EXPLORING EMOTIONAL SELF-MEDICATION DURING EXTINCTION OF ESCAPE BEHAVIOR IN RATS

By

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Introduction to Emotional Self-Medication

Individuals are often confronted with physiological and psychological stressors. To cope with such situations, humans have used psychoactive substances such as anxiolytics, opioids, and analgesics (Torres & Papini, 2016). These substances are used to alleviate the negative emotional states that stem from emotional stressors. Common examples of such substances include alcohol (ethanol) and prescription anxiolytics such as chlordiazepoxide (CDP). These substances, while capable of diminishing stress, also possess addictive potential (Briand & Blendy, 2010). Though the term "addiction" itself is not currently featured as a diagnostic category in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5; American Psychiatric Association, 2013) a subset of behaviors that can be classified as addictive are defined as substance use disorders (SUDs). SUDs are outlined in the DSM-5 as neurobehavioral disorders with symptoms of excessive consumption, craving, tolerance, and withdrawal, among others. Due to the addictive potential of substances that are capable of attenuating negative emotions, a targeted study of emotional self-medication (ESM) could contribute to a better understanding of the initial development of SUDs. A current hypothesis concerning the initial stages of SUDs is Khantzian's (1985, 2013) ESM hypothesis.

ESM is characterized by the consumption of psychoactive substances that reduce negative emotions that are brought on by stress, psychiatric conditions, and distress associated with withdrawal from drugs (Torres & Papini, 2016). Khantzian (1985, 2013) suggested the ESM hypothesis as a valuable clinical tool with implications for the treatment of SUDs. This hypothesis posits that a person's inability to cope with negative life events is a primary source of motivation to consume substances with addictive potential. An inability to cope can be described as dysfunction in day to day behavior, irregularities in performance, and lacking the ability to

adjust to changes. Khantzian's (1985) original ESM hypothesis suggested that the behavior of self-medicating could be predicted by previous reinforcement history as substance use involves the reduction of symptoms (Blume, 2001). Reinforcement plays a special role in influencing the likelihood of recurrent substance use and abuse. Specifically, the behavior of self-medication can be conceptualized as negatively reinforcing substance use through the attenuation of aversive and negative emotional states. Therefore, aversive emotions attenuated by using substances may provide a framework to understand how SUDs develop.

There are two important assumptions of the ESM hypothesis of addiction—the psychopathology postulate and the drug specificity postulate (Darke, 2013). The psychopathology postulate assumes that substance use relieves symptoms of negative emotions. The drug specificity postulate suggests that ESM would involve a substance capable of reducing the particular symptoms experienced. An example of drug specificity is the voluntary consumption of an anxiolytic drug, including alcohol, as a means of coping with anxiety. The anxiolytic properties of alcohol may perhaps serve to attenuate frustration that stems from incentive downshift. Becker and Flaherty (1982) explored how ethanol affected consummatory behavior using a successive negative contrast paradigm. Animals were exposed to either 32% sucrose for 10 days and then downshifted to 4% or exposed to 4% sucrose throughout the experiment. Forced administration of ethanol or saline on postshift days 1 and 2 yielded interesting results. The animals that received ethanol exhibited a reduction in the negative contrast effect on postshift day 2, but not postshift day 1. These results suggested that ethanol was able to reduce the negative emotions experienced during downshift that affected the observed negative contrast effect.

Further, Manzo et al. (2014) and Matson and Grahame (2015) suggested that the voluntary, rather than forced consumption of ethanol has the potential to inhibit negative emotional states and increase reinforcement by alleviating symptoms of anxiety.

Testing the ESM hypothesis

Briand and Blendy (2010) describe several studies suggesting that painful emotional situations are often precursors to the initial use of substances and relapse of drug abuse. Similar to these findings, experiencing acute or chronic emotional stress was found to contribute to the consumption of alcohol (Keyes et al., 2011). While this information is valuable, a test of the ESM hypothesis requires an experimental approach. To experimentally test the ESM hypothesis, one protocol involves an induction task that triggers an aversive emotional state and a secondary task that assesses ESM. Manzo et al. (2014) evaluated the voluntary oral consumption of and preference for ethanol following a situation involving incentive loss (appetitive extinction), using Roman low-avoidance and high-avoidance rat strains. In one experiment, consummatory extinction served as the induction task; rats were first exposed to 22% sucrose and then downshifted in extinction to 0% sucrose. Following each session of the induction, each animal was administered a 2-h, 2-bottle preference test in their home cage. For the experimental group in each strain, one bottle contained tap water and the other contained a 2% ethanol solution. For the control group in each strain, both bottles contained tap water. Bottles were weighed before and after the preference test to assess consumption. Researchers found that the voluntary postsession consumption of ethanol increased selectively after extinction sessions in Roman low-avoidance rats but not in Roman high-avoidance rats. Piras, Corda, and Giorgi (2006) tested emotionality and coping styles in these strains and found that high-avoidance rats exhibited proactive coping strategies whereas low-avoidance rats exhibit a more reactive coping style,

showing more freezing and self-grooming behaviors. These behaviors allow low-avoidance rats to be characterized as more emotional than their high-avoidance counterparts. As such, the selective increase in postsession ethanol consumption for low-avoidance rats was interpreted as an attempt to attenuate the relatively strong aversive emotional state induced by the incentive loss (downshift) in the induction task. A second experiment (Manzo et al., 2014) used instrumental extinction as the induction task with animals exposed to a 12-to-0 pellet downshift. Following each session in the induction task, animals were presented with the same preference test described above. Consistent with the results of the previous experiment, researchers found that the postsession consumption of ethanol was higher in the Roman low-avoidance rats after extinction sessions than in Roman high-avoidance rats. These results suggest that animals are capable of self-medicating in response to negative emotional states brought about by incentive loss. An important distinction between describing the voluntary oral ethanol consumption as self-medication as opposed to substance abuse is the transient nature of both the anxiety-inducing events and the increase in ethanol consumption. With prolonged ethanol consumption, drinking behavior may become habitual, which would be indicative of substance abuse.

Manzo et al. (2015) further tested the ESM hypothesis by exposing Wistar rats to a consummatory successive negative contrast as the induction task in which an incentive devaluation from 32-to-4% sucrose occurred for some animals (downshifted group) while others (unshifted control group) always received 4% sucrose. In the preference test, animals were given a choice between the anxiolytics CDP or ethanol, and water in a 2-h, 2-bottle preference test. One bottle contained either 1 mg/kg of CDP, 2% ethanol, or tap water, whereas the other bottle contained water. Preference for ethanol and CDP increased following the sucrose downshift; preference did not increase for animals with access to water or for unshifted control animals.

Again, these results suggest that preference for the voluntary consumption of anxiolytics can be influenced by situations involving reward loss.

An additional study evaluated the effects of partial reinforcement on anti-anxiety self-medication during an appetitive extinction situation. Manzo et al. (2015) used anxiety prone Roman low-avoidance rats and trained them to traverse a runway in the induction task. Each session was followed by a preference test with ethanol-water or water-water in independent groups. Animals received 10 sessions of training on the runway and 7 extinction sessions. Some animals were selected to receive only partial reinforcement (50%) in the induction task compared to control animals receiving continuous reinforcement. Partial reinforcement has been shown to elicit persistence during extinction (Amsel, 1992), an effect that can be interpreted as resilience to the disrupting effects of anxiety. The animals that received only partial reinforcement exhibited higher resistance to extinction compared to the continuously reinforced animals. Additionally, partially reinforced animals showed lower preference for ethanol than continuously reinforced animals. However, partial and continuous reinforcement in the induction task did not affect water consumption in the preference test. These results suggest that ESM can be attenuated in Roman low-avoidance rats by means of chronic exposure to reward uncertainty (partial reinforcement training). The uncertainty of the reward seems to build tolerance to the aversive emotional state brought about by reward loss. The aim of the current research was to further explore the relationship between psychological pain induced by reward loss and ESM.

Expectation of Reward

SNC involves the formation of expectations of reward and the subsequent violation of those expectations. The theoretical foundations of the proposed research can be illustrated with the SNC effect. Crespi (1942) provided a classic example of SNC by training rats to travel the

length of a straight runway to earn a small or a large quantity of food. The speed of running was recorded for all animals and the running speeds for rats with unshifted small reinforcement was compared to that of rats that received the same amount of reward following experience with a smaller reward or experience with a larger reward. Crespi labeled these effects in terms of emotion—using the word "depression" to describe how rats receiving a downshift in reinforcement ran slower than unshifted rats. Zeaman (1949) further explored the instrumental runway task in Wistar rats exposed to reward downshifts. Crespi's and Zeaman's results contrasted with Hull's (1943) view predicting that reward downshift should lead to a gradual reduction in the strength of a behavior. Instead, both Crespi and Zeaman found that decreasing the amount of food in their runway experiments caused animals to reach the goal much slower than animals consistently receiving the smaller reinforcer. These behavioral adjustments occurred faster and were more extreme than Hull's (1943) theory had predicted. Zeaman was the first to label this type of incentive contrast as "negative."

SNC has also been linked to frustration theory. Amsel's (1992) frustration theory suggests that the unexpected omission or devaluation of a reward induces a transient increase in drive and an aversive emotional state. Consistent with this view, anxiolytics, such as ethanol and benzodiazepines have been shown to attenuate the SNC effect (Flaherty, 1996).

Expectancy plays a crucial role in incentive contrast. When animals come to expect a certain reward, unexpected shifts to less preferred rewards are often accompanied by emotional activation and suppression of previously conditioned responding (Papini & Dudley, 1997). The aversive emotion induced by reward devaluation (as in SNC) or reward omission (as in appetitive extinction) is referred to as psychological pain (Papini et al., 2015).

Escape Extinction as Incentive Downshift

Previous ESM experiments (e.g. Manzo et al., 2015) used a reward downshift procedure based on devaluing a food outcome (e.g., a 32-to-4% sucrose devaluation). Immediately after each induction session, animals received access to ethanol, a substance with anxiolytic properties, but also with caloric content. Could an increase in ethanol consumption after reward downshift be due to caloric compensation, rather than anxiety reduction? This caloric compensation hypothesis cannot explain a similar increase in the consumption of a chlordiazepoxide solution that has no caloric content. Still, a more definitive test would be to induce a negative emotion in a procedure that does not involve food rewards. Escape extinction in the Barnes maze (BM) offers such an alternative. The BM task is an example of an instrumental maze task that can employ aversive or appetitive extinction which is not consummatory in nature. The incentive provided is escape, and during extinction no incentive or a partial incentive can be provided. This presents a key difference from other incentive downshifts that change from a large to a small food reward; however, the reward omission in the BM task is analogous to the appetitive extinction procedure used in previous ESM studies (e.g., Manzo et al., 2014) where animals went from reward to nonreward. The animal comes to expect the presence of the escape box through a series of acquisition trials, and the expectancy is violated during extinction when the escape box is inaccessible, or completely removed from the maze.

The BM was originally developed for use with rats as an alternative stress-inducing task to the Morris water maze (Barnes, 1979). The original BM was later adapted to provide access for smaller rodents, such as mice (Pompl, Mullan, Bjugstad, & Arendash, 1999). This BM task involves placing an animal in the center of the testing arena atop a circular platform containing

holes around the edge. Negative reinforcement is applied in the form of escape from intensely bright lights, exposure to an open environment, loud and unpleasant sound, and sometimes the use of pressurized air jets. Animals are motivated to escape into a box placed underneath one of the holes of the maze, which provides a quiet, dark place to retreat from the stimuli atop the maze (Kuzmin et al., 2012). The safety provided by the escape box, however, probably also provides a source of positive reinforcement. This is suggested by experiments based on a different, but analogous task: one-way avoidance (OWA). In the OWA situation, rats receive painful electric shocks in one box (analogous to open-space anxiety in the BM), unless they cross to a safety box (analogous to the escape box in the BM). Cándido et al. (1992) showed that a safety downshift from 30 s to 1 s in the OWA situation deteriorated avoidance relative to a group that always received 1 s of safety time—a successive negative contrast effect (SNC). This SNC effect suggests that the OWA situation also involves an incentive component (positive reinforcement), in addition to the aversive escape component (negative reinforcement).

Besides situations involving SNC, other tasks can include reward devaluation or omission. For example, in appetitive extinction, the removal of the reward is considered a devaluation in the form of complete reward omission. By rewarding escape behavior with access to safety in the BM situation, we can use its extinction as a source of loss-induced anxiety. The adapted BM protocol used in the current experiments consisted of two phases—acquisition and extinction. During the initial acquisition phase, the subject was introduced to the maze and allowed to explore for a predetermined maximum amount of time or until reaching the escape box. Learning during acquisition was assessed as a decrease in the latency to reach the escape box and a decrease in the number of holes checked before reaching the target hole containing the escape box (Sunyer, Patil, Hoger, & Lubee, 2007). Other supplemental measures

include the distance of the path traveled and the speed of ambulatory behavior. During extinction, the escape box remained in the same location, however access was blocked, which prevented escape. Critical components of the protocol included: properly applying the aversive stimuli necessary to motivate the animal to search for the escape box; ensuring conditions remained uniform (e.g., time of day, experimenters, control over external noise, extra-maze cues, and consistency of start position); and cleaning the maze with 70% ethanol (volume/volume) after every animal to minimize the effects of olfactory and visual cues from scent and possible fecal matter left on the maze (Rosenfeld & Ferguson, 2014).

While the BM is often used to assess spatial learning, it can also be a useful tool for inducing anxiety in an extinction situation. Vargas-Lopez, Lamprea, and Munera (2011) used an abbreviated BM protocol to evaluate escape extinction. Animals were trained to escape from the BM platform into a concealed box (escape box) during 8 trials of acquisition. Extinction was implemented for a subsequent 8 trials. Researchers found that during the extinction trials animals initially checked fewer holes (made fewer errors) and traveled shorter distances than they did during the acquisition phase, but that during later sessions of extinction animals checked more holes (made more errors) and traveled longer distances (explored more). Harloe, Thorpe, and Lichtman (2008) studied extinction in both appetitive and aversive BM tasks. The appetitive BM task involved access to water and the aversive BM task involved escaping from bright lights. The CB_1 (cannabinoid) receptor antagonist rimonabant was administered 30 min prior to each session of BM to evaluate potential effects on extinction learning. Rimonabant administration did not affect behaviors in acquisition for either aversive or appetitive BM tasks, but did disrupt extinction learning in the aversive BM task. Stemming from these findings, Harloe et al. (2008) posit that endogenous cannabinoids play a role in the extinction of aversively motivated

behaviors, but apparently not in the extinction based on appetitive motivation. The authors argued that the endocannabinoid system may be selectively activated in highly aversive situations.

Ortega et al. (2013) conducted a series of experiments using rats to test the aversive summation hypothesis with one of the experiments using extinction in the BM. The aversive summation hypothesis suggests that when two independently induced sources of stress are combined, the negative emotional state of the combined stressors is more intense than either source alone. The anxiety induction task was a session of restraint stress prior to BM trials. This was done to assess whether restraint stress affected behavior during extinction of escape behavior. The degree of incentive downshift is maximal due to the complete removal of the incentive (escape box) during the extinction phase. The key behavior was exploratory or ambulatory behavior while on the surface of the maze. Ortega et al. (2014) found that after being exposed to restraint stress, animals exhibited no difference in the latency to reach the location previously associated with the escape box, but did increase exploratory behavior after reaching the target location, relative to controls not exposed to restraint stress.

Extinction of escape behavior in the BM can be conceptualized as a reward-loss situation. Reward loss is most frequently studied in two basic situations: SNC and appetitive extinction (Papini, Fuchs, & Torres, 2015). Other situations involving partial reinforcement, extinction, one-way avoidance, and cSNC have also been used to study reward loss. In SNC, behavior deteriorates after a downshift from a large to a small reward, whereas in appetitive extinction, behavior is reduced after a downshift from reward to nonreward.

Current Research

The present research rested on the assumption that substances that reduce psychological pain are rewarding. We know that negative emotions induce the consumption of substances with addictive potential (e.g., ethanol) and we also know that the voluntary or forced administration of such substances often reduces signs of negative emotion. For example, anxiety is linked with higher oral ethanol consumption in rats (Pelloux, Costentin, & Boucher, 2015). Anxiolytics such as ethanol have also been found to modulate incentive downshift after systemic administration (Becker & Flaherty, 1982; Kamenetzky et al., 2009). In humans, the incidence of negative life events is associated with increased voluntary drinking and emotional reactivity (Keyes, Hatzenbuehler, & Hasin, 2011). This research questioned whether organisms could learn to regulate negative emotional states induced by reward loss through voluntarily consuming such substances. Previous studies have suggested that reward loss can induce negative emotion (e.g., frustration, anxiety) that can be attenuated by the postsession voluntary consumption of anxiolytics. A successful animal model of ESM could provide evidence supporting the hypothesis that ESM plays a role in the early development of addictive behaviors such as excessive alcohol consumption, and open the way to a neurobiological analysis of the early stages of SUDs.

As many of our environmental stressors stem from loss, it is important to consider how situations involving loss may translate into addictive behavior. Situational stressors associated with loss are capable of inducing psychological pain. Research is often focused on overt physiological changes. However, psychological or emotional changes are also important (Crane, 2015). Experiencing loss can elicit negative emotional states. The current studies attempted to provide an outline for an animal model of ESM by first establishing that the BM task is capable

of inducing negative emotion through a form of reward loss—extinction of escape behavior, and then demonstrating that rats will increase their voluntary consumption of the anxiolytic ethanol during the extinction phase of the BM protocol. This increase in voluntary oral ethanol consumption is thought to attenuate negative emotional reactivity, thus having a reinforcing effect.

Experiment 1

The primary objective of the first experiment was to evaluate the effects of escape extinction on behavior using a master-yoked design. An adapted BM protocol with 10 days of acquisition and 5 days of extinction was used where animals with access to escape during acquisition served as master animals, and animals with no access to escape during acquisition served as yoked (control) animals. Master-yoked pairs were matched in terms of their exposure to the BM task meaning that however long the master animal remained on the surface of the maze before entering the escape box was the length of time the yoked animal was allowed to remain on the surface of the maze to explore. Dependent variables of interest included: total holes checked, distance traveled in centimeters, and latency to goal (escape box). The latency to goal measure provided the criterion for yoking.

Method

Subjects. Subjects were 20 experimentally naïve female Wistar rats (*Rattus norvergicus*) bred from proven breeder parents purchased from Envigo Laboratories (Indianapolis, IN). All animals were maintained under standard animal colony conditions with an approved Institutional Animal Care and Use Committee (IACUC) protocol. The colony room was maintained on a 12:12 h light:dark cycle with lights on at 07:00 h. Access to the colony room was restricted during the 12 h of darkness. Temperature was kept between 18-22 °C, with humidity levels

ranging between 50% and 60%. Rat pups were housed with their mother for the first 21 days of life. On postnatal day (PND) 21, all animals were group housed based on litter in clear polycarbonate boxes, 45.4 x 24 x 20 cm, lined with a 2-cm corn cob bedding, a single 5 x 5 cm cotton nestlet, and a red plastic enrichment hut to provide exercise. On PND 31 all animals were single housed on wire bottom racks that included a red enrichment hut. All animals had ad libitum access to filtered water via a metal lick sipper tube in the home cage. Animals were fed Purina lab rat chow on an ad libitum basis until PND 90, at which point all subjects were food deprived to between 81-84% of their ad libitum body weight. Although not necessary, food deprivation was used for consistency with other ESM experiments.

Apparatus. A BM (Med-Associates, Richmond, VA) was used for behavioral testing. The maze was a circular, 1.3-cm thick, grey PVC slab with a diameter of 122 cm. It was located 140 cm from the floor, and had 18 holes, 9.5 cm in diameter each, around the perimeter, spaced 20 degrees apart at a distance of 2.5 cm from the edge. Surrounding the maze were four black curtains made of dense fleece material to temper light escaping and to provide a control for the amount of space around the maze. One extra-maze cue, a 30-cm white poster board equilateral triangle, was placed directly across from the escape box. Two 150 W LUMAPRO Clamp-On lights (Grainger, Fort Worth, TX) with 15 W LUMAPRO LED warm white lamp bulbs (Grainger, Fort Worth, TX) were mounted to the ceiling approximately 100 cm from the top of the maze to provide illumination. A Color Gigabyte Ethernet Camera (Noldus, Leesburg, VA) was mounted directly above the center of the testing arena and connected to EthoVision XT Version 11 Software (Noldus, Leesburg, VA). Pink noise was produced by a 16.51 x 4.06 x 6.35 cm SoundBot SB571 speaker (SoundBot, Hacienda Heights, CA) providing a manual aversive noise control for the experiment. A second experimenter manually started the pink noise once the

first experimenter brought the animal into the testing room and allowed it to play continuously, then once the animal reached the escape box (if available for that condition) the pink noise was terminated. All equipment was mounted above the testing arena using 5.08 cm wide Hook and Loop Adhesive (McMaster-Carr, Elmhurst, IL). After each rat was tested, the maze and the escape box were cleaned with a 70% ethanol solution (volume/volume) to minimize all odor or visual cues from previous subjects.

Procedure. Subjects were randomly assigned to either the yoked condition (Group Y), for which no escape from the maze was available during the acquisition or extinction phases, or the master condition (Group M), for which escape from the maze was possible only during the acquisition phase (n=10). For each master-yoked pair, the time that the master animal spent on the maze during acquisition determined the time that the yoked animal spent on the maze. Subjects experienced one trial per session with one session occurring each day of acquisition and extinction. Each session was video recorded.

One subject at a time was run on the maze with run-order counterbalanced across days to prevent order effects. All subjects were placed in the same start position in the center of the maze, facing the extra-maze cue each time they were tested. The session began once the experimenter released the rat at the start position. A second experimenter waited in an adjacent testing room to start the trial. When the first experimenter entered the testing room with the subject, the second experimenter began recording video data. The session was considered complete once the subject reached the escape box, or the appropriate yoked time elapsed. Subjects were allowed to remain in the escape box for 30 s during acquisition. The acquisition phase lasted a total of 10 days. The extinction phase began on Day 11 and ran through Day 15. During extinction sessions, the escape box was made inaccessible, but left in the same position.

All extinction sessions lasted 60 s. EthoVision XT software was used to assess the total holes checked, total distance traveled (in cm) on the maze, and the latency to reach the escape box. Immediately following the BM task, subjects were returned to their home cages and returned to the colony room. Subjects were fed at least 15 min after the study, during the light cycle, to maintain their 81-84% food deprivation levels.

Results

Results were analyzed using separate repeated measures analyses of variance (ANOVA) with sessions as the within subject factor and an alpha value set at the 0.05 level for the acquisition phase (Sessions 1 through 10) and the extinction phase (Sessions 11 through 15). All dependent variables were evaluated using Group (M, Y) x Session ANOVAs with the exception of the latency to goal measure, where a Group (M, Y) x Session (11-15) ANOVA was conducted. This analysis was different due to the yoked component being in place during the acquisition phase where Group Y animals were matched in terms of exposure to the BM and no latency measure would be relevant. During the extinction phase, both Groups M and Y had a fixed session length of 60 seconds, and animals in both groups experienced the same blocked goal hole location. For animals in Group M, this location used to contain the escape box. However, for animals in Group Y, this location was always blocked and inaccessible, though they were still free to check the hole. Thus, we used the latency to check the goal location as a measure only in extinction for Group Y.

Data for the total number of holes checked variable are shown in Figure 1. The acquisition ANOVA results revealed a nonsignificant two-way interaction, $F < 1$, as well as a nonsignificant main effect of group, $F(1, 18) = 1.78$, $p > 0.190$, but a significant session effect, $F(9, 162) = 5.17$, $p < 0.001$. The extinction ANOVA results revealed a significant two-way

interaction, $F(4, 72) = 4.95$, $p < 0.002$, a significant main effect of group, $F(1, 18) = 7.34$, $p < 0.001$, but a nonsignificant effect of session, $F(4, 72) = 1.99$, $p > 0.106$. Group comparisons based on the overall analysis and using pairwise LSD tests indicated that after exposure to escape extinction, master animals checked more total holes in sessions 11-13 compared to yoked control animals, Fs$(1, 18) > 5.77$, ps < 0.03.

Figure 2 shows the results for the distance traveled measure. The acquisition ANOVA results revealed a nonsignificant interaction and main effect of group, Fs < 1, but a significant effect of session, $F(9, 162) = 6.43$, $p < 0.001$. The extinction ANOVA results revealed a nonsignificant two way interaction as well as a nonsignificant effect of session, Fs$(4, 72) < 1.73$, ps > 0.152, but a significant main effect of group, $F(1, 18) = 9.43$, $p < 0.008$, indicating that master animals traveled more than yoked control animals following escape extinction.

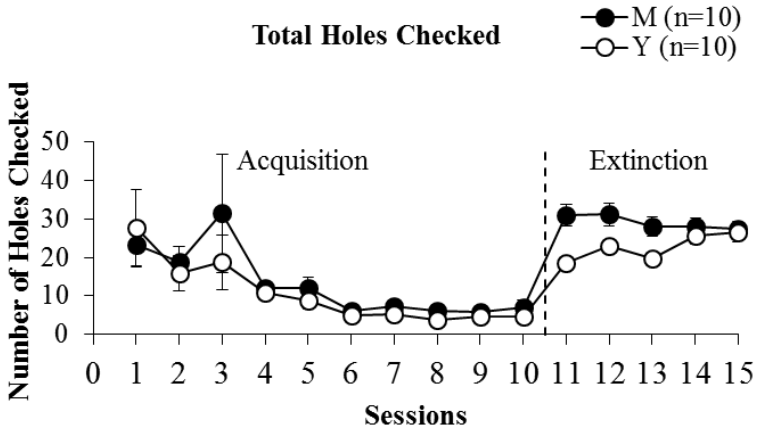

Figure 1. Total holes checked for Master and Yoked animals

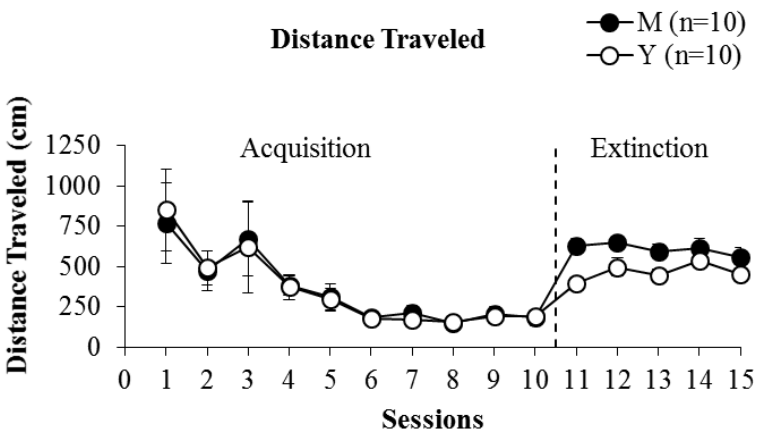

Figure 2. Distance traveled in centimeters for Master and Yoked animals

Figure 3 shows the results for the latency to goal measure. A Group (M, Y) x Session (11-15) ANOVA was conducted. The results revealed a significant two-way interaction, $F(4, 72) = 3.62$, $p < 0.02$, as well as a significant main effect of group, $F(1, 18) = 10.86$, $p < 0.004$, but not session $F < 1$. Group comparisons based on the overall analysis and using pairwise LSD tests indicated that after exposure to escape extinction, master animals reached the goal location quicker during sessions 11-13 compared to yoked control animals, $Fs(1, 18) > 10.91$, $ps < 0.005$. Extinction of escape behavior was apparent in the latencies exhibited by Group M. A one-way analysis of these latencies indicated a significant increase in latency across sessions 11-15, $F(4, 36) = 4.77$, $p < 0.004$.

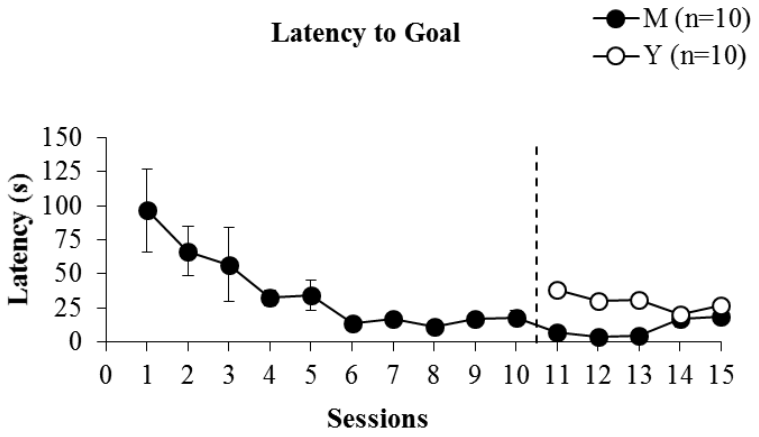

Figure 3. Latency to reach the blocked goal (escape box) hole. Data for yoked animals not shown during acquisition due to the mater-yoked design.

Experiment 2

The primary objective of the second experiment was to examine the effects of escape extinction on the voluntary consumption of the anxiolytic ethanol. Experiment 2 used the same BM protocol for master animals described in Experiment 1. Each session was followed by a daily 2-h, 2-bottle preference test where one bottle contained 8% ethanol (volume/volume) and the other distilled water (Group E). A control group had access to two bottles of water during the preference test (Group W). A concentration of 8% ethanol was selected based on unpublished pilot data from TCU resulting in higher preference for ethanol as the concentrations increased. This preference for higher concentrations of ethanol can be attributed to strain differences between the Wistar rats at TCU versus the Wistars used in Spain (e.g., Manzo et al., 2014), where the concentration used was 2%.

Method

Subjects. A total of 20 experimentally naïve female Wistar rats (*Rattus norvergicus*) bred from proven breeder parents purchased from Envigo Laboratories (Indianapolis, IN) served as subjects in this experiment, however, the final N = 19 due to an unexpected loss of one subject in

Group E. The same animal husbandry procedures from the preceding experiment were used for these subjects.

Apparatus. The preference testing took place in the animal's home cage, on a transport rack, in a designated room. Two 473-ml, wide-mouth bottles (Ancare, Bellmore, NY) with #8.5 rubber stoppers with 10.16 cm curved ball bearing metal sippers attached (Ancare, Bellmore, NY, United States) were secured to the home cage during the 2-h preference test. Bottles were labeled with plastic tape. All bottles were weighed before and after the preference test to assess the amount of consumption. The 8% ethanol (volume/volume) was made from 95% ethyl alcohol (Pharmco-Aaper, Shelbyville, KY) diluted in distilled water. The mixture contained 252.63 ml of 95% ethyl alcohol and 2747.37 ml of distilled water. Fresh ethanol was made as needed with the new mixture being combined with the original mixture each time.

Procedure. Subjects were randomly assigned to either Group E (n = 9*)* or W (n = 10). Immediately following the BM task, subjects were returned to their home cages located on a transport rack in the adjacent room and began the 2-h preference test. Two bottles were simultaneously presented to the animals, containing 8% ethanol (volume/volume) and distilled water for Group E or water in both bottles for Group W. All bottles were weighed prior to the preference test and after the completion of the 2-h task to assess consumption. Subjects were left unmonitored during the preference test in a room with the lights on. After the 2-h preference test was completed, subjects were returned to the colony room in their home cages and placed back on their home racks. Subjects were fed at least 15 min after the study, during the light cycle, to maintain their 81-84% food deprivation levels.

Results

Results were analyzed using separate repeated measures analyses of variance (ANOVA) for acquisition (Sessions 1 through 10) and extinction (Sessions 11 through 15) with sessions as the within subject factor and an alpha value set at the 0.05 level. All dependent variables were evaluated using Group (E, W) x Session (T-15) ANOVAs. A preference ratio greater than 0.5 (chance levels) indicated a preference for ethanol over distilled water. Though data from an unpublished pilot showed that animals still voluntarily consume higher concentrations than 8%, these were not selected due to the toxicity and organ damage associated with consumption of higher concentrations of ethanol (El-Guindy et al., 2010). The decision to use 8% ethanol vol/vol was based on it being the highest consecutive concentration used before the preference ratio decreases. This allowed for consumption to still increase, without being faced with ceiling effects.

Figure 4 shows the results for the total holes checked measure. The acquisition ANOVA revealed a nonsignificant two-way interaction, $F(9, 153) = 1.36$, $p > 0.223$, as well as a nonsignificant main effect of group, $F(1, 17) = 2.42$, $p > 0.14$, but a significant effect of session, $F(9, 153) = 14.27$, $p < 0.001$. Similarly, the extinction ANOVA revealed a nonsignificant two-way interaction as well as a nonsignificant main effect of group, Fs < 1, but a significant session effect, $F(4, 68) = 6.19$, $p < 0.001$.

Figure 5 shows the results for the distance traveled in cm. The acquisition ANOVA revealed a nonsignificant two-way interaction, $F(9, 153) = 1.36$, $p > 0.222$, as well as a nonsignificant main effect of group, $F < 1$, but a significant session effect, $F(9, 153) = 9.75$, $p < 0.001$. The extinction ANOVA also revealed a nonsignificant two-way interaction, $F(4, 68) =$

1.52, $p > 0.207$, as well as nonsignificant main effect of group, $F < 1$, but a significant session effect, $F(4, 68) = 9.65$, $p < 0.03$.

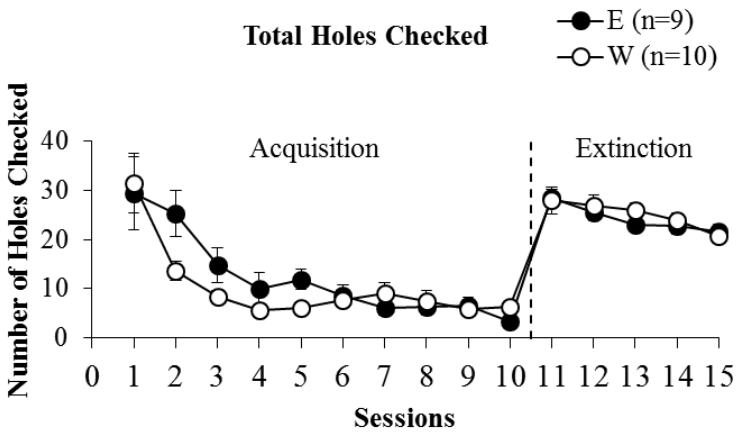

Figure 4. Total holes checked for Group E and Group W

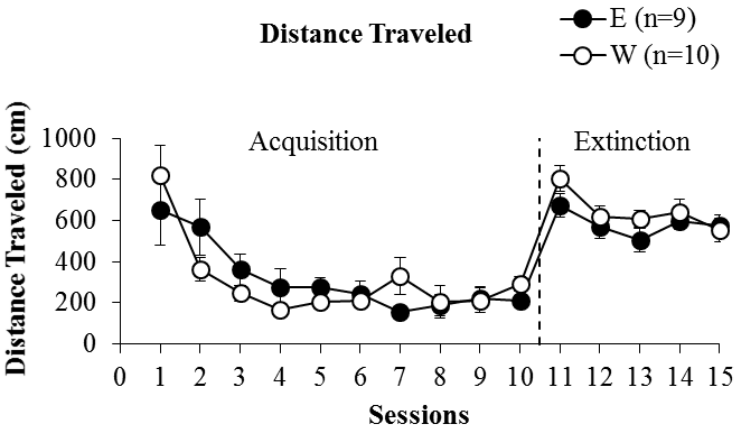

Figure 5. Distance traveled in cm for Group E and Group W

Figure 6 shows the results for the latency to goal measure. The acquisition ANOVA revealed a nonsignificant two-way interaction, $F(9, 153) = 1.25$, $p > 0.267$ as well as a nonsignificant main effect of group, $F(1, 17) = 1.10$, $p > 0.308$, but a significant effect of

session, $F(9, 153) = 14.85$, $p < 0.001$. The extinction ANOVA also revealed a nonsignificant two-way interaction, $F(4, 68) = 1.72$, $p > 0.155$, as well as a nonsignificant main effect of group, $F < 1$, but a significant effect of session, $F(4, 68) = 2.95$, $p < 0.027$.

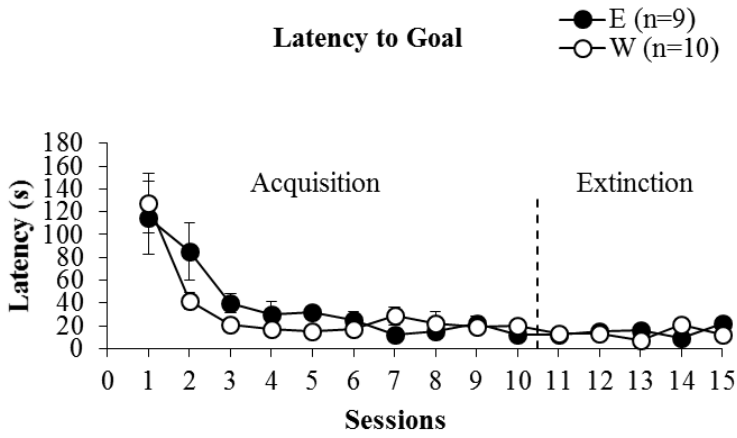

Figure 6. Latency to reach the goal (escape box) for Group E and Group W.

For ethanol consumption in Group W, one of the two bottles was designated as the ethanol control bottle despite containing distilled water. For the water consumption in Group E, the water bottle was used. The ANOVA results for ethanol consumption (Figure 7, left panel) during acquisition revealed a significant two-way interaction, $F(9, 153) = 2.08$, $p < 0.035$ as well as significant main effects of session, $F(9, 153) = 7.64$, $p < 0.001$, and group, $F(1, 17) = 42.83$, $p < 0.001$. Group comparisons based on the overall analysis and pairwise LSD tests showed that animals consumed more ethanol than water during all acquisition sessions except for Session 9, Fs$(1, 17) > 4.45$, ps < 0.05. The ANOVA for ethanol consumption during extinction revealed a nonsignificant two-way interaction, $F(4, 68) = 1.31$, $p > 0.277$, but significant main effects of session, $F(4, 68) = 12.77$, $p < 0.001$, and group, $F(1, 17) = 17.63$, $p < 0.002$. There were significant changes in water consumption (Figure 7, right panel) across sessions, $F(9, 153) = 10.39$, $p < 0.001$, but nonsignificant effects for group and for the interaction, Fs < 1, during

acquisition. There was also a significant change across sessions, $F(4, 68) = 18.53$, $p < 0.001$, but nonsignificant group and interaction effects, Fs < 1, during extinction.

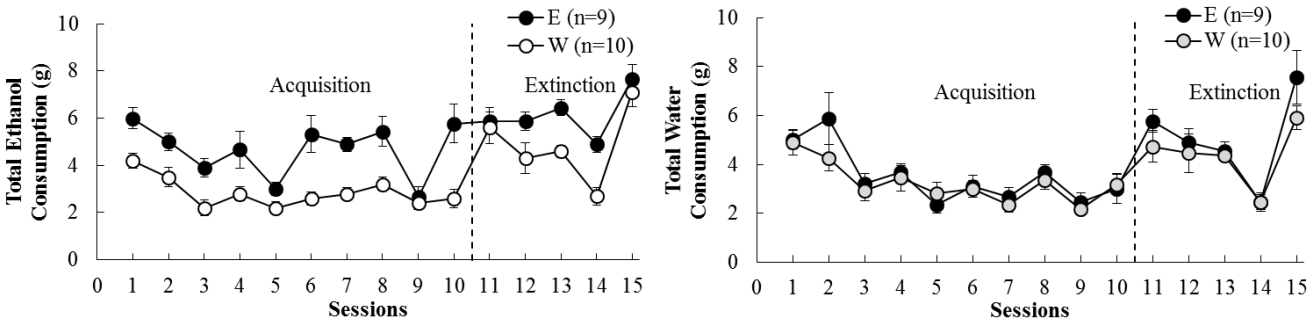

Figure 7. Data for ethanol (left) and water consumption (right) for Groups E and W.

Data for the preference ratio were obtained by calculating the amount of ethanol (or water) consumed during the 2-h, 2-bottle preference test, and dividing that amount by the total consumption. Figure 8 shows the results for preference of ethanol. Results from the acquisition ANOVA revealed a a nonsignificant two-way interaction, $F(9, 153) = 1.61$, $p > 0.117$, but a significant main effect of session, $F(9, 153) = 2.23$, $p < 0.03$, and group, $F(1, 17) = 34.75$, $p < 0.001$. The extinction ANOVA revealed a significant two-way interaction between group and session, $F(4, 68) = 3.55$, $p < 0.011$, as well as a significant main effect of group, $F(1, 17) = 15.96$, $p < 0.002$, but not session, $F(4, 68) = 1.55$, $p < 0.199$. Group comparisons based on the overall analysis and pairwise LSD tests showed that animals preferred ethanol to water following escape extinction during sessions 12-14, Fs(1, 17) > 4.49, ps < 0.05.

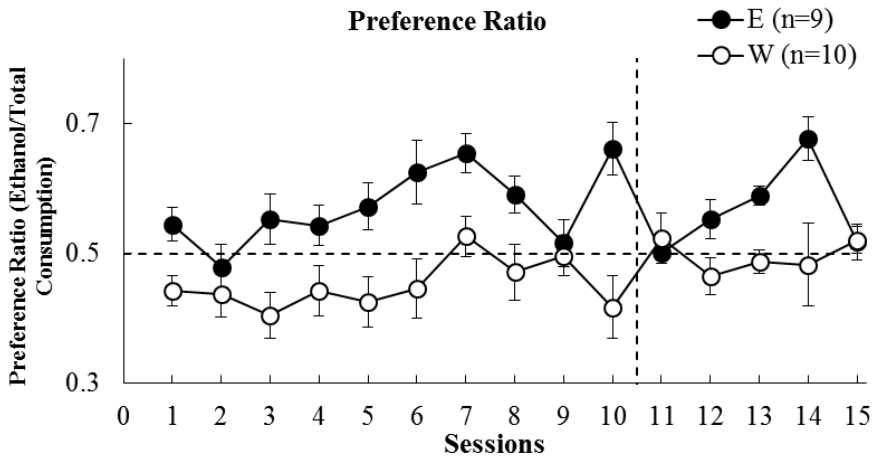

Figure 8. Preference Ratio for Groups E and W obtained by dividing the amount of ethanol (or water) consumed by the total consumption.

General Discussion

The current experiments were designed to validate escape extinction in the BM as an effective means of inducing a negative emotional state and to test the hypothesis that escape extinction would support an ESM effect. I explored the relationship between frustration induced by escape extinction and ESM by following procedures used in previous ESM experiments where the consumption of anxiolytic substances such as ethanol increased following a reward loss task (Manzo et al., 2014; Manzo et al., 2015). Consistent with frustration theory (Amsel, 1992), the negative emotional state induced by a situation involving loss is transient and dissipates over time. The results from the current experiments can be summarized in this manner: (1) Escape extinction in the BM task was effective at inducing an increase in exploratory behavior in Master animals. (2) Escape extinction followed by access to ethanol or water produced a seemingly transient increase in preference for ethanol following the first exposure (session 11) which dissipated by session 15.

Though the BM task is typically used to assess spatial learning and memory, it has also been used to induce negative emotional states (Ortega et al, 2013). First, testing the BM task

with a master-yoked design showed enhanced latencies to goal after exposure to escape extinction for master animals relative to their yoked controls. In the master-yoked design, master animals also exhibited increases in the total holes checked and distance traveled measures which is taken as another indication of escape extinction inducing a negative emotional state for these animals. These findings were consistent with the hypothesis that exposure to an instrumental downshift (escape extinction) would induce a negative emotional state, or a state of anxiety. Increases in exploratory behavior observed through total holes checked, distance traveled, and latencies to goal measures were interpreted as animals increasing their efforts to escape from the threatening, well-lit and open surface of the BM itself by searching for other avenues of escaping which is indicative of anxiety.

Second, the analyses of variance for Experiment 2 yielded non-significant interactions for all dependent variables aside from preference. This evidence suggests that animals given access to ethanol exhibited no significant differences in behavioral responding following escape extinction, but instead exhibited marked differences in the voluntary consumption of both ethanol and water. These findings are consistent with Manzo et al., 2014 where following an instrumental downshift animals exhibited an increase in their consumption of and preference for ethanol. The current research used a higher concentration of ethanol (8%) but the sequence of induction task followed by preference test was consistent.

Water consumption was variable during the extinction phase (See Figure 7). Upon visual inspection, the consumption appears to increase following terminal acquisition and fluctuate upwards and downwards. Any increases in water consumption are inconsistent with the drug specificity postulate which states that drug choice reflects the ability of the substance to reduce specific, relevant symptoms (Darke, 2013). Water lacks anxiety-ameliorating properties, thus it

is unclear why the consumption of water fluctuated after exposure to escape extinction. The peak of preference for ethanol occurred during session 14 (see Figure 8) and the effects of escape extinction appeared to dissipate by session 15. While the preference for ethanol seems to be a transient effect, the experimental parameters need to be extended to see if the effects are truly transient in nature. Extending the extinction phase by an additional 5 sessions could yield clearer results. While the use of 5 incentive downshift (escape extinction) sessions was chosen to remain consistent with the length of previous cSNC studies (Manzo et al., 2015), it appears that in the case of escape extinction in the BM, 5 sessions may not be sufficient to demonstrate a clearly transient effect.

Another way to alter the BM induction task is to introduce a complete removal of the escape box during the extinction phase, rather than leaving it in the same position and making the escape box inaccessible. Through this total removal we could assess if there is a further increase in exploratory behavior than that observed in the current research. Harloe, Thorpe, and Lichtman (2008) employed an adapted BM procedure where the escape box was completely removed during the extinction phase of the experiment. Their results suggest that the complete removal of the escape box facilitated a significant increase in exploratory behavior around the escape location during the first session of extinction which dissipated over subsequent extinction sessions. One unique measure they used was the percentage of time spent checking holes near the former location of the escape box. During the later sessions of escape extinction, they observed an almost complete behavioral extinction of hole checking behavior near the escape box.

To provide a more thorough test of the ESM hypothesis using escape extinction, future studies should employ an amalgamation of the current research where a master-yoked design is

used. Using this new design, a comparison could be made between master animals and yoked animals in terms of their consumption of ethanol and water. If yoked control animals consumed less ethanol relative to the master animals, this would be consistent with the two main assumptions of the ESM hypothesis. Specifically, we could test the psychopathology postulate through the use of yoked control animals because these animals, never having been exposed to escape, should not experience the same negative affect as their master counterparts. The drug-specificity postulate which states that drugs will be chosen based on the specific properties related to the relief of symptoms could be tested using this design as well. If master animals are selectively choosing to consume more ethanol compared to water, then it could be interpreted as animals choosing to self-medicate following exposure to extinction of escape behavior due to the specific anxiolytic properties that ethanol possesses. Anxiolytics are capable of attenuating negative emotions such as anxiety. Water, on the other hand, is free of any anxiety attenuating properties. Consistent with the drug-specificity postulate, water should not be able to attenuate a negative emotional state. A careful analysis of the patterns of consumption and preference for ethanol and water using this new paradigm could provide further support of the ESM hypothesis.

Conclusions

Extinction of escape behavior in the BM can be employed as another form of incentive downshift induction task to precede preference testing in ESM research. Experiment 1 helped validate the BM as a tool for inducing anxiety through the extinction of escape behavior. The increase for master animals compared to yoked control animals in the total holes checked measure is indicative of anxiety. The change in latencies to goal and the increase in distance traveled supports an interpretation of the escape extinction procedure being useful in inducing anxiety.

Experiment 2 expanded upon these findings by adding a 2-h, 2-bottle preference test component following the anxiety induction task. While animals in Group E exhibited a higher preference ratio as predicted, their preference and consumption were consistently high across phases. The preference ratio for Group E decreased on the first downshift session (Session 11) before exhibiting a transient increase which dissipated by Session 15. However, taking into account the variability of preference ratios during acquisition, the interpretation that the ratios were transient in extinction must be taken with caution.

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VITA

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While completing on her second thesis in Experimental Psychology, she worked under the mentorship of Dr. Mauricio R. Papini. She held a University Teaching Assistantship during her time at TCU. She is a member of the Association for Behavior Analysis International, the Louisiana Behavior Analysis Association, Psi Chi the International Honor Society in Psychology, the Southwestern Psychological Association, and the Texas Association for Behavior Analysis.

ABSTRACT

EXPLORING EMOTIONAL SELF-MEDICATION DURING EXTINCTION OF ESCAPE BEHAVIOR IN RATS

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Precursor behaviors that may lead to the development of addiction were explored using the emotional self-medication hypothesis. Experiment 1 involved a master-yoked design which tested the effects of escape extinction using the Barnes maze (BM). Experiment 2 tested the BM as an anxiety induction task which was immediately followed by a 2-h, 2-bottle preference test where ethanol, an anxiolytic substance was made available (i.e. ethanol/water in one group vs. water/water in the control). Increases in exploratory behavior were taken as evidence of anxiety in the escape extinction phase of the BM task. Transient increases in preference for ethanol were observed in Experiment 2 which provide support for the drug specificity postulate of the emotional self-medication hypothesis.