose (Timberlake & Engle, 1995).

Comparison between ANC and SNC. A SNC procedure produces an anxious state when the animal directly experiences a downshift in the rewarding solution. ANC, however, is not accompanied by an emotional component as evidenced by the lack of a CDP effect in

ameliorating the contrast effect (Flaherty & Rowan, 1986). That is, while ANC is not affected by benzodiazepines, the SNC effect is reduced by anxiolytic substances and the downshift produces significant increases in corticosterone (Rowan & Flaherty, 1991). Since corticosterone administration and anxiolytic substances do not affect the ANC effect; it is likely that this procedure does not induce an emotional state (Ruetti et al., 2009). The differential effects of anxiolytic drugs on SNC and ANC indicates that two procedures result in negative contrast effects that are mediated by different processes and only SNC appears to be mediated by an emotional reaction to a reduction in reward (Flaherty & Rowan, 1986). Since there is no negative emotional state induced by ANC, there is no opportunity for negative reinforcement.

Hypotheses. The present experiment will examine the anti-anxiety self-medication hypothesis using a SNC (SNC) procedure and an ANC procedure (ANC). Although both procedures are expected to produce a negative contrast effect, only the SNC procedure is expected to be accompanied by a negative emotional reaction. Therefore an anti-anxiety self-medication effect should be observed in the rats experiencing SNC, but not ANC. Both groups will be given access to ethanol (and water) after the contrast procedures in an ethanol choice test. It is hypothesized that downshifted rats will consume less saccharin solution than control rats (i.e., will show a SNC effect; (Flaherty & Rowan, 1986). And, due to the anxiolytic effect of ethanol, it is hypothesized that these rats will consume more ethanol in the ethanol choice test after the downshift than a control group that did not experience the shift.

It is also hypothesized that there will be no differences in ethanol consumption in rats exposed to a within-subjects ANC procedure since the reduced consumption of saccharin on days it is followed by a highly preferred sucrose solution is not accompanied by a negative emotional reaction (Flaherty & Rowan, 1986). Although the proposed experiment aims to

replicate the work of Manzo et al (2015) it is also novel because it proposes to use different negative contrast procedures to better isolate the importance of negative emotional distress in tests of the self-medication hypothesis. The finding that ethanol consumption increases in SNC but not in ANC rats would support the anti-anxiety self-medication hypothesis. However, the finding that ethanol consumption increases following SNC and ANC would suggest that the experience of reward contrast itself leads to enhanced ethanol consumption. This latter result would suggest that the consummatory contrast procedures may not be an appropriate procedure for testing the self-medication hypothesis.

Experiment 1

Method

Subjects. Twenty-four experimentally-naive male Long Evans rats were purchased from Harlan (Indianapolis, IN) and pair-housed in standard clear plastic cages with corncob bedding on a 12 hour light/dark schedule. One week prior to the start of the experiment the rats' daily food intake was restricted to maintain about 85% of their free-feeding body weight. The rats were approximately 70 days old at the beginning of the procedure. The rats received their daily food ration approximately 30 minutes after completing all daily test procedures. All rats were handled and weighed daily starting the week before the experiment. This procedure was approved by the Seton Hall Institutional Animal Care and Use Committee prior to initiation of the experimental procedures.

Apparatus. The contrast procedures were performed in a small room with 6 stainless steel cages measuring 17.8 X 24.4 X 17.9 cm (W X L X H) mounted in a row on a wooden frame. The two-hour ethanol test took place in the same type of cage mounted on a large steel rack in a different room in the laboratory, which can house all 24 rats at one time. These cages had a wire mesh front, sides and floor, and the back was a solid stainless steel wall with a circular opening that measured 2.5 cm in diameter. Solutions were mounted in the front on the wood rack and through the back opening in the ethanol choice test cages. Solutions were delivered by modified 100 mL graduated cylinders. Home cages were clear plastic standard paired-housing chambers measuring 23.6 X 45.3 X 20.3 cm (W X L X H) with corncob bedding.

Procedure. All rats were first adapted to the two sets of test cages. On days 1-4 all rats were placed in the ethanol choice test cages for 30 minutes with access to water at the front and back of the cages. On the fifth day they were habituated to the contrast test cages for a 5 minute

session without water or sucrose bottles prior to adaptation in the ethanol choice test cages, to familiarize the rats with the testing chambers. The rats were then matched by weight and randomly assigned to 3 groups of 8. These three groups included SNC experimental and control groups and a within-subjects ANC (WS-ANC) group (see below for details). Since there were 8 rats in each group, each group was separated into 2 squads of 4 rats which were transported to the contrast chambers for the 5 minute sessions at a time. All solution intakes were determined by weighing the bottles before and after the sessions.

Ethanol choice tests. After each session in the contrast chambers (see below), the rats were transported to the ethanol choice test cages in the larger laboratory room where they received 2 hours of access to water and ethanol bottles side by side at the front of the cage. Bottles were alternated each day to prevent the rats from developing a side preference. After 2 hours the rats were transported back to their home cages. Ethanol consumption by weight (g) was measured, and ethanol preference was calculated based on the ratio between ethanol and water consumption.

Successive negative contrast procedure. The SNC procedure was a between subjects design, with experimental and control groups. The procedure was divided into pre-shift and post-shift sessions. There were 14 pre-shift sessions, followed by 4 post-shift sessions. The rats were placed in testing chambers with 5 minutes access to a sipper tube bottle at the front of the cage containing 32% sucrose solution (commercial sugar dissolved in tap water) for the contrast group, and 0.15% saccharin solution for the control group. Each session began with the rats placed in the chamber. Then the contrast bottle holder rack was moved forward to begin the 5 minute session at the same time for each rat. After 5 minutes the rats were transported to the other testing room for the 2 hour ethanol choice test. For the post-shift sessions, the

experimental group's solution was replaced with the same 0.15% saccharin as the control group to induce a contrast effect. The transition from sucrose to saccharin solution marked the downshift, separating the 14 pre-shift sessions and 4 post-shift sessions. The 2 hour ethanol choice test was the same in these SNC experimental and control sessions. The amount of sucrose and saccharin solutions consumed was measured, the amount of ethanol and water solutions consumed was measured, and ratios were calculated between ethanol and water consumption to determine ethanol preference.

Intermittent ethanol exposure procedure. After 11 pre-shift sessions, all groups were consuming very low amounts of ethanol, so the procedure was suspended temporarily to increase the rat's ethanol consumption. The self-medication hypothesis requires that rats are willing to consume some ethanol in order to experience a pharmacological effect. Because the rats were not drinking much alcohol at all there was a concern that there would be no opportunity for the rats to experience a pharmacological effect. Increasing the rats' ethanol consumption was achieved with an intermittent ethanol exposure procedure, known to enhance consumption of ethanol in rodents (Rosenwasser, Fixaris, Crabbe, Brookes, & Ascheid, 2013). The rats received an ethanol solution in a bottle in their home cages for 24 hours, which was removed for the following 24 hours, and repeated. Consumption was measured by weighing the ethanol bottle before and after each session. Once the 3 groups were consuming a higher amount of ethanol in their home cages (5 ethanol exposure sessions; 10 total days) they returned to the procedure with 3 additional pre-shift sessions to regain stable consumption prior to the downshift in the SNC contrast group.

Anticipatory negative contrast procedure. The within-subjects version of the ANC procedure was used (Flaherty & Rowan, 1986). The rats were placed in a test chamber with 5 minutes of access to a 0.15% saccharin solution in the front of the cage. The first bottle was then

removed from the front of the cage and the second bottle was immediately presented through the opening at the back of the cage. The second bottle contained the same 0.15% saccharin solution for 3 minutes on control days (Monday, Wednesdays, and Thursdays), and 32% sucrose on the contrast days (Tuesdays, Thursdays, and Saturdays). To help the rats distinguish contrast days from control days, discriminatory context cues were provided. The context was background white noise and dim light for the control days and normal conditions for the contrast days (overhead florescent lights on, no added noise). This procedure was repeated for 30 days with 15 negative contrast test sessions and 15 control sessions, for a total of 15 two-day blocks. After the contrast procedure the rats were placed in the ethanol choice cages for the 2 hour ethanol choice test. Saccharin and sucrose solution consumption was measured by weighing the bottles before and after consumption, as well as water and ethanol consumption in the two hour ethanol choice test after each session, and the ratio calculated between ethanol and water in order to determine ethanol preference.

Experiment 1 Procedure	**Contrast Session**																			
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
SNC Contrast	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	SUCR	**SACC**	**SACC**	**SACC**	**SACC**
SNC Control	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC	SACC
WS-ANC (2nd Bottle)	SUCR	SACC	SUCR	SACC	SUCR	SACC	SUCR	SACC	SUCR	SACC	SUCR	SACC	SUCR	SACC	SUCR	SACC	SUCR	SACC	SUCR

Table 1. Experiment 1 Procedure. This table indicates the solutions available during each contrast session for the three groups throughout Experiment 1. The SNC Contrast group had daily access to a 32% sucrose (SUCR) solution, followed by 0.15% saccharin (SACC) for the four post-shift days. The SNC Control group experienced the SACC solution for every session of the experiment. The 2nd bottle for the WS-ANC group alternated daily between SUCR and SACC.

Statistical Analyses. SPSS statistical software was used for all statistical analyses. In the contrast sessions the dependent variable was the amount of solution consumed in the 5 minute sessions. In the ethanol choice test the dependent variables were ethanol consumed and the ethanol preference ratio (ethanol consumption divided by total consumption of ethanol

solution and water). The independent variables were contrast procedure and daily sessions. Partial eta-squared (η^2_p) was used as the measure of effect size for all analyses of variance (ANOVAs), with small ($\eta^2_p = 0.009$), medium ($\eta^2_p = 0.09$), and large ($\eta^2_p = 0.25$) effect sizes (Field, 2013; Levine & Hullett, 2013). ANOVAs were calculated for each dependent variable and statistical significance considered at $p < 0.05$. Any marginally significant results were followed up when the effect sizes were considered large.

Results

Pre-shift Successive Negative Contrast Group Consumption. The SNC procedure divides into pre-shift and post-shift phases, separated by the downshift for the SNC Contrast group. Figure 1 shows the intakes during pre-shift and post-shift days. While the SNC pre-shift phase lasted 14 days, only the final 6 are included for analysis to demonstrate the steady, significantly higher intake in the SNC Contrast group prior to the downshift. Separate mixed ANOVAs were calculated between the two SNC groups for the 6 pre-shift days and 4 post-shift days. A 2 x 6 (Group [contrast, control] x Days) mixed ANOVA with group as the between-subject's factor and days as the repeated measure was calculated for the 6 pre-shift sessions before the downshift. Intakes during the last 6 pre-shift days before the downshift in the SNC contrast group (Figure 1, left side) were stable as indicated by a non-significant main effect of day on consumption, $F(5, 70) = 0.385$, $p = 0.858$, $\eta^2_p = 0.03$. The main effect of group on sucrose/saccharin consumption was significant, $F(1, 14) = 79.79$, $p < 0.001$, $\eta^2_p = 0.851$, indicating the groups differed in solution consumption in pre-shift sessions. The SNC contrast group consumed more of the 32% sucrose solution in the pre-shift days than 0.15% saccharin solution consumed by the control group (Mean Difference = 6.1, SE = 0.7, 95% CI: 4.597, 7.503). The interaction between days and groups was not significant, $F(5, 70) = 1.77$, $p = 0.13$,

$\eta^2_p = 0.112$, indicating consumption was consistently higher for the SNC Contrast group in the 6 pre-shift days.

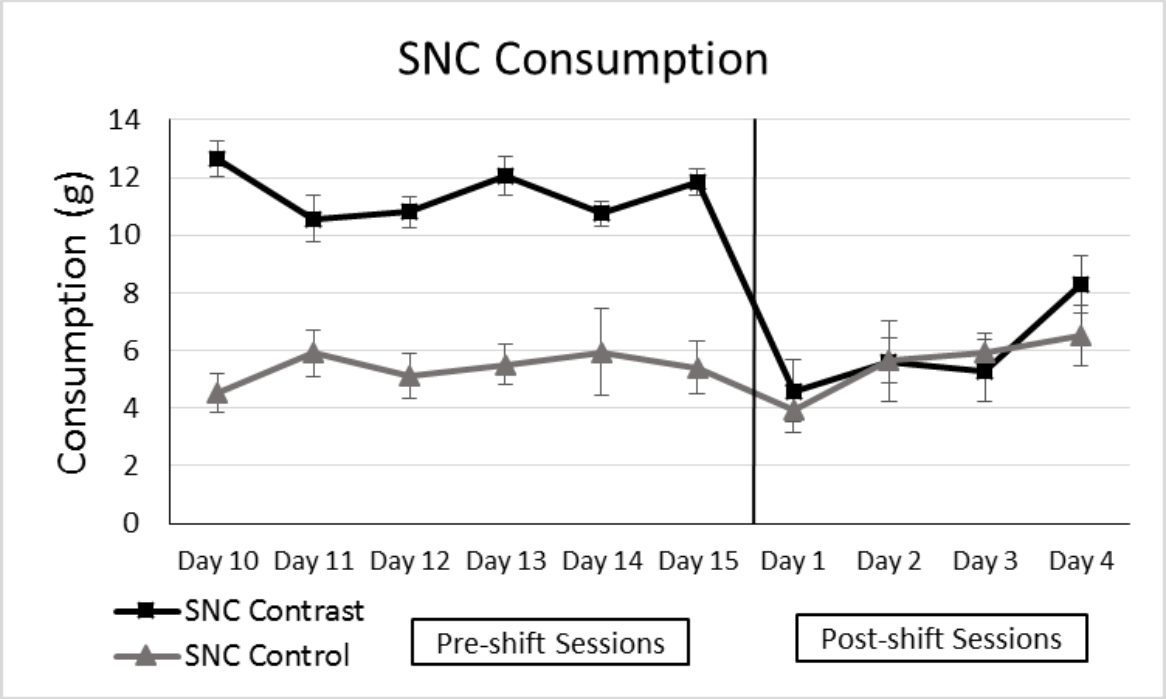

Figure 1. SNC Consumption. Intake (g) of 32% sucrose (SNC Contrast group) and 0.15% saccharin (SNC Control) consumed across the last 6 pre-shift days preceding the downshift, and saccharin intake (g) for both SNC groups across the 4 post-shift days. The vertical line marks the downshift. Error bars are standard error of the mean.

Post-shift Successive Negative Contrast Groups Consumption. Figure 1 (right side) shows the intakes during the four post-shift days. The change from 32% sucrose (pre-shift days) to a saccharin solution (post-shift days) substantially reduced solution intake in the SNC Contrast group. This substantial drop in intake can be explained by the replacement of a highly preferred solution (32% sucrose) with a much less preferred solution (0.15% saccharin). A SNC effect, however, requires a comparison of saccharin intake in groups with different prior experiences with the solutions. Thus a SNC effect would be indicated in the post-shift data as a significant

interaction with significantly lower saccharin intake in the shifted group relative to the control group at least on day 1.

The 4 post-shift days were analyzed as a 2 (Groups) x 4 (Days) mixed factorial design, to determine the effects of group and day on post-shift 0.15% saccharin intake. However, the interaction of days and groups was not significant, $F(3, 42) = 2.028$, $p = 0.125$, $\eta^2_p = 0.125$. The main effect of groups on saccharin consumption was also not significant, $F(1, 14) = 0.120$, $p = 0.734$, $\eta^2_p = 0.008$, indicating that both groups consumed similar amounts of saccharin in post-shift days. The main effect of days on saccharin consumption was significant, $F(3, 42) = 12.715$, $p < 0.001$, $\eta^2_p = 0.476$, as both groups showed increasing saccharin intake over the 4 post-shift saccharin days. Taken together, the SNC Contrast and SNC Control groups consumed a similar amount of 0.15% saccharin in each of the 4 post-shift days, indicating a lack of an SNC effect.

The absence of a group difference in saccharin intake on the first post-shift day may be partly due to the slight reduction in saccharin intake in the non-shifted control group (SNC-SACC). This is surprising since the SNC Control group demonstrated stable saccharin consumption over the last 5 pre-shift days, and, since the solution was unchanged, should not have experienced a decline in saccharin intake on the first post-shift day. A dependent *t*-test comparing the mean pre-shift intake with the first post-shift intake yielded a marginally significant decline in the SNC Control group, $p = 0.08$, suggesting that there was a small but unexpected drop in performance in the control animals that may have obscured the measure of SNC. A similar *t*-test with the SNC Contrast group yielded a statistically significant drop in solution intake, $p < 0.001$.

Because of this unexpected performance in the control animals I evaluated the post-shift data in the two groups with separate one-way ANOVAs. These ANOVAs revealed significantly

increased consumption across the 4 post-shift sessions in both groups. There was a significant main effect of post-shift day on SNC-control group intake, $F(3, 21) = 5.815$, $p = 0.005$, $\eta^2_p = 0.454$, indicating consumption of the saccharin solution depended on post-shift day. The SNC control group consumed significantly less saccharin on the first day than the second (Mean Difference = 1.7, SE = 0.4, $p = 0.026$) and fourth (Mean Difference = 2.6, SE = 0.6, $p = 0.02$) post-shift days, indicating post-shift consumption was not stable. There was also a significant main effect of post-shift day on SNC Contrast group intake, $F(1, 10) = 8.409$, $p = 0.010$, $\eta^2_p = 0.546$, indicating consumption of the saccharin solution depended on post-shift day. The SNC Contrast group consumed significantly more saccharin on the fourth post-shift day than the first (Mean Difference = 3.7, SE = 0.4, $p < 0.001$) and second day (Mean Difference = 2.7, SE = 0.7, $p = 0.031$). Taken together, both SNC groups demonstrated increased consumption across the 4 post-shift days, which was expected in only the SNC Contrast group as they recovered from the experience of negative contrast.

WS-ANC Contrast Group. A within-subjects ANC group of rats received access to a 0.15% saccharin solution daily, followed by access to the same solution (SACC/SACC) or a 32% sucrose solution (SACC/SUCR) on alternating days, with context cues used for each type of day to indicate the solution in the second bottle. Figure 2 plots the saccharin intakes of the ANC group during ANC training. A control day (SACC/SACC) and a contrast day (SACC/SUCR) is defined as a block of training. Therefore there were 15 2-day blocks of training. A 2 (second bottle) X 15 (block) repeated measures ANOVA was calculated to determine the effect of the second bottle on consumption of the first bottle across the 15 blocks. The main effect of day was significant, $F(14, 98) = 3.751$, $p < 0.001$, $\eta^2_p = 0.349$ indicating that in general intakes were lower very early in training compared to later in training. More importantly, the main effect of

second bottle on first bottle consumption was also significant, $F(1, 7) = 42.868$, $p < 0.001$, $\eta^2_p = 0.860$. On SACC/SACC days the WS-ANC group (Mean = 5.3, SE = 0.4) consumed significantly more saccharin from the first bottle than on SACC/SUCR days (Mean = 4.5, SE = 0.4) supporting the presence of an ANC effect. The interaction between training block and second bottle was also significant, $F(14, 98) = 2.412$, $p = 0.006$, $\eta^2_p = 0.256$, indicating that saccharin consumption from the first bottle depended on both second bottle content and block of training.

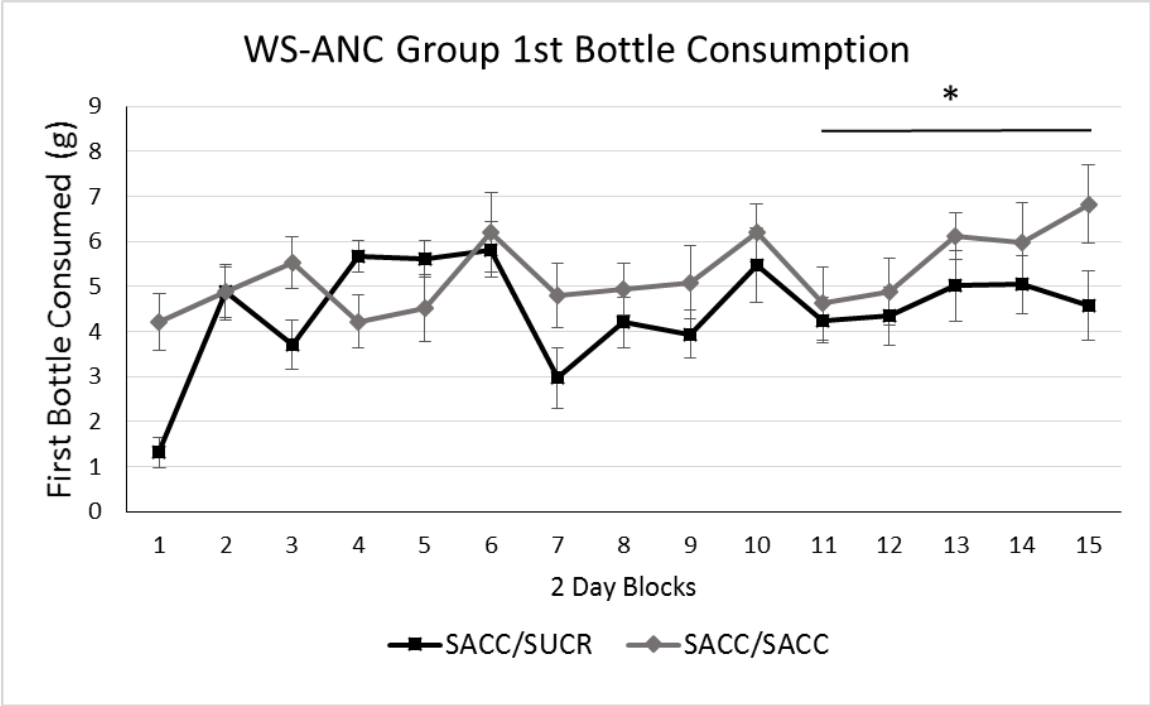

Figure 2. WS-ANC Group 1st Bottle Consumption. Amount (g) of the first bottle consumed in the WS-ANC group in the 15 2-day blocks (SACC/SUCR; SACC/SACC). Error bars are standard error of the mean. (*) indicates a significant difference between groups.

The nature of this interaction can be clearly seen in Figure 2. During the first 6 blocks of training the intakes during the two treatment conditions (SACC/SUCR or SACC/SACC) were inconsistent, with neither condition consistently producing greater intake over the other. Although intake on SAC-SUCR intake was substantially lower than on the SACC-SACC day in

Block 1, (Mean Difference = 4.4, SE = 0.6, $p = 0.017$), this difference cannot be an anticipatory contrast effect because ANC is acquired over several days of training (Flaherty & Rowan, 1986). The difference in block 1 is most likely due to unfamiliarity with the procedure since the bottles were presented for the first time on the SACC-SUCR day of block 1. Beginning in the 7th 2-day block, however, the WS-ANC group consistently consumed more saccharin from the first bottle on SACC/SACC days than on SACC/SUCR days, suggesting that by Block 7 ANC was acquired.

WS-ANC First Bottle Consumption in the Final 5 blocks. The final 5 blocks were examined separately to confirm the presence of an ANC effect. In the last 5 blocks of 2 days, consumption of the first bottle in the WS-ANC group was consistently lower by about 1 gram when the second bottle was sucrose (Figure 2). To confirm that a consistent ANC effect was acquired by the end of training the last 5 blocks were analyzed as a 2 (second bottle) x 5 (blocks) repeated measures ANOVA. The main effect of block was not significant, $F(4, 28) = 2.231$, $p = 0.091$, $\eta^2_p = 0.242$, indicating consumption did not vary across the final 5 blocks in the WS-ANC group. The main effect of second bottle was significant, $F(1, 7) = 13.932$, $p = 0.007$, $\eta^2_p = 0.666$, indicating the second bottle influenced consumption of the first in the WS-ANC group. In the final 5 blocks, the WS-ANC group consumed on average about 1 gram less of the first bottle on days it was followed by sucrose (Mean Difference = 1.0, SE = 0.3, $p = 0.007$). The interaction between second bottle and block was not significant, $F(4, 28) = 1.261$, $p = 0.308$, $\eta^2_p = 0.153$, indicating the effect of second bottle on consumption of the first bottle was stable and did not vary by block.

Intermittent Ethanol Exposure Sessions. Prior to the post-shift manipulation the ethanol intake in all groups was very low. To address this problem all rats were given

intermittent exposure to ethanol in their home cages overnight. Figure 3 displays the pattern of home cage intermittent ethanol consumption across the 5 sessions. All groups appeared to steadily increase ethanol intake across these 5 sessions.

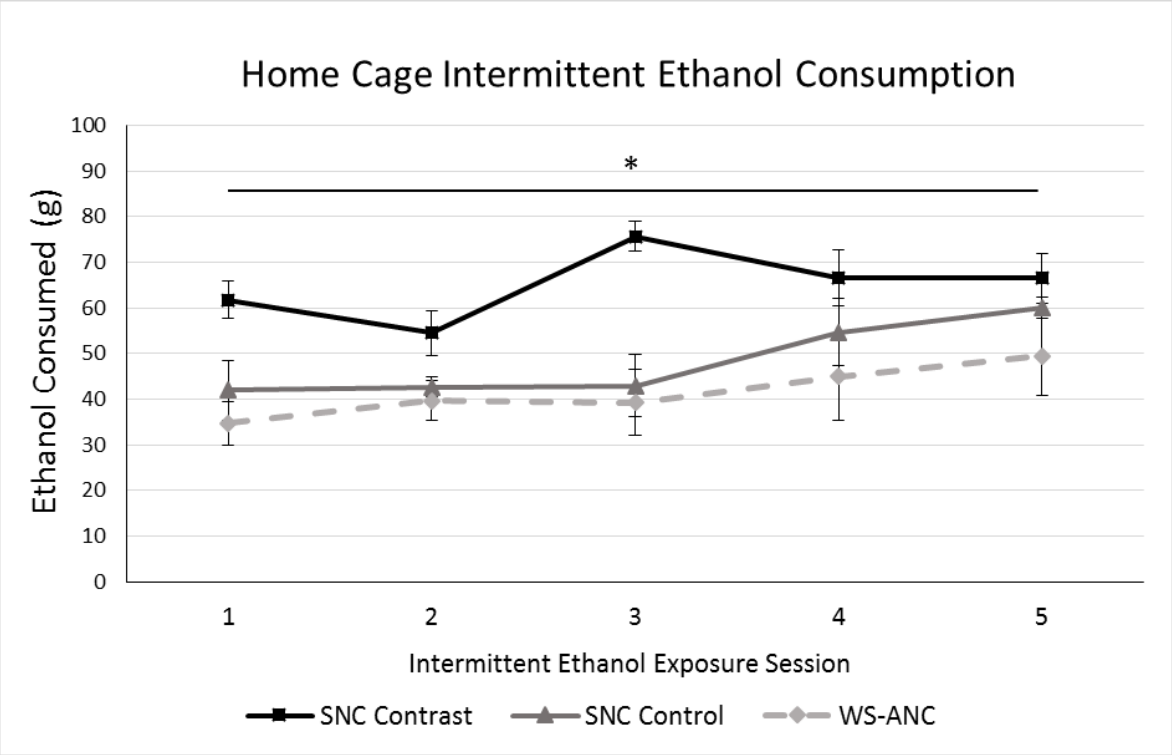

Figure 3. Home Cage Intermittent Ethanol Consumption. Ethanol (g) consumption by the SNC Contrast, SNC Control, and WS-ANC groups in 5 home cage intermittent ethanol exposure sessions across 10 days. Intake (g) is for combined total of the pair-housed rats in each 24 hour session. The pairs of rats increased ethanol intake over the 5 intermittent sessions. Error bars are standard error of the mean.

To determine if there were any group differences in ethanol intake during this experience the daily intake data were analyzed with a 3 (Group [SNC Contrast, SNC Control, WS-ANC]) x 5 (intermittent sessions) mixed ANOVA. The main effect of day on intermittent ethanol consumption was significant, $F(4, 36) = 3.57$, $p = 0.015$, $\eta^2_p = 0.284$, indicating the overall ethanol intakes increased over days. On day 5 of the intermittent ethanol exposure procedure, the groups consumed significantly more ethanol than on days 1 and 2 (Day 1: Mean Difference =

12.5, SE = 4.4, $p = 0.018$; Day 2: Mean Difference = 13.0, SE = 3.2, $p = 0.003$). Thus the intermittent ethanol exposure procedure successfully increased ethanol intake. Although the interaction of day and group was not significant, $F(8, 36) = 1.172$, $p = 0.342$, $\eta^2_p = 0.207$, the main effect of group was statistically significant, $F(2, 9) = 10.323$, $p = 0.005$, $\eta^2_p = 0.696$. The SNC contrast group consumed significantly more ethanol during the intermittent exposure days than the SNC control group (Mean Difference = 16.5, SE = 5.3, $p = 0.037$), and WS-ANC group (Mean Difference = 23.4, SE = 5.3, $p = 0.005$). The groups were returned to their respective procedures for 3 days before the downshift, and consumption of all solutions returned to similar levels as before the intermittent sessions.

Absolute Ethanol Intake Measure. Ethanol intake was examined separately from ethanol preference, to determine if the groups would change absolute ethanol intake in the experiment.

Post-shift Successive Negative Contrast Ethanol Consumption. Figure 4 shows the absolute ethanol intake of the SNC groups during the post-shift days. Intakes on the last 6 pre-shift days are also included for comparison. A 2 (Group [contrast, control] X 4 (Days) mixed ANOVA with group as the between-subjects factor and days as the repeated measure was calculated for ethanol intake in post-shift sessions. Mauchly's test of sphericity was significant for days, $\chi^2(5) = 24.610$, $p < 0.001$, therefore the Greenhouse-Geisser estimate ($\varepsilon = 0.436$) was used to correct for degrees of freedom. The main effect of days was significant, $F(1, 14) = 7.281$, $p = 0.012$, $\eta^2_p = 0.398$, indicating ethanol intake varied across the four post-shift days. The main effect of group was not significant, $F(1, 11) = 1.198$, $p = 0.297$, $\eta^2_p = 0.098$, but the interaction between day and group was significant, $F(3, 33) = 4.766$, $p = 0.037$, $\eta^2_p = 0.302$, indicating ethanol intake varied between groups across post-shift days. As can be seen in Figure

4 the largest group differences occurred on the first 2 post-shift days with the SNC-Contrast groups showing the greatest intakes. Additional one-way ANOVAs revealed a non-significant difference between groups on the first post-shift day, $F(1, 13) = 2.271$, $p = 0.156$, but a significant difference on the second post-shift day, $F(1, 14) = 5.386$, $p = 0.036$. This result supports the central hypothesis, as the SNC Contrast group increased ethanol intake in post-shift sessions compared to the SNC Control group.

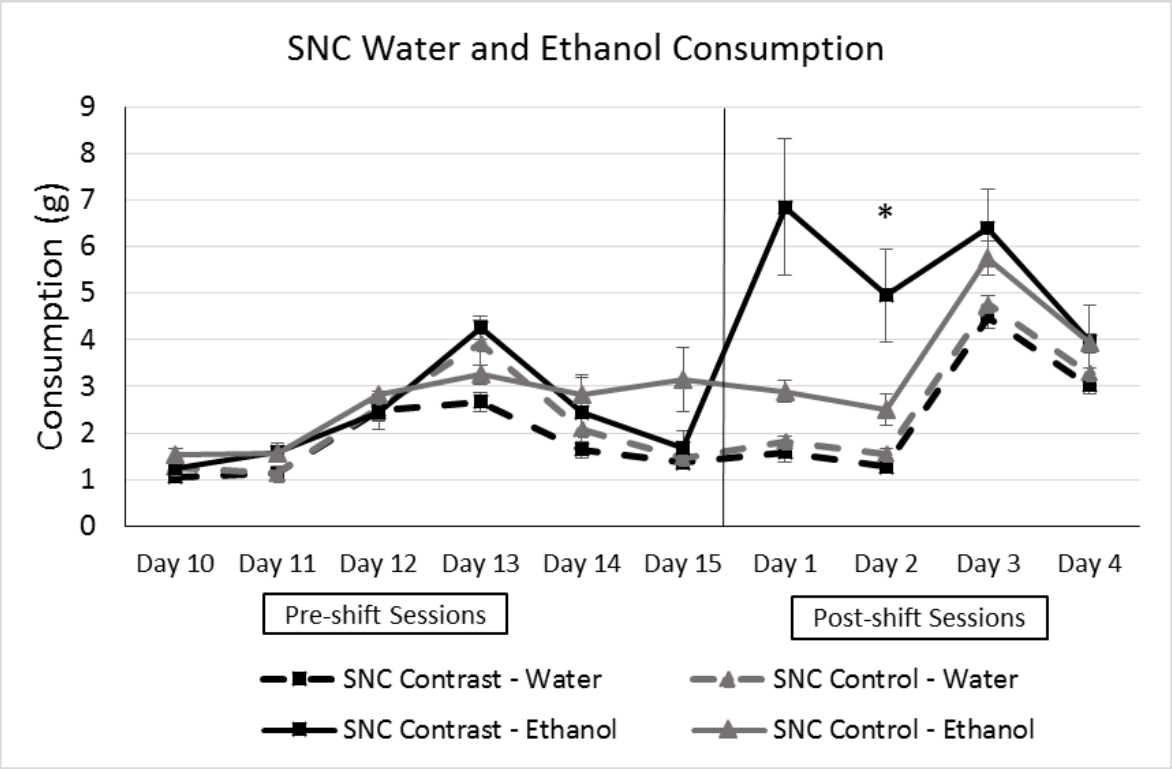

Figure 4. SNC Water and Ethanol Consumption. Consumption by weight (g) of water and ethanol by the SNC Contrast and Control groups in the 6 pre-shift ethanol choice test sessions and 4 post-shift sessions. The vertical line marks the downshift. Error bars are standard error of the mean. (*) indicates a significant difference between groups.

Ethanol Preference. Absolute intake is based on the weight of solution (water or ethanol) consumed by each rat in the post-session ethanol choice tests. However, a related yet distinct measure to further examine ethanol drinking behavior is ethanol preference, which is the

ratio of ethanol consumed to total consumption of both solutions during the post-session 2 hour period of access.

Successive negative contrast groups. The main hypothesis of this experiment is that the SNC Contrast group would increase ethanol preference in a 2 hour ethanol choice test following its daily access to a saccharin solution which was downshifted from a more rewarding sucrose solution. An increase in ethanol preference would be interpreted as self-medication due to the negative reaction induced by the decrease in reward (Manzo et al., 2015).

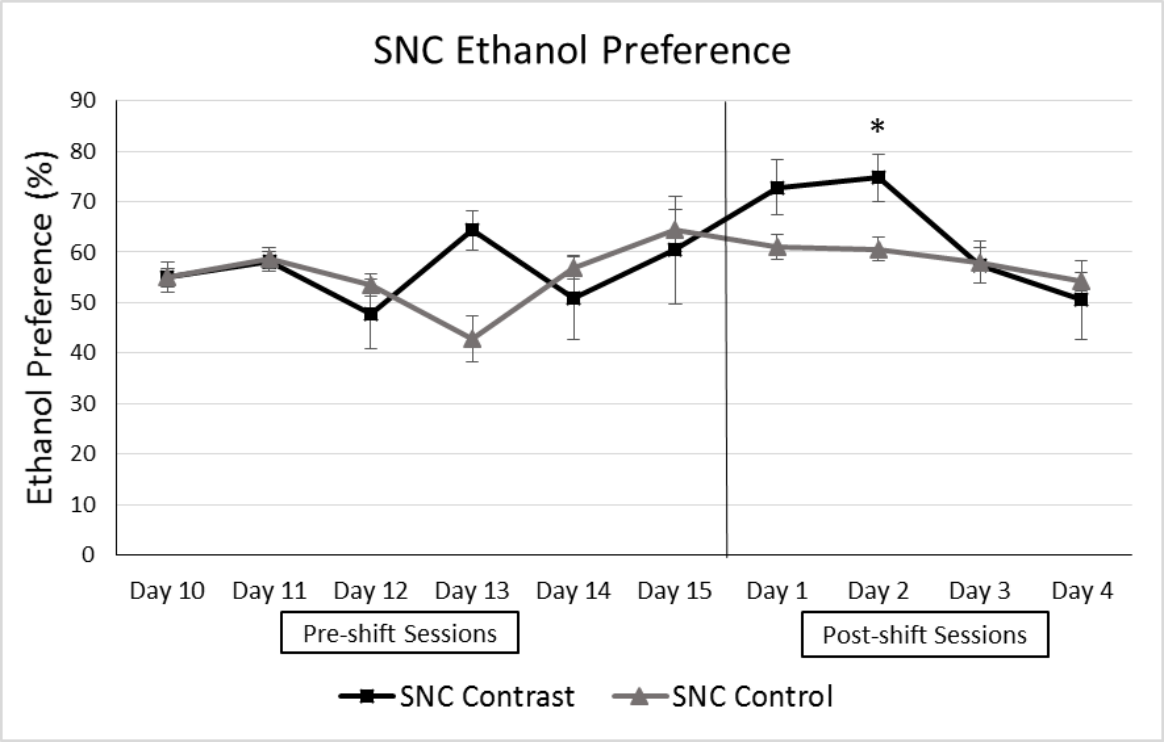

Figure 5. SNC Ethanol Preference. Ethanol Preference in the SNC Groups during the last 6 pre-shift sessions and 4 post-shift sessions in the SNC Contrast and Control groups. The vertical line marks the downshift. Error bars are standard error. (*) indicates a significant difference between groups.

Figure 5 shows the preference for Ethanol (ethanol intake relative to water intake) during the pre-shift days when the SNC groups were receiving their respective solutions. Preference appears stable for both groups during the pre-shift phase. To confirm that ethanol preference did

not change significantly during the pre-shift phase, a 2 x 6 (Group [contrast, control] x Days) mixed ANOVA was calculated, with group as the between-subjects factor and days as the repeated measure. Mauchly's test of sphericity was significant for days, $\chi^2(14) = 70.607$, $p < 0.001$, therefore, the Greenhouse-Geisser estimate was reported ($\varepsilon = 0.302$). The main effect of days on pre-shift ethanol preference was not significant, $F(5, 70) = 0.982$, $p = 0.369$, $\eta^2_p = 0.066$, indicating no difference in ethanol preference for the 6 pre-shift days. The main effect of group, $F(1, 14) = 0.875$, $p = 0.366$, $\eta^2_p = 0.059$, and the interaction between groups and days was not significant, $F(5, 70) = 1.191$, $p = 0.322$, $\eta^2_p = 0.078$, indicating no difference in ethanol preference between contrast and control groups across the 6 pre-shift days. Taken together, ethanol preference was stable across pre-shift days and did not differ between the SNC groups.

Successive negative contrast groups on post-shift days. To determine if ethanol preference changed during the post-shift phase of the experiment a 2 x 4 (Group [contrast, control] x Days) mixed ANOVA with group as the between-subjects factor and days as the repeated measure was calculated. The main effect of day on ethanol preference was significant, $F(3, 33) = 23.309$, $p < 0.001$, $\eta^2_p = 0.679$, indicating ethanol preference varied by post-shift day. Although the main effect of group was not significant, $F(1, 11) = 1.615$, $p = 0.230$, $\eta^2_p = 0.128$, the interaction between days and groups was significant, $F(3, 33) = 4.234$, $p = 0.012$, $\eta^2_p = 0.278$, indicating ethanol preference across days differed between SNC groups in the post-shift sessions, which can be seen in Figure 5. While the groups did not differ in preference in the first post-shift day, an independent-samples *t*-test indicated increased preference in the SNC Contrast group compared to the SNC control group on the second post-shift day, $t(14) = 2.682$, $p = 0.018$.

SNC Ethanol Preference compared with the ANC group. I also compared ethanol preference between the SNC groups and the WS-ANC group to determine if preference would be

similar between groups on pre-shift days, and if preference during down-shift days would be higher in the SNC Contrast group compared to the WS-ANC group, as hypothesized.

The WS-ANC group did not experience a pre-shift phase followed by a post-shift phase since they experienced a within-subject procedure. To compare ethanol preference in this group to the SNC groups, the last 4 SACC-SUCR days for the WS-ANC group was compared with the 4 post-shift days of the SNC Contrast and Control groups (Figure 6) using a 3 (Groups) x 4 (Days) mixed factorial ANOVA. Mauchly's test of sphericity was significant for day, ($\chi^2(5) = 21.312$, $p = 0.001$), therefore the Greenhouse-Geisser estimate ($\varepsilon = 0.645$) was used to adjust degrees of freedom. There was a significant main effect of day on ethanol preference, $F(1, 40) = 6.333$, $p = 0.004$, $\eta^2_p = 0.232$. The main effect of group on ethanol preference was also significant, $F(2, 21) = 5.835$, $p = 0.010$, $\eta^2_p = 0.357$. More importantly, there was a significant interaction between day and group, $F(3, 40) = 4.057$, $p = 0.008$, $\eta^2_p = 0.279$. On the first post-shift day the SNC contrast group increased ethanol preference compared to the WS-ANC group (Mean Difference = 21.2, SE = 5.0, $p = 0.001$). On the second post-shift day the SNC contrast group increased ethanol preference compared to the SNC control (Mean Difference = 14.1, SE = 4.5, $p = 0.016$) and WS-ANC group (Mean Difference = 22.4, SE = 4.5, $p < 0.001$). Taken together, ethanol preference in post-shift sessions was increased in the SNC Contrast group compared to the SNC Control and WS-ANC groups (Figure 6), supporting the hypothesis that the downshift induces a negative reaction which leads to self-medicating behavior, as indicated by increased preference for ethanol. There was hypothesized to be no negative reaction in the SNC Control or WS-ANC groups, and ethanol preference was similar between these groups and stable across days.

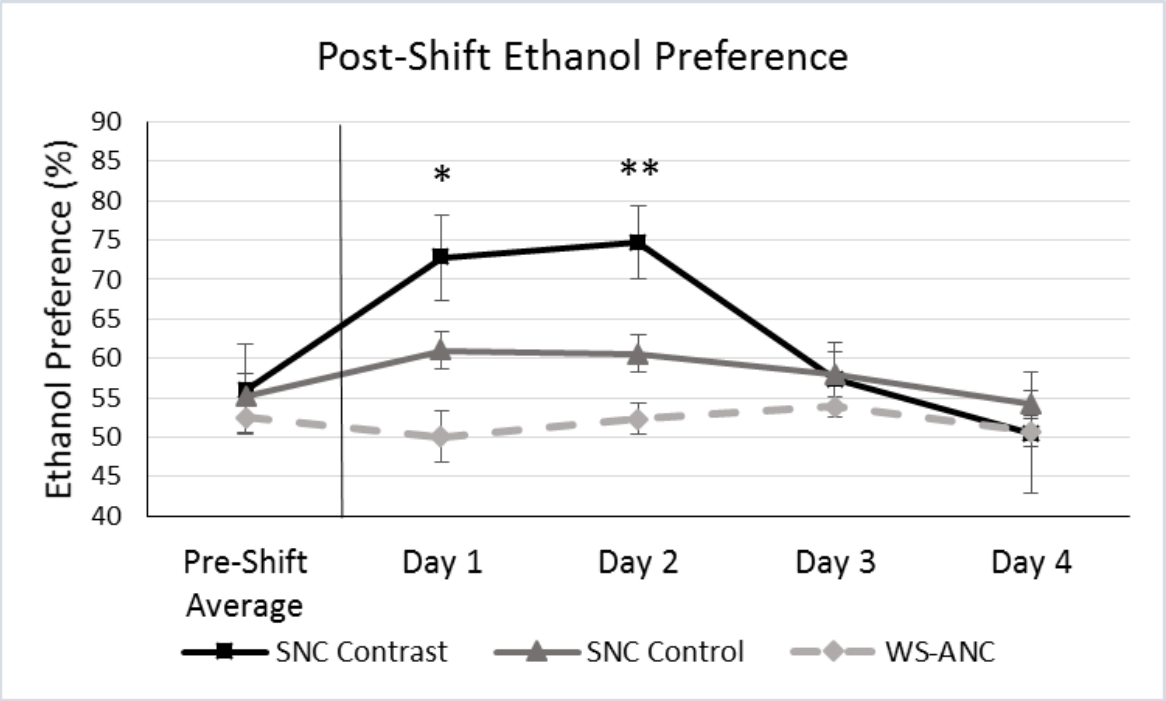

Figure 6. Post-shift Ethanol Preference. Ethanol preference for the SNC Contrast, SNC Control, and WS-ANC groups in the 4 post-shift sessions. Also included is the average ethanol preference for these groups in pre-shift sessions. Data for WS-ANC group is from contrast (SACC-SUCR) days. The vertical line marks the downshift. Error bars are standard error of the mean. (*) indicates significant difference between SNC groups. (**) indicates significant difference between the SNC Contrast and both the SNC Control and WS-ANC groups.

WS-ANC ethanol preference. Because the WS-ANC group experienced contrast and control conditions on alternating days it is of interest to determine if ethanol preference changed depending on their experience in the contrast chambers. The self-medication hypothesis predicts increased ethanol preference only when there is an emotional response that can provide an opportunity for negative reinforcement. Because the ANC effect is due to the anticipation of a highly desired reward rather than due to the elicitation of an aversive emotional state an increase in ethanol preference on a SACC-SUCR day relative to a SACC-SACC day is not predicted. As displayed in Figure 7 (below), ethanol preference did not differ between these 2 days in the WS-ANC group.

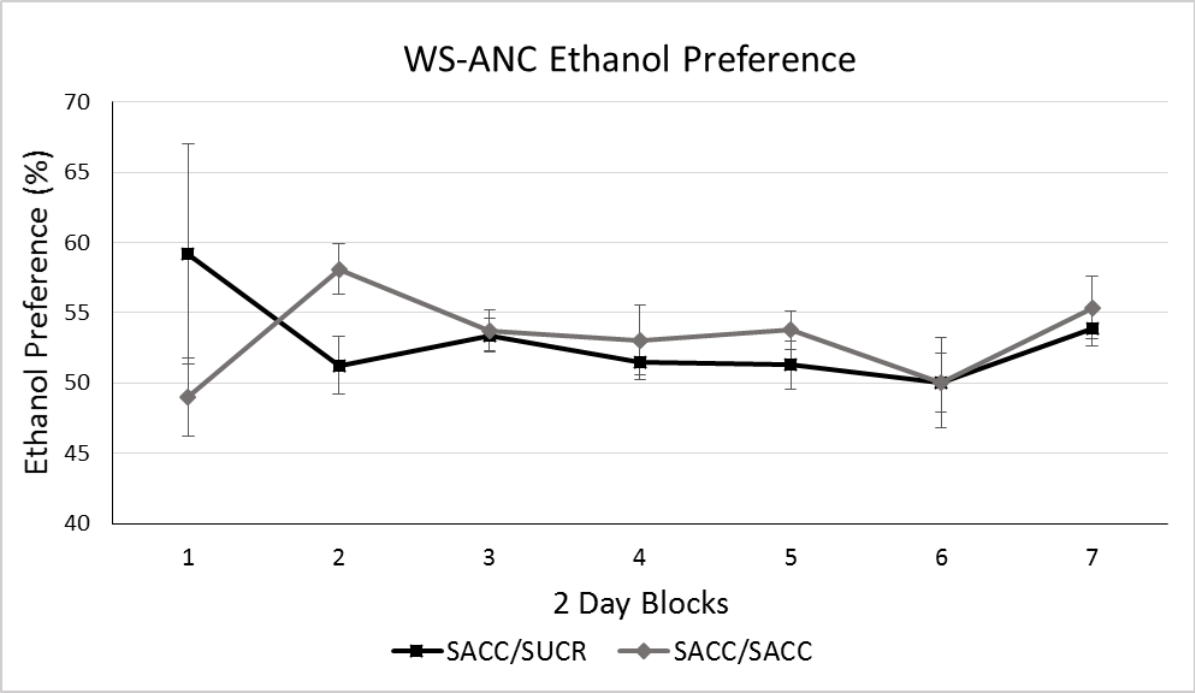

Figure 7. WS-ANC Ethanol Preference. Ethanol preference for the within-subjects ANC procedure across 15 two day blocks of Contrast (SACC/SUCR), and Control (SACC/SACC) days. Error bars are standard error of the mean.

To determine the effect of second bottle and blocks on WS-ANC ethanol preference a 2 (second bottle) X 7 (2 day block) repeated measures ANOVA was calculated. Mauchly's test of sphericity was violated for the effect of 2 day blocks, $\chi^2(20) = 39.618$, $p = 0.007$, therefore the Greenhouse-Geisser estimate ($\varepsilon = 0.429$) was used to correct for degrees of freedom. The main effects of 2 day block, $F(2, 35) = 0.761$, $p = 0.505$, $\eta^2_p = 0.052$ and second bottle, $F(1, 14) = 0.031$, $p = 0.863$, $\eta^2_p = 0.002$, were not significant. Neither was the interaction between second bottle and 2 day block was not significant, $F(2, 35) = 1.847$, $p = 0.163$, $\eta^2_p = 0.117$. Taken together, there were no differences in ethanol preference across 2 day blocks in the WS-ANC group, and no effect of alternating the solution in the second bottle (sucrose or saccharin) on ethanol preference.

Discussion

Successive Negative Contrast Groups. On pre-shift days the SNC Contrast group consistently consumed more sucrose than saccharin consumed by the SNC Control group, as sucrose is move rewarding and preferred by rats (Flaherty et al., 1989). However, the SNC Contrast group did not decrease saccharin consumption to levels below the SNC Control group in the post-shift days, which is necessary to unequivocally demonstrate an SNC effect. The SNC Contrast group did consume more saccharin on the fourth day than on the first and second days, demonstrating the rapid recovery of intake typical in post-shift sessions (Rowan & Flaherty, 1991). However, the SNC Control group also consumed more on the second and fourth days than the first. The low intake on the first day and the gradual increase over post-shift days was unexpected (Figure 1), as this group displayed stable consumption on pre-shift days. Perhaps because of this changing baseline in consumption in the control group there was not a significant difference between groups on this important first post-shift day. This may not have a consequential impact on the overall hypothesis however, as the SNC Contrast group did experience a very large drop in intake despite the absence of a stable control group needed to detect the emotion-mediated contrast in consummatory behavior.

Ethanol consumption and preference were stable and did not differ between groups in the 6 pre-shift sessions. The SNC contrast group consumed more ethanol during the initial post-shift days than the SNC Control group. Ethanol preference was also higher in the SNC Contrast group. These results support the central hypothesis of the current study and confirm previous research (Manzo et al., 2015). The SNC downshift induced a negative reaction which caused the rats to self-medicate, increasing intake of ethanol for its anxiolytic effect. This pattern of consumption may be interpreted as negative reinforcement (Ameringer et al., 2015), as the rats

increased ethanol intake on the post-shift second day when the negative reaction is the greatest (Flaherty & Rowan, 1991).

Within-subjects Anticipatory Negative Contrast Group. Consumption of the first bottle (saccharin) in the WS-ANC group in the final 5 blocks was examined separately from the first 10 blocks in order to characterize the ANC effect when it became present in the last third of the procedure. Consumption of the initial saccharin bottle during the final 5 blocks was consistently lower on SACC-SUCR days (Figure 2) which does demonstrate this ANC effect, which is important because the WS-ANC group was used as a second control group. The assumption is that an unpleasant emotional response causes negative contrast behavior in the SNC procedure and it is the presence of the emotion that provides the opportunity for self-medication with ethanol. However, it is possible that there are other characteristics of negative contrast procedures that contribute to subsequent changes in ethanol intake independent of an emotional reaction. For example, experiencing variety among tasty solutions or engaging in the contrast behavior may in general increase exploratory behavior and therefore greater sampling of any available resources. The WS-ANC procedure as a control includes these other possible characteristics but not the emotional component. I predicted the WS-ANC group would show similar preference for and intake of ethanol after both types of sessions (SACC-SACC; SACC-SUCR), as this type of contrast procedure is not thought to induce a negative emotion (Flaherty & Rowan, 1986; Ruetti et al., 2009). As shown in Figure 7, ethanol preference remained stable in the WS-ANC group for both types of sessions, supporting the hypothesis that ethanol preference would not be different for the two types of sessions experienced in the WS-ANC group.

These results validate the use of the WS-ANC group as a second control group for a test of the self-medication hypothesis. The SNC contrast group consumed more ethanol on the first and second post-shift days than the WS-ANC group. The self-medicating behavior in the SNC contrast group is further supported by the increase in ethanol intake compared to the WS-ANC group. The SNC contrast group also increased ethanol preference on the first and second post-shift days compared the WS-ANC group.

Taken together, rats increased ethanol intake in post-shift sessions due to the negative reaction induced by the downshift in an SNC procedure. This self-medicating behavior is present only in the SNC Contrast group, as ethanol intake did not vary for the SNC Control or WS-ANC groups. While the SNC Contrast group did not demonstrate the decrease in saccharin intake relative to the SNC Control group, consumption dropped significantly compared to pre-shift days. Increased ethanol intake is also observed when a highly palatable solution is simply removed (e.g., extinction of a consummatory task, Manzo et al 2014). The WS-ANC group demonstrated an ANC effect on the final five blocks, as consumption of the initial saccharin was significantly decreased on contrast days when the more rewarding sucrose was in the second bottle. Further, this group did not consume different amounts of ethanol or change ethanol preference on SACC-SUCR and SACC-SUCR days, which allowed this group to be a second control group to further characterize the self-medicating behavior in the SNC Contrast group.

Experiment 2

The results from Experiment 1 support the central hypothesis that rats increase ethanol consumption and preference on post-shift days after experiencing a decrease in reward in a SNC procedure. This was supported by comparison to the WS-ANC group which served as a second control group and further confirmed the hypothesis. However, possibly because of unstable baseline in the SNC-control group I was not able to confirm a SNC effect. In Experiment 2 the procedures for the 3 groups (SNC Contrast, SNC Control, and WS-ANC) were extended and modified in order to further characterize the hypothesized self-medicating behavior, and to support the results from Experiment 1. The SNC Contrast and Control groups were switched for 7 additional pre-shift days, followed by two post-shift days. Thus, the previous control group now served as the contrast group which was possible because they had not previously consumed sucrose. The previous contrast group now served as the control group. SNC would be demonstrated as less saccharin consumption in the new contrast group relative to the new control group on the post-shift day. Moreover the new contrast group should now drink more ethanol on the post-shift day. The WS-ANC procedure was also continued but an SNC downshift to the second bottle on contrast days (from sucrose to saccharin) was introduced to examine if this group would demonstrate an SNC effect.

Method

Subjects. The same twenty-four male Long Evans rats from Experiment 1 were used in Experiment 2. All rats were pair-housed in standard clear plastic cages with corncob bedding on a 12 hour light/dark schedule, and maintained at about 85% of their free-feeding body weight, receiving their daily food ration approximately 30 minutes after completing all daily test procedures.

Apparatus. The contrast and two-hour ethanol choice test procedures were performed in the same rooms as discussed previously for Experiment 1.

Procedure. Two new lab assistants conducted the procedures in Experiment 2. The assistants weighed and handled the rats for a week in order for the rats to become familiar with these individuals. The general procedure was the same as Experiment 1. For each group 2 squads of 4 rats were transported to the contrast chambers for the SNC and ANC sessions. Then the squads were transported to the choice test chambers for 2 hours access to water and 2% ethanol solutions. All intakes were determined by weighing the bottles before and after the sessions.

Successive negative contrast procedure. The SNC procedure was a between-subjects design, with 7 pre-shift and 2 post-shift sessions. The previous SNC Contrast group became the new control group, and the previous SNC Control group became the new contrast group. The groups received access to sucrose or saccharin for a 5 minute session, followed by the 2 hour ethanol choice test.

Anticipatory negative contrast procedure. The within-subjects version of the ANC procedure was continued with the WS-ANC group from Experiment 1. However, a SNC procedure was superimposed on the standard within-subjects ANC procedure. As in experiment 1 the rats received 5 minutes of access to saccharin, followed by 3 minutes access to a bottle alternating between sucrose and saccharin on contrast and control days, respectively. Unlike in Experiment 1, the second bottle on contrast days (sucrose) was replaced with saccharin to implement a SNC downshift within an ANC procedure. The context was background white noise and dim light for the control days and normal conditions for the contrast days. This procedure was repeated for 10 days with 5 contrast sessions (SACC-SUCR) and 5 control

sessions (SACC-SUCR), followed by 2 post-shift blocks with 2 contrast sessions (SUCR downshifted to SACC in the second bottle) and 2 control sessions. After the WS-ANC procedure, the rats were placed in the ethanol choice cages for the 2 hour ethanol choice test.

WS-ANC Group Procedure		
Day	**Bottle 1**	**Bottle 2**
Day 1 (Contrast)	Saccharin	Sucrose
Day 2 (Control)	Saccharin	Saccharin
Day 3 (Contrast)	Saccharin	Sucrose
Day 4 (Control)	Saccharin	Saccharin
Day 11 (Contrast)	Saccharin	**Saccharin**
Day 12 (Control)	Saccharin	Saccharin
Day 13 (Contrast)	Saccharin	**Saccharin**
Day 14 (Control)	Saccharin	Saccharin

Table 2. WS-ANC Group Procedure. The second bottle on contrast days (Sucrose) was downshifted to Saccharin for the final 2 blocks (Days 11 and 13).

Ethanol choice tests. After each SNC or ANC session in the contrast chambers, the rats were moved to the ethanol choice test cages for the 2 hour ethanol choice test, and then transported back to their home cages. Ethanol intake was measured by weight (g), and ethanol preference was calculated as the ratio between ethanol and total consumption of both bottles in the choice test.

Statistical Analyses. All statistical analyses were similar to Experiment 1, and SPSS statistical software was used for all statistical analyses. Analysis of variance (ANOVA) was calculated for each dependent variable with significance considered at $p < 0.05$.

Results

The SNC Contrast and Control groups were reversed for Experiment 2. Therefore, the SNC Contrast group became the Control group and the SNC Control group became the Contrast group.

Successive Negative Contrast Groups. Figure 8 shows the intakes for both groups during the 7 days of SNC training, followed by the downshift from sucrose to saccharin, which lasted two additional days. The new SNC Contrast group steadily increased consumption of sucrose during these 7 pre-shift days (Figure 8, below), consuming consistently more than the new SNC Control group consuming saccharin.

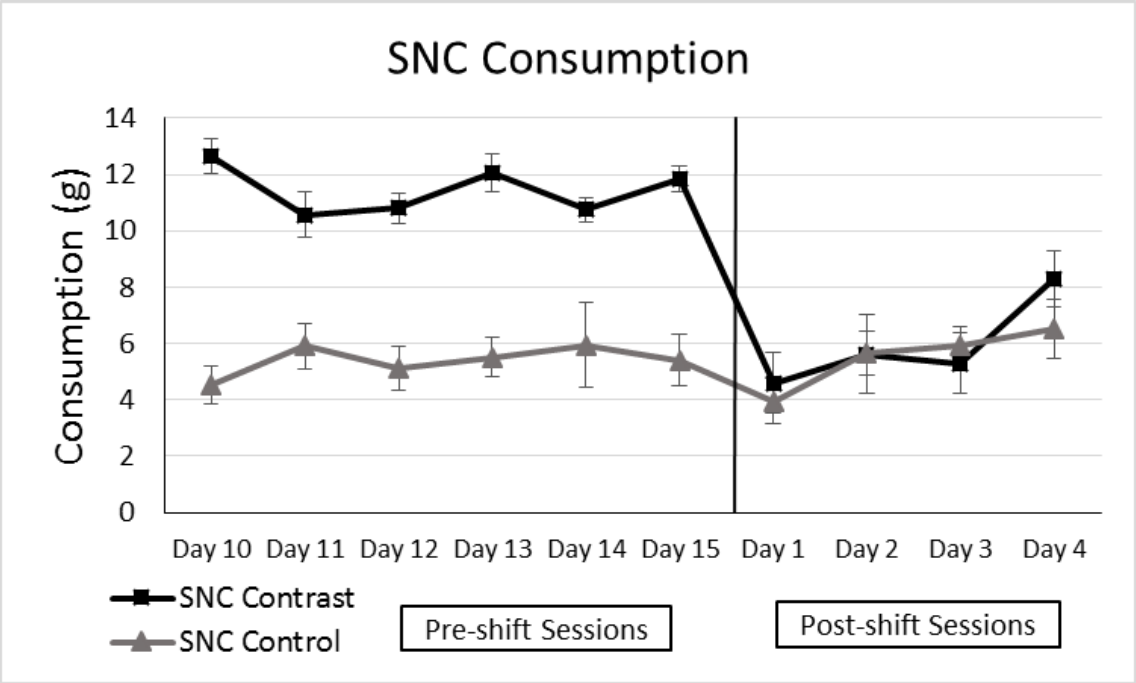

Figure 8. SNC Consumption. The SNC Contrast group steadily increased consumption of sucrose, while the SNC Control group maintained consistent, low consumption of saccharin. On the post-shift days, consumption of saccharin was similar for both groups.

A 2 (group) X 7 (day) mixed ANOVA was calculated for SNC pre-shift sessions to examine consumption before the downshift. Mauchly's test of sphericity was violated for days, $\chi^2(20) = 42.411$, $p = 0.003$, therefore the Greenhouse-Geisser estimate ($\varepsilon = 0.441$) was used to correct for degrees of freedom. The main effect of group was significant, $F(1, 14) = 31.065$, $p < 0.001$, $\eta^2_p = 0.689$, indicating pre-shift consumption of sucrose was significantly higher than consumption of saccharin in the respective groups. Although the main effect of day was not

significant, $F(2, 37) = 2.788$, $p = 0.060$, $\eta^2_p = 0.166$, a statistically significant interaction between day and group, $F(2, 37) = 6.705$, $p = 0.001$, $\eta^2_p = 0.324$, indicated that the group differences in pre-shift consumption depended on day. Pre-shift sucrose consumption increased steadily over the 7 days for the SNC Contrast group. This pattern may be due to learning the caloric value of sucrose.

As Figure 8 displays, the SNC Contrast group decreases post-shift saccharin intake to levels below the SNC Control group. A 2 (group) X 2 (day) mixed factorial ANOVA was calculated for post-shift consumption to determine of the downshifted SNC Contrast group displayed an SNC contrast effect, consuming less saccharin on post-shift days than the SNC Control group. Both the main effects of group ($F(1, 13) = 0.242$, $p = 0.631$, $\eta^2_p = 0.018$), and day ($F(1, 13) = 2.503$, $p = 0.138$, $\eta^2_p = 0.161$), as well as the interaction between day and group ($F(1, 13) = 4.091$, $p = 0.064$, $\eta^2_p = 0.239$) were not significant, indicating no difference in post-shift saccharin consumption. However, this interaction was marginally significant, and the effect size was large, indicating a significant interaction may be present with a larger sample size, and suggesting a marginally significant SNC effect. In addition, saccharin consumption was very low in the new control group suggesting that a floor effect precluded the confirmation of a SNC effect (see discussion).

SNC Absolute Ethanol Intake. Figure 9 shows the absolute intake of the two SNC groups during the pre-shift and the post-shift days. The SNC Contrast and SNC Control groups received 2 hours of access to water and ethanol bottles in the post-session tests. Separate ANOVAs were calculated for pre-shift and post-shift ethanol consumption and preference to determine if the downshift increased intake of ethanol in the SNC Contrast group compared to the SNC Control group.

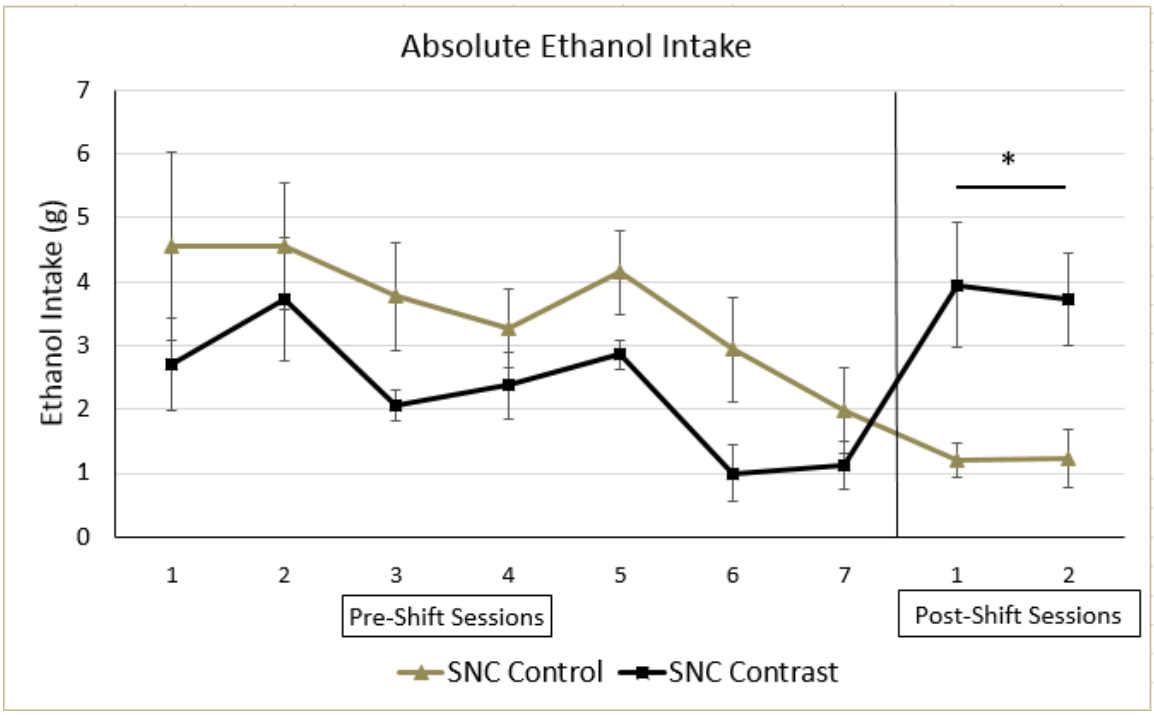

Figure 9. Absolute Ethanol Intake. Absolute ethanol intake in pre-shift and post-shift sessions by the SNC groups. Intake increased significantly in the SNC Contrast group in post-shift sessions compared to the SNC Control group. (*) Indicates a significant difference between groups.

A 2 (groups) X 7 (days) mixed ANOVA was calculated for pre-shift ethanol consumption. Mauchly's test of sphericity was significant for day, $\chi^2(20) = 39.447$, $p = 0.008$, therefore the Greenhouse-Geisser estimate ($\varepsilon = 0.430$) was used to correct for degrees of freedom for days. The main effect of day was significant, $F(2, 30) = 5.902$, $p = 0.004$, $\eta^2_p = 0.330$, indicating consumption decreased over the pre-shift days. The main effect of group was not significant, $F(1, 12) = 0.779$, $p = 0.395$, $\eta^2_p = 0.061$, indicating ethanol intake did not differ between the groups in pre-shift sessions. The interaction between group and day was not significant, $F(2, 30) = 0.503$, $p = 0.656$, $\eta^2_p = 0.040$, indicating the difference in ethanol intake by day did not depend on group. Taken together, the SNC Contrast and Control groups consumed similar amounts of ethanol on pre-shift days.

A 2 (group) X 2 (day) mixed ANOVA was calculated for post-shift ethanol intake. There was a non-significant main effect of day, $F(1, 14) = 0.036$, $p = 0.851$, $\eta^2_p = 0.003$, indicating ethanol intake was not different between the post-shift days. The main effect of group was significant, $F(1, 14) = 11.674$, $p = 0.004$, $\eta^2_p = 0.455$, indicating intake was higher for the SNC Contrast group on post-shift days. The interaction between day and group was not significant, $F(1, 14) = 0.054$, $p = 0.819$, $\eta^2_p = 0.004$. This finding confirms the results from Experiment 1 and the central hypothesis, that the downshift induces a negative emotional reaction which may be ameliorated by post-session increases in ethanol intake.

SNC Ethanol Preference. Pre-shift ethanol preference was examined separately to further determine ethanol consumption behavior in the SNC groups

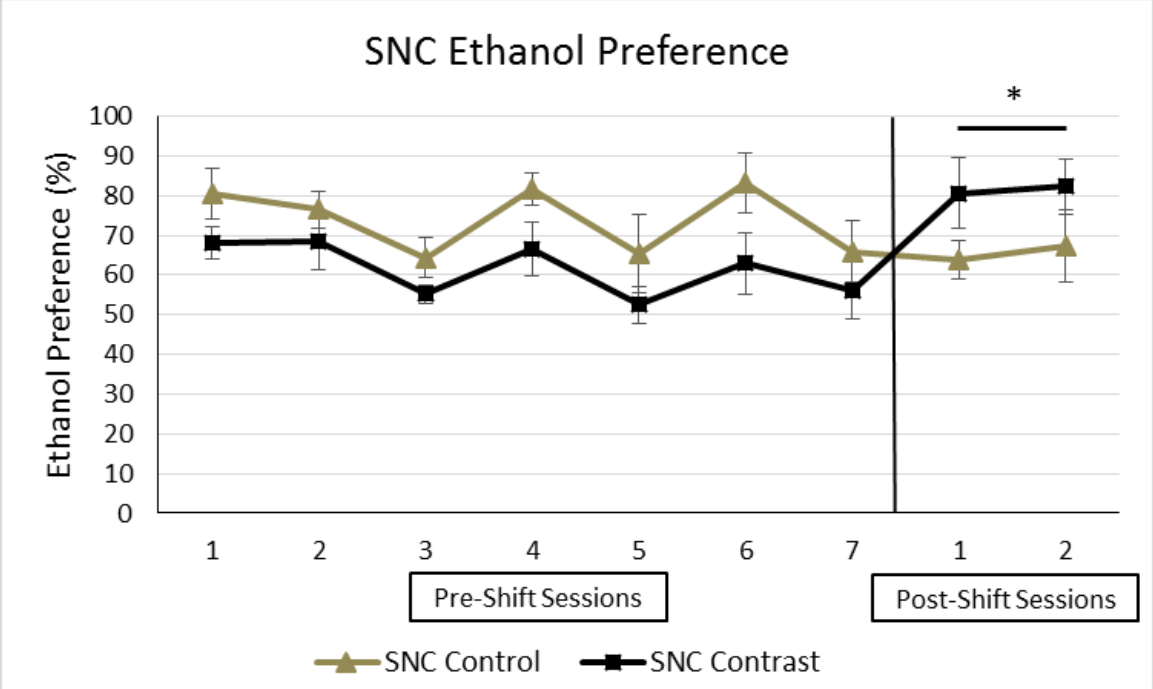

Figure 10. SNC Ethanol Preference. Ethanol preference for the SNC groups in the 7 pre-shift and 2 post-shift sessions. Preference increased in the SNC contrast group after the downshift from sucrose to saccharin.

A 2 (groups) X 7 (days) mixed ANOVA was calculated for ethanol preference in pre-shift sessions. As can be seen in Figure 10 both groups preferred ethanol, drinking reliably above 50%. Although the SNC Control group appears to show a generally stronger preference for ethanol than the SNC Contrast group, neither the main effect of group, $F(1, 12) = 3.339$, $p = 0.093$, $\eta^2_p = 0.218$, nor the interaction, $F(6, 72) = 0.396$, $p = 0.773$, $\eta^2_p = 0.032$, were statistically significant. Some day-to-day fluctuation during pre-shift days may explain the statistically significant main effect of days, $F(6, 72) = 3.594$, $p = 0.004$, $\eta^2_p = 0.230$. Preference on pre-shift day 3 was significantly lower than on day 1 (Mean Difference = 12.8, SE = 3.3, $p = 0.046$).

Post-shift ethanol preference was examined as well, to further determine the pattern of ethanol intake in the SNC groups. A 2 (group) X 2 (day) mixed ANOVA yielded only a statistically significant main effect of group, $F(1, 12) = 5.449$, $p = 0.038$, $\eta^2_p = 0.312$, during the post-shift days again supporting the result from Experiment 1, as well as the self-medication hypothesis.

WS-ANC Group. In the WS-ANC group, the sucrose bottle was replaced with saccharin on the final two SACC-SUCR days to implement a SNC procedure within the ANC procedure. Therefore the SACC-SUCR sessions were replaced with SACC-SACC in these final two blocks. Downshifting the sucrose in the second bottle to saccharin should disrupt ANC because the rats should no longer reduce their intake in anticipation of the sucrose.

First Bottle Intake. To examine if ANC was disrupted after a post-shift reduction in sucrose reward the intakes of saccharin on the two post-shift blocks were plotted in Figure 11 with the intakes from the last pre-shift block included for comparison. A downshift would not alter ANC on the first post-shift block because the rats would not have yet experienced the decrease in reward in the second bottle (from sucrose to saccharin). Therefore, ANC should not

be disrupted until after the first post-shift experience. This pattern of consummatory behavior is clearly seen in Figure 11. The differences in saccharin intake in the first bottle seen during the ANC procedure was still present during the first post-shift block but was no longer observed in the second post-shift bloc. A 2 (post-shift block; 1 or 2) X 2 (day; SACC-SACC or SACC-SUCR) mixed ANOVA was calculated for first bottle intakes to confirm this interpretation of the post-shift intakes. As expected, the interaction between post-shift block and original contrast condition was statistically significant, $F(2, 14) = 7.98$, $p = 0.020$, $\eta^2_p = 0.418$, confirming ANC on the first post-shift block, but not the second post-shift block. This disruption of ANC contrast by removing the anticipated sucrose solution is consistent with the assumption that the within-subjects ANC effect is due to the rats' anticipation of the preferred sucrose solution.

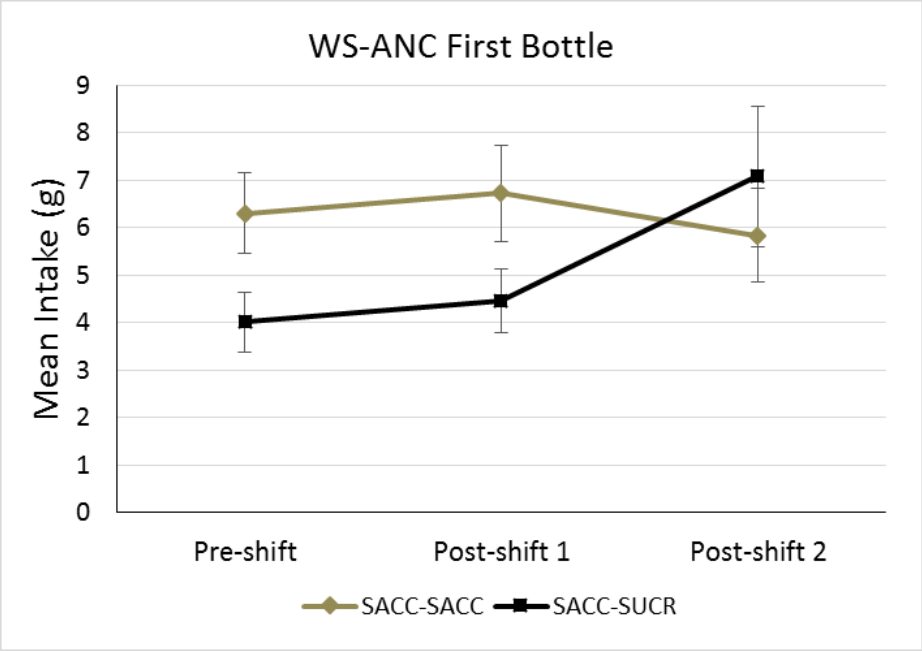

Figure 11. WS-ANC First Bottle. The ANC effect was present in the first post-shift block, but not the second block.

Second Bottle Intake. The first bottle intakes confirmed that the anticipation of a sucrose reward was no longer influencing the rats' consummatory behavior after the post-shift. The

intake of the second bottle was examined in Experiment 2 because of the SNC downshift implemented in this procedure. The next question is whether the rats would show a SNC effect, and the associated negative emotional reaction, when the second bottle was downshifted from SUCR to SACC during the last to session blocks?

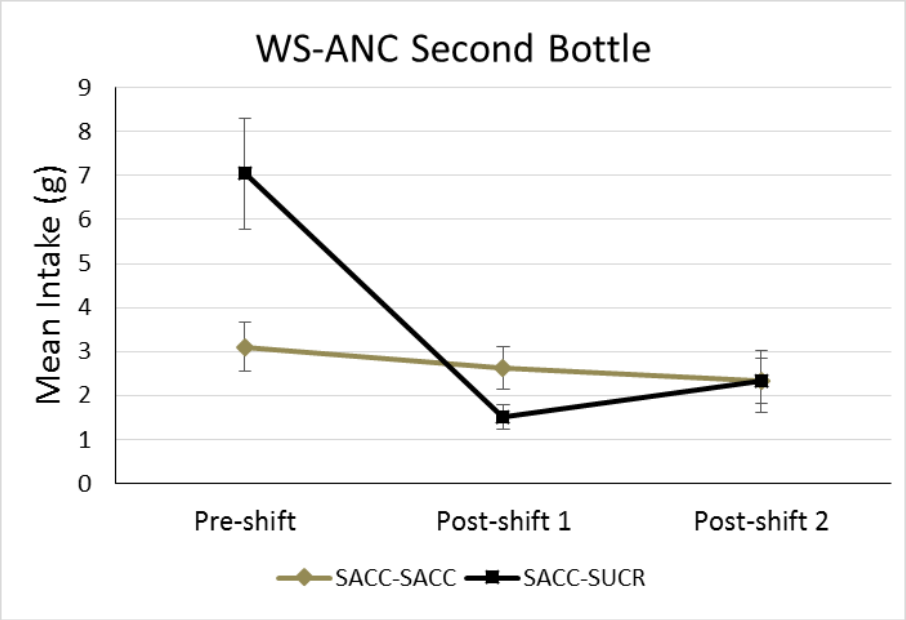

Figure 12. WS-ANC Second Bottle. Intake (g) of the second bottle by the WS-ANC group in the final pre-shift block, and the 2 post-shift blocks.

Intakes on the last pre-shift block and the two post-shift blocks are shown in Figure 12. A 1 (block) X 2 (day [SACC-SACC; SACC-SUCR]) mixed ANOVA was calculated to determine differences in pre-shift second bottle consumption in the WS-ANC group. The main effect of day was significant, $F(1, 7) = 14.945$, $p = 0.006$, $\eta^2_p = 0.681$, indicating higher consumption on the pre-shift SACC-SUCR day, which is expected, as sucrose is more preferable and rewarding to the rats than saccharin. This pre-shift block was analyzed separately from the two post-shift blocks. A 2 (block [post-shift block 1; post-shift block 2) X 2 (day [SACC-SACC; SACC-SUCR]) mixed ANOVA was calculated to determine if there was an SNC effect

within the ANC procedure. The main effects of block, $F(1, 7) = 0.785$, $p = 0.405$, $\eta^2_p = 0.101$, and day, $F(1, 7) = 1.904$, $p = 0.210$, $\eta^2_p = 0.214$, were not significant, nor was the interaction between block and day, $F(1, 7) = 1.411$, $p = 0.274$, $\eta^2_p = 0.168$. This indicates no difference in second bottle consumption between SACC-SACC and SACC-SUCR days in the post-shift blocks, which means the SNC downshift did not elicit an SNC effect in the WS-ANC group.

Absolute Ethanol Intake. Ethanol consumption (Figure 13) and preference (Figure 14) was also measured after all ANC sessions to characterize the effect of the SNC downshift within the ANC procedure on post-session ethanol intake in the WS-ANC group. Ethanol consumption data shows a drop in ethanol intake after experiencing a post-shift decline in the reward rather than the expected increase. However, ethanol preference, which compares ethanol intake relative to water intake, suggests an increase in preference on the post-shift contrast days.

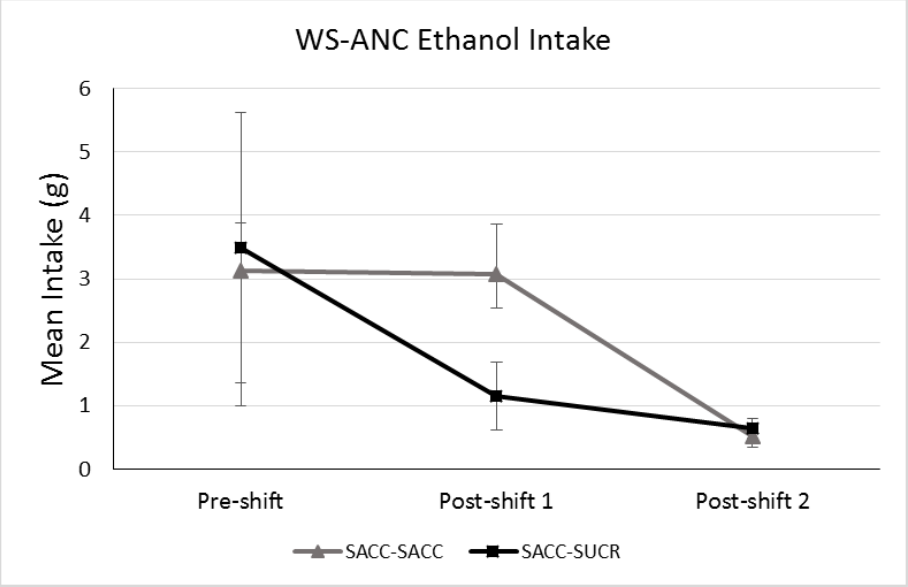

Figure 13. WS-ANC Ethanol Intake. Absolute ethanol intake varied across the blocks in the WS-ANC group by type of session (SACC-SACC; SACC-SUCR).

Consumption of ethanol did not differ between control (SACC-SACC) and contrast (SACC-SUCR) days in the pre-shift block. A 2 (block) X 2 (second bottle) repeated measures ANOVA was calculated to determine differences in ethanol consumption in the WS-ANC group

in the 2 post-shift blocks. The main effects of block ($F(1, 7) = 6.453$, $p = 0.039$, $\eta^2_p = 0.480$), day ($F(1, 7) = 16.093$, $p = 0.005$, $\eta^2_p = 0.697$) and the interaction between block and day ($F(1, 7) = 23.827$, $p = 0.002$, $\eta^2_p = 0.773$) were significant. While consumption actually increased on the first post-shift control day there was no difference by the WS-ANC group in ethanol intake between days in the second post-shift block.

Ethanol Preference. Ethanol preference was examined separately in the final pre-shift block and two post-shift blocks to characterize the pattern of ethanol preference in the WS-ANC group between SACC-SACC and SACC-SUCR days, and the change in preference induced by the downshift from SUCR to SACC on contrast days.

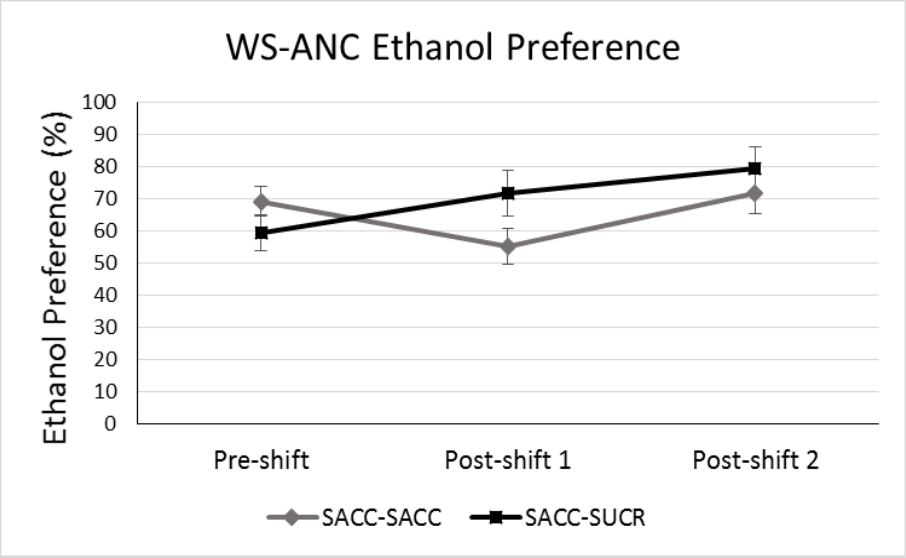

Figure 14. WS-ANC Ethanol preference. Ethanol preference increased in the WS-ANC group on the first post-shift contrast day (SACC-SUCR).

A 2 (block) X 2 (second bottle) repeated measures ANOVA was calculated for ethanol preference in the two post-shift blocks. While the main effect of block was significant ($F(1, 7) = 5.802$, $p = 0.047$, $\eta^2_p = 0.453$), the main effect of day ($F(1, 7) = 3.319$, $p = 0.111$, $\eta^2_p = 0.322$) and the interaction between block and day ($F(1, 7) = 0.847$, $p = 0.388$, $\eta^2_p = 0.108$) were not

significant. Ethanol preference was increased in the WS-ANC group on the first post-shift day when the rats experienced saccharin instead of sucrose in the second bottle, suggesting that the rats may self-medicate for this decrease in reward by increasing preference for ethanol.

Discussion

The SNC procedure in Experiment 1 was continued in Experiment 2, but the two groups were switched. The SNC Contrast group became the new control group, and the SNC Control group became the new contrast group. I hypothesized these two groups would rapidly change their consumption of the sucrose or saccharin, and that the downshift would similarly induce a negative reaction in the new contrast group, indicated by both decreased saccharin consumption, but also increased ethanol consumption. These results would confirm the results from Experiment 1 and further support the central hypothesis of the current study: that rats self-medicate by increasing ethanol consumption after experiencing a negative reaction from a decrease in reward in a SNC procedure.

The SNC contrast group increased consumption and preference for ethanol in the post-shift sessions, which supports Experiment 1 and Manzo et al (2015). This indicates the negative reaction induced by the downshift in an SNC procedure may cause self-medicating behavior in order to reduce the negative state from decrease in reward, as the rats increased ethanol intake. As in Experiment 1, however, a SNC effect, which would be indicated by significantly reduced consumption of the post-shift saccharin compared to the SNC control group was not observed. This failure to demonstrate a SNC effect again appears to be due to the control group. Intake of saccharin declined to a very low level resulting in a possible floor effect as consumption for both groups was very low.

The WS-ANC procedure was continued from Experiment 1 with the addition of an SNC downshift in the second bottle on contrast days. I hypothesized the WS-ANC group would demonstrate a SNC effect, consuming less saccharin on contrast days than control days in post-shift sessions. If a SNC effect is observed I also hypothesized this group would increase ethanol consumption on post-shift contrast days. While the WS-ANC group displayed an ANC effect, reducing consumption of saccharin on the first post-shift contrast day, consumption of the second bottle did not differ between days in post-shift blocks. The effect on intake was equivocal however. Absolute intake of ethanol decreased following the post-shift, but there was an increased preference for ethanol on one post-shift block.

General Discussion

The purpose of the current study was to determine if rats increase ethanol intake after experiencing a negative reaction induced by the downshift in a SNC procedure. This was examined with SNC procedures in Experiments 1 and 2. The over-arching concept behind this experimental procedure is that rats may be capable of self-medication, consuming ethanol for its anxiolytic effect to reduce the negative current state (Manzo et al., 2015). I hypothesized that after experiencing access to a rewarding sucrose solution, the SNC Contrast group would decrease intake of the less-rewarding saccharin on post-shift days, and that this effect would not be observed in the SNC Control group which was exposed only to saccharin on all days. I also hypothesized the negative reaction induced by this downshift would cause a significant increase in consumption of ethanol in the 2 hour post-session ethanol choice test.

The WS-ANC group was also included to demonstrate an ANC effect, but was not predicted to show a difference in ethanol intake, as this procedure is not associated with a negative emotional reaction. In Experiment 2 another manipulation was introduced to seek a further test of the self-medication hypothesis. A SNC downshift was superimposed on the ANC procedure by downshifting the second bottle on contrast days, to determine if this group would reduce consumption of saccharin on these downshifted days, and if the group would increase ethanol intake.

Successive Negative Contrast Groups

In an SNC procedure groups of rats receive access to sucrose (SNC Contrast) or saccharin (SNC Control) for a number of days, and then only saccharin for 4 additional days. In pre-shift trials the SNC Contrast group will typically demonstrate stable consumption of sucrose while the SNC Control group will demonstrate stable consumption of saccharin (Crespi, 1942;

Flaherty, 1996). When the SNC Contrast group experiences the downshift (sucrose replaced by saccharin), the rats significantly reduce consumption in post-shift days compared to the control group, which received access to the same saccharin during the pre-shift sessions. This is the SNC effect and the reduction in intake during post-shift days by the SNC Contrast group is evidence of a negative reaction to the decrease in reward with the downshift. However, consumption quickly recovers to levels of the SNC control group by the third or fourth post-shift day (Phelps, Mitchell, Robinson, Nutt, & Marston, 2015; Rowan & Flaherty, 1991). While the SNC Contrast group did not demonstrate this significant reduction in consumption on post-shift days compared to the SNC Control group, consumption did decrease substantially, and may have caused a negative reaction due to the decrease in reward from sucrose to saccharin. This negative reaction is important in interpreting the increased ethanol intake and preference in post-shift sessions by the SNC Contrast group in both experiments as self-medicating behavior by negative reinforcement, due to the negative reaction caused by the decrease in reward from the downshift in the SNC procedure.

Within-Subjects Anticipatory Negative Contrast Group

In a WS-ANC procedure a group of rats receives access to a saccharin solution daily followed by access to the same saccharin or sucrose on alternate, context-cued days. An ANC effect is the decrease in consumption of the initial saccharin on the days it is followed by the more rewarding sucrose. Supporting previous research (Rowan & Flaherty, 1991; Flaherty et al., 1995), the WS-ANC group in the current study consumed consistently less saccharin on contrast days, when the rewarding and preferred sucrose solution followed saccharin (see Figure 2).

The current study demonstrated that rats increase ethanol consumption and preference after experiencing a downshift in a SNC procedure, replicating Manzo et al (2015), while adding

a WS-ANC group as a second control group to further determine that this response is due to the negative reaction induced by the downshift rather than to some other characteristic of a contrast procedure such as increasing the consumption of any available resource through the activation of exploratory behavior. The increase of ethanol consumption in SNC procedure but not the ANC procedure may be interpreted as self-medication due to the negative reaction from the decrease in reward in the SNC Contrast group. Experiment 2 confirmed this pattern of ethanol consumption and provides evidence of self-medication in rats due to a decrease in reward in an SNC experiment.

While the current study examined the effect of the downshift in a SNC procedure on subsequent ethanol intake in rats, previous researchers (Kamenetzky, Mustaca, & Papini, 2008; Becker & Flaherty, 1982), switched these variables to determine the effect of anxiolytic substances on post-shift saccharin consumption. In both experiments (Becker & Flahety, 1983; Flaherty, Clarke, Coppotelli, 1996), 1 g/kg of 15% ethanol and CDP before the post-shift sessions prevented the SNC contrast effect. This demonstrates that the downshift induces an anxiogenic, negative reaction which is ameliorated by acute administration of substances with anxiolytic properties like ethanol.

Similar to the current study, Manzo et al (2015) demonstrated that the downshift from sucrose to saccharin induces increases ethanol consumption during a post-session ethanol choice test in rats experiencing this reward devaluation. These authors used 32% sucrose in pre-shift sessions and 4% sucrose in post-shift sessions, while the current study used 0.15% saccharin in post-shift sessions; however, the effect is similar (Mitchell & Flaherty, 2005). Manzo et al (2015) further demonstrated that rats increased consumption of a CDP solution in post-shift

sessions as well, which supports the hypothesis that rats may self-medicate for this negative state by consuming anxiolytic substances.

Manzo et al (2014) found increased post-session ethanol consumption by rats as well. This experiment implemented food pellet and 22% sucrose consummatory reward loss procedures. Like reward devaluation, this reward loss induced similar self-medicating behavior, as ethanol intake increased during the extinction sessions. This incentive loss produced by the loss of reward induces an aversive, anxiogenic state which leads to increased consumption of ethanol (Manzo et al., 2014). Taken together, the current study confirms that rats increase ethanol consumption after experiencing a downshift in reward, which supports the self-medication hypothesis (Manzo et al., 2014; Manzo et al., 2015).

Limitations

There were two major limitations to this experiment. First, there was not a robust, significant SNC effect between the SNC contrast and SNC control groups in either experiment. One possibility is that the downshift from sucrose to saccharin may not have produced a significant negative reaction in the SNC contrast group. Without this negative reaction, interpreting the increase in ethanol consumption and preference in post-shift ethanol choice test sessions as being due to self-medication of this induced negative reaction becomes tenuous. However, intake of the solutions was significantly and substantially reduced in post-shift sessions in both Experiment 1 and Experiment 2, to still induce the negative reaction. The other more likely explanation is that the control group takes were not effective in revealing the contrast effects because of a shifting baseline (Experiment 1) or a very low intake resulting in a possible floor effect (Experiment 2). Although saccharin has been used previously as the downshifted

solution in the SNC procedure (Mitchell & Flaherty, 2005) perhaps a reduced sucrose value (e.g., 4%) may produce higher and more consistent intakes in the control condition.

Second, all 3 groups were slow to consume ethanol on pre-shift days in Experiment 1. This was unexpected, as Long-Evans rats are more likely to drink ethanol than the more common Sprague Dawley rat (Khanna, Kalant, Shah, & Sharma 1990). Therefore, I implemented an intermittent ethanol exposure procedure, which increases voluntary ethanol consumption in rodents (Rosenwasser et al., 2013). This may be important in order to test the self-medication hypothesis. That is, if the rats do not experience the anxiolytic effect of ethanol, due to too low of an intake, they may not learn this association. The rats were exposed to ethanol for 24 hours in their home cages followed by another 24 hours without ethanol access, for a total of 5 sessions lasting 10 days. The rats consumed more home cage ethanol on the fifth exposure session than on the first two intermittent exposure sessions. I then returned the rats to their respective procedures for 3 days, until consumption of all solutions stabilized, and proceeded with the downshift from sucrose to saccharin for the SNC Contrast group. Because all rats were given this intermittent exposure to ethanol at the same time, this manipulation was not confounded with the contrast manipulations. Nevertheless, a strain of rats that more readily accepts ethanol would eliminate the need for this intermittent exposure to ethanol.

Conclusions

The results of the current study support the hypothesis that when experiencing a negative reaction from a decrease in reward, rats self-medicate by increasing consumption and preference for an ethanol solution. This is confirmed by the results from both Experiments 1 and 2, as well as by comparison to a second control group, as the WS-ANC group displayed no changes in ethanol consumption. By further characterizing ethanol consumption in rats experiencing SNC

and ANC procedures, this study provides additional evidence the negative reaction induced by the SNC downshift is the cause of the change in ethanol intake. This suggests reward contrast in an SNC procedure causes rats to self-medicate, consuming more ethanol and increasing ethanol preference.

References

Acevedo, M. B., Nizhnikov, M. E., Molina, J. C., & Pautassi, R. M. (2014). Research report: Relationship between ethanol-induced activity and anxiolysis in the open field, elevated plus maze, light-dark box, and ethanol intake in adolescent rats. *Behavioural Brain Research,* 265203-215. doi:10.1016/j.bbr.2014.02.032.

Ameringer, K. J., Chou, C., & Leventhal, A. M. (2015). Shared versus specific features of psychological symptoms and cigarettes per day: Structural relations and mediation by negative- and positive-reinforcement smoking. *Journal of Behavioral Medicine, 38*(*2*), 224-236. doi:10.1007/s10865-014-9597-y.

Andrews, N., Papakosta, M., & Barnes, N. (2014). Review: Discovery of novel anxiolytic agents - The trials and tribulations of pre-clinical models of anxiety. *Neurobiology Of Disease, 61(Neurological drug development: from bench to bedside),* 72-78. doi:10.1016/j.nbd.2013.10.006.

Arabo, A., Potier, C., Ollivier, G., Lorivel, T., & Roy, V. (2014). Temporal analysis of free exploration of an elevated plus-maze in mice. *Journal Of Experimental Psychology: Animal Learning And Cognition, 40*(*4*), 457-466. doi:10.1037/xan0000031.

Bahi, A., & Dreyer, J. (2014). Chronic psychosocial stress causes delayed extinction and exacerbates reinstatement of ethanol-induced conditioned place preference in mice. *Psychopharmacology, 231*(2), 367-377.

Becker, H. C., & Flaherty, C. F. (1982). Influence of ethanol on contrast in consummatory behavior. *Psychopharmacology, 77*(3), 253-258.

Becker, H. C., & Flaherty, C. F. (1983). Chlordiazepoxide and ethanol additively reduce gustatory negative contrast. *Psychopharmacology, 80*(1), 35-37.

Becker, H., Lopez, M., & Doremus-Fitzwater, T. (2011). Effects of stress on alcohol drinking: a review of animal studies. *Psychopharmacology, 218*(1), 131-156.

Bentosela, M., Ruetti, E., Muzio, R. N., Mustaca, A. E., & Papini, M. R. (2006). Administration of corticosterone after the first downshift trial enhances consummatory successive negative contrast. *Behavioral Neuroscience, 120*(*2*), 371-376. doi:10.1037/0735-7044.120.2.371.

Bertholomey, M. L. (2012). Alterations in the seeking and self-administration of ethanol and anxiety-like behavior following exposure to yohimbine in rats selectively bred for high alcohol intake. *Dissertation Abstracts International, 73*, 608.

Bertholomey, M., West, C., Jensen, M., Li, T., Stewart, R., Weiss, J., & Lumeng, L. (2011). Genetic propensities to increase ethanol intake in response to stress: studies with selectively bred swim test susceptible (SUS), alcohol-preferring (P), and non-preferring (NP) lines of rats. *Psychopharmacology, 218*(1), 157-167.

Butler, E. A. (2011). Three views of emotion regulation and health. *Social & Personality Psychology Compass, 5*(8), 563-577.

Caldwell, E. E., & Riccio, D. C. (2010). Alcohol self-administration in rats: Modulation by temporal parameters related to repeated mild social defeat stress. *Alcohol, 44*265-274. doi: 10.1016/j.alcohol.2010.02.012.

Conger, J. J. (1956). Reinforcement theory and the dynamics of alcoholism. *Quarterly journal of studies on alcohol*.

Crespi, L.P.(1942). Quantitative variation in incentive and performance in the white rat, *Am. J. Psychol.,* 55, 467-517.

Crum, R. M., La Flair, L., Storr, C. L., Green, K. M., Stuart, E. A., Alvanzo, A. A. H., & Mojtabai, R. (2013). Reports of drinking to self-medicate anxiety symptoms: Longitudinal

assessment for subgroups of individuals with alcohol dependence. *Depression & Anxiety (1091-4269), 30*(2), 174-183.

Cuenya, L., Sabariego, M., Donaire, R., Fernández-Teruel, A., Tobeña, A., Gómez, M., & ... Torres, C. (2012). The effect of partial reinforcement on instrumental successive negative contrast in inbred Roman High- (RHA-I) and Low- (RLA-I) Avoidance rats. *Physiology & Behavior,* 1051112-1116. doi:10.1016/j.physbeh.2011.12.006.

Dermody, S. S., Cheong, J., & Manuck, S. (2013). An evaluation of the stress-negative affect model in explaining alcohol use: The role of components of negative affect and coping style. *Substance use & misuse, 48*(4), 297-308. Doi: 10.3109/10826084.2012.761713

Drusch, K., Lowe, A., Fisahn, K., Brinkmeyer, J., Musso, F., Mobascher, A., & Wölwer, W. (2013). Effects of nicotine on social cognition, social competence and self-reported stress in schizophrenia patients and healthy controls. *European Archives of Psychiatry & Clinical Neuroscience, 263*(6), 519-527. doi: 10.1007/s00406-012-0377-9.

Field, A. P. (2013). *Discovering statistics using IBM SPSS statistics*. Los Angeles : Sage, [2013].

Flaherty, C. F. (1982). Incentive contrast: A review of behavioral changes following shifts in reward. *Animal Learning & Behavior, 10*(4), 409-440. doi:10.3758/BF03212282.

Flaherty, C. F., & Rowan, G. A. (1986). Successive, simultaneous, and anticipatory contrast in the consumption of saccharin solutions. *Journal Of Experimental Psychology: Animal Behavior Processes, 12*(4), 381-393. doi:10.1037/0097-7403.12.4.381.

Flaherty, C. F., Becker, H. C., & Pohorecky, L. (1985). Correlation of corticosterone elevation and negative contrast varies as a function of post-shift day. *Animal Learning & Behavior, 13*(*3*), 309-314. doi:10.3758/BF03200025.

Flaherty, C. F., Clarke, S., & Coppotelli, C. (1996). Lack of tolerance to contrast-reducing actions of chlordiazepoxide with repeated reward reductions. *Physiology & behavior*, *60*(2), 645-652.

Flaherty, C. F., Rowan, G. A., Emerich, D. F., & Walsh, T. J. (1989). Effects of intrahippocampal administration of colchicine on incentive contrast and on radial maze performance. *Behavioral Neuroscience*, *103*(2), 319-328. doi:10.1037/0735-7044.103.2.319.

Genn, R. F., Tucci, S., Parikh, S., & File, S. E. (2004). Effects of nicotine and a cannabinoid receptor agonist on negative contrast: distinction between anxiety and disappointment? *Psychopharmacology*, 177(1/2), 93-99.

Gross, J. J. (2013). Emotion regulation: Taking stock and moving forward. *Emotion, 13*(3), 359-365.

Hahn, B., Harvey, A. N., Concheiro-Guisan, M., Huestis, M. A., Holcomb, H. H., & Gold, J. M. (2013). *Archival report: A test of the cognitive self-medication hypothesis of tobacco smoking in schizophrenia* Elsevier Inc.

Kamenetzky, G. V., Mustaca, A. E., & Papini, M. R. (2008). An analysis of the anxiolytic effects of ethanol on consummatory successive negative contrast. *Avances En Psicología Latinoamericana*, 26(2), 135-144.

Kamenetzky, G. V., Mustaca, A. E., Pedron, V. T., Cuenya, L., & Papini, M. R. (2009). Ethanol facilitates consummatory extinction. *Behavioural Processes*, 82(3), 352-354. doi:10.1016/j.beproc.2009.08.001.

Kampov-Polevoy, A., Overstreet, D., Rezvani, A., & Janowsky, D. (1995). Article: Suppression of ethanol intake in alcohol-preferring rats by prior voluntary saccharin consumption. *Pharmacology, Biochemistry And Behavior*, *52*59-64. doi:10.1016/0091-3057(94)00430-Q.

Khanna, J. M., Kalant, H., Shah, G., & Sharma, H. (1990). Comparison of sensitivity and alcohol consumption in four outbred strains of rats. *Alcohol, 7*(5), 429-434. doi:10.1016/0741-8329(90)90027-A.

Khantzian EJ. Treating Addiction as a Human Process. Northvale, NJ: Library of Substance Abuse and Addiction Treatment, 1999; 687.

Khantzian, E. J. (1985). The self-medication hypothesis of addictive disorders: Focus on heroin and cocaine dependence. *The American Journal Of Psychiatry, 142*(11), 1259-1264.

Khantzian, E. J. (2013). Addiction as a self-regulation disorder and the role of self-medication. *Addiction*, *108*(4), 668-669. doi: 10.1111/add.12004.

Knapp, D., Overstreet, D., Huang, M., Wills, T., Whitman, B., Angel, R., & ... Breese, G. (2011). Effects of a stressor and corticotrophin releasing factor on ethanol deprivation-induced ethanol intake and anxiety-like behavior in alcohol-preferring P rats. *Psychopharmacology*, *218*(1), 179-189.

Kumar, V., Bhat, Z. A., & Kumar, D. (2013). Review: Animal models of anxiety: A comprehensive review. *Journal of Pharmacological And Toxicological Methods*, 68175-183. doi:10.1016/j.vascn.2013.05.003.

Lembke, A. (2012). Time to abandon the self-medication hypothesis in patients with psychiatric disorders. *American Journal of Drug & Alcohol Abuse, 38*(6), 524-529.

Levine, T. R., & Hullett, C. R. (2002). Eta squared, partial eta squared, and misreporting of effect size in communication research. *Human Communication Research*, *28*(4), 612-625. doi:10.1111/j.1468-2958.2002.tb00828.x.

Markou, A., Kosten, T. R., & Koob, G. F. (1998). Neurobiological similarities in depression and drug dependence: a self-medication hypothesis. *Neuropsychopharmacology, 18*(3), 135-174. doi:10.1016/S0893-133X(97)00113-9.

Makowska, I. J., & Weary, D. M. (2013). Review: Assessing the emotions of laboratory rats. *Applied Animal Behaviour Science*, 1481-12. doi:10.1016/j.applanim.2013.07.017.

Manzo, L., Donaire, R., Sabariego, M., Papini, M. R., & Torres, C. (2015). Anti-anxiety self-medication in rats: Oral consumption of chlordiazepoxide and ethanol after reward devaluation. *Behavioural Brain Research, 278*90-97.

Manzo, L., Gómez, M. J., Callejas-Aguilera, J. E., Fernández-Teruel, A., Papini, M. R., & Torres, C. (2014). Anti-anxiety self-medication induced by incentive loss in rats. *Physiology & Behavior, 12 3*86-92.

Meyer, J. S., & Quenzer, L. F. (2013). Psychopharmacology: Drugs, the brain, and behavior (2nd ed.). Sunderland, MA, US: Sinauer Associates.

Mitchell, C. P., & Flaherty, C. F. (2005). Differential effects of removing the glucose or saccharin components of a glucose–saccharin mixture in a successive negative contrast paradigm. *Physiology & behavior*, *84*(4), 579-583.

Noori, H. R., Helinski, S., & Spanagel, R. (2014). Cluster and meta-analyses on factors influencing stress-induced alcohol drinking and relapse in rodents. *Addiction Biology, 19*(2), 225-232. doi:10.1111/adb.12125.

Ortega, L. A., Uhelski, M., Fuchs, P. N., & Papini, M. R. (2011). Impairment of recovery from incentive downshift after lesions of the anterior cingulate cortex: Emotional or cognitive deficits? *Behavioral Neuroscience, 125*(6), 988-995. doi:10.1037/a0025769.

Papini, M. R. (2009). Role of Opioid Receptors in Incentive Contrast. *International Journal Of Comparative Psychology*, *22*(*3*), 170-187.

Phelps, C., Mitchell, E., Robinson, E., Nutt, D., & Marston, H. (2015). Psychopharmacological characterisation of the successive negative contrast effect in rats. *Psychopharmacology*, *232*(15), 2697-2709. doi:10.1007/s00213-015-3905-2

Rosenwasser, A.M., Fixaris, M.c', Crabbe, J.C., Brookes, P.C., & Ascheid, S. (2013). Escalation of intake under intermittent ethanol access in diverse mouse genotypes. *Addiction Biology, 18(3),*496-507. doi: 10.1111/j.1369-1600.2012.00481.x.

Rowan, G. A., & Flaherty, C. F. (1991). Behavior of Maudsley Reactive and Nonreactive Rats (*Rattus norvegicus*) in Three Consummatory Contrast Paradigms. *Journal of Comparative Psychology, 105* (2), 115-124.

Rowan, G. A., & Flaherty, C. F. (1991). Behavior of Maudsley reactive and nonreactive rats (Rattus norvegicus) in three consummatory contrast paradigms. *Journal of Comparative Psychology*, *105*(2), 115. Doi: 10.1037/0735-7036.105.2.115.

Ruetti, E., Justel, N., Mustaca, A. E., & Papini, M. R. (2009). Posttrial corticosterone administration enhances the effects of incentive downshift: Exploring the boundaries of this effect. *Behavioral Neuroscience*, *123*(*1*), 137-144. doi:10.1037/a0013805.

Stopponi, S., Somaini, L., Cippitelli, A., Guglielmo, G., Kallupi, M., Cannella, N., & ... Ciccocioppo, R. (2012). Pregabalin reduces alcohol drinking and relapse to alcohol seeking in the rat. *Psychopharmacology*, *220*(*1*), 87-96.

Timberlake, W., & Engle, M. (1995). Decremental carryover effects of sucrose ingestion in the negative anticipatory contrast procedure in rats. *Journal Of Experimental Psychology: Animal Behavior Processes, 21(4),* 304-317. doi:10.1037/0097-7403.21.4.304.

Wright, R. L., Gilmour, G., & Dwyer, D. M. (2013). Microstructural analysis of negative anticipatory contrast: A reconsideration of the devaluation account. *Learning & Behavior, 41*(4), 353-359. doi:10.3758/s13420-013-0110-1.

Xiaobai, L. (2012). Using the conditioned fear stress (CFS) animal model to understand the neurobiological mechanisms and pharmacological treatment of anxiety. *Shanghai Archives Of Psychiatry, 24(5),* 241-249. doi:10.3969/j.issn.1002-0829.2012.05.001.

Zentall, T. R. (2007). Reinforcers following greater effort are preferred: A within-trial contrast effect. *Behavior Analyst Today, 8*(4), 512-527.