*Journal of Behavioral and Neuroscience Research*
2011, Vol. 9(1), 1-6


**BRIEF REPORT**

# The Addictive Properties of Sweetener Consumption Using a Conditioned Taste Aversion Animal Model for Dependency

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This study investigated sweetener dependency using the American Psychiatric Association's (APA) seventh criteria for dependency, namely continued use despite adverse consequences, using a rodent model behavioral paradigm. For a period of 14 days, animals orally self-administered a water mixture with either Sugar cane, Stevia®, Splenda®, Agave® or regular tap water. On day 15, the rats were exposed to a 10% apple juice mixture, and received an intraperitoneal injection of .15M lithium chloride at 2% body weight to induce a conditioned taste aversion. On day 16, the rats were given simultaneous access to the apple juice/sweetener mixture and the water mixture, and the amount consumed of each was recorded. The results showed the animal's demonstrated addiction by consuming the sweetener mixture despite conditioned taste aversion (CTA), which supports the APA's seventh criteria for dependency. These findings suggest that different types of sweetener have addictive properties.

Key Terms: *Sweetener, Adiction, Dependence, Rats, Conditioned Taste Aversion®, Stevia, Splenda®, Agave®*

The rise in addictive substances continue to be a topic of concern for government officials, doctors, employers and parents (Cummins, Freeman, Hughes, Miller & Hock, 2010). Consequences from drug abuse and dependency not only impact the individual user both physically and mentally but can also put an emotional strain on relationships. For a drug to be classified as a "drug of abuse," it must meet three of seven criterion established by the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR, 2000) criterion for substance dependency: (1) tolerance, (2) withdrawal, (3) increased drug administration for longer periods than intended, (4) inability to reduce or control drug use-binging, (5) spending considerable amount of time acquiring, using, and/or recovering from drug use, (6) significant impact on daily activities, (7) continued use despite adverse physical or psychological effects. Prior to Schmidt, Schmidt and Hock (2008), which tested the 7th criteria in a rodent model of dependency, animal models most commonly tested the first four criteria, primarily focusing on tolerance and withdrawal. However, the behavioral component, and not just the physiological components, should be equally considered as an important factor when empirically measuring dependency.

## Measuring Dependency

There can be considerable differences when using animal models as a tool to mimic human behavior (Willner, 1997). The methodologies used to study human psychopathology and animal behavior usually involve surgically implanting intravenous catheters and/or advanced training procedures (Schmidt et al., 2008; Willner, 1997). By contrast, we have previously proposed a way to measure dependency that is more proficient, non-invasive and requires minimal training (Schmidt et al., 2008). Previously, this behavioral paradigm has been successful for investigating the addictive properties of soft drinks, (Schmidt et al.; Cummins, Freeman, Hughes, Miller & Hock, 2010) and uses conditioned taste aversion (CTA) as the behavioral component in order to measure dependency based on the APA's seventh criteria. This model attempts to mimic some

of the effects of human dependency by allowing the animal's free access to a possible substance of abuse daily over a period of fourteen days. On day fifteen a novel substance is paired with lithium chloride (LiCl) which induces illness similar to food poisoning in humans. On day sixteen the illness producing substance is mixed with the substance of abuse and offered along with an alternative. Dependency will be determined if the animals continue to drink the mixture despite the previous day when CTA was administered causing illness (Cummins et al., 2010). Although this behavioral paradigm is limited to drugs that can only be administered orally (Schmidt et. al, 2008), this model not only calls attention to consumption but stresses motivation as an identifier in substance abuse as well (Willner, 1997).

## Sweeteners and Health Consequences

Artificial sweeteners are used as a primary substitute for sugar (Yang, 2010). They can be found in a variety of foods and have the added benefit of providing sweetness without the added calories (Whitehouse, Boullata & McCauley, 2008). This can be especially beneficial to those with particular dietary needs such as diabetics and those that are on a calorie restricted diet. However, there is much controversy over the safety of these products. Most artificial sweeteners are not fully metabolized in the body. For example, the "metabolites" are the byproduct of what is not fully metabolized and exposure to this in rats, dogs and humans has been shown to potentially induce cancer (Whitehouse et al., 2008).

Sucrolose is marketed under the brand name Splenda® and is fairly new to the family of artificial sweeteners (Binns, 2003). The process of making sucrolose involves the hydroxyl groups of chlorine, which delivers a product that is 600 times sweeter than sugar (Binns, 2003). Sucrolose does not dechlorinate after ingestion and is therefore considered safe for consumption (Binns, 2003). As aforementioned, sucrolose may be considered safe for consumption; however, the "metabolites" that are left behind increase the risk for cancer development. (Whitehouse et al., 2008).

Agave® nectar has recently emerged as a "healthy" alternative to sugar and/or high fructose corn syrup (Fallon & Nagel, 2009). However, agave nectar is made from the starch of the pineapple-like root bulb of the yucca plant. The primary ingredient of this root is starch and a complex carbohydrate called inulin. The manufacturer chemically processes this root into "nectar" much in the same way that corn starch is converted into corn syrup. This chemical processing produces a product which results in more concentrated fructose (77%) than which high fructose corn syrup contains (55%) (Fallon & Nagel, 2009).

Stevia® is another popular sweetener that has been proposed to be a more "safe" alternative to other artificial sweeteners. Stevia® is made from the Stevia Rebaudiana plant, found in Brazil and Paraguay (Koyama, Kitazawa, Ohori, Kakegawa, Fujino & Ui, 2002). Glycosides of the derivative steviol added with other components, including the dry leaves of the plant make up the key ingredients in the dietary supplement Stevia®. Moreover, compared to sucralose it is 200-300 times sweeter, and like sucralose, is stable in all degrees of temperature (Koyama et al., 2002). However, just like Splenda® and Agave® nectar, Stevia® is not created without added chemical processing and because of this should not be looked upon as a healthy alternative to sugar. What must be considered is the fact that all three of these products attempt to mimic the sweetness of sugar and uses similar processing that may result in unhealthy consequences.

## Artificial Sweeteners, Sugar and Dependency

Research has proposed sugar as a substance of abuse, and previously identified that, after intermittent access to sugar, behavioral and neurochemical changes were evident (Avena, Rada & Hoebel, 2008). Behavioral and neurochemical changes are common with drugs of abuse and sugar has been found to mimic these changes. For example, previous studies demonstrated that under certain conditions of abstinence-binging, sugar could produce behavioral and neurochemical changes similar to those of other substances of abuse, such as amphetamines (Avena, 2007; Avena et al., 2008). It has been hypothesized by Avena et al., (2008) that any sweet taste consumed is a candidate for producing signs of dependency, but different types of sweeteners have not been tested. Considering the fact that most commercial food and drink contains sugar and/or artificial sweetener, and that sweetener can mimic the effects of drug abuse, this suggests support for additional investigation into the addictive properties of sweeteners.

One of the main reasons for the substitution of artificial sweeteners for sugar is the need to control caloric intake. Research has shown that sweet taste enhances the appetite and does not differentiate between natural sugar and artificial sweetener. Artificial sweeteners were produced to mimic sugar in sweetness and contribute to sugar craving and

possible sugar dependency (Yang, 2010). Considering the fact that most commercial food and drink contains sugar and/or artificial sweetener, and that sugar can mimic the effects of drug abuse, this suggests support for additional investigation into the addictive properties of sweeteners.

The current study extended the Cummins et al., (2010) investigation of the role of caffeine addiction in soft drink consumption based on the APA's seventh criteria of dependency. This same rodent behavioral model for dependency developed by our lab and described above was used, and aims to differentiate between the addictive nature of different sugars and sweeteners. We intended to identify which of the following sweeteners, Sugarcane, Stevia®, Splenda®, or Agave®, were addictive using our behavioral model and hypothesized that Stevia® and Agave® use would not be addictive.

## Methods

### Subjects

The study used 75 Long-Evans female (Harlan) rats that were 75 days old at the start of the experiment. There were five different groups with 15 rats assigned to each group. Group A received 6% Agave® nectar while Group B received 4% Stevia®. Group C was given 1.2% Splenda®, Group D received 10% pure cane sugar, and Group E was the control group; all groups received their sugar treatments daily via test tubes with drinking spouts (AnCare) for 14 days, (all the groups treatments were mixed with tap water). The animals were housed in the animal vivarium where the lights were kept on a 15:9 light/dark cycle starting at 7am. The rats were housed in Plexiglas cages and were given free access to food with water restricted as noted below for 23 hours every day. This experiment had full IACUC approval before the start.

### Procedure

All five groups received a 15-min exposure of 20.0 mls of their drinking mediums for 2 weeks in graduated drinking tubes. The amounts of the solutions ingested were recorded daily. After day 14, the rats were again water deprived for 23 hours. On the 15$^{th}$ day, all animals were given a 15-minute exposure to 10.0%, no sugar added apple juice (Wal-Mart) mixed with tap water. Immediately following, the rats were given a 0.15 M lithium chloride (Carolina Biological Supply) intraperitoneal (IP) injection at 2.0% of their body weight. The IP injection was used to provide a conditioned taste aversion. On day 16 of the study, the four groups were given simultaneous 15-minute access to both 10.0% apple juice/treatment and tap water. Therefore, Group A was given a choice between apple juice/6% Agave® mixture vs. tap water, Group B given apple juice/4% Stevia® mixture vs. tap water, Group C given apple juice/1.2% Splenda® mixture vs. tap water, Group D given apple juice/10% pure cane sugar vs. tap water, and Group E was given 10% apple juice vs. tap water. Sweetener concentrations were based on strengths compared to sugar cane (Koyama et al., 2002). A one-way ANOVA was used with Tukey HSD post comparisons to identify any significant differences, defined at $p<.05$.

## Results

This study used one independent variable with five levels given prior to CTA: Group A Agave®, Group B Stevia®, Group C Splenda®, Group D Sugar Cane, and Group E Control group. The two dependent variables were the Pre-CTA/Post-CTA difference score and the preference score between the two different solutions Post CTA. The Pre-CTA/Post-CTA difference score was calculated by determining the difference between consumption of the 10.0% apple juice before CTA and subtracting the 10.0% apple juice/sugar mixture between all the groups tested. The preference score between the two different solutions, post CTA, was measured by consumption of the 10.0% apple juice/sugar mixture Post-CTA minus the tap water. The data from one rat in the Agave® group on day 12 was excluded from analysis due to spillage of the mixture. The data from one rat in the Splenda® group and one rat from the control group on day 16 was also excluded from analysis for lack of water and mixture consumption.

The one-way ANOVA for the Pre/Post CTA difference score data was significant $F(4,68) =5.722$, $P =.<001$. A subsequent Tukey HSD was performed and showed that the Splenda® group ($M=3.607$ $SD= 3.696$) significantly decreased their post-CTA apple juice mixture consumption ($p<.017$) than the Sugar cane group ($M= -0.2$, $SD=4.174$; See Table 1 and Figure 1). All other group comparisons were not significant (See Figure 1). The control group was significantly different from all groups ($p<.05$) except Stevia® and Splenda® ($p>.10$). Furthermore, an investigation of the group means in Table 1 show that all groups difference scores, except the Splenda® and Control groups, had mean differences that were less than one, demonstrating little difference between the pre- vs post-CTA consumption of the 10% apple juice and 10% apple juice/sugar mixture.

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Table 1

| Sweetener | Day 15 AJ | Day 16 AJ | Day 16 H2O | Difference | Preference |
|---|---|---|---|---|---|
| **Agave** | 9.8 | 10 | 4.83 | -.02 | 5.17 |
| | (3.44) | (3.94) | (2.71) | (4.17) | (3.66) |
| **Stevia** | 10.27 | 9.6 | 3.63 | 0.667 | 5.97 |
| | (2.48) | (3.50) | (2.91) | (3.50) | (5.15) |
| **Splenda** | 11.96 | 8.36 | 4.18 | 3.607 | 4.18 |
| | (1.78) | (3.56) | (3.69) | (3.70) | (6.85) |
| **Sugar Cane** | 9.8 | 11 | 4.27 | -0.97 | -1.3 |
| | (2.42) | (3.22) | (2.24) | (4.36) | (5.68) |
| **Control Group** | 9.25 | 4.64 | 5.89 | 4.607 | 5.17 |
| | (1.87) | (2.73) | (3.22) | (3.39) | (3.66) |

The means and standard deviations from the AJ consumption on days 15 & 16, water consumed on day 16, as well as the difference and preference scores. For Difference scores, a positive number means rats drank more 10% apple juice before CTA than after, whereas a negative number means rats drank more 10% apple juice/sugar mixture after CTA than 10% apple juice before CTA. For Preference scores, a positive number means rats drank more 10% apple juice/sugar treatment than tap water after CTA, whereas a negative number means rats drank more tap water than 10% apple juice/sweetener treatment after CTA.

The one-way ANOVA for the preference score data was also significant $F(4,68)=4.808$, $p=.002$. A subsequent Tukey HSD test was performed and showed that Agave® group ($p=.017$), Stevia® group ($p=.005$), and Sugar Cane group ($p=.002$) all were significantly different from the control group (See Table 1 for group means and standard deviations). However, the Splenda® group ($M= 4.18$ $SD= 6.85$) approached but failed to significantly prefer the apple juice/Splenda® mixture ($p=.070$) over the tap water (See Figure 2). Furthermore, an investigation of the group means in Table 1 show that all groups preference scores, except the control group, had mean preferences that were positive values, demonstrating preference for the 10% apple juice/sugar mixture over the tap water, despite the apple juice making the rats ill the day before.

The preference results of this study show that all groups except Splenda® ($p=.070$) failed to drink significantly less apple juice/sugar mixture than tap water following CTA versus control group. However, the Splenda® group did reach significance in the difference score ($p<.017$). All the sweetener consuming groups continued to drink the apple juice/sweetener mixture despite the fact they became ill from the apple juice suggesting sweetener dependence.

## Discussion

This study utilized a rodent behavioral paradigm model which used conditioned taste aversion and which had been proposed previously by Schmidt et al., to test the seventh criteria of dependency. The American Psychiatric Association (2000) outlines the seventh criteria of dependency as the continued use of a substance of abuse, despite known adverse consequences to the user. There were four different types of sweetener used, which allowed for a comparison of dependency to be differentiated: Agave®, Splenda®, Stevia® and Sugar Cane. The pre-test/post-test differences in this study suggest that sweeteners, with the possible exception of Splenda, have an addictive component. However, the retention test also lends stronger support to the preference for sweeteners. It should be noted that there were three rats out of the fifteen in the Splenda® group that failed to prefer the apple juice/sweetener mixture over the tap water post CTA, and this explains the reason this group approached significance in the preference score.

Previous studies have suggested that sugar could mimic behavioral and neurochemical changes similar to those found with other substances of abuse such as amphetamines (Avena 2007; Avena et al., 2008). This study suggests that different types of sweeteners, not limited to cane sugar, can be a potential substance of abuse according to the seventh behavioral criteria of addiction. This study lends further support to previous studies of sweetener dependency.

The limitations of this study are that 1) it utilized rats and therefore, the generalizability to humans requires caution, and 2) it is limited to substances that can be orally consumed. However, this model has been replicated now with three separate substances: Soft Drinks (Schmidt et al., 2008; Cummins et al, 2010); sweeteners (current study); and alcohol (current laboratory unpublished findings).

In conclusion, this behavioral model for measuring dependency has reproduced findings by Schmidt et al. (2008) and Cummins et al. (2010). In

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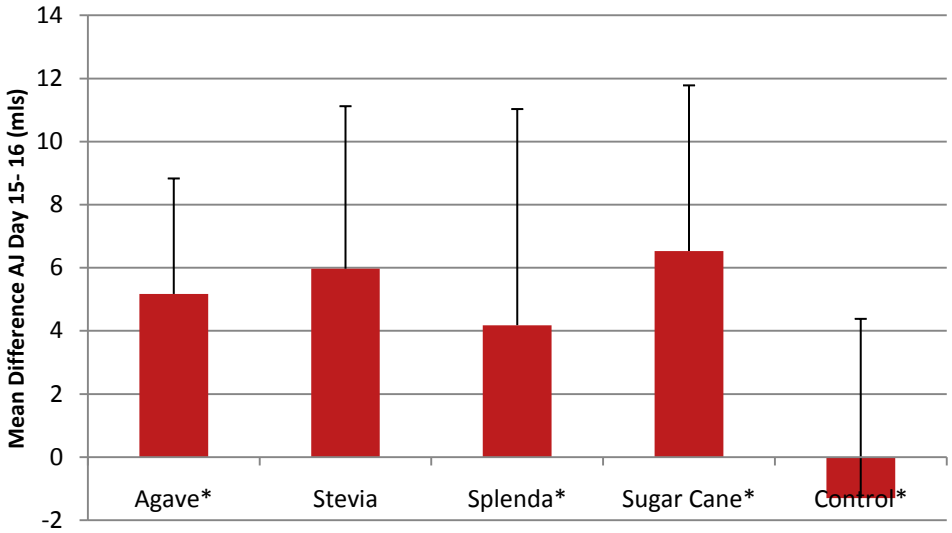


*Figure 1.* Mean difference between consumption of 10% apple juice before CTA minus 10% apple juice/sweetener treatment after CTA. Error bars represent the standard deviation in milliliters. The one-way ANOVA for the Pre/Post CTA difference score data was significant. The control group was significantly different from all groups except the Stevia and Splenda groups.

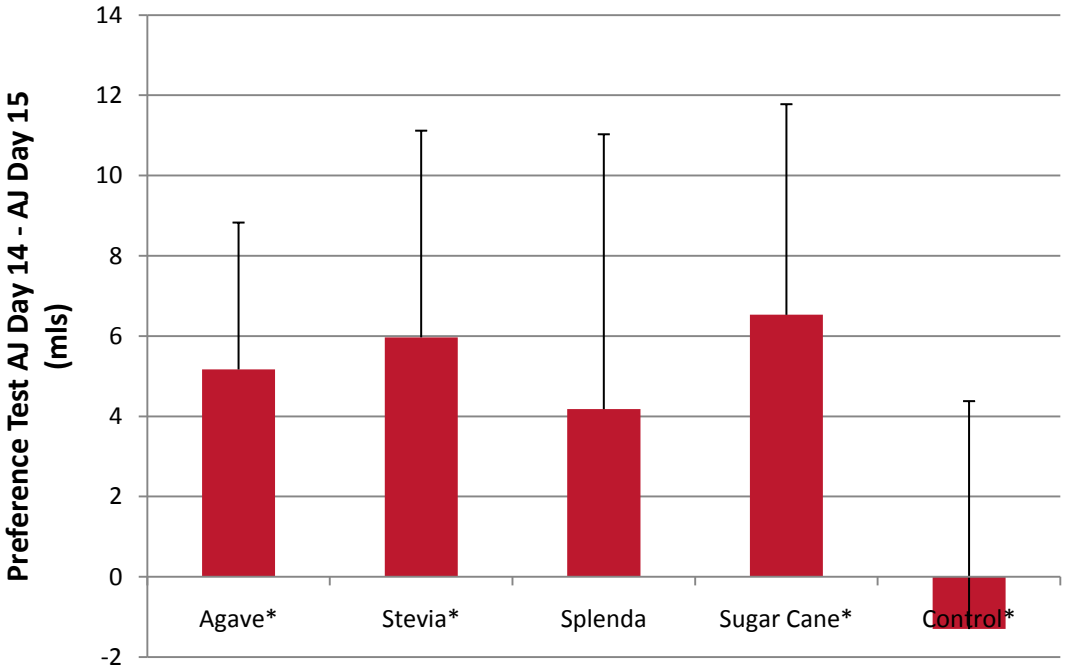


*Figure 2.* Preference for the different solutions Post-CTA. The error bars represent the standard deviation in milliliters. The one-way ANOVA for the preference score data was significant; all groups were significantly different from the control group.

addition, this model is an efficient way in which to measure for the seventh criteria of dependence (American Psychiatric Association, 2000; Schmidt et al., 2008 & Cummins et al., 2010). Furthermore, motivational factors that exist with substance abuse can be measured using this model.

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