COCA-COLA ADDICTION IN THE PRESENCE OF A CONDITIONED TASTE AVERSION IN RATS

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by

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Abstract

The following study examined the addictive nature of Coca-Cola® by providing either diet, caffeine free Coca-Cola® or Coca-Cola® for two weeks to adolescent rats. On day 15, rats were given 10% apple juice followed by a 0.15M lithium chloride. On day 16, the controls was given a choice between 10% apple/diet caffeine free Coca-Cola® vs. Coca-Cola® with the experimental group given 10% apple/Coca-Cola® vs. diet caffeine free Coca-Cola®. The study found that the experimental rats continued to drink Coca-Cola®, despite it being paired with the illness producing apple juice, and preferred it over a non-illness producing substance, thus demonstrating addiction. Equally important, this experiment also provides a novel behavioral paradigm for measuring addiction in animals.

Coca-Cola Addiction in the Presence of a Conditioned Taste Aversion in Rats

There is much debate about whether caffeine should be considered a drug of abuse (Griffiths & Mumford, 1994; Satel, 2006). Currently the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) does not list caffeine as a drug of dependence or abuse, but instead gives it an intoxication status (American Psychiatric Association, 2000). During the last decade the argument has continued to gain momentum due to the vast increases in childhood obesity and type two diabetes (Schulze, et al., 2007) and their positive correlation with increased soft drink consumption over the last three decades (French, Lin, & Guthrie, 2003). While much of the research surrounding soft drinks examines the health effects of the beverage, little research has been done to identify any addictive qualities of soft drinks, despite the mean intake of soft drink consumption within the population more than doubling within a twenty year span (French, Lin, & Guthrie, 2003). One study that examined the reinforcing and subjective effects of caffeine in adolescents found that 22% of adolescent participants reliably self-administered caffeine during a choice period, choosing caffeinated over non-caffeinated colas (Hale, Hughes, Oliveto, & Higgins, 1995). Reliably self-administering caffeine suggests the possibility of psychological and or physical dependence. Hughes and Hale (1998) call attention to the need for more research examining the dependence-producing effects of caffeine in children. More information in this area could greatly impact the way society views the unrestricted use of caffeine, especially its use in products, such as soft-drinks, that are heavily used by children.

Health Concerns

Another important aspect of investigating the possible addictive nature of soft-drinks containing caffeine is the many health risks associated with soda consumption. A study by

Harnack, Stang, and Story (1999) examining children ages 2 to 18 years, found that soft drink users consumed more dietary calories per day than non-soft drink users, and that children rated as high soft drink users consumed less milk and fruit juice when compared to non-soft drink users. These findings suggest that children consuming soft-drinks decrease their intake of other more nutritious options, possibly decreasing the amount of nutrients consumed during childhood development; this could have strong health implications for children later in life. Other studies have positively correlated soft-drinks and other sugar-sweetened beverages with childhood obesity, weight gain, and type 2 diabetes in young and middle aged women (Ludwig, Peterson, & Gortmaker, 2001; Schulze, Manson, Ludwig, et al., 2007). A range of other studies have linked consumption of soft-drinks and other sugar-sweetened beverages to negative health outcomes such as hypocalcemia, decreased bone mineral density, increase risk of bone fractures, dental caries, kidney stones, increased risk of hypertension, and most consistently associated with increased energy intake of which individuals do not adequately compensate (Vartanian, Schwartz, & Brownell, 2007).

Similar health risks have been identified in animal studies examining soft-drink consumption in rats. Belpoggi and colleagues (2006) found statistically significant increase of body weight, malignant mammary tumors in female rats, exocrine ademonas of the pancreas in males and females, as well as a non-significant increase of rare pancreatic carcinomas in female rats administered Coca-Cola® as a substitute for drinking water. Other animal studies examining soft-drink consumption in rats found evidence of hypocalcemia and lower femoral mineral density (García-Contreras, Paniagua, Avila-Díaz, et al., 2000) as well as dental caries, hyperuresis, diarrhea, and decreased hair gloss (Tamura, Fujii, & Kusaba, 1979). Such a large number of studies finding evidence of negative health outcomes associated with soft-drink

consumption strengthens the need for further investigations into the addictive nature of soft drinks.

Animal Models of Addiction

There are many studies examining drugs of abuse, such as alcohol, cocaine and heroin, in animals. To date the primary animal models of addiction consists of operant intravenous drug self-administration, drug discrimination, brain stimulation reward, and place preference (Koob, 1994; Willner, 1997). While animal models of addiction have been shown to be reliable, valid, and widely accepted tools used to investigate drug-taking behavior in humans (Koob, 1994; Willner, 1997), there are many disadvantages to using these currently accepted animal models.

In order to use the drug self-administration model, researchers must perform surgeries on the animals to insert intravenous catheters for drug delivery. After surgery, the catheter site is monitored consistently to maintain the general health of the animal(s). Animals are trained to carry out some behavior, such as lever pressing, in order for the drug to be administered using a chosen schedule of reinforcement. Some of the disadvantages to using the drug self-administration model are that this model requires a surgical procedure to insert an intravenous catheter as well as, long periods of time for training animals. According to Koob (1994), besides the costly surgery and complex testing equipment, individuals working with the animals require training to acquire the appropriate skills necessary to maintain the proper function of the catheter. Another weakness of the self-administration model is that many drugs such as caffeine, nicotine, and alcohol are not intravenously injected by humans and therefore this type of model does not accurately mimic human use of such drugs which are ingested orally.

Studies using the drug discrimination model also require training animals to exhibit a particular behavior, such as lever pressing, escape from T-maze procedure, or escape from shock

procedure. In the lever pressing procedure, animals are given a choice between two levers; one lever produces food following drug administration, and the other lever produces food following administration of a control medium. The T-maze procedure requires training animals to turn left under the influence of one drug, and to turn right under the influence of another drug. Animals in tested with the T-maze procedure attempt to escape shock by making the correct turn in order to find the safe box. The final drug discrimination procedure, known as the escape from shock procedure, requires training animals to press a lever to escape shock. Animals are placed in a cage that delivers intermittent shock to a grid floor and the animals can avoid shock by pressing one of two possible levers. Disadvantages to the drug discrimination animal models are similar in that lengthy training is involved and in addition, any effects on the nervous system from the large quantities of the training drug administered during the training period cannot be measured (Koob, 1994).

Procedures for brain stimulation studies begin by surgically implanting electrodes into the areas of the animal's brain responsible for reinforcement processes. These electrodes deliver electrical stimulation to the brain and act as a reward when the animal performs a particular trained behavior. The disadvantages of brain stimulation procedures are also similar to those of drug self-administration models in that they require surgery to implant electrodes that deliver stimulation to areas of the brain. Also, training of lab personnel is also required to properly train the animals to administer electrical brain stimulation.

In animal place preference testing, after the delivery of a drug, the animal is placed in an environment that is distinctively different from the absence of drug environment. Animals are then given a choice between environments, one previously paired with drug exposure, and one without previous drug exposure. One disadvantage to this model is the lengthy periods of time

required for animal place preference procedures in order to identify any preference for one environment over another before the start of testing. Another disadvantage to place preference testing is the large number of both independent and dependent variables that can impact the results (Koob, 1994).

While self-administered drug testing is often used in animal research with drugs of abuse due to the reinforcing qualities of many drugs (Grigson, 1997), there is limited use of behavior models of addiction such as the use of lithium chloride (LiCl) induced conditioned taste aversion (CTA) drug testing. Much of the research using CTA drug testing uses the drug of abuse in place of LiCl to induce a CTA, which has been found to be ineffective if the drug of abuse has positive reinforcing effects (Grigson, 1997). There are many advantages to using a CTA animal model of drug addiction. CTA testing is efficient and inexpensive; there is no training time required for the animals and no expensive surgeries or equipment is required to perform testing. Another advantage to using a CTA model is drugs such as caffeine can be ingested orally, mimicking human consumption routes.

It was the intention of this study to test a new behavioral animal model of addiction using a LiCl-induced CTA to a novel substance such as a (10%) apple juice solution to support the notion that the addictive nature of Coca-Cola® is such that rats in the experimental group will ingest the Coca-Cola® despite its paring with CTA substance, while the rats in the control group will refrain from drinking the control solution paired with the CTA substance. The success of this study investigating the addictive nature of caffeine provides support for using a behavior model of addiction in animals, and also provides support for the notion that caffeine and soft-drinks such as Coca-Cola® that contain caffeine are addictive.

Method

Subjects

The study used 16 adolescent Long-Evans (Harlan) rats 60 days old (Levin, Rezvani, & Montoya, 2003). Eight rats were assigned to one of two groups: group one was the control group, which received diet, caffeine free Coca-Cola® and group two was the experimental group, which received the treatment of Coca-Cola® containing caffeine at 23mg/8fl ounces purchased from Wal-Mart. Coca-Cola® and the control medium were self-administered daily via test tubes with drinking spouts for 14 days. The animals were located in the animal lab of Clement Hall. The lights were kept on a 15-9 light/dark cycle, coming on at 7AM. Rats were housed in Plexiglas cages and were given free access to food and water unless otherwise noted.

Procedure

The Control group received 10mls of the control medium (diet, caffeine-free Coca-Cola®), the treatment group received 10mls of regular Coca-Cola® (containing 23mg of caffeine/8 fl ounces) daily for 14 days. The amounts of control or treatment solutions ingested were recorded daily. After administration of control and treatment on day 14, rats were water deprived for 23 hours. On the 15th day, all animals were given a 30-minute exposure to a (10%) apple juice/water mixture followed by a 0.15 M lithium chloride IP injection, which served as the unconditioned stimulus (US), 10 minutes later to provoke a CTA (Domjan, 1977; Spear & Riccio, 1994). All animal ingested 10mls of the (10%) apple juice/water mixture before the LiCl injection. On day 16 through 18 of the study, the control group was given a choice between a (10%) apple juice/control medium mixture and regular Coca-Cola®, while the rats in the treatment group were given a choice between (10%) apple juice/Coca-Cola® mixture and diet, caffeine-free Coca-Cola®. All rats were exposed to the described choices for 30 minutes (See

Table 1). It was hypothesized that the rats in the experimental group would ingest the Coca-Cola® despite its paring with CTA substance, while the rats in the control group would refrain from drinking the control solution paired with the CTA substance.

Results

The data collected from the experiment included amount of Coca-Cola® drank prior to training (days 1 thru 14, prior to LiCl injection) for the treatment group; the amount of diet, caffeine free Coca-Cola® drank prior to training (days 1 thru 14, prior to LiCl injection) for the control group; the amount of (10%) apple water drank at training (day 15) prior to the LiCl injection used for the acquisition of a CTA to the (%10) apple solution; the amount of Coca-Cola®/(10%) apple juice solution versus amount of diet, caffeine free Coca-Cola® drank at test 1 (days 16 and 17, after the LiCl injection) for the treatment group; and the amount of diet, caffeine free Coca-Cola®/(10%) apple juice solution versus amount of Coca-Cola® drank at test 1 (days 16 and 17, after the LiCl injection) for the control group. Other data collected from the study include the amount Coca-Cola® drank verses the amount of (10%) apple water drank at test 2 (day 18) for the treatment group; and the amount of diet, caffeine free Coca-Cola® drank verses the amount of (10%) apple water drank at test 2 (day 18) for the control group.

Difference scores were calculated using the (10%) apple water data from training and subtracting the soda/(10%) apple solution data from test for both the groups to determine differences between the groups, if any, in the amount of (10%) apple water solutions drank at training and the amount of soda/(10%) apple solution drank at test. The study found that there was a significant reduction of drinking a (10%) apple solution between controls (M=4.68, SD=0.61) and the treatment group (M=1.91, SD=1.69; t(14)=-4.35; p=<.01; d = 2.33, r = .79). This outcome shows that the rats in the control group drank considerably less (10%) apple

solution at test than did the treatment group after twenty-four hours following exposure to the LiCl injection and suggests, therefore that only the experimental rats drank the apple solution, even with the possibility it would make them ill (See Figure 1). An analysis of the data suggests that the results are unlikely to have occurred by chance.

Although the control group demonstrated a preference for the non-(10%) apple solution over the (10%) apple solution (M=-.24, SD=0.13), the treatment group demonstrated a statistically significant difference in preference of the (10%) apple solution over non-(10%) apple solution (M=0.46, SD=0.17; $t(14)=9.28$; $p=<0.01$; $d = 4.96$, $r = .93$) when compared to the control group. This result shows that the rats in the control group avoided the (10%) apple solution while the rats in the treatment group did not, providing more evidence that the rats that previously consumed the Coca-Cola®, continued to do so even when paired with a solution that previously made them ill (See Figure 2). Analyses of the data suggest that these differences are unlikely to have occurred by chance.

This study demonstrates that rats previously given Coca-Cola® will continue to drink the beverage, even when paired with a substance that previously made them ill, supporting the definition of addiction in animals. Furthermore, these rats preferred the Coca-Cola® paired with the apple juice over a substance that never made them ill.

Discussion

This study used a new, efficient, and inexpensive behavior model of addiction using a LiCl-induced CTA to examine the addictive nature of Coca-Cola® in rats. The results of this study provide evidence for the notion that soft drinks that contain caffeine, such as Coca-Cola®, are addictive in adolescent rats. The results show that the rats in the control group drank significantly less (10%) apple solution at test than did the treatment group after twenty-three

hours following exposure to the US, and that the rats in the control group significantly avoided the (10%) apple solution while the rats in the treatment group significantly preferred the (10%) apple solution after the acquisition of a CTA to (10%) apple juice/water.

These findings have many implications for the widely accepted availability and use of caffeinated beverages, like Coca-Cola®, by children and adolescents. Recent attention has been placed on the epidemic of obesity across the nation, especially in our children, and the association between obesity and soft drink consumption (Ludwig, Peterson, & Gortmaker, 2001). This association has lead to some school districts choosing to remove soft drinks from their schools. However, children have easy access to soft drinks in other areas as well, with the largest source of soft drinks coming from the home environment, restaurants, and vending machines (French, Lin, & Guthrie, 2003) despite the consistent association between soft drink consumption and negative health outcomes (Schulze et al., 2004; Vartanian, Schwartz, & Brownell, 2007). While much of the research surrounding soft drinks examines the health effects of the beverage, such as its consistent positive correlation with increased energy intake (Harnack, Stang, & Story, 1999), which may be associated with increased body mass index (BMI) and obesity (Ludwig, Peterson, & Gortmaker, 2001), little research has been done to identify any addictive qualities of soft drinks despite the mean intake of soft drinks in the U.S. population more than doubling within the last 20 years (French, Lin, & Guthrie, 2003). French and colleagues (2003) further found that soft drink consumption increased 48% among American children and adolescents from 1977 to 1998.

The results of this study provide support for the addictive nature of Coca-Cola® in adolescent rats. Koob (1994) and Willner (1997) both point out that animal models of addiction are widely accepted methods to investigating drug-taking behavior in humans. That being said,

based on the results of this study, further research in the area of caffeine addiction as it relates to soda consumption in children and adolescents is strongly recommended in order to protect the health and wellbeing of our youth. While some school districts have decided to remove soda and other sugar-sweetened beverages from schools in recent years, there may be a need to reevaluate the general availability of these beverages to our youth outside of school if these caffeinated beverages are, in fact, found to be addictive in humans. Availability of soft drinks to children may need limited by imposing a minimum age requirement for purchase of the beverage, thereby allowing parents to control soft drink consumption for their children.

The success of this study also calls attention to a new animal model of addiction testing that is both efficient and inexpensive. To date there are four primary models of testing animal addiction, which include self-administered drug testing, animal place preference testing, brain stimulation testing, and drug discrimination testing. While these methods are widely accepted by the research community to investigate drug dependence behavior in humans, most of them require lengthy training times and expensive surgeries to prepare the animals for testing. Other disadvantages to these methods include the inability of the method to truly mimic drug use in humans, such as a drug being administered via catheter as opposed to oral ingestion used most often by human for certain drugs such as caffeine; the failure to measure training drug effects on the nervous system during the training period; and not having enough control over many independent and dependent variables that can impact results. The behavior model of addiction used in this study employed the use of a CTA to (10%) apple juice solution, which was then coupled with the drug being tested for addictive qualities. This model did not require any behavioral training to test the animals; the 14 days prior to testing was used to allow the animals to orally administer the drug being tested in order to allow the animals time to demonstrate a

tolerance to caffeine (Griffiths & Woodson, 1988). Also, the method used for this study did not require any expensive equipment or surgical procedures to prepare the animals for testing.

This study is a good starting point for investigating the addictive nature of soft drinks in animals in that this study attempted to remove two variables from the control, caffeine and sugar. Further research should look closely at separating the three variables of soft drinks (caffeine, sugar, and carbonation) and controlling for each. Creating more testing groups that control for each variable separately would allow future studies to more thoroughly investigate which variable, or combination of variables, is responsible for the addictive nature of the beverage.

In conclusion, this study used a new, efficient, and inexpensive behavioral model of animal addiction to investigate the addictive nature of Coca-Cola®. This new behavioral animal model of addiction is free of the many disadvantages associated with the more widely used animal models of addiction. The results of this study found that the rats in the control group drank significantly less (10%) apple solution at test than did the treatment group after twenty-four hours following exposure to the LiCl injection, and that the rats in the control group significantly avoided the (10%) apple solution while the rats in the treatment group significantly preferred the (10%) apple solution. The results show a significant difference in the preference for a (10%) apple solution despite the acquisition of the conditioned taste aversion in both groups, which provides support for the addictive nature of the caffeine found in Coca-Cola®. Further research into the addictive nature of soft drinks containing caffeine should be studied in order to identify a possible need to limit consumption of these beverages in children and adolescents due to their possible addictive nature and high correlation with negative health outcomes.

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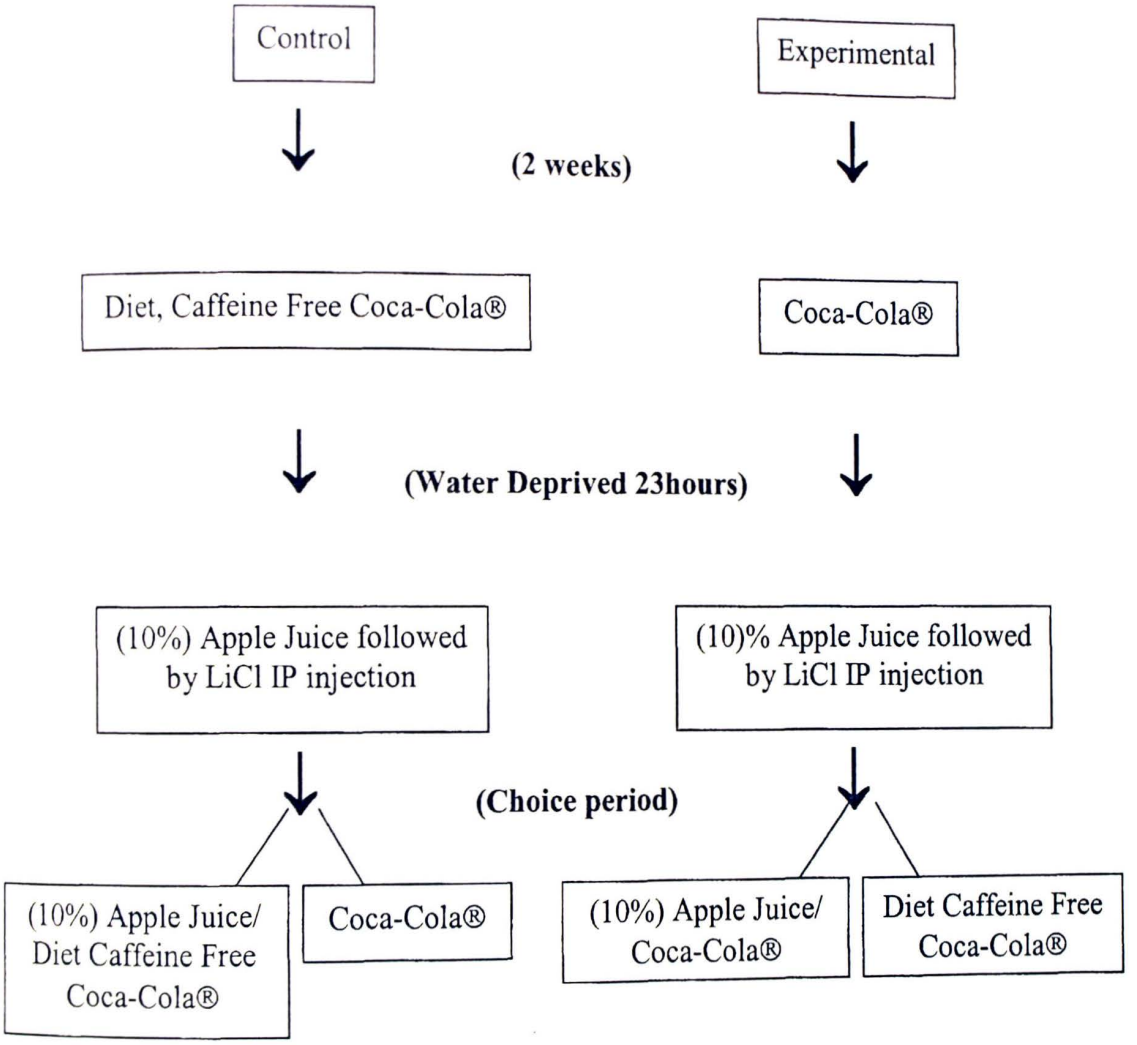
Figure Captions

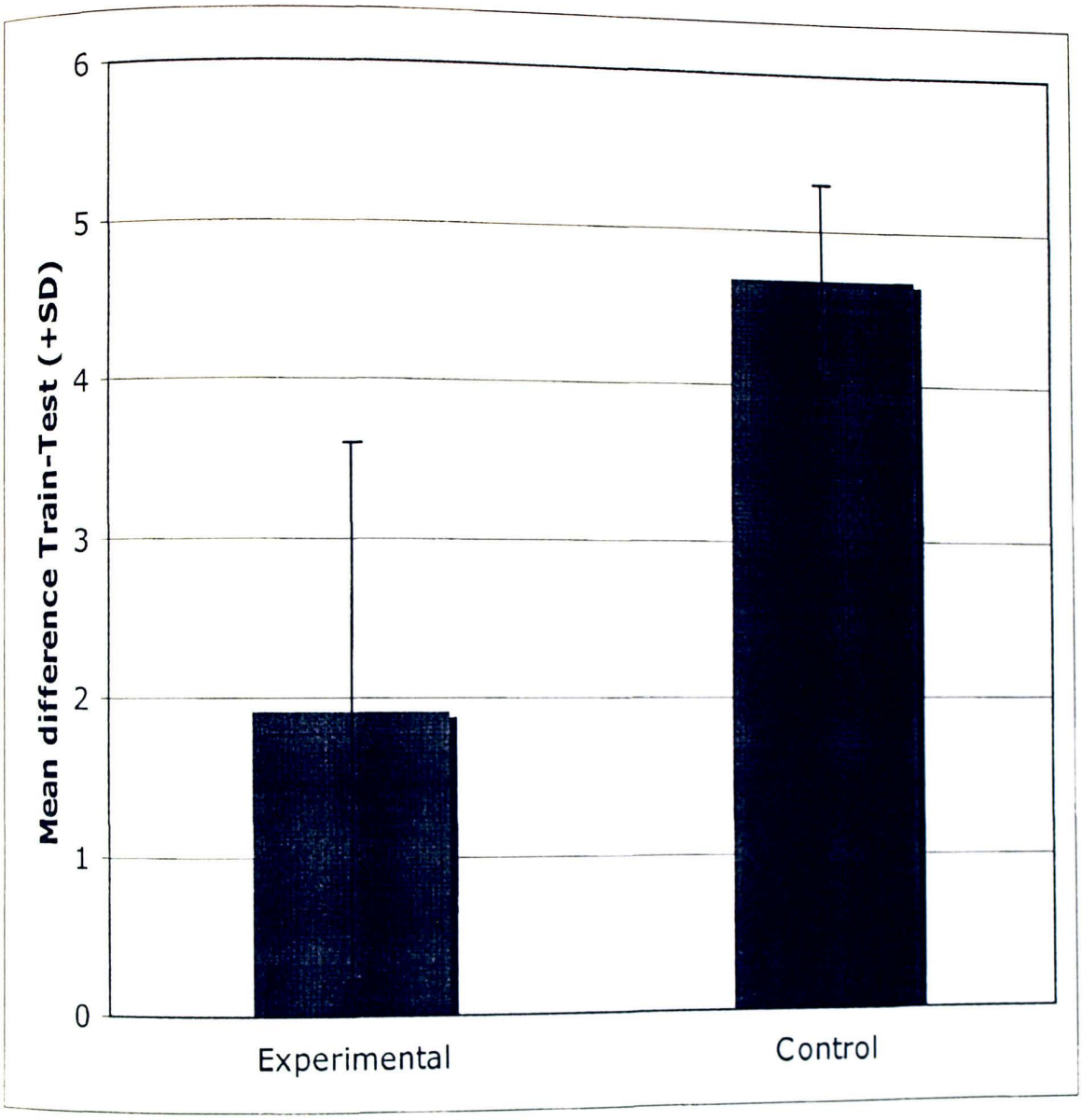
Figure 1: The mean differences (mls) between 10% apple water drank at training and 10% apple/soda solution drank at test for each group can be found by looking at the y-axis, with group condition on the x-axis. The error bars in this figure represents the standard deviation of the difference between the 10% apple water drank at the train condition and the 10% apple/soda solution drank at the test condition for each group. The study found that there was a significant reduction of drinking a 10% apple solution between controls and the treatment group, which demonstrates that the rats in the control group drank considerably less 10% apple solution at test than did the treatment group, after twenty-four hours following exposure to the LiCl injection.

Figure 2: The mean difference between the amount of a 10% apple solution and a non-10% apple solution drank at test for each group for the purpose of showing preference, can be found by looking at the y-axis, while the group condition can be found by looking at the x-axis. The error bars in this figure represents the standard deviation of the difference between the amount of a 10% apple solution and a non-10% apple solution drank at test condition for each group. The control group demonstrated a statistically significant difference in the preference for the non-10% apple solution over the 10% apple solution, while the treatment group demonstrated a statistically significant difference in the preference of the 10% apple solution over non-10% apple solution. This shows that the rats in the control group avoided the 10% apple solution while the rats in the treatment group did not.

Table 1

Experimental Design




Mean difference Train-Test (+SD)
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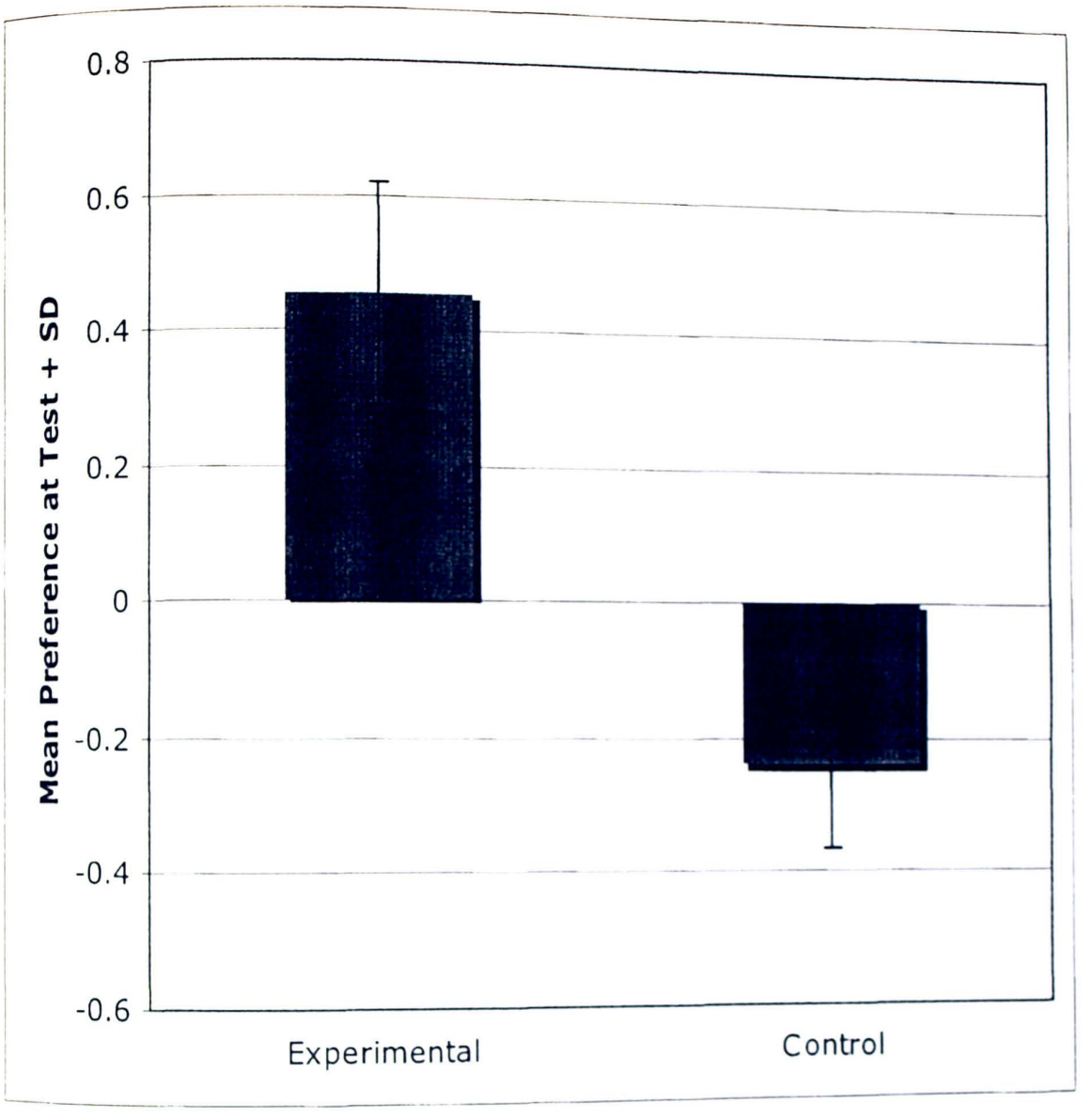
5
4
3
2
1
0
Experimental
Control


0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
Mean Preference at Test + SD
Experimental
Control