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The Effects of Taurine and Caffeine Alone and in Combination on Locomotor Activity in the Rat

Marissa Dombovy-Johnson, Class of 2010

Background Information

History of Energy Drinks

Energy drinks were first marketed in Europe and Asia in the 1960's, but they did not reach the United States market until the 1980's. Red Bull, the leader of energy drink sales since its introduction, was created by Dietrich Mateschitz and released in Austria in 1987 and in the United States ten years later (Ressig, Strain, and Griffiths, 2009). By 2006 there were over 500 brands worldwide (Johnson, 2006) and approximately 31% of American teens consumed energy drinks (Lord, 2007). In 2008 the leaders in the energy drink market were Red Bull, Monster, Rockstar, and AMP. They had 40, 23, 12.3, and 8 percent of the sales, respectively (Energy Fiend, 2007).

Energy drinks, unlike their caffeine counterparts, coffee and soda, were associated with the emerging lifestyle of action/extreme sports (Ho, 2006). They are marketed towards those wanting increased alertness and energy, particularly in athletic endeavors. Thus today's youth, especially young males, have become the target for energy drink companies. Miller (2008) surveyed undergraduate students in 2008 and found that masculinity and risk taking behaviors were positively correlated with energy drink consumption. Red Bull "vitalizes body and mind," while "AMP

energy gets you focused and ready for everything life throws at you each day," according to each of their websites (AMP Energy, 2010; Red Bull, 2010).

The ingredients in energy drinks are designed to create activating effects on the human body. The most common ingredients found in energy drinks are ginseng, taurine, caffeine, glucose, B-vitamins, and glucuronolactone. Each energy drink is unique in its combination of ingredients; however, caffeine and taurine are present in all of the above mentioned energy drinks. Glucuronolactone and glucose are present in Monster and Red Bull, ginseng in AMP, while B-vitamins are in Red Bull and AMP (Amp Energy, 2010; Monster Energy Drink, 2010; Red Bull, 2010; Rockstar, 2010).

The above ingredients are included because of proven or hypothesized effects of increased alertness, stimulation, and energy (Clauson, Shields, McQueen, and Persad, 2008). Glucose, which is a monosaccharide, is the main source of energy in the human body. In addition to providing energy, glucose has been found to enhance memory, and concentration (Clauson et al., 2008). Panax ginseng

grows naturally and has been used to prevent fatigue, liver disease, amnesia, and colds. It has been suggested that has immuno-modulating activity and can enhance mental capacity (Kiefer and Pantuso, 2003). B-vitamins include a wide variety of vitamins, such as folate, B12, and B6. Deficiencies in B-vitamins can lead to impaired neurological and psychological functioning (Selhub, Bagley, Miller, and Rosenberg, 2009). A naturally occurring substance in the body, glucuronolactone is believed by energy drink manufacturers to detoxify the body. However, no studies have studied this (Kim, 2003). Taurine, an essential amino acid that is not used to build proteins (Birdsall, 1998), has many different effects, possibly because of its interaction at the GABA, glycine, and “unknown” taurine receptors (Ramanathan, Chung, Giacomini, and Brett, 1997; El Idrissi et al., 2008). This amino acid’s best known role involves the synthesis of taurine bile acid conjugates (Clauson et al., 2008). However, its function in improving activity level and mental performance has been controversial. The last main ingredient is caffeine, which stimulates the central nervous system, heart, and skeletal muscle, and has a diuretic effect (Clauson et al., 2008).

As shown above, the ingredients found in energy drinks have separate potentially different effects on the brain and body. As these effects are likely to interact, the effects of ingesting an energy drink are likely due to the combined interaction of these substances on the brain and body systems. The inclusion of taurine in energy drinks

maybe due to its suggested stimulant effects or, when combined with caffeine it further increases the stimulant properties of caffeine. However, Whirley and Einat (2008) concluded that taurine’s stimulant properties were hypothetical because they failed to find increased activity in mice. However, they studied taurine alone and not in combination with caffeine, thus further research is needed to verify this possibility. Furthermore, Zeratsky (2008) states that athletic and maybe mental performance is increased by the interaction of taurine and caffeine. If caffeine and taurine acted in a synergistic way then it would support their dual inclusion in energy drinks. In this study I examined whether or not the locomotor activity of a rat, as measured by the number of rotations in a rodent wheel and activity in the open field test, increased in the presence of combined taurine and caffeine, when compared to taurine or caffeine alone or control saline. As well as measuring activity, the open field test is also useful in evaluating anxiety, a potentially detrimental side effect of caffeine. Before stating the details of this experiment, research involving taurine and caffeine alone and in combination will be further examined.

Caffeine

Caffeine, the most commonly used psychoactive substance, is believed by most researchers to cause the increase in energy, memory and alertness associated with energy drinks. Caffeine is a competitive A₁ and A₂ adenosine receptor antagonist (Hughes, McHugh, and Holtzman, 1998; Smit and Rogers,

2000; Angleucci, Cesário, Hiror, Rosalen, and Da Cunha, 2002; Karcz-Kubicha et al., 2003). Adenosine slows down neural activity and, by preventing this inhibitory effect, caffeine stimulates brain activity (Rao, Hu, and Nobre et al., 2005). The A₁ adenosine receptors are expressed in the cerebral cortex, while the A₂ receptors are present in the hippocampus and the striatum (Angleucci et al., 2002). Angelucci et al. (2002) reported that acetylcholine (ACh) release is inhibited by activating the A₁ receptor, thus caffeine positively impacts memory storage. Motor activity is believed to be regulated by adenosine's interaction with both the A₁ and A₂ receptors (Karcz-Kubicha et al., 2003). Synder, Katims, Annau, Bruns, and Daly (1981) showed that the A₁ receptors mediated the motor-activity effects of caffeine. However, later studies have determined the A₂ receptors as the cause of the activity increase (Ferré, Fuxem von Euler, Johansson, and Fredholm, 1992).

The ingestion of caffeine has many effects on the human body. This substance helps prevent fatigue (Smit and Rogers, 2000), decreases reaction time (Clubley, Bye, Henson, Peck, and Riddington, 1979; Lieberman, Wurtman, Emde, Roberts, and Coviella, 1987; Kerr, Sherwood, and Hindmarch, 1991), facilitates memory attention and stimulates the heart, and voluntary muscle (Angleucci et al., 2002). Angleucci et al. (2002) found that the effects of caffeine are dose related, with peak stimulating effects occurring at moderate doses. Behavioral activity, learning, and memory were actually suppressed when rats were administered

high doses of caffeine. The timing of the dosing was another important finding because the rats who received caffeine after training experienced more of an impact on memory than those who received it before training, implying a possible effect on memory consolidation. Enhancement in memory and other higher level cognitive functions possibly results from caffeine's antagonism by enhancing cholinergic release (Smit and Rogers, 2000).

Numerous past studies have been conducted on caffeine's effects both alone or in association with other energy drink ingredients. Scholey and Kennedy (2004) found improved memory and speed in attention tests, but no other mood or cognitive effects, when humans drank a mixture of caffeine, glucose, and herbal flavoring fractions. Similarly, Kennedy and Scholey (2004) found that after cognitive demand, a caffeine and glucose drink improves cognitive performance and decreases fatigue. Increased behavioral performance in a selective-attention task, specifically improved accuracy and reaction times, was discovered after individuals consumed a caffeine, glucose drink (Rao et al., 2005). These researchers postulate that caffeine and glucose, the major source of energy in the body, may interact in a synergistic way. Various studies (Johnson, Spinweber, and Gomez, 1990; Rusted, 1999; Walburton, 1995) found increased alertness after caffeine was administered. Frewer and Lader (1991) and Fine et al. (1994) replicated the findings that caffeine has beneficial effects on psychomotor speed and vigilance.

As mentioned above, caffeine has been found to be a competitive adenosine antagonist and have dose related effects. Karcz-Kubicha et al. (2003) found that administering caffeine acutely produced motor-activating effects by blocking both the A₁ and A_{2a} receptors. Despite being an antagonist of both adenosine subtypes, Ferré et al. (1992) determined that the A_{2a} receptors are responsible for the motor-activating effects of caffeine in rodents. Therefore, they may also be the cause for decreased reaction times experienced by humans after caffeine ingestion. Past research has shown positive effects on arousal when lower doses of caffeine were used (12.5-100mg) (Smit and Rogers, 2000, 2002; Smit, Cotton, Hughes, and Rogers, 2004), while a dose of 500mg produces negative effects by impairing performance on tests of attention and vigilance (Frewer and Lader, 1991). The best interpretation of the above literature review is that caffeine exerts stimulant effects in a dose-dependent manner.

Taurine

Taurine is the most abundant amino acid in many tissues and, most importantly, in the brain's extracellular fluid (Birdsall, 1998; Olive, 2002). Cysteine sulfinic acid decarboxylase (CSAD) synthesizes taurine from cysteine (Olive, 2002). Since it is not incorporated into proteins (Birdsall, 1998), taurine is found free in tissues, for example the brain and muscles (Whirley and Einat, 2008). The consumption in food and synthesis by astrocytes are the main ways taurine enters the body

(Whirley and Einat, 2008). High levels of this amino acid can be found in the cerebral cortex, basal ganglia, hippocampus, hypothalamus, cerebellum, and the caudate-putamen (Olive, 2002; El Idrissi et al., 2008; Whirley and Einat, 2008). In order to reach the brain, taurine must cross the blood-brain-barrier by way of a sodium and chloride dependent carrier (Benrabh, Bourre, and Lefauconnier, 1995).

Taurine has been found to impact many different biochemical actions (Huxtable, 1992), thus it has been difficult to locate the exact taurine receptors. It can act as a trophic factor and inhibitory modulator (Chepkova, Sergeeva, and Hass, 2005), a neuroprotectant (Kim, 2003), as well as playing a role in detoxification, membrane stabilization, and osmoregulation (Birdsall, 1998; Imagawa et al., 2009). Taurine levels were altered in some patients with neuropsychiatric illness, suggesting a possible use as medication. In addition it already has been used to treat people with seizure disorders (Whirley and Einat, 2008).

Since taurine is structurally similar to GABA, it may mimic GABA's effects in enhancing chloride ion flux or possibly displace the agonists associated with the GABA_A receptor complex (Olive, 2002). Furthermore, its interaction with GABA receptors, as well as glycine, cholinergic, and adrenergic receptors, may provide support for its role in stress, mood, and behavior (El Idrissi et al., 2008). The interaction at GABA receptors is important for taurine's anxiolytic effect because

anxiolytic drugs interact at the same receptor (Whirley and Einat, 2008). Similar to taurine's use in treating people with seizure disorders, its interaction at GABA receptors demonstrate is sedative effect when used alone. However, when combined with caffeine it may interact in a synergistic way to further increase caffeine's stimulant properties.

El Idrissi et al. (2008) found that the way taurine was administered, either injected (43 mg/kg) or supplemented in drinking water, resulted in suppressed or increased locomotor activity, respectively. The injection produced an anti-anxiety effect, while supplementation induces the opposite effect, anxiety. In another study in mice, Whirley and Einat (2008) gave two different doses of taurine that were significantly higher than the amounts in energy drinks. However, they stated that mice, when compared to humans, are given higher doses of drugs due to metabolism differences. Since the high doses had no significant stimulant effects, they speculated that lower doses maybe more effective. Furthermore, they only tested taurine, and other research implies a possible synergistic effect with caffeine. Even though they found that sub-chronic or chronic administration did not have a stimulant effect, they saw an initial reduction in locomotor activity, which is typical of other stimulants, followed by an increase in activity. They propose that this may be due to taurine affecting different pathways. After analysis of their findings and past research, they consider the claim of taurine as a stimulant to be speculative and further research is needed.

Other behavioral studies have produced conflicting results regarding taurine's role in locomotor activity as measured through the open-field test. Baskin et al. (1974) found a dose-dependent decrease in activity following i.p. injections of taurine (0.3-3.0 mg/kg). Similarly, Sanberg and Ossenkopp (1977) found that increasing doses of taurine (25-200 mg/kg) decreased ambulation in rats, with doses greater than 50 mg/kg causing a significant decrease. Mice exhibited the same decrease in activity following i.p. injections (Hruska, Thut, Huxtable, and Bressler, 1975). Contradictory to the Sanberg and Ossenkopp's (1977) finding, Kong et al. (2006) found that a dose of 126 mg/kg increased the number of times a rat entered the center during the open field test. Comparable to Kong et al.'s finding, Imagawa et al. (2009) found an increase in endurance performance following two weeks of taurine administration. Possible explanations for these differences could be environmental conditions, duration of administration, and age of the animals. Higgins, Jones, and Oakley (1992) and Serrano et al. (2002) have stated that environmental conditions and the age impact the way taurine interacts in the rodent body.

Caffeine and Taurine

As energy drinks often contain caffeine and taurine it is vital to examine past research involving the combination. Many researchers have looked at the impact of Red Bull, which contains caffeine and taurine, in humans. Alford, Cox, and Wescott (2001) found that a

Red Bull energy drink caused increased diastolic blood pressure, aerobic endurance and anaerobic performance. The observed increase in blood pressure may suggest an interaction between taurine and caffeine because taurine is an antihypertensive, thus opposing the caffeine mediated increase in both the diastolic and systolic blood pressure. No effect on short term recall memory in college students was found after they ingested a Red Bull drink (Birchler, Swenson, and Harris, 2006). The heart rate in subjects was decreased, and cardiac stroke volume was increased (Baum and Weis, 2001). Baum and Weis (2001) suggested that the increase in ventricular function in young athletes may be due to the combined effects of caffeine and taurine. However, these effects may also be due to caffeine, since they did not look at that substance alone.

Walburton, Bersellini, and Sweeney (2001) looked at a glucose, caffeine, and taurine drink and discovered that information processing increased after consumption of caffeine and taurine. Glucose was not included because there was no difference between the control drinks, one sugar (glucose) free and the other with just glucose. However, similar to the Baum and Weis (2001) study, caffeine was only looked at in association with taurine, therefore it could be caffeine's effect alone.

Imagawa et al. (2009), as far as I know, were the only study to examine the effect of taurine and caffeine, alone and in combination, on performance. They found that taurine use for two weeks significantly increased endurance

performance in mice. In addition, their results also show that caffeine and taurine use for two weeks had an additive effect, when compared to both substances alone and to administration for only a week. Therefore, the duration of administration could be essential to caffeine's and taurine's effects in energy drinks.

The research on the components of energy drinks is very limited. Since the literature suggests a possible synergistic effect between taurine and caffeine on causing the increased alertness and faster processing associated with energy drinks, it is important to study this in further detail. Based on past research I hypothesize that rats will display increases in locomotor activity after an injection caffeine alone and an additive effect when caffeine and taurine are combined.

Methods

Animals

Adult male Sprague-Dawley rats raised at Colgate University weighing 472-578g (avg. 514g) at the beginning of the experiment were used. The rats were born in either February or March 2009 making them an average of a year old during the experiment. The animals were housed on a 12hr light/dark cycle (lights on at 08:00) at $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with food and water available *ad libitum*, except from the time of the injection until the beginning of the exercise wheel. The rats were housed in cages (47 X 26 X 20 cm) with the same cagemates during the duration of the experiments. The cages were switched every week to allow the

rats to receive equal distribution of light. After the habituation period, which will be explained in the procedure subsection, the rats were broken into four groups: control (C), taurine (T), caffeine (F), and taurine and caffeine, the combined group (B). The experiments and treatments were approved by Colgate University's Animal Care Committee.

Drugs

Taurine (cell culture tested), 2-aminoethanesulfonic acid, and caffeine, 1,3,7-trimethylxanthine, were purchased from Aldrich Supply Company. The average weight of 514g was used as the weight multiplied by the drug dose, which was 50 mg/kg taurine and 30 mg/kg caffeine. After the drugs completely dissolved, 1mL aliquots were transferred to twelve 1mL micro-vials and frozen until needed. This ensured that the doses were as equal as possible. All of the drugs were injected intraperitoneally (i.p.) in a 1mL amount 30 min before the open field test. Control rats received an i.p. injection of 1 mL of 0.9% NaCl and the combined group received the same amount of both drugs.

Open-field Apparatus

A rectangular (105 X 90 cm) open field divided into 42 equally sized squares (15 X 15 cm) with a plexiglass floor and 28 cm high walls was used. A video camera was positioned above the arena, high enough to cover just the field.

Exercise Wheel

The exercise wheel consists of a rodent wheel containing an automated counting device secured to a plastic storage bin covered with bedding and supplied with water and food. Thus, while in the wheel, each full rotation was automatically recorded.

Procedure

Twenty four rats went through three 16-hr habituation periods (which included their active period) in the exercise wheels before the experiment began in order to choose twelve that ran an average of 5 complete spins. Rats that never ran or ran over 15 times in any habituation period were eliminated. After the habituation period, the twelve rats chosen experienced each treatment group. In order to prevent order effects, the rats were divided into the four groups described above with three rats in each. Thus, each rat went through four trials, one for each treatment group. There was at least 36 hours in between injections on the same rat. The open field test began thirty minutes after the injection, which occurred in the same room as the open field. The interval of 30-min was chosen because methylxanthine, a metabolite of caffeine formed in the liver, has a half-life of 0.7-1.2 hr in rats, compared to 2.5-4.5 hr for humans (Morgan, Stults, and Zabik, 1982). Taurine has a longer full body half-life at 18.6 days (Huxtable, 2000). If a longer duration was chosen, then caffeine would be mostly metabolized, thus the results would not display an accurate portrayal of caffeine's effects.

Furthermore, past research using the open-field test waited thirty minutes after the injection before beginning the experiment (Kong et al., 2006; Whirley and Einat, 2008). The rats were placed in the center to start and were videotaped for ten minutes, after which they were placed in the exercise wheel for four hours. The open-field was cleaned after each rat with 70% ethanol to remove any odor traces. The open field test was analyzed for total number of squares entered with all four paws, number of rears (forelegs raised from the floor or against the wall), total duration of grooming (total time in seconds spent grooming; considered as any time the paws are touching fur or whiskers), and percentage of perimeter squares entered compared to the middle squares (rats usually move around by hugging the walls with one side of their body). Perimeter squares were considered the squares that directly bordered a wall. Videotapes were later scored by 3 observers blind to the treatments, allowing an average of the 3 to be used for each trial (every rat went through 4 trials, one for each drug). See figure 1 for the sheet used by the observers. After the open field, the rats were immediately placed into an exercise wheel in an adjacent room for 4-hrs. At the end of the 4-hrs, the number of rotations were recorded and the rat was returned to its original cage in the housing room. All the behavioral experiments occurred during the hours of 10:00 and 16:00h, besides the habituation period, which occurred during a 16-hour window encompassing their active period.

Statistical Analysis

Results are reported as means \pm SEM. Data were analyzed by means of repeated measures analysis of variance (ANOVA). Whenever the ANOVA was significant, further multiple comparisons were made using the paired t-test. All analyses were performed using the software SPSS V16.0 for windows. The level of statistical significance adopted for the ANOVA was $P < 0.05$, while for the paired t-test it was $P < 0.0083$, which corresponds to the Bonferroni correction for 6 pair-wise comparisons.

Results

Open Field Test

Table 1 shows the complete data for the open field test and the exercise wheel. The results only include the data from ten of the twelve rats. One rat died after receiving only two drugs, while the other suffered a hematoma after the injection, thus did not participate in one trial. Therefore, in order to make sure the results were from rats that experienced all the drugs, the data for these two rats were eliminated. The results for the open field test are also shown in figure 2. The ANOVA for total squares showed a significant drug-induced effect [$F(3,27) = 12.9, P < 0.05$]. The paired t-test showed a significant difference when the combined group of taurine and caffeine was compared to the control [$t(10) = 6.9, P < 0.0083$] and taurine groups [$t(10) = 5.8, P < 0.0083$]. Caffeine showed a tendency to increase the number of squares entered when compared to the control [$t(10) = 3.2, P <$

0.05] and taurine groups [$t(10) = 2.6, P < 0.05$]. There was no significant difference between the number of squares entered for the combined group when compared to caffeine [$t(10) = 0.9, P > 0.0083$]. Both taurine and saline have no significant effect on the locomotor activity, as shown through total squares entered.

Figure 2 shows the results for the number of rears. The repeated measures ANOVA showed a significant drug-induced effect [$F(3,27) = 5.54, P < 0.05$]. The use of a paired t-test showed that both the caffeine and combined groups had a tendency to increase the number of rears when compared to the control and taurine groups [$P < 0.05$]. No pairings showed a significant difference.

For grooming [$F(3,27) = 2.8, P > 0.05$] and the percentage of outer squares [$F(3,27) = 2.9, P > 0.05$] the ANOVAs failed to show a significant difference among treatment groups.

Exercise Wheel

There was no significant difference among treatment groups for the exercise wheel data [$F(3,27) = 0.78, P > 0.05$].

Discussion

The interaction of caffeine and taurine has not been a well studied in past research, despite their presence together in energy drinks. Past research has concluded that caffeine alone increases alertness (Smit and Rogers, 2000) and decreases reaction time (Clubley et al., 1979). Past research on

taurine's stimulant properties has produced conflicting results possibly due to large differences in the dosages used (Sanberg and Ossenkopp, 1977; Kong et al., 2006). Examination of their combined effect has been sparse with the majority finding an increase in activity (Alford et al., 2001; Walburton et al., 2001). Only one study to my knowledge has considered the drugs in combination, as well as alone. In this study, Imagawa et al. (2009) found that taurine and caffeine had an additive effect on endurance.

Based on past research and conflicting data, the present study was designed to evaluate the potential stimulant interaction caffeine and taurine may have. The open-field test and the exercise wheel were chosen as the apparatuses because of their ability to show multiple behaviors at once.

One dose each of caffeine and taurine were tested, 30 and 50 mg/kg, respectively. These dosages were chosen based on previous research (Sanberg and Ossenkopp, 1977; Smit and Rogers, 2000, 2002; Smit et al., 2004; Kong et al., 2006). Both of these doses are higher than the ones contained in a typical energy drink. An 8.0 oz can of Rockstar contains 80mg caffeine and 1000mg taurine, thus for a 70kg person the doses would be 1.14 and 14.3 mg/kg, respectively. Even though these doses are significantly greater, it is typical for rats to be given greater amounts because they metabolize the drugs at a faster rate (Einat, Yuan, and Manji, 2001).

The open-field test, ever since its introduction by Calvin Hall in the 1930's, has been one of the most common apparatuses used to study rat behavior because of the various measures one is able to analyze after one trial in the field (Walsh and Cummins, 1976). In this study, the open-field was used to assess how four different behaviors were affected by saline, taurine, caffeine, and both drugs. The behaviors considered were total squares entered or total locomotion, rearing, grooming, and percentage of perimeter squares entered.

Total locomotion is an important factor for determining whether or not a substance causes a stimulant effect because stimulants increase activity. The opposite of locomotion is called freezing and it coincides with high amounts of stress (Walsh and Cummins, 1976). If caffeine and taurine interact in a synergistic way to strengthen caffeine's stimulant properties, then the total locomotion (total squares entered) experienced by this group would be greater than for any of the other groups. The finding that the combined administration of caffeine and taurine produced a significant increase in locomotion when compared to the control and taurine groups, suggests that caffeine and taurine somehow interact. However, there was no difference between the results of the combined group versus caffeine alone. Rearing, like total locomotion, is another measure of activity specifically, excitability (Walsh and Cummins, 1976). When the rat is less anxious, they are more likely to explore the open-field. Rearing is a way for them to explore vertically. The ANOVA

reached significance [$F(3,27) = 5.54$, $P < 0.05$], but none of the pairwise comparisons were statistically different ($P < 0.0083$). However, there was a tendency for the combined and caffeine groups to increase the number of rears when both were compared to control and taurine groups. As with total locomotion, there was no significant difference between the combined and caffeine groups.

Grooming has long been seen as an anxiogenic, or anxious response (Choleris, Thomas, Kavaliers, and Prato, 2001; Nosek et al., 2008). In other words, when a rat grooms itself, it is anxious. Stimulants, such as caffeine, have been found to promote an anxious state, thus the total time of grooming would be expected to be the highest from the combined and caffeine groups. Despite there not being a significant difference between the different groups [$F(3,27) = 2.8$], caffeine's average was the highest amongst the 4 groups (65.3 ± 22.8).

The last measure of the open-field test was the percentage of perimeter squares entered. Rats are typical wall-huggers because it allows them to not enter open and dangerous spaces (Wilson et al., 1976). Thus, when they are placed into the open-field rats remain in close proximity to the walls. When rats travel away from the perimeter of the open-field it is normally because they are not as anxious and feel more comfortable exploring the remaining field (Lister, 1990). The ANOVA for the percentage of perimeter squares entered failed to reach significance [$F(3,27) = 2.9$].

The exercise wheel is a measure of activity because the rat has free access to the wheel. Before the experiment began, the rats went through three habituation periods in order to become acquainted with surroundings and figure out how to work the wheel. The ANOVA failed to show any statistically significant difference among groups for the exercise wheel data [$F(3,27) = 0.78$]. This could be due to the fact that all of the rats ran very little, thus making comparisons difficult. Reasons for the lack of running in the exercise wheel could include the nocturnal nature of rats (i.e. rats are more active at night). The rats chosen ran an average of 1.5-8.5 spins during the habituation period, which encompassed their active period. However, when the rats were placed into the exercise wheel after the open-field test, they were in there for a 4-hr period that was during their normal sleep time. Furthermore, the rats only experienced the exercise wheels three times before the experiment, so it is possible that further habituation time could have led to more spins during the experiment.

There are limitations of the present study. First, there was only one dose of each drug used, which raises a possibility that different amounts of caffeine and taurine could have an additive effect. The best way to determine this would be to replicate the study, but add a lower and higher dose of each drug, in addition to the one used. Even though this proposal will dramatically increase the number of pairing between the drugs, it will allow a detailed analysis to better conclude if a

synergistic effect does indeed happen and at what doses.

Second, the rats used in this experiment were around 12 months old, which brings in the issue of age. Most experiments use rats that are younger because as they get older their activity levels are not as high. Future research should use a group of rats that are younger, in order to produce more accurate results. In addition to activity levels, many drugs exert different effects depending on the age of the test animals (Serrano et al., 2002). In addition, the data was only from ten rats, which is a very small sample size. Larger samples tend to increase the likelihood of finding a significant difference.

The caffeine treatment group had high variability, specifically for the total number of squares entered. The standard deviation for this measure was 96.79. Therefore, if the rats were grouped based on their response to caffeine (little response, average, high response) and then compared to the control, there might have been a significant difference. A significant difference between the high response group and control would most likely occur. Increasing the sample size, as mentioned above, will help to diminish the variability amongst treatment groups.

Each rat experienced all four conditions, thus experienced the open-field on four separate occasions, which allows the rat to habituate to the field. The more the rat experiences the open-field, more the locomotion tends to decrease (Walsh and Cummins, 1976).

The procedure used in this experiment corrected for this habituation by each group experiencing the different treatments in a varied order, thus eliminating order effects.

There was a minimum of 36 hours in between injections; however the taurine's half-life exceeds this time frame. Future studies should increase the time in between injections in order for taurine to be almost completely metabolized before the next injection occurs. There may have been taurine present from the previous injection when the next injection occurred, which may have affected the results. However, administering the treatments in different orders for each group should have taken this into consideration. Furthermore, by increasing time between injections, the rat will also have a longer period in between the open-field test, which may decrease habituation effects.

Imagawa et al. (2009) found an additive effect on endurance performance when caffeine and taurine were supplemented in drinking water for two weeks. Therefore, if the rats received daily injections of taurine and caffeine for two weeks there maybe an additive effect on activity levels compared to caffeine alone.

In summary, the present study provides further research into the interaction between caffeine and taurine. Even though there wasn't a significant difference between the combined group and caffeine, there might be under different experimental parameters (e.g., use of younger rats, varying dosages of

the drugs, and longer habituation periods in the exercise wheel). This study provides results that justify the inclusion of caffeine in energy drinks, but does not provide evidence for a synergistic relationship between caffeine and taurine.

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Appendix

RAT # _____

TREATMENT GROUP _____

TOT

AL # of Squares Entered _____

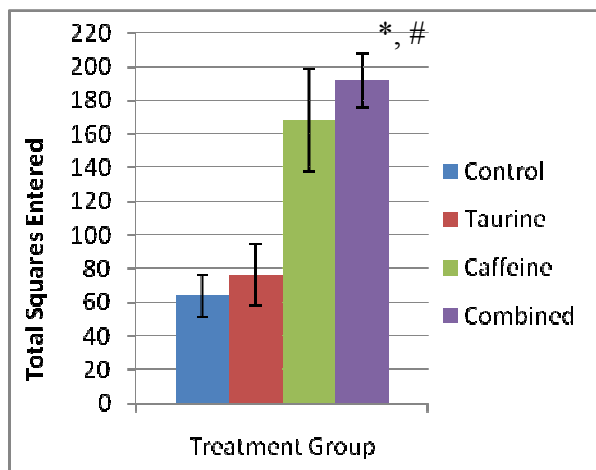
TOTAL # of Rears _____

TOTAL Cleaning Time (secs) _____

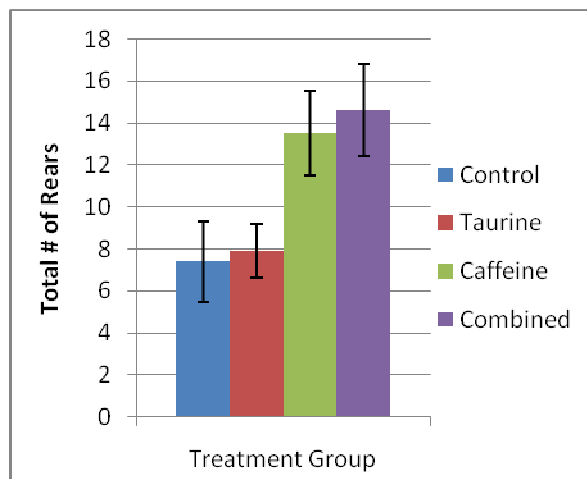
Figure 1: Coding Sheet Used By the Blind Observers.

Test	Measure	Control (mean±SEM)	Taurine (mean±SEM)	Caffeine (mean±SEM)	Combined (mean±SEM)
Open Field	Total Squares (4 paws)	63.7 ± 12.4	76.2 ± 17.9	168.1 ± 30.6	191.8 ± 15.8*, #
	Rears (front paws off floor)	7.4 ± 1.9	7.9 ± 1.3	13.5 ± 2.0	14.6 ± 2.2
	Grooming Time (secs)	16.7 ± 4.1	38.2 ± 8.8	65.3 ± 22.8	25.6 ± 4.5
	% of Perimeter Squares	96.7 ± 1.6	92.7 ± 1.7	94.8 ± 1.3	91.5 ± 1.7
Exercise Wheel	Total Rotations	0.1 ± 0.1	0.5 ± 0.3	0.7 ± 0.4	0.3 ± 0.2

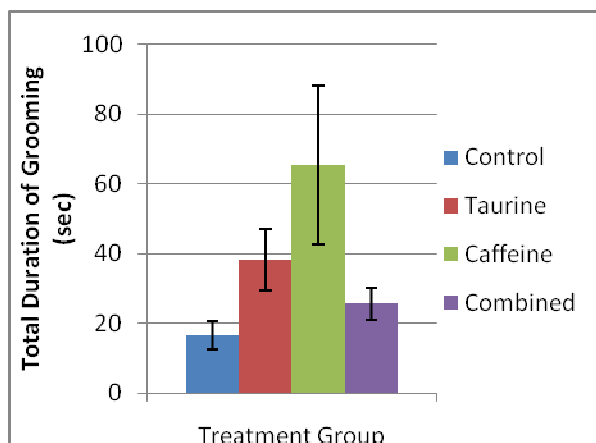
Table 1: Mean Values For Total Squares, Total Rears, Cleaning Time, % of Perimeter Squares, and Total Rotations. Taurine (50 mg/kg), caffeine (30mg/kg), combined (same amounts), or control (0.9% saline) was injected 30 min prior to the start of the open field test. * $P < 0.0083$ (Bonferroni correction) combined group vs. control. # $P < 0.0083$ combined group vs. taurine. Repeated measures ANOVA followed by paired t-test. $n = 10$.



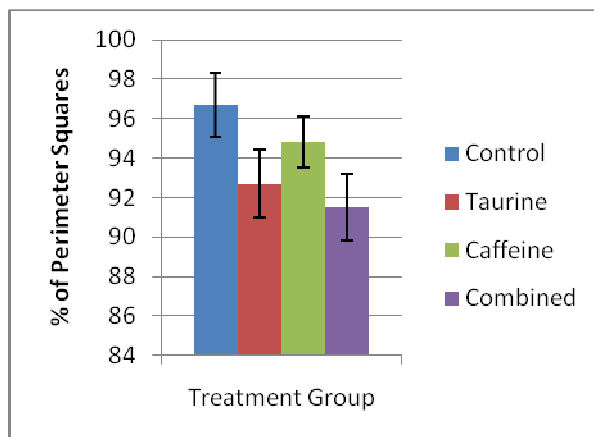
A. Total Squares Entered



B. Total # of Rears



C. Total Duration of Grooming



D. % of Perimeter Squares

Figure 2: Effect of taurine (50 mg/kg), caffeine (30 mg/kg), control, and the combined treatment groups on rat behavior in the open-field test: (A) the total number of squares entered; (B) the total number of rears; (C) the total duration of grooming; (D) the percentage of perimeter squares entered. All drugs were injected 30 min prior to the start of the open-field test. Results are expressed as mean ± SEM. Significant differences: compared to control * $P < 0.0083$; compared to taurine # $P < 0.0083$. $n = 10$.