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Exploring the Link between Drug Addiction Propensity and Improper Top- Down Processing via Sustained Attention Tasks

by

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Abstract

Sustained attention, or the ability to direct and focus cognitive activity on specific stimuli, has been inextricably linked with neuroscience research dealing with disorders ranging from ADHD and Schizophrenia to drug addictions including, but not limited to, alcohol and cocaine. However, until recently, much of this research has been geared towards the behavioral platform of sustained attention. As the propensity to attribute incentive salience to reward cues has been hypothesized to indicate vulnerability to addiction, in this study, the sustained attentional capabilities were investigated in rats showing high propensity in attributing salience to cues classified as "sign-trackers" (ST), and in their counterpart "goal trackers" (GT) which did not readily attribute incentive salience to cues during a Pavlovian conditioned approach test.

Both groups were trained on Sustained Attention Tasks (SAT). After reaching stable performance on criteria, the effects on SAT performance of systemic injections of mecamylamine a nicotinic receptor antagonist; scopolamine hydrobromide, a muscarinic antagonist; and MK801, an NMDA receptor antagonist were monitored. Though our results have confirmed that the level of performance of ST rats is significantly lower compared to their GT counterparts, injections of mecamylamine, scopolamine hydrobromide, and MK801 equally impaired both groups.

Key words: Sign Tracking, Goal Tracking, Sustained Attention Task, Top- down processing, Drug addiction

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The cholinergic network in attention

Sustained attention, or the ability to direct and focus cognitive activity on specific stimuli, has been inextricably linked with neuroscience research dealing with disorders ranging from ADHD and Schizophrenia to drug addictions including, but not limited to, alcohol and cocaine (Belgrove et. al.; 2006; Seok et. al; 2012; Coles et. al.; 2002; Goldstein et. al.; 2007). Though much research has been done on the behavioral platform of sustained attention, it is only recently that the neural mechanisms behind the concept have begun to be identified and understood. In this experiment, a rat model was used to help elucidate the intricacies of sustained attention and its part in proper top-down processing.

Sign Tracking v.s. Goal Tracking tendencies in rats

Pavlovian auto-shaping procedure consisting of a brief presentation of an illuminated retractable lever, the conditioned stimulus (CS), followed by the response-independent delivery of a food pellet, the unconditioned stimulus (US), has yielded two rat phenotypes: Sign Tracking (ST) and Goal Tracking (GT) (Flagel et. al.; 2008). For ST rats, incentive salience becomes intertwined with the cue for reward. GT rats on the other hand, do not approach the cue nor exhibit incentive salience, but rather, when the cue is presented, go to the food delivery location. It is important to note that both ST's and GT's develop the conditioned responses at approximately the same rate and present roughly equivalent degrees of motivation, implying that learning rate is not a variable that contributes to the divergent controlled response development between the two rat groups during Pavlovian Conditioned Approach (PCA) (Robinson, Flagel; 2008). This rat model has shown to be useful in elucidating the mechanisms underlying the

propensity in attributing incentive salience to reward cues, hypothesized to indicate vulnerability for addiction-like behavior. In this study, we explored whether sign-tracking behavior is related to a breakdown in proper top-down control as tested for via sustained attention tasks- tasks known to stimulate the cholinergic system (Kozak et al.; 2005) .

It has recently been reported that the intrinsic differences in ST and GT rats significantly affects their attentional capabilities. In a study involving ST and GT rats trained on SAT, ST rats reported reduced capability in cue detection. In fact, they reported fewer hits (see methods) when contrasted to their GT counterparts. Furthermore, their performance was very inconsistent, fluctuating from good to near chance performance (Paolone et. al., submitted). In this study, to explore the role of prefrontal cholinergic neurotransmission, task-associated releases were monitored via microdialysis in both groups. As expected, the lower levels of performance reported by ST rats was associated with reduced increase of task associated ACh releases compared to GT rats. This attenuation in Acetylcholine release may help explain the decreased ST rat capabilities in task rule maintenance as well as observed deficits in behavioral goals in working memory when compared to GT rats - a deficit that can help clarify the reason for fluctuating performance levels observed in ST rats.

ST and GT SAT performance

Top-down control of attention, also known as cognitive or executive control of attention reflects neural mechanisms and circuits which work together to provide individuals with sustained attention performance capabilities in the face of possible distracters. Sustained attention is not simply a singular idea, but rather, a series of different complimentary capabilities. The subcategories that make up sustained attention include the ability to maintain task-rules and

behavioral goals in working memory, monitor error performance, augment cue processing and distractor filtering in situations characterized by performance decline, choose between reward and reward loss in terms of levels of motivation, and lastly, ignore competing behaviors and pre-potent behaviors (Sarter, Paolone; 2011). It is when these various capabilities under the umbrella of "sustained attention" are altered or prove to be attenuated, is there a marked difference in sustained attention capabilities as well as a higher propensity for addictive behavior.

It has been showed that a major neurological aspect of sustained attention as previously described revolves around tonic, or, minute- based Acetylcholine efflux. This release has been observed to reach as high as 140 percent of baseline levels in rats performed a SAT. This can be compared to the much smaller 50 percent augmentation to baseline in Acetylcholine release in rats performing a control, non- cognitively taxing tasks (Sarter, Bruno; 2001). This has strengthened the hypothesis that it is in fact the attentional demands rather than lever pressing frequency, reward delivery, or other possible task variables in a sustained attention task that are causing the selective activation of the basal forebrain corticopetal cholinergic system (Arnold et. al.; 2002). It is also interesting to note that it is primarily the right hemisphere- the anterior cingulate, dorsolateral prefrontal, and parietal cortical regions- that is found to be activated in test subjects involved in tasks requiring sustained attention (Pardo et. al.; 1991). It is currently believed that it is specifically tonic releases of Acetylcholine that are responsible for the mediation of previously described top-down attentional control in the frontoparietal cortex (Demeter et. al.; 2013).

Studies using new technology- choline sensitive microelectrodes- have helped demonstrate the intricacies of Acetylcholine releases and have also identified a second type of cholinergic neurotransmission: sub-second phasic outputs, also known as Acetylcholine

transients. Cholinergic transients in the basal forebrain have been implicated in providing the necessary capabilities required for the initiation of sustained attention, also known as cue detection (Howe et. al.; 2010). The resulting hypothesis pinpoints specifically α4β2 and α7 nicotinic receptors as being the integral receptors involved in cholinergic action resulting in ACh transients. The infusion of selective agonists for α4β2 nicotinic acetylcholine receptors into the basal forebrain has shown to induce faster rise and decay times for ACh transients, a discovery that has a correlation with improved results in cue detection when compared to control groups not receiving agonist treatment. Furthermore, α4β2 receptors appear to be integral in controlling the amplitude of Acetylcholine transients. It has also been reported that sole stimulation of α4β2 nicotinic ACh receptors is sufficient to induce cholinergic transients (Parikh et. al., 2010). Stimulation of α7 receptors on the other hand is responsible for between 10 to15 fold longer ACh release times in transients than that observed by stimulation of α4β2 receptor agonists. Furthermore, in a different study (Howe et. al.; 2010), it has been shown that the beneficial effects of nicotine on SAT performance are obtained after blockade of α7 nAChRs.

NMDAr glutamatergic receptors in attention

Another hypothesis refers to the importance of the glutamate receptor NMDA in attention capabilities. It has been reported that intra-Nucleus Accumbens NMDA receptor infusions with CPP (3-((±) 2-carboxypiperazin-4yl) propyl-1-phosphate), an NMDA antagonist, or even NMDA itself, causes Acetylcholine stimulation in the Prefrontal Cortex (Zmarowski et. al.; 2007). Though there are no cholinergic neurons in the Nucleus Accumbens, it is considered an important part of the cholinergic system and top-down processing because it receives both direct and indirect cholinergic projections going from the prefrontal cortex to the basal forebrain (Demeter et. al.; 2013).

These findings have provided a basis for the hypothesis that glutamatergic receptor activity plays a role in the cholinergic fluxes responsible for attention capabilities. More recently, it has been found that CPP, when co-administered with nicotine, causes an increase in omission errors and response latencies as well as reduction in anticipatory responding in rats running 5-CSRTT tasks (Quarta et. al.; 2007). This has helped implicate a role for NMDA receptors in sustained attention with impaired glutamatergic networks. Interestingly, a relationship between NMDA receptors and Acetycholine release has also been reported. In a study using bilateral infusions of APV ((2R)-amino-5-phosphonovaleric acid; (2R)-amino-5-phosphonopentanoate), an NMDA receptor antagonist, into the basal forebrain of rats, it was found that animal signal detection during SAT decreased. During infusions of APV, rats exhibited increases in medial Prefrontal Cortex cholinergic efflux when compared to efflux levels prior to drug administration (Kozak et. al.; 2005). It is believed that performance-associated increases in medial Prefrontal Cortex cholinergic transmission may be important for the mediation of increased demands on attentional effort. In other words, increased cholinergic transmission is important for the maintenance of performance levels under challenging conditions. The data suggests a joint glutamatergic-cholinergic network involved in sustained attention that encompasses receptors such as previously mentioned NMDA receptors. Further evidence for this hypothesis has come from results showing that NMDA receptor blockades attenuate some of the effects of nicotine on rat attention performance, namely on 5- CSRTT (Quarta et. al; 2007). The identification of cholinergic inputs to the medial prefrontal cortex as being integral to successful 5 CSRTT performances shows the important nature of nAChR and ionotropic glutamate receptor interaction for cognitive processing (Dalley et. al.; 2004). NMDA receptor stimulation is important for nicotinic acetylcholine receptor agonist activation, as the

use of APV to antagonize NMDA receptors has resulted in attenuated cholinergic signaling even in the presence of ABT-089, an α4β2 neuronal nicotinic receptor partial agonist (Parikh et. al.; 2008). While APV is a selective antagonist for NMDA channels, MK-801 was used in this experiment because it is a non-selective NMDA receptor agonist, and thus provides a greater scope in NMDA receptor antagonization.

The rat model and SAT

Since drugs of abuse have been shown to disrupt normal attention processing in the Prefrontal Cortex and preliminary research has shown that ST rats are more impaired than GT rats in their attentional capabilities, this research study was designed to try and better understand the neuropharmacological pathways responsible for proper top-down control and sustained attention maintenance. The drugs mecamylamine, scopolamine hydrobromide, and MK801 were used in IP injections to antagonize cholinergic nicotinic receptors, muscarinic receptors, and glutamatergic NMDA receptors, respectively. It was hypothesized that only injections of mecamylamine would result in significantly more potent SAT score attenuation in ST groups when compared to GT group counterparts due to a possible deficit in α4β2 receptors as well as other Acetylcholine receptors.

Experiment 1: Mecamylamine injections

Mecamylamine is a non-selective, non-competitive antagonist of acetylcholine receptors. Mecamylamine IP injections in two dose rounds: .02 mg/kg, and 3 mg/kg were used. These injections were meant to target nicotinic receptors because these receptors have been implicated in the past for being important for cholinergic modulation, a currently hypothesized characteristic of sustained attention initiation. Specific antagonists for nicotinic α4β2 receptors

were not used because they have been identified as being highly unstable, and could potentially lead to inconsistent results. In this study, it was hypothesized that though both ST and GT rats would exhibit a significant drop in SAT scores due to the drug's antagonistic actions, ST rats would be even more adversely affected by the injection than GT rats. Previous administration of ABT-089, a nicotinic Acetylcholine receptor partial agonist improved SAT scores in ST rats while Nicotine did not (Parikh et. al.; 2010). Once again, this points to the importance of α4β2 receptors and may indicate that in ST rats, there may be an initial deficit in the number of α4β2 receptors when compared to GT rats. In this study, it was believed that this was the case and thus, mecamylamine injections were expected to cause more significant attenuation in ST rat SAT performance when compared to that of GT rats.

Experiment 2: Scopolamine hydrobromide injections

In the second experiment, scopolamine hydrobromide (muscarinic antagonist) were administered (IP) to explore the potential involvement of muscarinic neuromodulation on SAT performance on both groups. Previous studies have refined the role played by muscarinic receptors in attention related tasks. Using a circular Morris water maze with a trapezoidal Plexiglas insert to conduct visual discrimination testing, it has been observed that the activation of muscarinic receptors is significant in cases where a maintenance of visual discrimination performance is required under conditions in which visual information availability is reduced (Tsui, Dringenberg; 2012). Though both mecamylamine and scopolamine hydrobromide have been shown to affect both P(hits) and P(fa) in SAT tasks, it was hypothesized that there would not be a statistically significant difference in SAT performance between the ST and GT groups with scopolamine hydrobromide injections because it is believed that it is primarily nicotinic receptors that are involved in sustained attention (Howe et. al.; 2010). Furthermore, it appears

that scopolamine hydrobromide produces a more noticeable effect on tasks employing loss of visual information at lower dose and since this experiment does not employ the use of visual cue reduction, it was not believed that this experimental design would show a significant difference between ST and GT abilities after systemic injections of scopolamine hydrobromide in a dose of .03 mg/kg. The reason scopolamine hydrobromide was used was to make sure that all cholinergic receptor variants were tested to help pin point the true reason for the noticeable difference in ST and GT behavior.

Experiment 3: MK-801 injections

In the third experiment, MK-801, a non-competitive antagonist of the N-methyl-d-aspartate (NMDA) receptor was used to determine whether or not ionotropic glutamate receptors may be responsible for the degree of attentional deficits prevalent in ST rats in comparison to GT rats. MK-801 was used due to research previously mentioned showing the significance of NMDA receptors in cases of attentional deficits [(Kozak et. al.; 2005), (Quarta et. al.; 2007)]. It was hypothesized that administration of MK-801, antagonizing the NMDA receptors in both groups of rats, would significantly impair attentional performance in both the ST and GT groups because we did not expect any difference in glutamatergic neurotransmission between groups. The procedure for the third experiment mirrored that of the previous two. However, in this experiment, the dose for MK-801 IP injections was set at .15mg/kg.

Methods and Procedures

Subjects

Adult male Sprague Dawley rats (Harlan Laboratories), aged 9–11 months and weighing between 350 and 400 g at the beginning of the pharmacological treatments, were used. Each animal was individually housed with climate control set to a temperature of 23°C and the humidity set to 45%. The living environment ran under a 12 h light/dark (12:12 LD) schedule. Animals were handled extensively before the beginning of task training process. All animals were water deprived with 7-minute limits on water access following each behavioral (operant) training session. However, water was also provided as a reward for correct responses during task performance. On days not tested (Saturdays), the duration of water access was increased to 30 min. Food (5001 Rodent Diet; Lab Diet) was available ad libitum. The rat body weights were recorded every week. All procedures were conducted in adherence with protocols approved by the University Committee on Use and Care of Animals at the University of Michigan and in laboratories accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. For experiment 1, there were a total of 24 rats, for experiment 2, 22 rats, and for experiment 3, 19 rats. The reason for decreasing group numbers was due to rat deaths determined to be of natural causes.

PCA

Pavlovian Conditioned Approach (PCA) index score was used to identify the degree to which rat behavior was lever or food cup directed. Pavlovian training procedures were similar to those described by Meyer et. al.; 2012. Two days prior to PCA testing, ~25 banana-flavored sucrose pellets (45 mg, BioServ; Frenchtown, NJ) were given to the rats in their home cages.

Rats were tested in conditioning chambers (MedAssociates Inc., St. Albans, VT; 20.5 x 24.1 cm floor area, 29.2 cm high).

The PCA chamber

In the center of one side of the rat home cages was located food magazine which received sucrose pellets directly from an automatic feeder. These magazine entries were detected via an infrared photo beam. On one of the magazine's two sides was a retractable lever that contained an LED backlight that became illuminated when the lever was inserted in the chamber. Lever deflections either in the up or down vertical directions was used as a method of recording lever contacts. On the opposite side of the rat cage chamber from the lever was red house light located near the ceiling. The red light's illumination signaled the beginning of the test sessions.

PCA training

On the first day of testing ("pre-training"), rats were placed into transport boxes, transported to the testing room, and placed into the conditioning chambers. After a five-minute habituation period, the house light was illuminated. Twenty-five sucrose pellets were delivered on a VI-30 (0-60 s) schedule post house light illumination. The lever was retracted throughout this session. During this PCA session as well as during those that were administered subsequently, the rats consumed all the delivered sucrose pellets. For the 5 daily PCA sessions, the houselight was used to signal the session start after one minute. After this houselight signal, the rats went through 25 lever-pellet pairings administered on a VI-90 (30-150 s) schedule. The conditioned stimulus (CS) for each trial was the extension of the illuminated lever into the chamber for 8 seconds. Once the lever was retracted, the rat received a sucrose pellet via the magazine. These sessions lasted, on average, 37.5 min.

The two main variables of interest for the PCA were lever presses and magazine entries during the conditioned response periods. To record rat results from the PCA, the PCA index was employed. From the lever-press and magazine-entry data, three measured of approach were determined and calculated.

1. Response Bias

Response Bias was determined to be the difference between lever presses and magazine entries during the CS, expressed as a proportion of the total responses [(Lever Presses - magazine entries)/(Lever presses + magazine entries)].

2. Latency Score

Latency score was calculated as the difference between the latencies to approach the lever and the magazine upon CS presentation. The difference between the two was accounted for by dividing by the maximum 8-s latency [(Magazine Latency - Lever Latency)/8]. 3. Probability Difference was calculated as the difference between the probability of pressing the lever during the CS (i.e., # of trials with a lever press/25 trials) minus the probability of entering the magazine. Thus, PCA index score consisted of [probability difference score + response bias score + latency difference score)/3]. The values attainable from this formula range from - 1.0 to +1.0. While a score of +1 means the rat approached and contacted the lever on every trial, a score of -1 indicates magazine entry on every trial. A score of 0 means that lever contacts and magazine entries were equally distributed across trials.

Determining ST/GT characterization

The PCA index scores calculated on the fifth day of PCA trials were used to classify rats as sign tracking (ST) or goal tracking (GT). Rats were considered ST if they obtained scores from +.5 to +1.0, indicating that lever- directed behavior was more than twice as frequent as food cup-directed behavior. Rats with scores ranging between -.5 and -1.0 were classified as GT. Rats that received intermediate scores were not used in this experiment. Approach responses (response probability, # of contacts, and latency) were analyzed with repeated measures ANOVA with Phenotype (ST, GT) as the between groups measure and training Day (1-5).

Drug preparation and administration

All drug and vehicle injections done on the animals were intra-peritoneal (IP) and were administered at the same time: 14:00 EST. All drugs were prepared in saline solution and on days pre and post drug administration, rats received 1 mg/kg saline IP injections. All rats were placed in operant chambers immediately after drug administration. For mecamylamine, rats began SAT testing 10 minutes after drug administration. The mecamylamine doses for the rats were .02 mg/kg and 3 mg/kg and were administered 3 days apart. For scopolamine hydrobromide, rats began testing 20 minutes after drug administration. A dose of .03 mg/kg was used for injections and was administered 7 days after the second round of mecamylamine injections. For MK 801, a dose of .15 mg/kg was used for drug administration. Post drug administration, 20 minutes elapsed between injections and initiation of SAT. The MK 801 drug injections were done 30 days after the scopolamine hydrobromide injections.

Measures of SAT performance

For each session, hits, misses, correct rejections, false alarms, and omissions were recorded. The relative number of hits (hits/hits + misses) was calculated for each signal length. Additionally, the relative number of correct rejections (correct rejections/correct rejections + false alarms) was calculated. As an overall measure of attentional performance that integrates both the relative number of hits (h) and the relative number of false alarms (f), an overall performance score (SAT score) was calculated in accordance to the following: SAT = (h − f)/[2(h + f) − (h + f)2]. This index is based off of the sensitivity index as proposed by Frey and Colliver (Frey, Colliver; 1973) except that the SAR score in this experiment was based off of the relative number of hits and false alarms, as opposed to using probabilities for hits and false alarms. This allows the experiment not to be confounded by errors of omission. SAT scores range from +1.0 to −1.0. A score of +1.0 indicates that all responses were hits and correct rejections, 0 indicates an inability to discriminate between signal and non-signal events, and −1.0 indicates that all responses were misses and false alarms. SAT scores were calculated for all signal duration (SAT 500, 50, 25) as well as averaged over the three durations. Errors of omission were recorded separately. Performance measures were calculated for each of the five task blocks.

Results

Experiment 1: Effects of systemic injections of mecamylamine.

The ANOVA run to explore the effects of two doses of mecamylamine (.02 and 3.0 mg/kg) administered to both phenotypes (GT, n = 13 and ST, n = 11) on SAT score revealed a main effect of dose $F(2, 32) = 7.76$; $p = .002$. We then explored the effects of this compounds on percent of hits and correct rejections. While the ANOVA over percent hits revealed main effect of dose $F(2, 32) = 3.58$; $p = .04$ the correct rejection responses were unaffected by this treatment

$F(2, 32) = .503$; $p = .610$. Furthermore this treatment did not increase the number of omissions $F(2, 32) = .321$; $p = .728$. The effects of systemic injections of this compound are shown in fig 1: A,B,C,D. It should be noted that no main effect of group was found suggesting that both groups were affected equally.

For GT and ST rats, a paired t- test showed that none of the parameters of testing were significantly affected by the .02 mg/kg dose of mecamylamine. For GT rats, results were [$t(8) = .575$; $p = .581$], [$t(8) = .074$; $p = .943$], [$t(8) = .60$; $p = .954$], [$t(8) = 1.224$; $p = .954$], for SAT score, percent hits, percent correct rejections, and percent omissions, respectively. For ST rats, results were [$t(8) = .380$; $p = .714$], [$t(8) = .762$; $p = .468$], [$t(8) = .429$; $p = .680$], [$t(8) = -.869$; $p = .410$], for SAT score, percent hits, percent correct rejections, and percent omissions, respectively.

For the 3 mg/kg dose, GT rats exhibited a significant change in only SAT scores [$t(8) = 3.248$; $p = .012$] and percent hits [$t(8) = 2.828$; $p = .022$]. All other parameters were not significant (correct rejections [$t(8) = -.644$; $p = .538$] and percent omissions [$t(8) = 1.063$; $p = .319$]. For ST rats, 3 mg/kg injections of mecamylamine showed no significant change in SAT performance in any parameter [$t(8) = 2.120$; $p = .067$], [$t(8) = 1.866$; $p = .099$], [$t(8) = 1.118$; $p = .296$], [$t(8) = -1.332$; $p = .219$] for SAT score, percent hits, percent correct rejections, and percent omissions, respectively.

To explore the effects of each dose, a paired t-test was performed per both phenotypes. It was observed that between the two doses, GT rat SAT scores [$t(8) = 2.807$; $p = .023$] and percent hits [$t(8) = 2.529$; $p = .035$] were significantly attenuated by the increase in dose while percent correct rejections [$t(8) = .147$; $p = .887$] and percent omissions [$t(8) = .505$; $p = .627$] did not

show any significant change. For ST rats, none of the test parameters showed any significant change with increasing dose (SAT score [$t(8) = 1.638$; $p = .140$], percent hits [$t(8) = .449$; $p = .665$], percent correct rejections [$t(8) = -.134$; $p = .896$], percent omissions [$t(8) = .389$; $p = .708$)].

Furthermore, an independent t-test was run to observe any differences in initial vehicle SAT performance between the two groups. No significant difference was observed for any of the study parameters: [$t(8) = -1.798$; $p = .091$], [$t(8) = -1.812$; $p = .089$], [$t(8) = .102$; $p = .920$], [$t(8) = -.869$; $p = .398$] for SAT score, percent hits, percent correct rejections, and percent omissions, respectively.

Exepriment 2: Effects of a low dose of .03 mg/kg of scopolamine hydrobromide

The repeated measures ANOVA performed to explore the effects of systemic administration of scopolamine hydrobromide revealed a main effect of dose for SAT score (GT, n = 12; ST, n = 10; [$F(1, 14) = 84.137$; $p < .0001$]. A more detailed analysis showed that even the ability to detect cues, reflected in the percent hits scored [$F(1, 14) = 13.261$; $p < .0001$] and response on the non- signal trials were significantly affected [$F(1, 14) = 16.205$; $p = .001$]. Furthermore we found that the administration of this muscarinic antagonist scopolamine significantly affected the error of omissions [$F(1, 14) = 58.749$; $p < .0001$]. The administration of this compound impaired the performance of both ST and GT rats. The effects of scopolamine are shown on fig. 2: A,B,C,D

A paired t-test was performed to investigate the effect of the drug on both groups. For GT rats, a paired t- test showed that all parameters of testing were significantly affected (SAT score: [$t(8) = -6.599$; $p = .001$], percent hits: [$t(8) = -4.560$; $p = .004$], percent correct rejections [t(8) = -

3.199; p = .019)], percent omissions [$t(8) = 6.505$; $p = .001$], by drug administration. A paired t-test showed that for ST rats, changes in performance were significant in only SAT score [t(8) = 6.07; p < .0001] and percent omissions [$t(8) = -4.839$; $p = .001$]. For percent hits and percent correct rejections, [$t(8) = 1.376$; $p = .206$] and [$t(8) = 1.887$; $p = .096$], respectively.

Experiment 3: Administration of .15mg/kg of MK-801

The aim of the last pharmacological treatment was to investigate the effect of the NMDA antagonist MK-801 on SAT performance in ST and GT rats. We were able to collect data from just one dose of this compound as during our pilot studies we encountered many difficulties in identifying the dose that did not induced significant increase in locomotor hyperactive response. The ANOVA revealed a main effect of dose for the SAT score [GT, n = 10, ST, n = 7; $F(1, 13) = 32.425$; $p < .0001$], percent hits [$F(1, 13) = 32.376$; $p < .0001$], and correct rejections [$F(1, 13) = 31.397$; $p < .0001$]. Systemic administration of MK-801 significantly increased error of omissions [$F(1, 13) = 33.887$; $p < .0001$].

The effects of systemic administration of MK-801 are reported in fig. 3: A,B,C,D.

Discussion

The present study aimed to explore the top down control deficits and alterations in a rat model reported to be a valid tool for exploring the neurobiological basis for subjects manifesting a higher propensity for developing addictive-like behavior. Furthermore, this study allowed us to explore whether the differences on SAT performance between ST and GT rats were related to a higher vulnerability of nicotinic and muscarinic neurotransmission. In addition, we decided to investigate the involvement of the glutamatergic system by injecting animals with the NMDA antagonist MK-801 prior to SAT practice. Our results indicated that, although we were able to

replicate the lower level of performance of ST rats, administration of mecamylamine, scopolamine hydrobromide, and MK-801, equally impaired both phenotypes.

Experiment 1: Mecamylamine

Injections of both doses of mecamylamine did not produce the expected results of significant attenuation of SAT performance for both ST and GT rats. We did not see the hypothesized more severe attenuation in the SAT performance of ST rats when compared to GT rats. These findings were unexpected as it has been shown that stimulation of α4β2 and α7 nicotinic receptors are important in the initiation and continuation of SAT and mecamylamine is a nicotinic receptor antagonist [(Howe et. al; 2010), (Parikh et. al.; 2010)]. In fact, both doses did not cause any significant change in ST rats' SAT scores, percent hits, percent correct rejections, and even percent omissions. While GT rats also experienced this lack of significant attenuation in all parameters with the .02 mg/kg dose, GT rats did experience significant attenuation in SAT scores and percent hits with the 3 mg/kg dose. Though it is odd that GT rats were more affected by the drug than ST rats based on prior knowledge, it may be that it was a chance occurrence and both doses were not high enough to bring about the necessary SAT performance attenuation necessary to notice a visible difference between the performances of both rat groups. In future studies it may be prudent to retry mecamylamine IP injections with slightly higher doses. If further studies continue to show no more severe performance attenuations during SAT for ST rats when compared to GT rats, it may mean that ST rats do not in fact suffer from a deficit in Acetylcholine receptors, namely α4β2 and α7 receptors- or at least those receptors do not play an active role in the incentive salience being experienced by the ST rats.

Experiment 2: Scopolamine hydrobromide

In agreement with previous studies, administration of one dose of scopolamine hydrobromide injections confirmed that the blockade of muscarinic receptors affects SAT performance. In fact, SAT score and percent hits were significantly attenuated and percent omissions significantly increased in ST and GT rats when compared to saline control injections. However, it should be noted that while percent correct rejections significantly decreased for GT rats with scopolamine injections, the same was not the case for ST rats. As was the case with mecamylamine, a significant difference in SAT performance attenuation between the two groups with drug injections was not found. The significant attenuation in SAT performance in both rat group sans the insignificant change in ST rat percent correct rejections when looked at together with the data showing no significant difference in rat performance across groups with injections leads us to believe that ST rats do not suffer from deficits of muscarinic receptors as their performance trend was very close to that of GT rats. Furthermore, we do not believe that muscarinic receptors are involved in the incentive salience visible in ST rats.

Experiment 3: MK801

NMDA receptors and the glutamatergic system have been established to interact with the cholinergic system in maintaining proper top down control and more specifically, sustained attention (Parikh et. al, 2010). Thus, with MK801, an NMDA antagonist, it was expected that both ST and GT rats would be significantly affected by the drug injections. This was in fact the case as ST and GT rats exhibited a significant attenuation in SAT score and percent hits when compared to their respective baseline levels. However, it must be noted that for percent omissions and correct rejections, only GT rats experienced significantly attenuated performance.

It is interesting to note that GT rats' SAT scores were more significantly attenuated than that of their ST counterparts. Though this may very likely be a product of chance- both groups recorded scores that had a mean below .17, a marker regarded as the threshold of chance occurrence- if it is not, then it may allude to a possible alteration in importance and role of the glutamatergic system in sustained attention tasks in individuals experiencing incentive salience. In the future it would be prudent to once again repeat the MK 801 experiment with a larger sample size as well as with more MK 801 doses to see if these results repeat themselves outside of the chance threshold.

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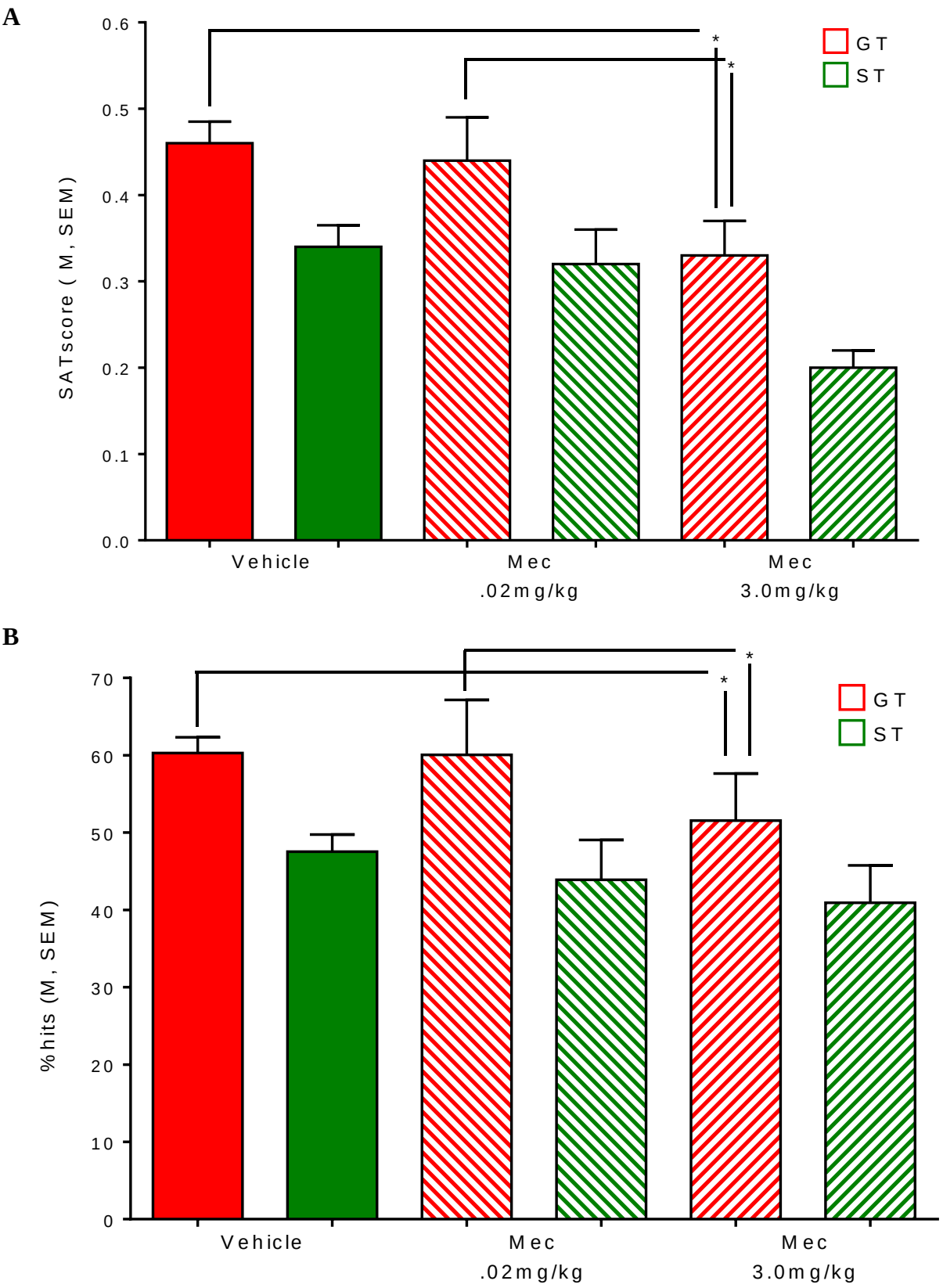
Figure 1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Mec
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B
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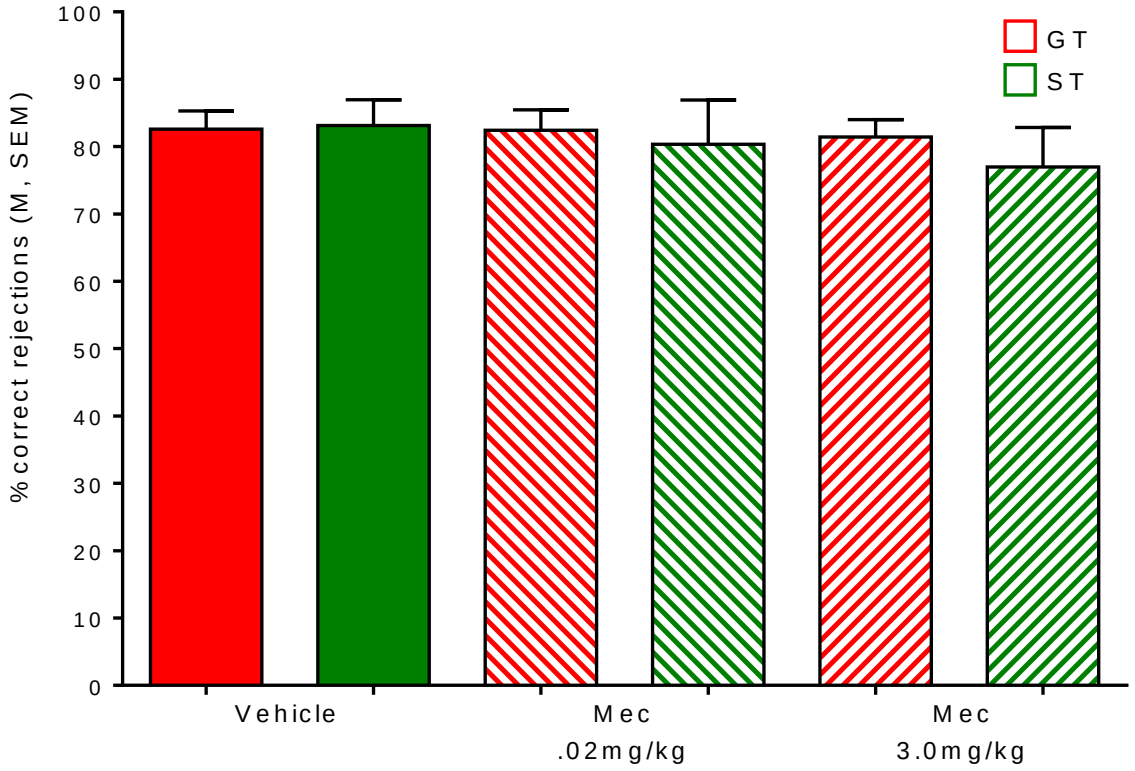
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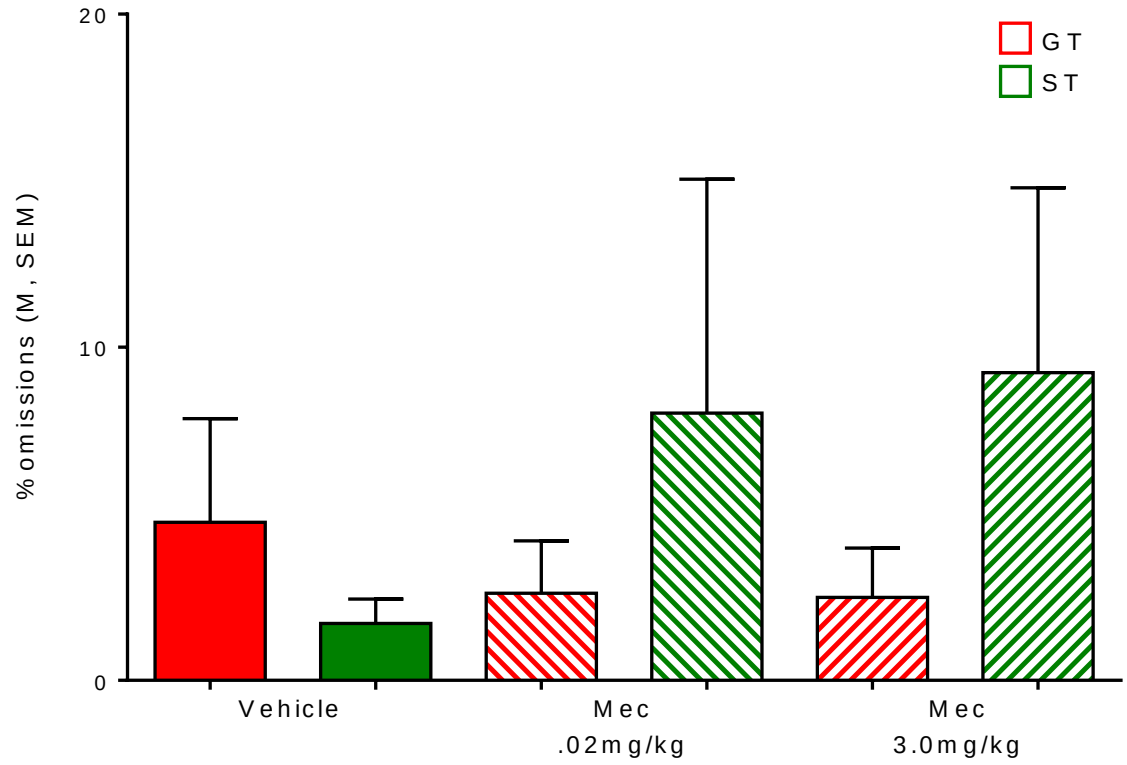
D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% omissions (M, SEM)
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Figure 1- SAT results for .02 mg/kg & 3 mg/kg injections of mecamylamine and 1 mg/kg vehicle (saline).

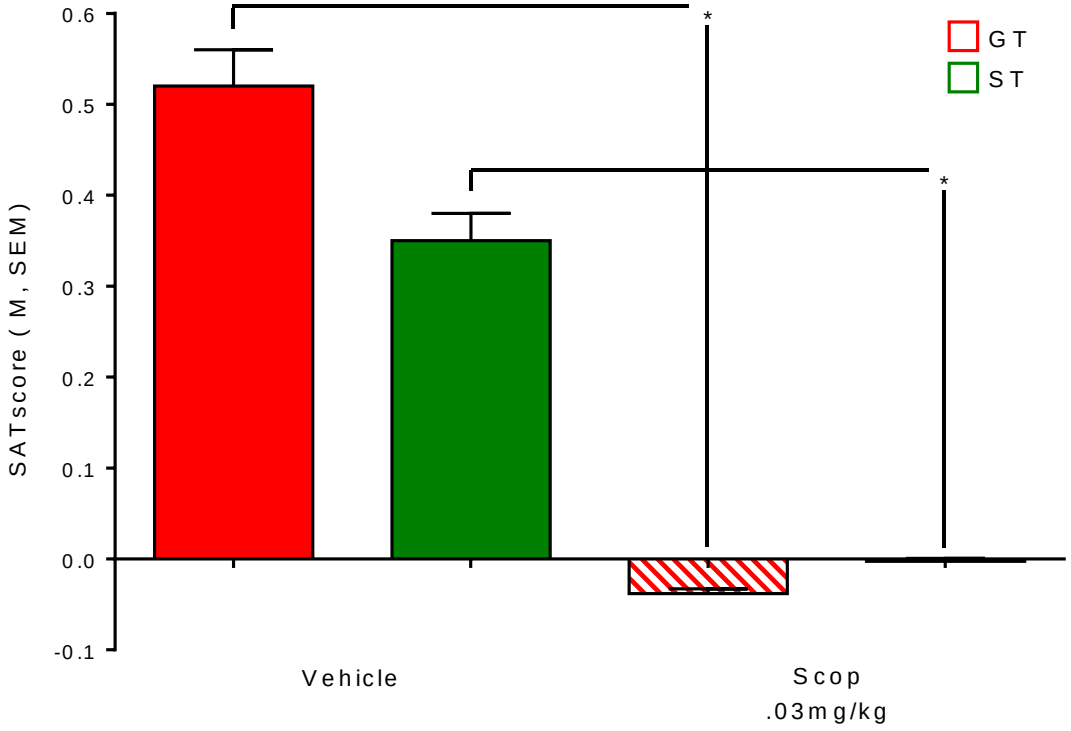
A. For SAT scores, mecamylamine injections were not significant within GT and ST groups when compared to vehicle for injections of .02 mg/kg mecamylamine ($p > .05$). However, for injections of 3 mg/kg of mecamylamine, GT group SAT scores were significantly attenuated compared to vehicle ($p < .05$). The vehicle GT group SAT scores had a mean of .46 ± .03 whereas once injected with .02 mg/kg mecamylamine, their scores had a mean of .44 ± .05. With injections of 3 mg/kg mecamylamine, the GT group SAT scores had a mean of .33 ± .04. The vehicle ST group SAT scores had a mean of .32 ± .04 whereas once injected with .02 mg/kg mecamylamine, their scores had a mean of .44 ± .05. With injections of 3 mg/kg mecamylamine, the ST group SAT scores had a mean of .20 ± .02.
B. For SAT percent hits, mecamylamine injections were not significant within GT and ST groups when compared to vehicle for injections of .02 mg/kg mecamylamine ($p > .05$). However, for injections of 3 mg/kg of mecamylamine, GT group SAT percent hits were significantly attenuated ($p < .05$) compared to vehicle. The vehicle GT group SAT percent hits had a mean of 60.28 ± 2.04 whereas once injected with .02 mg/kg mecamylamine, their percent hits had a mean of 60.07 ± 7.08. With injections of 3 mg/kg mecamylamine, the GT group SAT percent hits had a mean of 51.56 ± 6.08. The vehicle ST group SAT percent hits had a mean of 47.53 ± 2.23 whereas once injected with .02 mg/kg mecamylamine, their scores had a percent hits of 43.89 ± 5.17. With injections of 3 mg/kg mecamylamine, the ST group SAT percent hits had a mean of 40.94 ± 4.83.

C. For SAT percent correct rejections, mecamylamine injections were not significant within GT and ST groups when compared to vehicle for injections of both .02 mg/kg mecamylamine and 3 mg/kg mecamylamine ($p > .05$). The vehicle GT group SAT percent correct rejections had a mean of 82.61 ± 2.68 whereas once injected with .02 mg/kg mecamylamine, their percent correct rejections had a mean of 82.42 ± 3.04. With injections of 3 mg/kg mecamylamine, the GT group SAT percent correct rejections had a mean of 81.43 ± 2.59. The vehicle ST group SAT percent correct rejections had a mean of 83.12 ± 3.85 whereas once injected with .02 mg/kg mecamylamine, their percent correct rejections had a mean of 80.34 ± 6.56. With injections of 3 mg/kg mecamylamine, the ST group SAT percent correct rejections had a mean of 76.97 ± 5.88.

D. For SAT percent omissions, mecamylamine injections were not significant within GT and ST groups when compared to vehicle for injections of both .02 mg/kg mecamylamine and 3 mg/kg mecamylamine ($p > .05$). The vehicle GT group SAT percent omissions had a mean of 4.75 ± 3.11 whereas once injected with .02 mg/kg mecamylamine, their percent omissions had a mean of 2.61 ± 1.57. With injections of 3 mg/kg mecamylamine, the GT group SAT percent omissions had a mean of 2.49 ± 1.47. The vehicle ST group SAT percent omissions had a mean of 1.71 ± .73 whereas once injected with .02 mg/kg mecamylamine, their percent omissions had a mean of 8.02 ± 7.02. With injections of 3 mg/kg mecamylamine, the ST group SAT percent omissions had a mean of 9.24 ± 5.54.

Figure 2

A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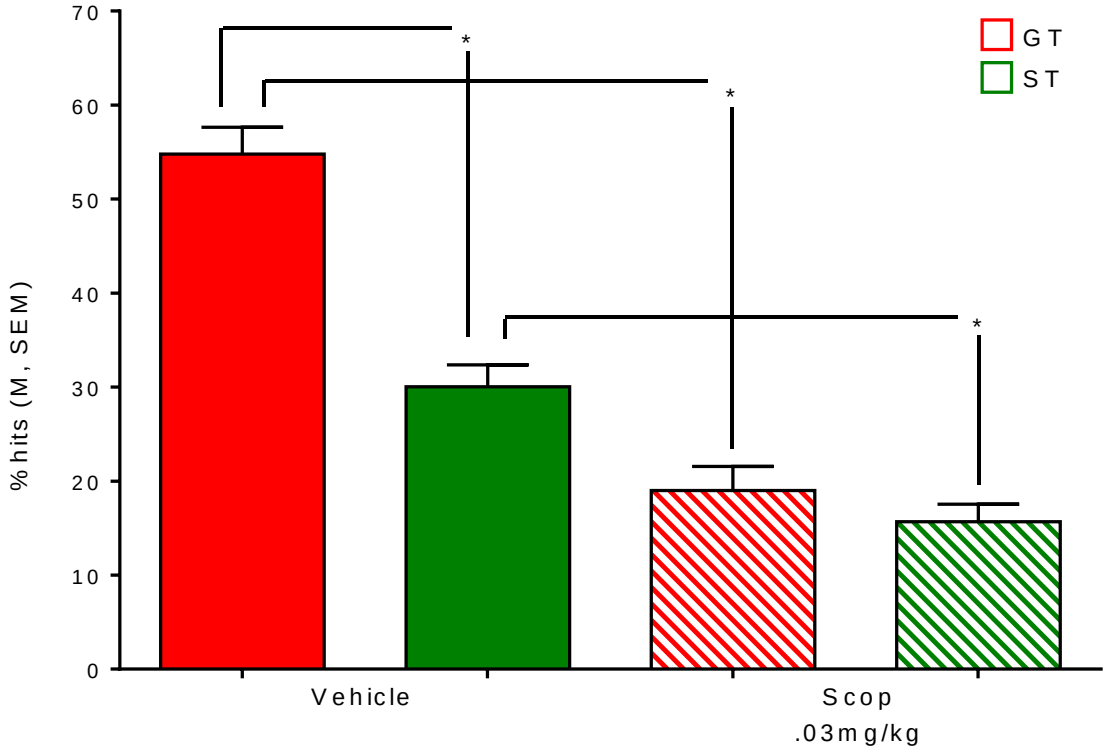
Vehicle
Scop
.03mg/kg

B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% hits (M, SEM)
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Vehicle
Scop
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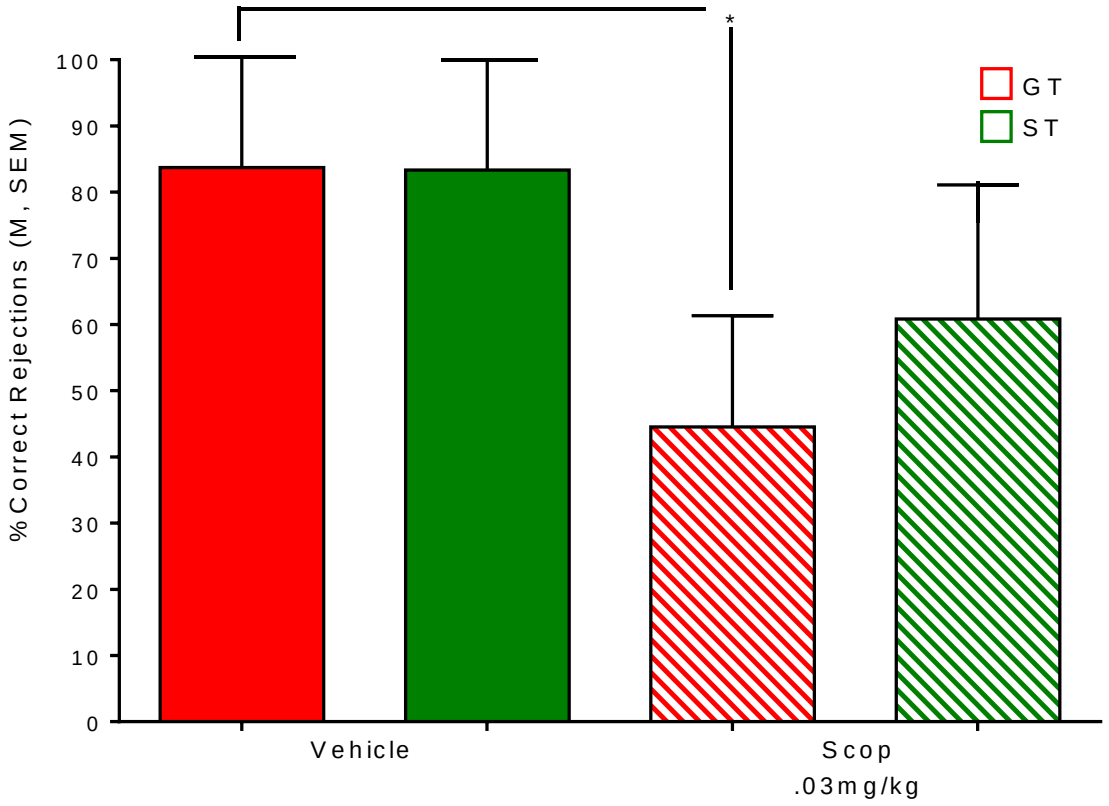
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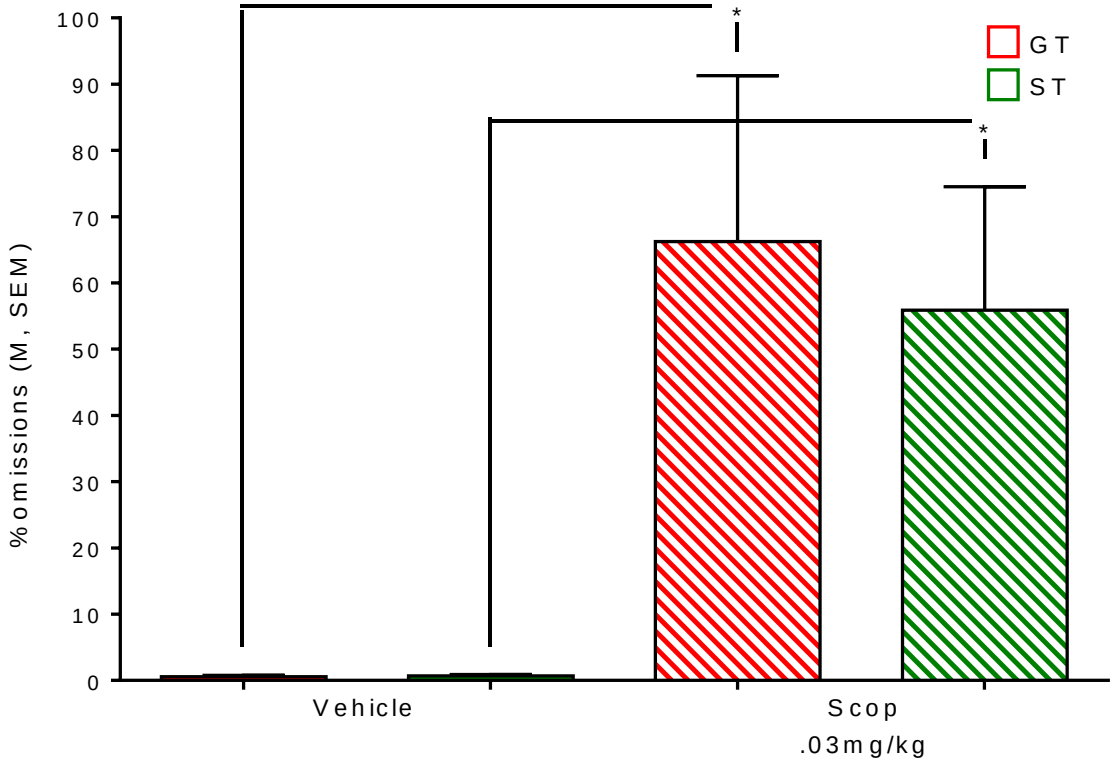
GT
ST
Vehicle
Scop
.03mg/kg

D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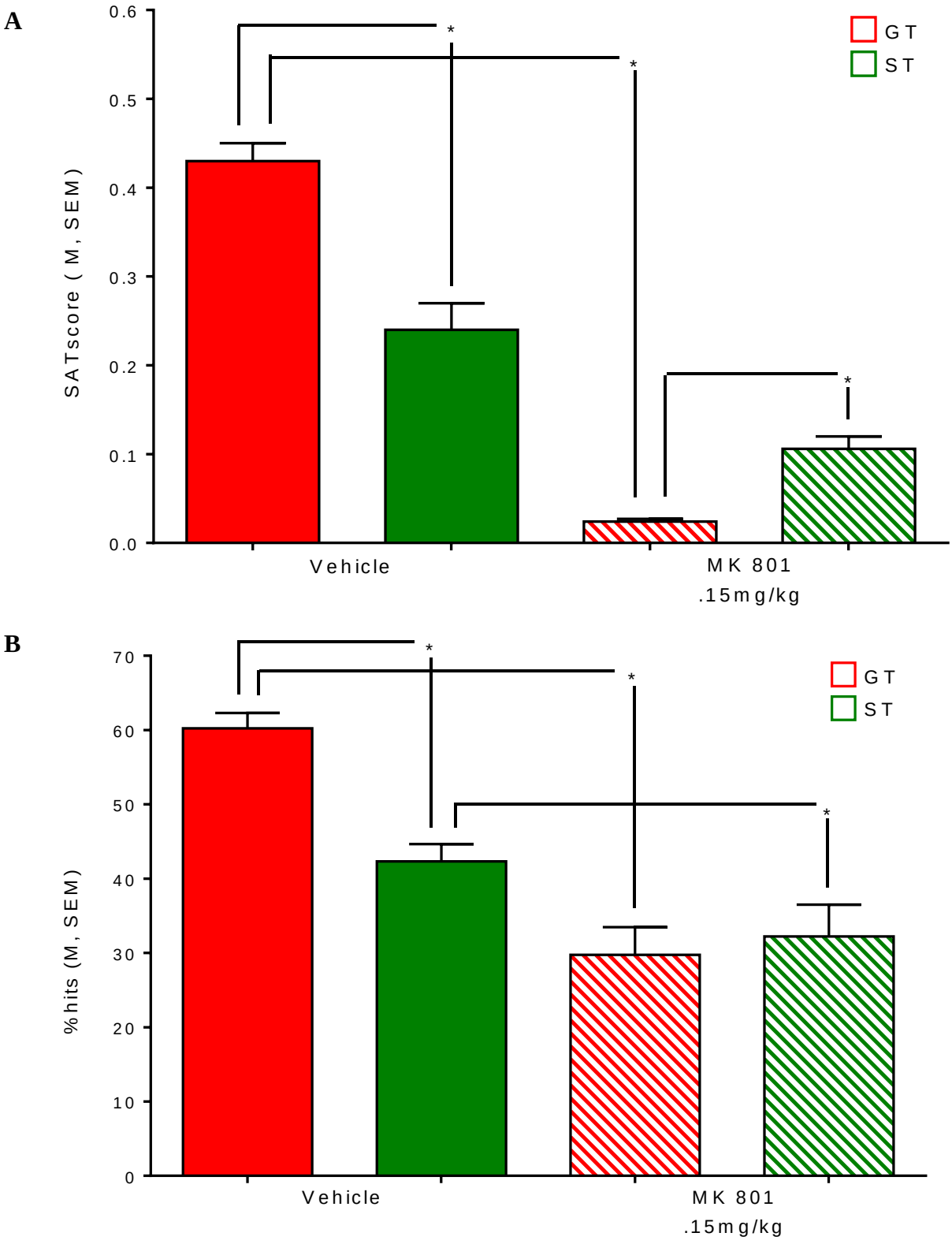
Scop
.03mg/kg

Figure 2- SAT results for .03 mg/kg injections of scopolamine hydrobromide and 1 mg/kg vehicle (saline).

A. For SAT scores, scopolamine hydrobromide injections were significant within both groups when compared to vehicle ($p < .05$). The vehicle GT group SAT scores had a mean of .52 ± .04 whereas once injected with .03 mg/kg scopolamine hydrobromide, their scores had a mean of -.038 ± .005. The vehicle ST group SAT scores had a mean of .35 ± .03 whereas once injected with .03 mg/kg scopolamine hydrobromide, their scores had a mean of -.002 ± .03.
B. For SAT percent hits, scopolamine hydrobromide injections were significant within both groups when compared to vehicle ($p < .05$). The vehicle GT group percent hits had a mean of 54.80 ± 2.85 whereas once injected with .03 mg/kg scopolamine hydrobromide, their percent hits had a mean of 19.01 ± 2.54. The vehicle ST group percent hits had a mean of 30.04 ± 2.33 whereas once injected with .03 mg/kg scopolamine hydrobromide, their percent hits had a mean of 15.68 ± 1.85.
C. For SAT correct rejections, scopolamine hydrobromide injections were significant only within the GT group ($p < .05$). The vehicle GT group percent correct rejections had a mean of 83.71 ± 31.64 whereas once injected with .03 mg/kg scopolamine hydrobromide, their percent correct rejections had a mean of 44.52 ± 16.83. The vehicle ST group percent correct rejections had a mean of 83.33 ± 27.78 whereas once injected with .03 mg/kg scopolamine hydrobromide, their percent correct rejections had a mean of 60.81 ± 20.27.
D. For SAT percent omissions, scopolamine hydrobromide injections were significant within both groups when compared to vehicle ($p < .05$). The vehicle GT group percent

omissions had a mean of .57 ± .22 whereas once injected with .03 mg/kg scopolamine hydrobromide, their percent omissions had a mean of 66.23 ± 25.03. The vehicle ST group percent omissions had a mean of .67 ± .22 whereas once injected with .03 mg/kg scopolamine hydrobromide, their percent omissions had a mean of 55.89 ± 18.63.

Figure 3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Vehicle
MK 801
.15mg/kg
B
%hits (M, SEM)
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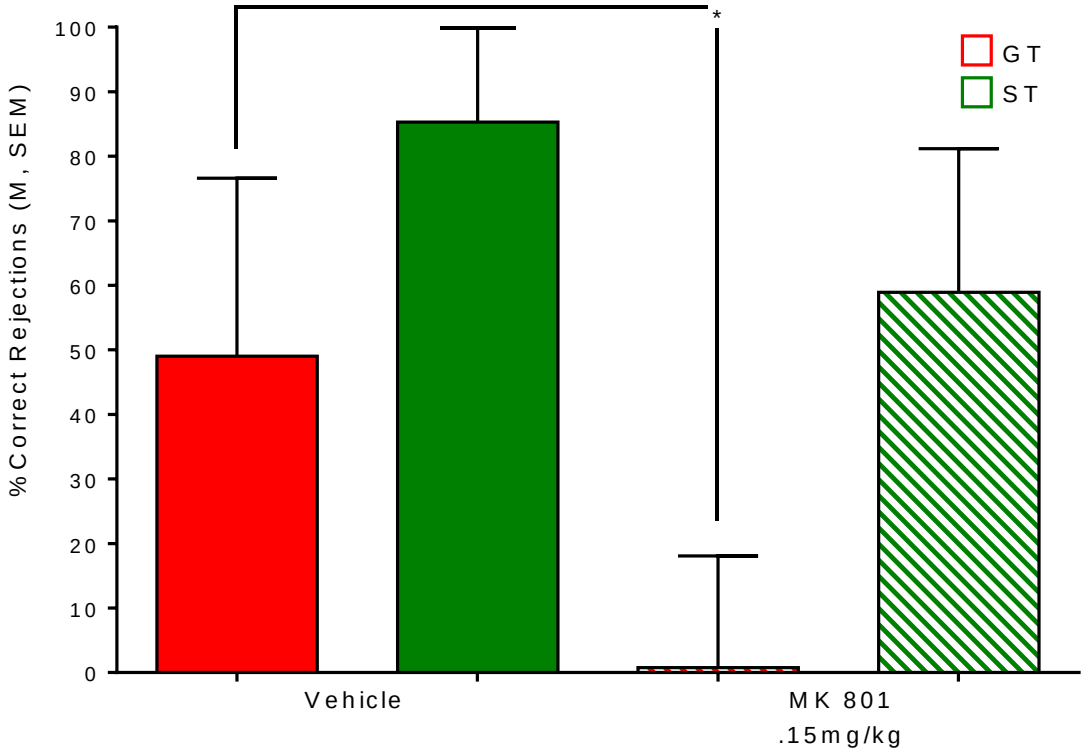
% Correct Rejections (M, SEM)
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GT
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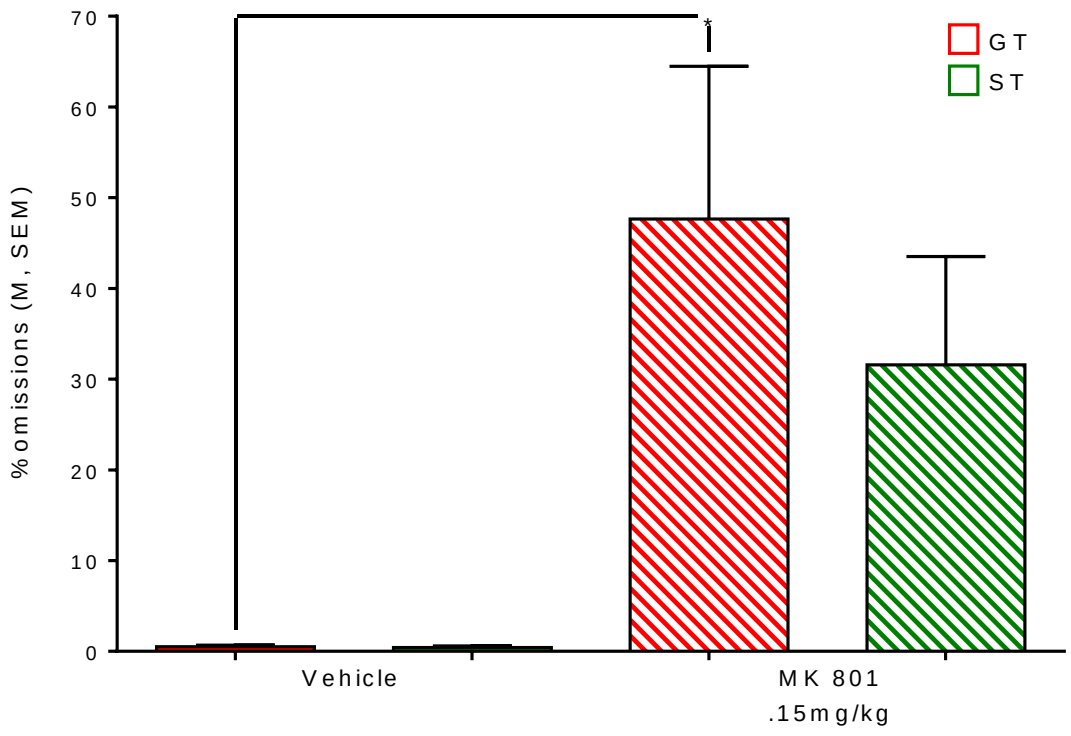
Vehicle
MK 801
.15mg/kg

D


% omissions (M, SEM)
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.15mg/kg

Figure 3- SAT results for .15 mg/kg injections of MK 801 and 1 mg/kg vehicle (saline).

A. For SAT scores, MK 801 injections were significant within only GT when compared to vehicle ($p < .05$). The vehicle GT group SAT scores had a mean of .43 ± .02 whereas once injected with .15 mg/kg MK 801, their scores had a mean of .024 ± .03. The vehicle ST group SAT scores had a mean of .24 ± .03 whereas once injected with .15 mg/kg MK 801, their scores had a mean of .106 ± .014. It should be noted that a significant difference ($p < .05$) was found for vehicle SAT scores between ST and GT rats.
B. For SAT percent hits, MK 801 injections were significant within both groups when compared to vehicle ($p < .05$). The vehicle GT group percent hits had a mean of 60.22 ± 2.09 whereas once injected with .15 mg/kg MK 801, their percent hits had a mean of 29.75 ± 3.72. The vehicle ST group SAT scores had a mean of 42.32 ± 2.33 whereas once injected with .15 mg/kg MK 801, their scores had a mean of 32.21 ± 4.30. It should be noted that a significant difference ($p < .05$) was found for vehicle SAT percent hits between ST and GT rats.
C. For SAT percent correct rejections, MK 801 injections were significant within only GT rats when compared to vehicle ($p < .05$). The vehicle GT group percent correct rejections had a mean of 49.00 ± 27.58 whereas once injected with .15 mg/kg MK 801, their percent correct rejections had a mean of .75 ± 17.32. The vehicle ST group percent correct rejections had a mean of 85.28 ± 32.23 whereas once injected with .15 mg/kg MK 801, their percent correct rejections had a mean of 58.9 ± 22.26.
D. For SAT percent omissions, MK 801 injections were significant within only GT rats when compared to vehicle ($p < .05$). The vehicle GT group percent omissions had a mean of .5 ± .18 whereas once injected with .15 mg/kg MK 801, their percent omissions

had a mean of 47.65 ± 16.84. The vehicle ST group percent omissions had a mean of .43 ± .16 whereas once injected with .15 mg/kg MK 801, their percent omissions had a mean of 31.57 ± 11.93.