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Sign-tracking and its Relation to Food-Carrying Behaviors

by

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Abstract

Understanding the role of incentive salience in behavioral reward can be useful in drawing connections between sign-tracking and food-carrying behaviors. In Pavlovian conditioning, "sign-tracking" is characterized by approach and attempted consumption of a conditioned-stimulus [(CS) e.g. lever], while the "goal-tracking" response to the CS is characterized by approaching the location of the unconditioned-stimulus [(US) e.g. food-magazine tray]. Attribution of incentive salience to a lever in Pavlovian conditioning, and to food in food-carrying behaviors connects these two seemingly unrelated behaviors. The purpose of this study was to determine if these Pavlovian conditioned group differences correlate with attributions of incentive salience to food in food-carrying behaviors. Although all animals were expected to exhibit "carry-to-eat" behavior (retrieving and carrying food to a refuge to eat it) only sign-trackers were additionally expected to exhibit "carry-to-leave" (hoarding) behavior. Furthermore, it was hypothesized that the sign-tracking and goal-tracking phenotypes could be predicted solely based on food-carrying behaviors. Results showed that when Pavlovian conditioning occurred first, then food-carrying behavior could in fact be predicted in subsequent tasks; sign-trackers seemed to be more hyper overall, and hoarded significantly more food pellets than did goal-trackers. However, results also revealed that these phenotypes could not be predicted solely from food-carrying behavior. Learning effects and order effects were implicated as well to explain the individual results of this study.

Sign-tracking and its Relation to Food-Carrying Behaviors

Understanding the role of incentive salience in behavioral reward can be useful in drawing connections between sign-tracking and food-carrying behaviors. Because there are many factors that affect behavioral reward, parsing it into its component processes is necessary to understand which aspect is being studied at a given time. Without knowing which component is being manipulated or studied in an experiment, it is impossible to draw accurate conclusions or suggest implications of the results. Although related in some ways, the three components of behavioral reward, learning, liking, and wanting, are psychologically distinct processes (Berridge & Robinson, 2003). Focusing on wanting narrows the discussion of behavioral reward to the concept of motivation.

Incentive Salience and the Notion of "Wanting"

Wanting is speculated to have evolutionarily emerged as a basic form of stimulus-guided goal direction to mediate the pursuit of a few select unconditioned stimuli (e.g. food, sex) (Berridge & Robinson, 2003). Attribution of incentive salience to an object turns it into a wanted target of motivation, and although various brain substrates are important, it is primarily influenced by dopamine (DA) neurotransmission. The notion of incentive salience, a motivational component of reward first proposed by Berridge and Robinson (Berridge & Robinson, 1998; Robinson & Berridge, 2003), was based on the fact that manipulation of dopaminergic systems substantially alters motivated behavior.

Although learning can take many forms, and can be measured in various manners, associative learning as measured in Pavlovian conditioning (response-independent conditioning) is a strong example of the concept of incentive salience. The elements of associative learning (stimulus-stimulus [S–S] associations and stimulus-response [S–R] associations) contribute to

the products of this learning (procedural habits). In 'autoshaping' (a form of Pavlovian

conditioning), a conditioned stimulus ([CS] e.g. retractable lever) elicits a conditioned response

([CR] e.g. procedural habit). The CSs, now instilled with incentive salience, become

motivational magnets, eliciting appetitive approach as well as attempted consumption of the CS

(gnawing and biting). In this case, the CS (Pavlovian cue) has become an incentive wanted

stimulus that elicits a behavioral response. It is this very type of motivation and behavioral

response that is thought to rely on mesolimbic dopaminergic systems (Berridge & Robinson,

2003).

Dopaminergic Effects – Goal-tracking and Sign-tracking

Flagel et al. (2007) explained how rats trained under an identical Pavlovian conditioning

(autoshaping) paradigm have been found to display two distinct behavioral patterns. Sign-

trackers (ST) were those animals that responded to the CS by approaching and attempting to

consume it, while goal-trackers (GT) responded to the CS by approaching the location of the

unconditioned stimulus ([US] i.e. food-magazine tray). Although in-situ hybridizations revealed

higher levels of some types of DA mRNA in the nucleus accumbens (NAcc) of ST than in GT, it

remained unclear if these differences in DA were due to the autoshaping itself, or to intrinsic

genetic factors. Additionally, Tomie, Aguado, Pohorecky, and Benjamin (2000) reported a

positive correlation between DA levels in the NAcc and lever-pressing behavior, indicating that

ST might have increased dopaminergic tone relative to GT. Therefore, while the cause of this

increased dopaminergic tone is ambiguous; it is likely that the attribution of incentive salience to

Pavlovian cues in ST is closely tied to DA.

Introduction to Food Carrying Behaviors

While it is clear how incentive salience applies to ST behavior, it is interesting to note that it has also been implicated in food-carrying behaviors. One type of food-carrying behavior, "carry-to-eat behavior", describes a rat's tendency to carry food to a refuge to eat it, rather than eating in an open area (Whishaw & Oddie, 1989). In the experimental "refuge task", rats are trained to consistently perform in this manner, retrieving and eating one pellet after another. Another type of food-carrying behavior, "carry-to-leave behavior" (hoarding behavior), has been operationally defined as the transportation of objects, particularly food, from a source to a secluded area (Ross, Smith, & Woessner, 1955). Hoarding behavior can be described as carrying food to store it, not eat it. This carry-to-leave behavior is entirely distinct from the carry-to-eat behavior of the previously described refuge task.

The Motivation to Carry Food

Experimentally, there are certain conditions that significantly affect the motivation to both carry-to-eat and hoard food. The influences of handling time and pellet size have been investigated in previous studies. Characteristics of the food itself help to determine whether a rat will simply sit and eat it at the source, or will feel compelled to carry it back to the refuge to either eat or store it. Handling time was studied as a function of the hardness or softness of pellets (Whishaw, 1990), and results showed that when compared with soft pellets, rats took longer to eat hard pellets and also carried more hard pellets to the refuge. Additionally, Whishaw and Tomie (1989) showed that there was a direct relation between the size of food pellets and the probability that they were carried to the refuge to be eaten; the larger the pellet, the higher the carrying probability. The 500 mg pellet (largest size tested) was shown to consistently induce hoarding behavior, while Whishaw and Kornelsen (1993) found that the

1000 mg pellet[1] reliably induced carry-to-eat behavior. As direct as these interactions seemed to be, further experimentation revealed that the carrying probability of hard/soft or small/large pellets alike was primarily a function of eating time; therefore, a larger-soft pellet and a smaller-hard pellet might both be carried to the refuge (Whishaw).

Additionally, the environmental factor of travel distance from the refuge to the food source can impact the motivation to carry food. Based on the hypothesis of cognitive appraisals of rats based on travel distance, this effect on food-carrying behaviors was studied by Whishaw and Tomie (1989). Surprisingly, they reported a null effect; varying the distance between the refuge and the food source *did not* significantly change behavior patterns toward food pellets. Although Whishaw and Dringenberg (1991) overturned these results to show that distance *did* have an effect on food-carrying behavior, they found that this significant effect was only true for small to medium sized food pellets. They found that smaller pellets were no longer carried at longer distances, but the largest pellets (750-1000 mg) were always carried regardless of travel distance. Food carrying behavior can always be induced with a sufficiently large (1000 mg) food pellet.

Therefore, in light of the evidence, of pellet characteristics and travel distance, anticipated eating time emerges as a major factor for the motivation behind carry-to-eat behavior. The pellet's hardness/softness and size, combined with the distance to the food source forms the basis of a sort of cognitive appraisal of the situation. For both chickadees and gray squirrels, food-handling time (eating time) combined with the distance to the refuge determines whether food will be carried (Lima & Valone, 1986; Lima, Valone, & Caraco, 1985, as cited in Whishaw, 1990). Lima suggested a utilitarian tradeoff explanation; if eating time exceeded the return time from the food source to the refuge, then the animal would carry the food to the refuge.

Therefore, a sufficiently hard or large food pellet can be used to induce food-carrying behavior under any travel distance circumstance.

A rat's familiarity to its surrounding is also important to its food-carrying motivation. It has been found that rats eat and hoard little in strange situations (Bindra, 1948b). Acclimation to new stimuli (food, apparatus) is vital to eliminate this as a confounding variable. Familiarity is also closely tied to the concept of security. It has been argued that rats transport food from areas of less security to areas of greater security (Bindra; Viek & Miller, 1944). Dubbed the *security hypothesis*, it is also a widely held belief that environmental lighting affects food-carrying behavior such that rats transport food from more exposed to less exposed areas (Whishaw & Oddie, 1989). In a test apparatus consisting of a dark refuge and a highly lit alley, increasing ambient illumination within the alley serves to make it seem "less secure", and in turn make the dark refuge "more secure". In the refuge task, rats may move food to the refuge based on how they would act in natural environments. Carrying food to eat in the refuge minimizes the possibility of predation as well as the possibility of losing food to conspecifics (Whishaw, Oddie, McNamara, Harris, & Perry, 1990). In the hoarding situation, rats may move food to the refuge because it provides the maximum security and the least amount of anxiety. It is important to remember that it is a difference in security that elicits the onset of food-carrying behaviors (Whishaw & Oddie).

The Role of Food Deprivation

Although there are many common motivational features of carry-to-eat and carry-to-leave behaviors, it is important to remember that these behaviors are fundamentally distinct. Therefore, it is plausible that as unique as these behaviors are, so too may be the effect of an underlying motivational feature. In much of the literature on food-carrying behaviors, it seems

that the role of food deprivation has been under debate. Whether it is necessary, sufficient, or peripheral to bringing about these food-carrying behaviors has been a focus of the literature in the field. As far as eating behavior is concerned, rats are most likely to eat when deprived of food (Bindra, 1948a); therefore food-deprivation can help to produce more robust carry-to-eat behavior and experimental results.

While it is intuitive to think that a hungry animal is more likely to eat than a sated animal, this logic is not quite as intuitive when applied to hoarding behavior. When hoarding behavior in the rat was first studied in the late 1930s, the *deficit hypothesis* postulated that the behavior was motivated primarily by a dietary deficit (due to food deprivation) in the animal (Wolfe, 1939). Subsequent studies sought to more accurately characterize the role of food deprivation and satiety states in hoarding behavior. Although Morgan, Stellar, and Johnson (1943) demonstrated that food deprivation was neither sufficient nor necessary to initiate this behavior, they still supported Wolfe's theory based on results that hoarding behavior was found to *primarily* result from food deprivation.

The general paradigm that hoarding behavior was primarily a result of some sort of dietary deficit was sufficiently overturned when it was found that it was completely unnecessary to initiate this behavior (Bindra, 1948a). Bindra additionally showed that because rats were most likely to eat under conditions of food deprivation, they hoarded more as well. Decades later, Whishaw and Tomie (1989) challenged Bindra's results by demonstrating that varying the level of deprivation between 24 hours of food deprivation and complete satiation did not significantly affect behavior toward food presented in the experiment.

Therefore, the majority of the literature supports the conclusion that while food deprivation is unnecessary to either behavior, it increases the likelihood of eating behavior and

thus carry-to-eat behavior. However, this overall energizing effect is not as clearly defined for hoarding behavior. The most recent studies seem to support the conclusion that food deprivation has no significant effect on hoarding behavior.

Explanatory Hypotheses in Literature and the Role of Limbic Circuitry

It is clear that carry-to-eat and hoarding behaviors are distinct, and exploring the foundation of these differences is key to distinguishing and understanding both behaviors. Morgan (1947) suggested that some rats are constitutionally more prone than others to hoard food. This suggestion was based on Hunt's (1941) analysis of effects of infant food-deprivation on hoarding showing that some litters were significantly affected by deprivation while others were not. It was unclear whether this difference was due to constitutional factors present at birth and/or having a genetic linkage or differences in nursing care. Although inconclusive, Morgan's suggestion was the first of its kind, suggesting a potential genetic basis for variations in responses to deprivation and thus food-carrying behavior. Later studies described potential genetic differences in the dopaminergic systems of GT and ST (Flagel et al., 2007), the involvement of this system in mediating food-carrying behaviors, and the importance of this system's increased arousal to food-carrying behaviors (Kelley & Stinus, 1985).

Limbic circuitry has been implicated as an underlying feature of hoarding behavior. Kelley and Stinus (1985) discovered that hoarding behavior both disappeared after 6-hydroxydopamine lesions were made to mesolimbic DA neurons (ventral tegmental area or NAcc) and was reinstated with administration of L-dopa. They concluded that hoarding behavior was mediated by mesolimbic DA neurons and hypothesized this dopaminergic system to be necessary for the facilitation of certain types of food-carrying behaviors under high levels of arousal. This is just one of the many studies suggesting that damage or pharmacological

disruption of limbic structures can disrupt hoarding behavior (Whishaw, 1990). Whishaw

explains that because the structure of most hoarding tests is very simple (simply entailing

transportation of food from a source to a refuge), no contemporary theory of limbic function

would predict such a simple associative task to require limbic circuitry. He does acknowledge

that if animals are using processes that require knowledge of food (size, type), consciousness of

environmental characteristics (travel distance, ambient lighting), and estimates of anticipated

eating time, then the importance and involvement of limbic circuitry becomes identifiable.

Based on the presented research, the associative demands and decisions of food-handling and

carrying behaviors can be paralleled with the associative demands of behaviors that have been

shown to be dependent on limbic circuitry.

 With the role of limbic circuitry in mind, Whishaw and Kornelsen (1993) proposed an

incentive motivation hypothesis to explain the differences between carry-to-eat and hoarding

behaviors. Assuming two types of motivation, one that directs behavior to primary food cues

(drive-reducing or unconditioned motivation), and one that directs behavior to its secondary

features (incentive appetitive or conditioned), when hungry, all animals treated food as likable

and satisfying to eat; but when sated only control animals (in contrast to NAcc lesioned animals)

treated the food as valuable. Because the concept of incentive salience is neurobiologically

based on the mesolimbic dopaminergic system, Whishaw and Kornelsen extended their theory to

say that it was evidence for the selective involvement of the NAcc (and therefore DA) in

hoarding behavior. Furthering this idea, they postulated hoarding behavior to be more

encephalized (NAcc connecting to the forebrain) than the primary carry-to-eat behavior.

Purpose of the Carry-to-eat and Hoarding Tasks in the Present Study

Marx (1950) provided evidence of hoarding behavior to be learned rather than instinctive, which meant that the "pre-hoarding" behaviors described in his study were those that correlate to the behaviors prior to and during the refuge task in the present experiment. Marx's reported decreases in exploratory behavior and the latency to hoard are characteristics of learning effects. The purpose of the carry-to-eat task was twofold. Firstly, it was conducted to ensure that the animals were reliably carrying-to-eat the pellets presented in the hoarding apparatus. Essentially, it was a test of behavioral learning, ensuring the formation of a habit. Secondly, this behavioral learning was a precursor to the hoarding task. The hoarding task was another food-carrying task designed to aid in assessing group differences of food-carrying behavior.

Hypotheses

While it is clear that a rat's motivation to hoard is governed by many different aspects of the situation, some factors are essential, while the rest are peripheral. Bindra (1948b) argued the two determining factors of hoarding behavior to be (1) the difference in security offered by the testing apparatus and (2) the value of the object. Assuming the difference in security to be significant and the object to be valuable, all other things equal, if rats eat until satiation, then they should hoard as well. (Bindra, 1948a; Whishaw & Tomie, 1989).

The present experiment investigated the nature of this "all or nothing" statement of hoarding. It postulated that differences in attribution of incentive salience would affect hoarding behavior, and sought to parse out specific group differences in food-carrying behavior. Although all animals were expected to exhibit "carry-to-eat" behavior (retrieving and carrying food to a refuge to eat it), varying attributions of incentive salience to food post-satiation were expected to result in group differences in "carry-to-leave" hoarding behavior (Hypothesis I). In other words,

group differences from the Pavlovian conditioning were expected to carry through to the refuge task as well as to the hoarding task. The attribution of incentive salience to the lever in Pavlovian conditioning by ST was expected to parallel a similar attribution to food in the hoarding task. It was also hypothesized that the sign-tracking and goal-tracking phenotypes could be predicted solely based on food-carrying behaviors (Hypothesis II). The group differences expected in the refuge task were expected to correlate with group differences in the hoarding task; and these group differences were expected to carry through to the Pavlovian conditioning. The predictive validity of this test would be a novel way to determine the GT and ST phenotypes without the traditional Pavlovian conditioning. From a neurobiological standpoint, based on this hypothesized overactive dopaminergic system in ST (Tomie et al., 2000), if they do hoard more than GT, then this group difference would suggest that factors other than instinctive or even learning mechanisms govern these behaviors. This would show that Bindra's (1948a) hypothesis might only be partially true, and furthermore, only true in certain circumstances. Conclusions could then be drawn about the role of incentive salience and DA in food-carrying behavior, and provide further information about these group differences.

Method

Overview

This study was conducted in two parts. Experiment I began with the Pavlovian conditioning (determining the sign-tracking/goal-tracking phenotypes), and was followed by the food-carrying tests (refuge task followed by hoarding task). Experiment II nearly reversed the first experiment; it began with the food-carrying tests (refuge task followed by hoarding task), and was followed by the Pavlovian conditioning.

Experiment I

(Pavlovian Conditioning – Autoshaping)

   Subjects. Thirty healthy adult male[2] Sprague-Dawley rats from Charles River

(Wilmington, MA, USA) were used in this study. The animals were housed singly in

temperature and humidity controlled home cages (following UCUCA guidelines). Food and

water were available *ad libitum*. The animals had three days to acclimate to a 12-hour light/dark

cycle (lights off at 1900 hours) and daily human handling. To acclimate the rats to new stimuli

that would be used throughout the training, 45 mg banana-flavored food pellets (BioServe,

#F0059, Frenchtown, NJ, USA) were placed in the home cages for two days prior to the start of

the Pavlovian conditioning. This study followed the Guidelines for the Care and Use of

Mammals in Neuroscience and Behavioral Research as well as the Principles of Laboratory

Animal Care.

   Apparatus. Sixteen standard operant conditioning chambers ($20.5{\times}24.1$ cm^2 floor, 29.2

cm high; MED Associates, St. Albans, VT, USA) were used for the Pavlovian conditioning.

Each chamber was equipped with a red house light that remained on throughout the training.

The opposite side of the chamber was equipped with a retractable illuminated lever (6 cm above

stainless steel grid floor) and a food-magazine tray (3 cm above floor) where the 45 mg banana

flavored food pellets were presented. To illuminate the lever, A 0.635-cm-high output white

LED was flush mounted on its inside; and to eliminate side bias, the side of the lever with

respect to the food-magazine tray was counterbalanced across boxes (eight chambers had the

lever on left side of the tray, and eight had it on the right side). Any force greater than or equal

to 10-g registered as a "lever press." Each operant conditioning chamber was enclosed in

another sound-minimizing box; a ventilating fan provided white noise to mask external noise

(Flagel et al., 2007).

   Protocol.   All experiments were conducted between 1300 and 1700 hours (within the

light cycle).   Two waves of rats (15 rats / wave) were tested each day.   After the acclimation

period, the pre-training protocol began.   The red house light remained on throughout pre-

training, and the lever remained retracted.   The pre-training protocol was designed to familiarize

rats to the operant chamber and to ensure that they were reliably eating the banana pellets

delivered to the food-magazine tray.   Fifty banana pellets were delivered to the food-magazine

tray on a 90-second variable interval schedule; therefore each pre-training session lasted

approximately 25 minutes.   Flagel et al. (2006) showed that typically after two days of pre-

training the rats will have consumed all of the food pellets.   This result was supported by the

present study; therefore after two days, the Pavlovian conditioning began.   This protocol was

designed to develop Pavlovian conditioned approach (PCA) behavior.   An illuminated lever was

presented for eight seconds and then retracted; which was immediately followed by the response-

independent delivery of a 45 mg banana pellet into the food-magazine tray.   A single trial

consisted of lever presentation, retraction, and banana pellet presentation.   The beginning of the

next intertrial interval began immediately after the previous pellet had been delivered.   Twenty-

five banana pellets were delivered to the food-magazine tray on a 60-second variable interval

schedule; therefore each Pavlovian conditioning session lasted approximately 40 minutes[3].

Acquisition of the Pavlovian CR was achieved by repeatedly pairing the lever–banana pellets

(CS–US) for five consecutive days (Flagel et al., 2007).

   Data Collection.   The following dependent variables from the Pavlovian conditioning

were utilized in separating the animals into two groups: lever press frequency and CS-magazine

entry frequency. Because approach toward, and clutching/gnawing the lever are clear indications that the CS has acquired incentive salience, the dependent variable – average-lever-presses was used to characterize the animal groups. After the fifth day of Pavlovian conditioning, average-lever-press data across the five days of training for each rat were analyzed to determine which rats were sign-trackers and goal-trackers. Sign-trackers (ST) were those animals found to respond to the CS by approaching and attempting to consume it, while goal-trackers (GT) were found to respond to the CS by approaching the location of the US. The intermediates were those animals with no clear preference for either the lever or food-magazine tray.

(Refuge [carry-to-eat] task)

Subjects. The subjects for the refuge task were the 23 ST and GT isolated from the previous Pavlovian conditioning (the intermediates were sacrificed prior to this round of testing). The rats were housed singly for this experiment. Water was available *ad libitum*.

Apparatus. The hoarding apparatus was divided into two sections: a refuge and an alley, as depicted in Figure 1. The refuge was made of black Plexiglas and was 23 cm wide, 26 cm high, and 22 cm long. There was a small clear observation window on the side facing the experimenter, and an 8 cm wide by 16 cm high door that allowed access to the alley from the refuge. There was also a camera fixture in the ceiling that was connected to a TV monitor, allowing more accurate observation of activity (e.g. eating behavior) within the refuge. The alley was made of painted wood and was 18 cm wide, 26 cm high, and 152 cm long, with a clear Plexiglas removable ceiling panel. A 1 cm diameter circular opening on the wall (6 cm above the floor) at the end of the alley was the location of manual food pellet delivery into a small Plexiglas food-tray (4.5 cm above the floor) directly under the opening.

The refuge was almost completely devoid of light, providing a safe and comfortable environment for the rats. The alley on the other hand, was purposely overpowered with light. Two extra lamps were situated just above the alley, and were kept on throughout testing. This served to maximize the "difference in security" discussed earlier. A removable panel separated the refuge from the alley. Prior to the start of each session, the panel was in place, separating the two compartments. Rats were placed in the refuge to begin each session, and timing began once the panel was lifted, allowing access to the alley.

Food Deprivation and Habituation. Following the Pavlovian conditioning, the ST and GT rats were placed on a food deprivation schedule to reach and maintain 85–90% of their *ad libitum* body weight[4]. To maintain this weight, rats were food deprived day and night, and food rations (12–15g standard Purina lab chow) were placed in their home cages after daily experimentation. During the first week of food deprivation, the rats began to be habituated to the hoarding apparatus and the 1000 mg food pellets (Bioserve Inc, Frenchtown, NJ, USA). Each day, thirty 1000 mg food pellets were scattered throughout the alley, and rats were placed in the hoarding apparatus in pairs for 15 minutes. For the next three days, rats were placed individually in the hoarding apparatus, and food pellets were presented by hand (one pellet at a time) through the opening in the wall at the end of the alley. Each rat was placed in the apparatus for the time required to retrieve five food pellets. After three days of individual habituation, rats were spontaneously retrieving the pellets presented.

Protocol. All experiments were conducted within the light cycle, and each rat was tested individually. To begin the task, rats were placed in the refuge with the removable panel in place. After the panel was lifted and rats had access to the alley, timing for the first trial began. Each trial consisted of the delivery of a single food pellet into a food tray at the end of the alley. Rats

exited the refuge, retrieved the pellet, returned to the refuge, and ate the pellet. During the "eating time," another pellet was manually delivered into the food tray. For the first four days, each session consisted of the delivery of ten pellets; and for the next seven days, each session consisted of the delivery of eight pellets[5].

Data Collection. The following five dependent variables were manually recorded (see Appendix for the refuge task data sheet).

(1) Latency to exit refuge – the amount of time spent in the refuge prior to exiting[6] into the alley (either for initial excursion, or time after eating for each subsequent excursion).

(2) Run time – the amount of time from exiting the refuge until acquisition of the food pellet.

(3) Carry time – the amount of time from food-pellet-acquisition until reentry[7] into the refuge.

(4) Latency to eat – the amount of time spent between reentry into the refuge and beginning to eat the food pellet.

(5) Eating time – the amount of time taken to completely eat the pellet.

(Hoarding Task)

Subjects. Please see the *Refuge Task – Subjects* section for details.

Apparatus. Please see the *Refuge Task – Apparatus* section for details.

Food Deprivation and Habituation. The food deprivation regimen was maintained throughout this phase of testing as well. Rat weight was maintained at 85-90% of the *ad libitum* body weight. Reliable pellet retrieval in the refuge task was the habituation for the hoarding task.

Protocol. All experiments were conducted within the light cycle, and each rat was tested individually. Because the hoarding task was a 60-minute test, approximately 3–5 rats were tested each day. The remaining rats were simply maintained on the food deprivation regimen.

Each rat was tested one time. To begin the task, rats were placed in the refuge with the

removable panel in place. Seventy pellets were placed in a pile at the end of the alley. After the

panel was lifted and rats had access to the alley, timing for the session began. Rats proceeded to

perform the refuge task (retrieving and eating pellets one-by-one from the 70-pellet pile) until

satiation, without any external cues. Satiation was marked by subjective observation, by either

the start of hoarding behavior or lack of further food retrieval. After 60-minutes had elapsed or a

15-minute period of complete inactivity, the test was ended.

Data Collection. The following four dependent variables were recorded and / or

calculated (see Appendix for the hoarding task data sheet).

(1) Interval recording – the number of pellets remaining in the pile at ten-minute intervals.

(2) Pellets remaining in pile – the number of pellets remaining in the pile.

(3) Pellets hoarded – the number of pellets moved from the pile to the refuge (pellets hoarded).

(4) Pellets eaten – [70 – (# pellets hoarded + # pellets remaining in pile)].

Experiment II

(Refuge [carry-to-eat] Task)

Subjects. Thirty healthy adult male Sprague-Dawley rats from Charles River

(Wilmington, MA, USA) were used in this study. The animals were housed singly in

temperature and humidity controlled home cages (following UCUCA guidelines). Food and

water were available *ad libitum*. The animals had seven days to acclimate to a 12-hour light/dark

cycle and human handling (lights off at 1900 hours). This study followed the Guidelines for the

Care and Use of Mammals in Neuroscience and Behavioral Research as well as the Principles of

Laboratory Animal Care.

Apparatus. Please see the *Refuge Task – Apparatus* section from Experiment I for
details.

Food Deprivation and Habituation.   After the seven-day acclimation period, rats were placed on a food deprivation schedule to reach and maintain 85–90% of their *ad libitum* body weight[8].   Please see the *Refuge Task – Food Deprivation and Habituation* section from Experiment I for details about the food deprivation regimen and habituation to the hoarding apparatus. In Experiment II, five days of individual habituation were conducted.

Protocol. All sessions consisted of the delivery of five pellets.

Data Collection. Timing for the five specific actions listed in Experiment I were manually recorded for the final three days of testing. Please see the *Refuge Task – Data Collection* section from Experiment I for details.

(Hoarding Task)

Subjects. Please see the *Refuge Task – Subjects* section from Experiment II for details.

Apparatus. Please see the *Refuge Task – Apparatus* section from Experiment I for details.

Food Deprivation and Habituation.   Please see the *Hoarding Task – Food Deprivation and Habituation* section from Experiment I for details.

Protocol. Please see the *Hoarding Task – Protocol* section from Experiment I for details.

Data Collection. Please see the *Hoarding Task – Data Collection* section from Experiment I for details.

(Pavlovian Conditioning – Autoshaping)

Subjects. The subjects for the hoarding task were the same 30 animals that were previously tested in the hoarding task. Water was available *ad libitum*.

Food Deprivation and Habituation. Rats were taken off the food deprivation regimen at the conclusion of the hoarding task. To acclimate the rats to new stimuli that would be used

throughout the training, 45 mg banana-flavored food pellets were placed in the home cages for

three days prior the start of the Pavlovian conditioning.

      Apparatus. Please see the *Autoshaping – Apparatus* section from Experiment I for
details.

      Protocol. Please see the *Protocol* section from Experiment I for details. One day of pre-

training was conducted; and four days of Pavlovian conditioning were conducted.

      Data Collection. Please see the *Autoshaping – Data Collection* section from Experiment

I for details. The ten intermediates were not used in data analysis.

<div align="center">Results</div>

Group Assignments. After Pavlovian conditioning was conducted in both experiments I and II,

the two distinct groups (GT and ST) were identified. Lever-press and CS-magazine entry data

were averaged per rat, across each day's 25-trial session; and then these data from four – five

consecutive days of Pavlovian conditioning were averaged again. Animals were categorized as

GT or ST based on average lever press behavior and secondarily on average CS-magazine entry

behavior across four – five days of training. GT had a low number of lever-presses and a high

number of CS-magazine entries, while ST had a high number of lever-presses and a low number

of CS-magazine entries. See Tables 1 and 2 for individual subject Pavlovian conditioning data.

      Independent T-tests were used to assess group differences in PCA behavior within each

respective experiment (GT vs. ST) as well as between experiments (experiment I vs. experiment

II: GT and experiment I vs. experiment II: ST). Significance was set at $p \leq .05$ for all analyses.

Figures 2a and 3a show average lever press data and average CS-magazine entry data between

GT and ST for experiments I and II. Figures 2b and 3b show average lever press data and

average CS-magazine entry data between groups (Experiment I – GT vs. Experiment II GT and

Experiment I ST vs. Experiment II ST). GT (experiment I: $N = 13$, experiment II: $N = 9$) had the

lowest average number of lever presses (experiment I: 0 – 7, experiment II: 0 – 31), and a high

number of CS-magazine entries (experiment I: 8 – 163, experiment II: 35 – 84), and ST

(experiment I: $N = 10$, experiment II: $N = 11$) had the highest average number of lever-presses

(experiment I: 32.5 – 100.5, experiment II: 64 – 95), and a low number of CS-magazine entries

(experiment I: 1.5 – 42.5, experiment II: 4 – 45). Animals that did not demonstrate any clear

preference for, or aversion to the CS were labeled as intermediates, and were not used in further

testing or analysis.

Refuge Task. In both experiments I and II, the two groups characterized by the Pavlovian

conditioning, GT and ST, were carried through in the reported data for the food-carrying tasks as

well. Data were collected per rat daily, for the time required to retrieve eight pellets. Data were

averaged across all days of testing, for all rats in each group. All data were reported by

excursion number (Study I: eight excursions; Study II: five excursions).

Linear mixed-effect models were used to assess longitudinal trends across excursions

(experiment I: eight excursions, experiment II: five excursions) in carry-to-eat behavior. The

covariance structure for the longitudinal carry-to-eat data was explored and modeled

appropriately for each dependent variable (average latency to exit refuge, average run time,

average carry time, average latency to eat, average eating time). When significant main effects

or group*excursion interactions were revealed, Bonferonni post hoc comparisons were

conducted. Significance was set at $p \leq .05$ for all analyses (Flagel et al., 2007). Within-group

effects refer to significant effects from excursion to excursion for a particular group (GT or ST).

Overall group effects refer to overall differences between GT and ST for a particular dependent

variable. Group*excursion interactions reveal group differences during individual excursions.

The first measure that was analyzed across experiments I and II was the average latency to exit the refuge. Please see Table 3 for compiled results of this measure. For experiment I, as shown in Figure 4b, the average latency to exit the refuge was 23.258 s (SEM = 0.966 s) for GT and 9.039 s (SEM = 1.101 s) for ST. Within-group effects revealed that for GT, the average latency to exit the refuge decreased with some fluctuation from excursion one (M = 27.515 s, SEM = 3.336 s) to excursion eight (M = 23.724 s, SEM = 2.795 s), and for ST it decreased from excursion one (M = 10.517 s, SEM = 3.804 s) to excursion eight (M = 7.877 s, SEM = 3.187 s), as shown in Figure 4a. There was an overall group effect, $F(1,6)$ = 94.221, $p < .001$, showing that the GT and ST groups were entirely distinct across all eight excursions. The group*excursion (between-group) interaction revealed that GT were significantly slower than ST to exit the refuge during every single excursion, $10.155 < F(1,15\text{-}21) < 15.497$, $p < .005$. There was no significant within-group change in the average latency to exit the refuge across excursions for either GT, $F(7,27)$ = 1.977, $p = .096$, or ST, $F(7,27)$ = 0.167, $p = .990$.

For experiment II, as shown in Figure 4d, the average latency to exit was 6.941 s (SEM = 1.063 s) for GT and 8.188 s (SEM = 0.962 s) for ST. Within-group effects revealed that for GT, the average latency to exit the refuge increased with some fluctuation from excursion one (M = 5.963 s, SEM = 1.077 s) to excursion five (M = 10.407 s, SEM = 1.904 s), and for ST it increased from excursion one (M = 5.303 s, SEM = 0.974 s) to excursion five (M = 11.818 s, SEM = 1.722 s), as shown in Figure 4c. There was a significant within-group effect for GT between excursions two and five, $F(4,18)$ = 4.215, $p < .05$. There were significant within-group effects for ST between excursions one and three, $F(4,18)$ = 7.775, $p < .05$, and one and five, $F(4,18)$ = 7.775, $p < .05$. There was no overall group effect, $F(1,18)$ = 0.757, $p = .396$, or

group*excursion (between-group) effect for any excursion, $0.207 < F(1,18) < 1.594, .223 < p < .655$.

The next measure that was analyzed across experiments I and II was average run time. Please see Table 4 for compiled results of this measure. The first excursion of many trials was marked by exploration of the refuge and alley, thereby inflating the run time for this initial excursion. Once this excursion had occurred for each rat, this exploratory behavior diminished and subsequent excursions were much quicker. For experiment I, as shown in Figure 5b, the average run time was 10.674 s ($SEM = 1.497$ s) for GT and 6.571 s ($SEM = 1.707$ s) for ST. Within-group effects revealed that for GT, average run time decreased with some fluctuation from excursion one ($M = 24.405$ s, $SEM = 4.407$ s) to excursion eight ($M = 9.397$ s, $SEM = 2.962$ s), and for ST it decreased with some fluctuation from excursion one ($M = 13.317$ s, $SEM = 5.024$ s) to excursion eight ($M = 4.933$ s, $SEM = 3.377$ s). There were significant within-group effects for GT between excursions one and four, $F(7,21) = 3.756, p < .05$, one and five, $F(7,21) = 3.756, p < .05$, and one and six, $F(7,21) = 3.756, p < .05$. There was no significant within-group change in the average run time across excursions for ST, $F(7,21) = 0.541, p = 0.794$, overall group effect, $F(1,21) = 3.266, p = .085$, or group*excursion (between-group) effect, $0.016 < F(1,21) < 2.970, .100 < p < .901$, for any excursion.

For experiment II, as shown in Figure 5d, the average run time was 2.630 s ($SEM = 0.158$ s) for GT and 3.061 s ($SEM = 0.143$ s) for ST. Within-group effects revealed that for GT, average run time increased with some fluctuation from excursion one ($M = 2.667$ s, $SEM = 0.177$ s) to excursion five ($M = 2.778$ s, $SEM = 0.301$ s), and for ST it increased with some fluctuation from excursion one ($M = 2.606$ s, $SEM = 0.160$ s) to excursion five ($M = 3.424$ s, $SEM = 0.272$ s), as shown in Figure 5c. There was an overall group effect, $F(1,58) = 4.077, p < .05$, showing

that the GT and ST groups were entirely distinct across all eight excursions. There was no significant within-group change in the average run time across excursions for either GT, $F(4,39)$ = 0.285, p = .886, or ST, $F(4,39)$ = 2.370, p = .069, or group*excursion (between-group) effect for any excursion, $0.023 < F(1,18) < 3.082$, $.096 < p < .882$.

The next measure that was analyzed across experiments I and II was average carry time. Please see Table 5 for compiled results of this measure. For experiment I, as shown in Figure 6b, the average carry time was 3.699 s (SEM = 0.420 s) for GT and 3.464 s (SEM = 0.479 s) for ST. Within-group effects revealed that for GT, average carry time decreased with some fluctuation from excursion one (M = 5.764 s, SEM = 0.748 s) to excursion eight (M = 4.762 s, SEM = 0.748 s), and for ST it decreased with some fluctuation from excursion one (M = 5.163 s, SEM = 0.853 s) to excursion eight (M = 3.577 s, SEM = 0.853 s), as shown in Figure 6a. There was a significant within-group effect for GT between excursions one and three, $F(7,71)$ = 2.506, $p < .05$. There was an overall within-group effect in the average carry time across excursions for GT, $F(7,71)$ = 2.506, $p < .05$, but not for ST, $F(7,71)$ = 1.227, p = .299. There was no overall group effect, $F(1,18)$ = 0.136, p = .717, or group* excursion (between-group) effect for any excursion, $0.064 < F(1,111) < 1.090$, $.299 < p < .801$.

For experiment II, as shown in Figure 6d, the average carry time was 2.363 s (SEM = 0.092 s) for GT and 2.321 s (SEM = 0.083 s) for ST. Within-group effects revealed that for GT, average carry time increased with some fluctuation from excursion one (M = 2.407 s, SEM = 0.335 s) to excursion five (M = 2.593 s, SEM = 0.274 s), and for ST it decreased with some fluctuation from excursion one (M = 2.576 s, SEM = 0.303 s) to excursion five (M = 2.394 s, SEM = 0.248 s), as shown in Figure 6c. There was a significant within-group change in the average carry time across excursions for GT, $F(4,18)$ = 3.469, $p < .05$, but not for ST, $F(4,18)$ =

1.109, $p = .383$. There was no overall group effect, $F(1,18) = 0.114$, $p = .740$, or

group*excursion (between-group) effect, $0.139 < F(1,18) < 0.567$, $.461 < p < .714$.

The next measure that was analyzed across experiments I and II was average latency to

eat. Please see Table 6 for compiled results of this measure. For experiment I, as shown in

Figure 7b, the average latency to eat was 2.936 s ($SEM = 0.393$ s) for GT and 3.840 s ($SEM =$

0.448 s) for ST. Within-group effects revealed that for GT, average latency to eat decreased with

some fluctuation from excursion one ($M = 5.821$ s, $SEM = 2.341$ s) to excursion eight ($M = 2.796$

s, $SEM = 0.450$ s), and for ST it decreased with some fluctuation from excursion one ($M =$

13.890 s, $SEM = 2.670$ s) to excursion eight ($M = 2.193$ s, $SEM = 0.514$ s), as shown in Figure

7a. There were significant within-group effects for ST between excursions one and two, $F(7,21)$

$= 4.801$, $p < .005$, one and three, $F(7,21) = 4.801$, $p < .01$, one and four, $F(7,21) = 4.801$, $p <$

.005, one and five, $F(7,21) = 4.801$, $p < .005$, one and seven, $F(7,21) = 4.801$, $p < .01$, and one

and eight, $F(7,21) = 4.801$, $p < .01$. The group*excursion (between-group) interaction revealed

significant group differences during excursions one, $F(7,21) = 5.165$, $p < .05$, four, $F(7,21) =$

5.988, $p < .05$, and five, $F(7,21) = 7.583$, $p < .05$. There was no significant within-group effect

for GT, $F(7,21) = 1.111$, $p = .393$, or overall group effect, $F(1,21) = 2.302$, $p = .144$.

For experiment II, as shown in Figure 7d, the average latency to eat was 7.889 s ($SEM =$

1.392 s) for GT and 7.024 s ($SEM = 1.260$ s) for ST. Within-group effects revealed that for GT,

average latency to eat increased with some fluctuation from excursion one ($M = 9.370$ s, $SEM =$

2.813 s) to excursion five ($M = 14.037$ s, $SEM = 3.362$ s), and for ST it decreased with some

fluctuation from excursion one ($M = 10.545$ s, $SEM = 2.545$ s) to excursion five ($M = 6.970$ s,

$SEM = 3.041$ s), as shown in Figure 7c. There was a significant within-group effect for GT,

$F(4,18) = 7.827$, $p < .005$. There was no significant within-group effect for ST, $F(4,18) = 2.469$,

$p = .082$. There was no overall group effect, $F(1,18) = 0.212$, $p = .651$, or group*excursion

(between-group) effect, $0.096 < F(1,18) < 3.556$, $.076 < p < .760$.

The final measure that was analyzed across experiments I and II was average eating time.

Please see Table 7 for compiled results of this measure. For experiment I, as shown in Figure

8b, the average eating time was 69.149 s ($SEM = 2.960$ s) for GT and 60.288 s ($SEM = 3.364$ s)

for ST. Within-group effects revealed that for GT, average eating time increased with some

fluctuation from excursion one ($M = 66.815$ s, $SEM = 4.399$ s) to excursion eight ($M = 77.545$ s,

$SEM = 5.029$ s), as shown in Figure 8a. For ST, average eating time increased with some

fluctuation from excursion one ($M = 61.940$, $SEM = 5.016$ s) to excursion eight ($M = 71.860$ s,

$SEM = 5.299$ s). There were significant within-group effects for GT between excursions two and

six, $F(7,12) = 7.318$, $p < .001$, two and seven, $F(7,12) = 7.318$, $p < .005$, two and eight, $F(7,12) =$

7.318, $p < .01$, and four and seven, $F(7,12) = 7.318$, $p < .05$. There were also significant within-

group effects for ST between excursions two and five, $F(7,12) = 17.711$, $p < .01$, two and six,

$F(7,12) = 17.711$, $p < .001$, two and seven, $F(7,12) = 17.711$, $p < .001$, two and eight, $F(7,12) =$

17.711, $p < .001$, three and six, $F(7,12) = 17.711$, $p < .001$, three and seven, $F(7,12) = 17.711$, p

$< .01$, three and eight, $F(7,12) = 17.711$, $p < .001$, four and six, $F(7,12) = 17.711$, $p < .01$, four

and seven, $F(7,12) = 17.711$, $p < .005$, and four and eight, $F(7,12) = 17.711$, $p < .005$. The

group*excursion (between-group) interaction revealed that GT took significantly longer to eat

than ST during excursions two, $F(1,28) = 9.185$, $p < .05$, three, $F(1,26) = 7.782$, $p < .05$, and

four, $F(1,30) = 6.672$, $p < .05$. There was no overall group effect, $F(1,41) = 3.911$, $p = .055$.

For experiment II, as shown in Figure 8d, the average eating time was 54.852 s ($SEM =$

1.681 s) for GT and 53.115 s ($SEM = 1.520$ s) for ST. Within-group effects revealed that for GT,

average eating time increased from excursion one ($M = 49.889$ s, $SEM = 1.811$ s) to excursion

five (M = 59.037 s, SEM = 2.087 s), and for ST it increased from excursion one (M = 47.424 s,

SEM = 1.639 s) to excursion five (M = 60.121 s, SEM = 1.887 s), as shown in Figure 8c. There

were significant within-group effects for GT between excursions one and four, $F(1,18)$ = 7.087,

p < .005, one and five, $F(1,18)$ = 7.087, p < .05, two and four, $F(1,18)$ = 7.087, p < .005, two and

five $F(1,18)$ = 7.087, p < .01, and three and four, $F(1,18)$ = 7.087, p < .05. There were also

significant within-group effects for ST between excursions one and four, $F(4,18)$ = 16.163, p <

.005, one and five, $F(4,18)$ = 16.163, p < .001, two and four, $F(4,18)$ = 16.163, p < .001, two and

five, $F(4,18)$ = 16.163, p < .001, three and four, $F(4,18)$ = 16.163, p < .005, and three and five,

$F(4,18)$ = 16.163, p < .005. There was no overall group effect, $F(1,18)$ = 0.587, p = .453, or

significant group*excursion (between-group) effect, $0.148 < F(1,18) < 2.103, .164 < p < .704$.

Hoarding Task. Independent T-tests were used to assess group differences in hoarding behavior

within each respective experiment (GT vs. ST) as well as between experiments (experiment I vs.

experiment II: GT and experiment I vs. experiment II: ST). The following dependent variables

were tested: average number of pellets eaten, average number of pellets hoarded, and the average

time to complete the task. See Tables 1 and 2 for individual hoarding task data. Significance

was set at $p \le .05$ for all analyses.

In both experiments I and II, the GT-ST group separations from the Pavlovian

conditioning were carried through in the hoarding task. Figures 9a and 10a show the average

number of pellets eaten and the average number of pellets hoarded between GT and ST for

experiments I and II. Figures 9b and 10b show the average number of pellets eaten and the

average number of pellets hoarded between groups (Experiment I – GT vs. Experiment II GT

and Experiment I ST vs. Experiment II ST). For experiment I, there was no significant

difference (p = .838) in the number of pellets eaten by GT (M = 16.385 pellets, SEM = 0.917

pellets) and ST (M = 17.000 pellets, SEM = 1.000 pellets), but there was a significant difference (p < .001) in the number of pellets hoarded by GT (M = 1.615 pellets, SEM = 0.866 pellets) and ST (M = 22.400 pellets, SEM = 8.367 pellets). For experiment II, there was no significant difference (p = .167) in the number of pellets eaten by GT (M = 11.111 pellets, SEM = 0.455 pellets) and ST (M = 12.364 pellets, SEM = 0.789 pellets), or in the number of pellets hoarded (p = .188) by GT (M = 29.444 pellets, SEM = 8.283 pellets) and ST (M = 39.364 pellets, SEM = 6.171 pellets). Significantly more pellets (p < .05) were eaten by GT in experiment I (M = 16.385 pellets, SEM = .917 pellets) vs. experiment II (M = 11.111 pellets, SEM = 0.455 pellets), and significantly fewer pellets (p < .001) were hoarded by GT in experiment I (M = 1.615 s, SEM = 0.866 pellets) vs. experiment II (M = 29.444 pellets, SEM = 8.283 pellets). There was no significant difference (p = 0.543) in the number of pellets eaten for ST in experiment I (M = 17.000 pellets, SEM = 1.000 pellets) vs. experiment II (M = 12.364 pellets, SEM = 0.789 pellets), or in the number of pellets hoarded (p = .053) in experiment I (M = 22.400 pellets, SEM = 8.367 pellets) vs. experiment II (M = 39.364 pellets, SEM = 6.171 pellets). See Tables 8a and 8b for compiled results for the hoarding task.

Discussion

Food-carrying behavior, as postulated by Whishaw and Kornelsen (1993) can be dissociated into multiple forms. The results of experiment I not only support this conclusion (separating food-carrying behavior into carry-to-eat behavior and hoarding behavior), but also provide evidence of group differences for these behaviors.

Role of Learning

It has been suggested that food-carrying behavior has a foundation in common learning principles (Marx 1950). Although Marx's observations did not constitute complete and

sufficient evidence for the learned basis of food-carrying behavior, they were more than simply suggestive. Results of the present study corroborate this theory to a certain extent.

It is true that over the course of days, all animals learned to consistently carry food to the refuge and eat it. However, if carry-to-eat behavior was *solely* a learned behavior, all animals in both experiments should have performed equally in the refuge task. Only in experiment I did animals display decreased average latencies to exit the refuge, run times, and eating times from excursion one to excursion eight, evidence of learned behavior. And again, although all animals in experiment II did learn to carry food and eat it, the decreases in average latencies and times observed in experiment I did not carry over. Therefore, although all animals learned the carry-to-eat behavior itself, something else must have been responsible for the group differences seen in experiment I, as well as the lack of group differences seen in experiment II. It seems as though the learning effect was amplified in the ST of experiment I. Additionally, if hoarding was *solely* a learned behavior, then it follows that all animals in both experiments should have performed equally in the hoarding task as well. This was clearly not the case, because although all animals in experiment II hoarded food pellets, significant group differences in experiment I do not support this conclusion.

Role of Food Deprivation

While food deprivation energized all animals to eat in both experiments, in experiment I, significant group differences were found between GT and ST. As suggested by Morgan (1947), it is possible that there is a genetic basis for variations in responses to deprivation, and thus, food-carrying behaviors. Keeping this in mind, and merging the hypothesis that ST have an overactive dopaminergic system (Flagel et al., 2007) with the hypothesis that hoarding behavior is mediated by the mesolimbic DA neurons under high levels of arousal (Kelley & Stinus, 1985),

it is likely that the impact of food deprivation in this study resulted in increased arousal in an already hyper dopaminergic system in the ST of experiment I. In other words, overactive dopaminergic systems in the ST could have rendered them more susceptible to the impact of food deprivation. Selective impact on these ST is plausible because data show that they were quicker than GT to exit the refuge (average latency to exit the refuge) during every single excursion. Additionally, in experiment I, lower average run times, carry times, and eating times for ST provide more evidence for increased arousal in contrast to GT. Extending these results even further, hoarding task results for experiment I show that ST hoarded significantly more food pellets than did GT. The aforementioned hyperarousal affecting the dopaminergic system might also be responsible for this increased hoarding behavior. Experiment I refuge and hoarding task data confirm hypothesis I to be conceivably correct.

It is important to note that no definitive conclusions can be drawn about the effect of food deprivation from this study because all animals were equally food deprived or sated during testing. Having said that, previous research does seem to form a rather plausible backdrop for the effect of food deprivation in Experiment I.

Order Effects

While it is true that in experiment II, significant hoarding by all animals (GT and ST alike) is evidence that instinctive and learning components are involved in this behavior, by virtue of the existence of group differences in experiment I, Bindra's (1948b) two determining factors (satiation and value of object) for hoarding behavior, seem to only hold up in certain circumstances. It seems that Bindra's factors apply more consistently to carry-to-eat behavior, rather than to hoarding behavior, as all animals value the food enough to eat until satiation, but not enough to hoard it. Bindra's notion of overall hoarding after satiation does not leave room

for an explanation of group differences in hoarding behavior such as in Experiment I of the present study.

It is possible that order-effects in this study played a factor in the results, and therefore conclusions. Because experiments I and II were comprised of the same components (Pavlovian conditioning, refuge task, hoarding task) but differed in sequence, it is possible that the vast disparity in results between experiments was due to an order-effect. In Flagel et al. (2007) it was suggested that increased levels of D1 receptor mRNA in ST relative to GT may have contributed to the initial acquisition of the CR and the subsequent emergence of different behavioral patterns. It was unclear however, whether the group differences in mRNA levels were preexisting characteristics, or a consequence of the first day of Pavlovian conditioning. Essentially, the group differences in mRNA levels could have been due to the Pavlovian conditioning.

Applying this to the present study, it is possible that the group differences observed in the refuge and hoarding tasks of experiment I were a consequence of the Pavlovian conditioning, as these same group differences were not observed in experiment II where conditioning occurred after the food-carrying tasks. Pavlovian conditioning behaviorally measures the attribution of incentive salience to a cue, and has been shown to correlate with dopaminergic differences as well (Flagel et al., 2007, Tomie et al., 2000). Associative learning in ST results in a habit where these animals attribute incentive salience to and approach the CS prior to consuming the food. Therefore, it is possible that due to a dopaminergic system potentially influenced by this Pavlovian conditioning, ST in experiment I were more likely than the GT to attribute incentive salience to the food in the hoarding task, as they already viewed food as a valuable object. In Pavlovian conditioning, GT respond to the CS by approaching the US, and the only habit they form is to eat the food when it is dispensed. This corresponds to GT behavior in the hoarding

task, to only eat the food, and not hoard it. Additionally, it is possible that all animals in

experiment II learned the carry-to-eat and hoarding behaviors equally because Pavlovian

conditioning, and thus possible differentiation, did not occur until after the food-carrying tasks

had been completed. Due to the fact that group differences did not carry over from experiment I

to experiment II, it also follows that it was not possible to predict the ST and GT phenotypes

simply from food-carrying behaviors (carry-to-eat and hoarding behaviors); hypothesis II did not

hold up from experiment I to experiment II.

Value Judgments

As suggested by Morgan et al. (1943), the goal of hoarding might be the carrying activity

itself, rather than the eating of pellets. This suggests that the value of the food might have

changed for the ST in experiment I. Hoarding by ST (and significantly less hoarding in GT)

suggests that the animals attributed a secondary motivation to the food. Whishaw and Kornelsen

(1993) reported that the NAcc is not responsible for the primary motivation to eat, but rather is

involved in the secondary value judgments associated with hoarding behavior. This fits with the

hypothesis that the dopaminergic system of the ST is different than that of the GT in experiment

I. If these changing value judgments arose in part due to an order effect, it is understandable

why these same effects did not hold up in experiment II.

Conclusions

It is important to keep in mind that in both experiments of this study, the animals for each

experiment were obtained from a batch of identically bred animals. Therefore group differences

in behavior could have been due to intrinsic genetic differences, learning effects, or experimental

order effects. Additionally, it is clear that two types of motivation exist with respect to food

carrying behaviors. While the primary drive-reducing features of food are recognized and acted

upon by all rats, this motivation can be dissociated from that required to motivate carry-to-leave behavior.

Future Studies

Because the concept of incentive salience relies heavily on the over-activation of the mesolimbic dopaminergic system, DA was implicated as the mediating neurological factor between sign-tracking and hoarding behaviors. Understanding the role of DA in the neural circuits associated with reward can be useful in drawing connections among multiple, seemingly unrelated behaviors. Although behaviorally distinct, sign tracking and certain food-carrying behaviors share a common connection through involvement of the mesolimbic dopaminergic system.

Food deprivation has been shown to be unnecessary in initiating hoarding behavior; therefore, these same experiments could be repeated under free feeding conditions. Neurobiological studies (lesion studies) should also be conducted to conclusively determine differences in the mesolimbic dopaminergic system as they relate to sign-tracking and food-carrying behaviors.

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Author Note

Footnotes

[1] 1000 mg was chosen as the size because in Whishaw et al. (1990), results show that this pellet size *always* induced the rats to demonstrate "carry-to-eat" behavior (as opposed to eating in the alley). Once the rat was sated, this sized food pellet also reliably induced hoarding behavior. This sized food pellet was deemed by the rat too big to be eaten directly.

[2] Males were used because studies done to separate ST and GT are traditionally done with male rats only.

[3] The lever was presented on average every 60 seconds, but the actual time between lever presentations varied randomly between 30 and 90 seconds

[4] If weight did happen to vary significantly, daily food rations were adjusted to compensate for these changes.

[5] After the first four days of testing, preliminary analysis of the results showed leveling off of results for dependent variables for pellets eight – ten. Because results were so consistent for these pellets, only eight pellets were delivered for the following seven days of data collection.

[6] Exiting into the alley was defined as complete movement from the refuge to the alley (all four paws in the alley.)

[7] Reentry into the refuge was defined as complete movement from the alley into the refuge (all four paws in the refuge.)

[8] If weight did happen to vary significantly, daily food rations were adjusted to compensate for these changes.

Appendix: Refuge (carry-to-eat) Data Sheet and Hoarding (carry-to-leave) Data Sheet

Refuge (carry-to-eat) Task

Excursion	Exit	Retrieve Pellet	Enter Refuge	Begin Eating	End Eating
1					
2					
3					
4					
5					
6					
7					
8					

Hoarding (carry-to-leave) Task

Subject	# Eaten	# Remaining	# Hoarded
1			
2			
3			
4			
Etc…			

Table 1

Experiment I – Compiled Data - Characterization of ST and GT

	Pavlovian Conditioning Data		Hoarding Task Data	
Type	Average LP	Average CS-Mg-Entries	#Pellets Eaten	#Pellets Hoarded
GT	2	8	15	1
GT	4.5	41.5	17	0
GT	2.5	51.5	20	2
GT	0.5	51.5	18	11
GT	0	64	10	4
GT	0	72	23	0
GT	0	84	13	0
GT	0.5	96.5	15	0
GT	1	99.5	15	0
GT	0.5	114	18	0
GT	0	126	19	0
GT	0	163	15	0
GT	5.5	73	15	3
ST	93.5	1.5	17	0
ST	69	2	16	4
ST	100.5	2	21	49
ST	48.5	6.5	20	0
ST	66.5	9.5	15	2

ST	79.5	12	17	53
ST	55.5	15.5	16	54
ST	46.5	29	12	6
ST	53	38	14	56
ST	32.5	42.5	22	0

Note:
LP = Lever-Presses, CS-Mg = CS-Magazine

Table 2

Experiment II – Compiled Data - Characterization of ST and GT

	Pavlovian Conditioning Data		Hoarding Task Data	
Type	Average LP	Average CS-Mg-Entries	#Pellets Eaten	#Pellets Hoarded
GT	0	51.7	13	9
GT	0	57	11	12
GT	0	61.3	10	16
GT	2.7	83.7	13	40
GT	7.7	37.7	11	59
GT	12.7	31	10	59
GT	14	35.3	12	60
GT	22	48.7	11	0
GT	31	72.7	9	10
ST	64.3	19.3	14	2
ST	69.3	39	18	10
ST	70	25.7	15	27
ST	75.3	44.7	9	30
ST	77.3	5	9	40
ST	81.7	13.7	12	42
ST	82	17.3	13	43
ST	83	17	11	58
ST	91.7	8.3	11	59

ST	94.7	12.7	12	61
ST	95	3.7	12	61

Note:
LP = Lever-Presses, CS-Mg = CS-Magazine

Table 3

Major Results – Latency to Exit Refuge

	Experiment I	Experiment II
AvLatExit (GT)	23.258 ± 0.966 s	6.941 ± 1.063 s
AvLatExit (ST)	9.039 ± 1.101 s	8.188 ± 0.962 s
WithinGroup (GT) (overall)	$p = .096$	$p < .05^{a}$
WithinGroup (ST) (overall)	$p = .990$	
		$p < .05^{b}$
		$p < .05^{c}$
GroupEffect	$p < .001$	$p = .396$
Group*Excursion (all)	$p < .005$	$.223 < p < .655$

Note:
[a]   *Between excursions two and five*
[b]   *Between excursions one and three*
[c]   *Between excursions one and five*

Table 4

Major Results – Run Time

	Experiment I	Experiment II
AvRunTime (GT)	10.674 ± 1.497 s	2.630 ± 0.158 s
AvRunTime (ST)	6.571 ± 1.707 s	3.061 ± 0.143 s
WithinGroup (GT) (overall)		$p = .886$
	$p < .05^{a}$	
	$p < .05^{b}$	
	$p < .05^{c}$	
WithinGroup (ST) (overall)	$p = 0.794$	$p = .069$
GroupEffect	$p = .085$	$p < .05^{d}$
Group*Excursion (all)	$.100 < p < .901$	$.096 < p < .882$

Note:
[a]   *Between excursions one and four*
[b]   *Between excursions one and five*
[c]   *Between excursions one and six*
[d]   *ST slower overall*

Table 5

Major Results – Carry Time

	Experiment I	Experiment II
AvCarryTime (GT)	3.699 ± 0.420 s	2.363 ± 0.092 s
AvCarryTime (ST)	3.464 ± 0.479 s	2.321 ± 0.083 s
WithinGroup (GT) (overall)	$p < .05$[a]	$p < .05$[c]
	$p < .05$[b]	
WithinGroup (ST) (overall)	$p = .299$	$p = .383$
GroupEffect	$p = .717$	$p = .740$
Group*Excursion (all)	$299 < p < .801$	$461 < p < .714$

Note:
[a]   *Across pellets one – eight*
[b]   *Between excursions one and three*
[c]   *Across pellets one – five*

Table 6

Major Results – Latency to Eat

	Experiment I	Experiment II
AvLatEat (GT)	2.936 ± 0.393 s	7.889 ± 1.392 s
AvLatEat (ST)	3.840 ± 0.448 s	7.024 ± 1.260 s
WithinGroup (GT) (overall)	$p = .393$	$p < .005$
WithinGroup (ST) (overall)		$p = .082$
	$p < .005^a$	
	$p < .01^b$	
	$p < .005^c$	
	$p < .005^d$	
	$p < .01^e$	
	$p < .01^f$	
GroupEffect	$p = .144$	$p = .651$
Group*Excursion (all)		$.076 < p < .760$
	$p < .05^g$	
	$p < .05^h$	
	$p < .05^i$	

Note:
[a]   *Between excursions one and two*
[b]   *Between excursions one and three*
[c]   *Between excursions one and four*
[d]   *Between excursions one and five*
[e]   *Between excursions one and seven*
[f]   *Between excursions one and eight*

[g] *Excursion one, ST slower than GT*
[h] *Excursion four, GT slower than ST*
[i] *Excursion five, GT slower than ST*

Table 7

Major Results – Eating Time

	Experiment I	Experiment II
AvEatTime (GT)	69.149 ± 2.960 s	54.852 ± 1.681 s
AvEatTime (ST)	60.288 ± 3.364 s	53.115 ± 1.520 s
WithinGroup (GT)	$p < .001^{a}$	$p < .005^{r}$
	$p < .005^{b}$	$p < .05^{s}$
	$p < .01^{c}$	$p < .005^{t}$
	$p < .05^{d}$	$p < .01^{u}$
		$p < .05^{v}$
WithinGroup (ST)	$p < .01^{e}$	$p < .005^{w}$
	$p < .001^{f}$	$p < .001^{x}$
	$p < .001^{g}$	$p < .001^{y}$
	$p < .001^{h}$	$p < .001^{z}$
	$p < .001^{i}$	$p < .005^{aa}$
	$p < .01^{j}$	$p < .005^{bb}$
	$p < .001^{k}$	
	$p < .01^{l}$	
	$p < .005^{m}$	
	$p < .005^{n}$	
GroupEffect	$p = .055$	$p = .453$
Group*Excursion (all)		$.164 < p < .704$

$$p < .05^o$$

$$p < .05^p$$

$$p < .05^q$$

Note:

[a]   *Between excursions two and six*
[b]   *Between excursions two and seven*
[c]   *Between excursions two and eight*
[d]   *Between excursions four and seven*
[e]   *Between excursions two and five*
[f]   *Between excursions two and six*
[g]   *Between excursions two and seven*
[h]   *Between excursions two and eight*
[i]   *Between excursions three and six*
[j]   *Between excursions three and seven*
[k]   *Between excursions three and eight*
[l]   *Between excursions four and six*
[m]   *Between excursions four and seven*
[n]   *Between excursions four and eight*
[o]   *Excursion two, GT slower than ST*
[p]   *Excursion three, GT slower than ST*
[q]   *Excursion four, GT slower than ST*
[r]   *Between excursions one and four*
[s]   *Between excursions one and five*
[t]   *Between excursions two and four*
[u]   *Between excursions two and five*
[v]   *Between excursions three and four*
[w]   *Between excursions one and four*
[x]   *Between excursions one and five*
[y]   *Between excursions two and four*
[z]   *Between excursions two and five*
[aa]   *Between excursions three and four*
[bb]   *Between excursions three and five*

Table 8a

Major Results – Hoarding Task (I)

	Experiment I	Experiment II
# Pellets eaten (GT)	16.385 ± 0.917 pellets	11.111 ± 0.455 pellets
# Pellets eaten (ST)	17.000 ± 1.000 pellets	12.364 ± 0.789 pellets
# Pellets eaten (GT v. ST)	$p = .838$	$p = .167$
# Pellets hoarded (GT)	1.615 ± 0.866 pellets	29.444 ± 8.283 pellets
# Pellets hoarded (ST)	22.400 ± 8.367 pellets	39.364 ± 6.171 pellets
# Pellets hoarded (GT v. ST)	$p < .001$[a]	$p = .188$

Note:
[a]   *ST hoarded more than GT*

Table 8b

Major Results – Hoarding Task (II)

	# Pellets eaten	# Pellets hoarded
ExpI v. ExpII (GT)	$p < .05^b$	$p < .001^c$
ExpI v. ExpII (ST)	$p = 0.543$	$p = .053$

Note:
[b]   *More eaten by GT in Experiment I than Experiment II*
[c]   *More hoarded by GT in Experiment II than Experiment I*

Figure Captions

Figure 1.   Setup of apparatus used for the refuge and hoarding tasks. A computer with an audible timer was located near the apparatus. To facilitate more accurate observation, A TV monitor was connected to a built-in-camera situated just inside the ceiling of the refuge.

Figure 2 (a) and (b).   (a) Average Number of Lever Presses for GT and ST in experiment I and experiment II, (b) Average Number of Lever Presses for GT in experiment I/II and for ST in experiment I/II.

Figure 3 (a) and (b).   (a) Average Number of CS-Mg-Entries for GT and ST in experiment I and experiment II, (b) Average Number of CS-Mg-Entries for GT in experiment I/II and for ST in experiment I/II.

Figure 4 (a), (b), (c), and (d).   For experiment I: (a) Average Latency to Exit Refuge vs. Excursion, (b) Average Latency to Exit Refuge – GT / ST group separations. For experiment II: (c) Average Latency to Exit Refuge vs. Excursion, (d) Average Latency to Exit Refuge – GT / ST group separations.

Figure 5 (a), (b), (c), and (d).   For experiment I: (a) Average Run Time vs. Excursion, (b) Average Run Time – GT / ST group separations. For experiment II: (c) Average Run Time vs. Excursion, (d) Average Run Time – GT / ST group separations.

Figure 6 (a), (b), (c), and (d).   For experiment I: (a) Average Carry Time vs. Excursion, (b) Average Carry Time – GT / ST group separations. For experiment II: (c) Average Carry Time vs. Excursion, (d) Average Carry Time – GT / ST group separations.

Figure 7 (a), (b), (c), and (d).   For experiment I: (a) Average Latency to Eat vs. Excursion, (b) Average Latency to Eat – GT / ST group separations. For experiment II: (c) Average Latency to Eat vs. Excursion, (d) Average Latency to Eat – GT / ST group separations.

Figure 8 (a), (b), (c), and (d).   For experiment I: (a) Average Eating Time vs. Excursion, (b) Average Eating Time – GT / ST group separations. For experiment II: (c) Average Eating Time vs. Excursion, (d) Average Eating Time – GT / ST group separations.

Figure 9 (a) and (b).   (a) Average Number of Pellets Eaten for GT and ST in experiment I and experiment II, (b) Average Number of Pellets Eaten for GT in experiment I/II and for ST in experiment I/II.

Figure 10 (a) and (b). (a) Average Number of Pellets Hoarded for GT and ST in experiment I and experiment II, (b) Average Number of Pellets Hoarded for GT in experiment I/II and for ST in experiment I/II.
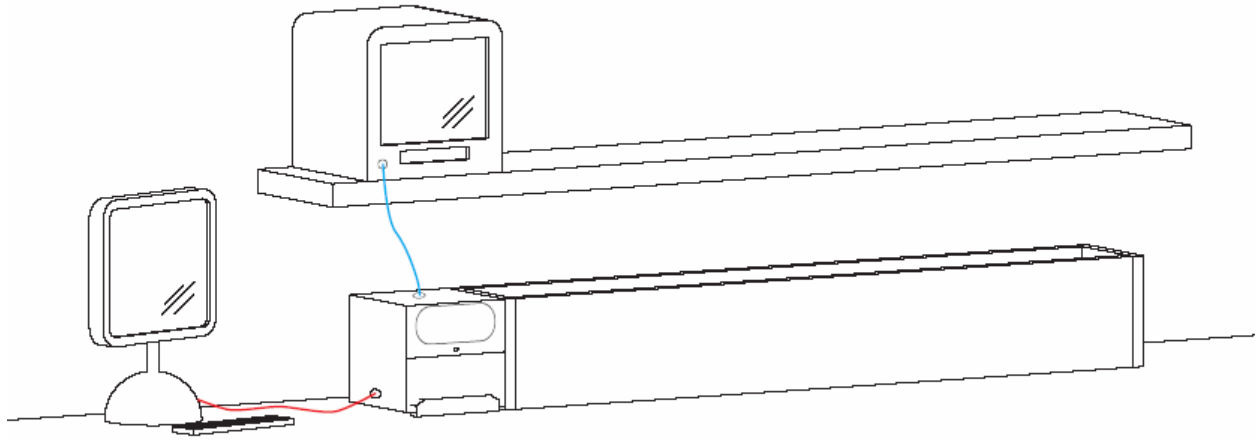
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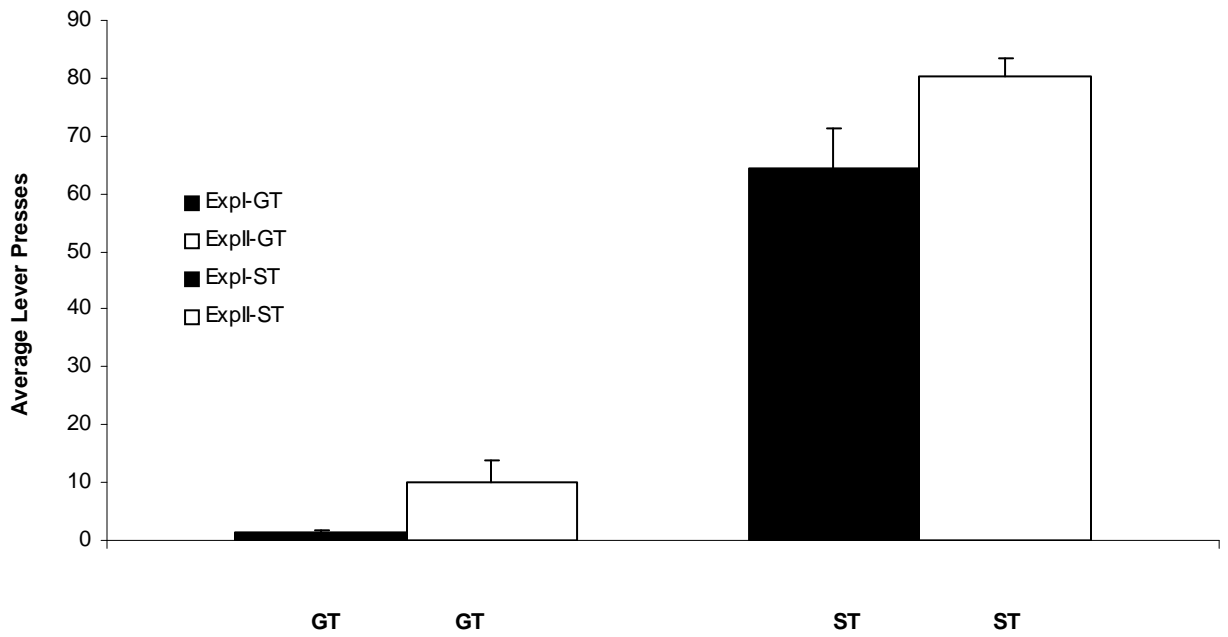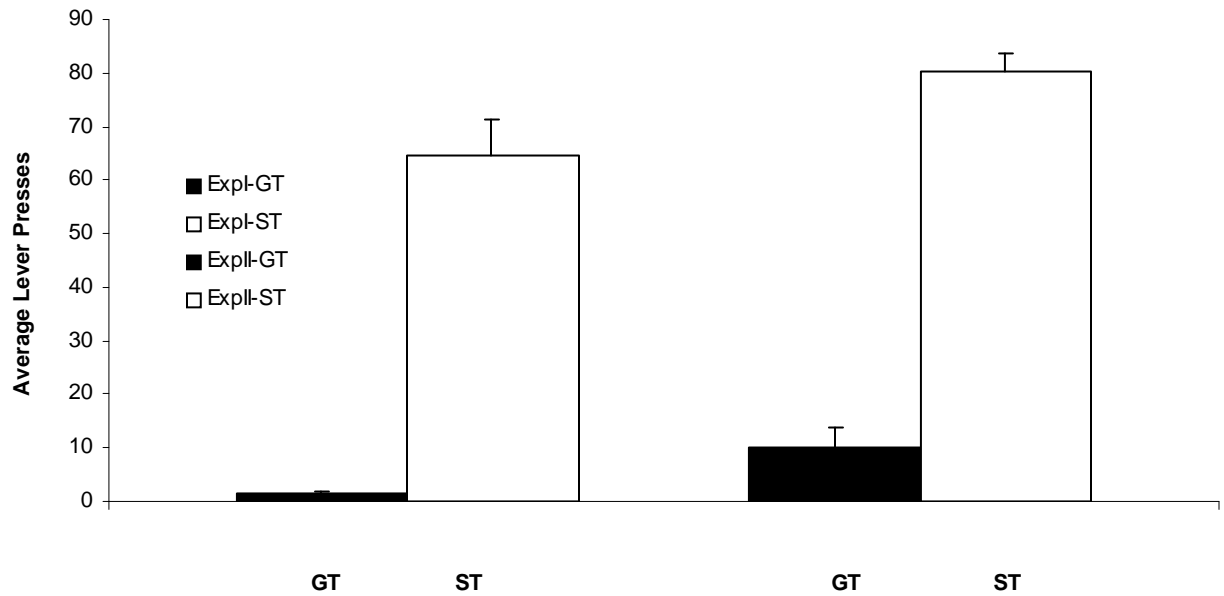
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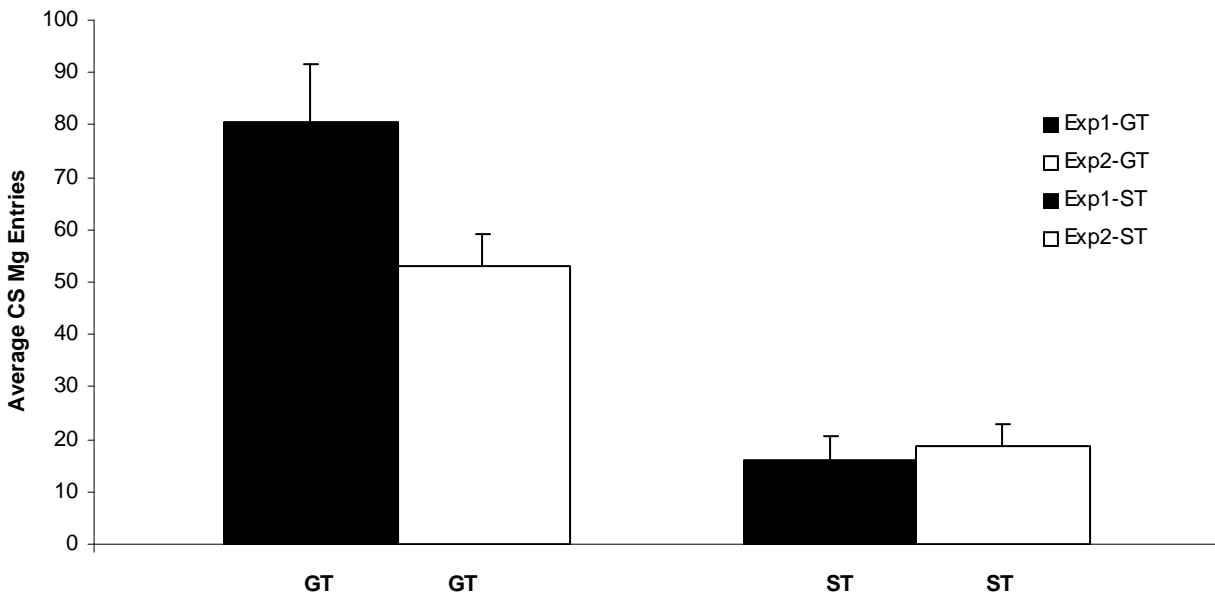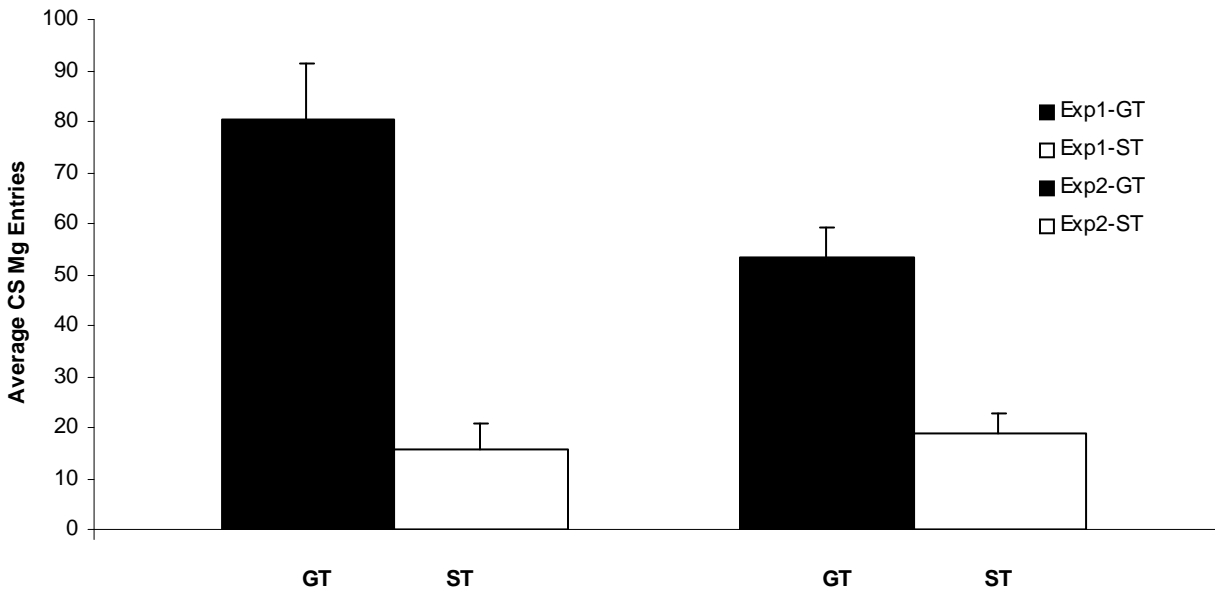
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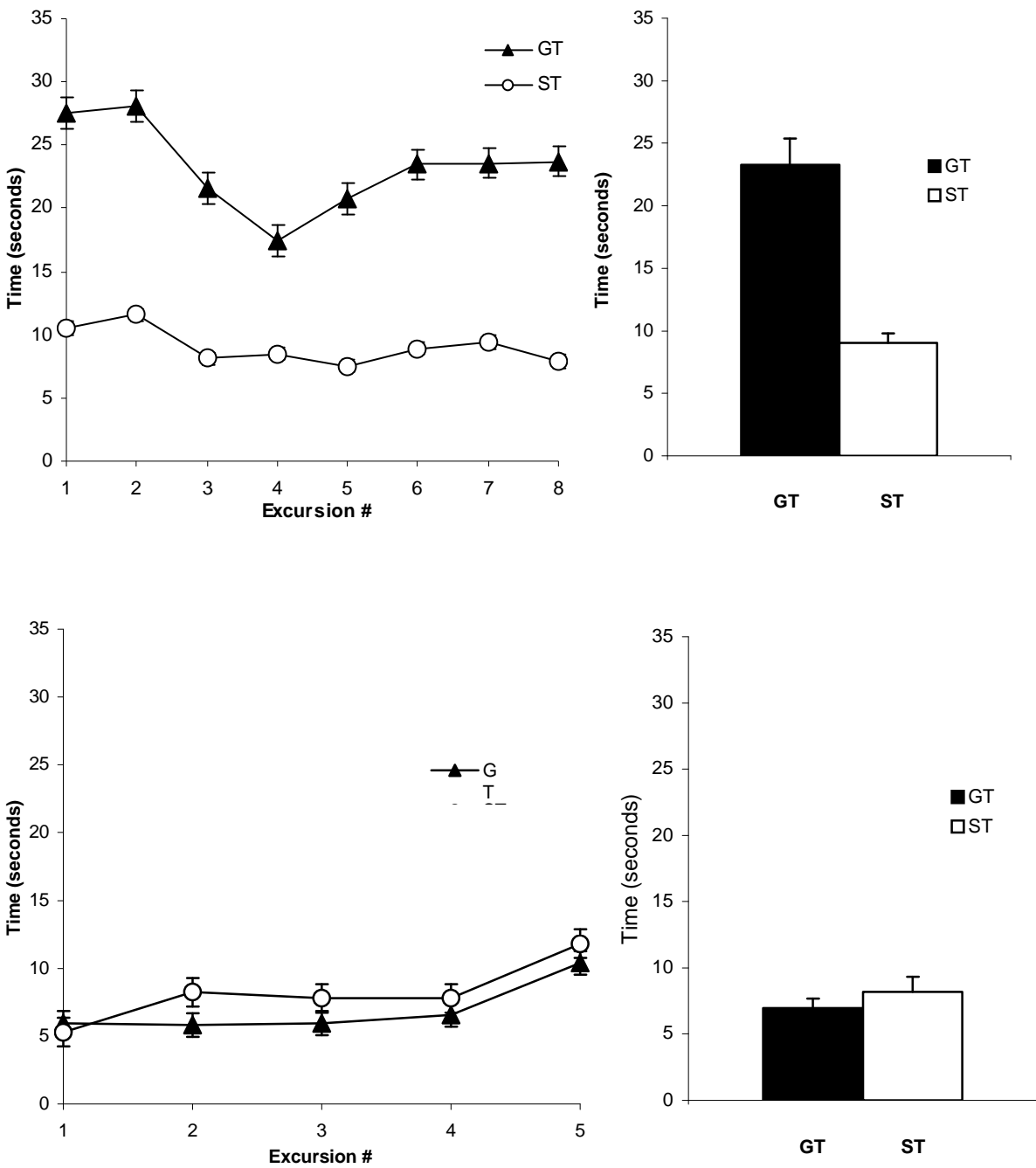
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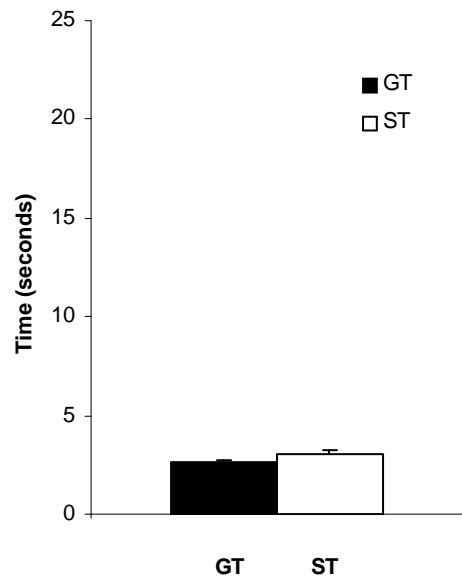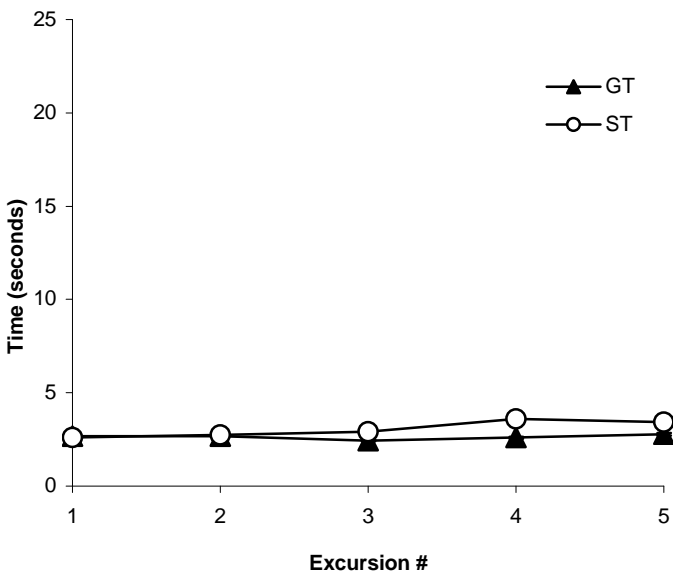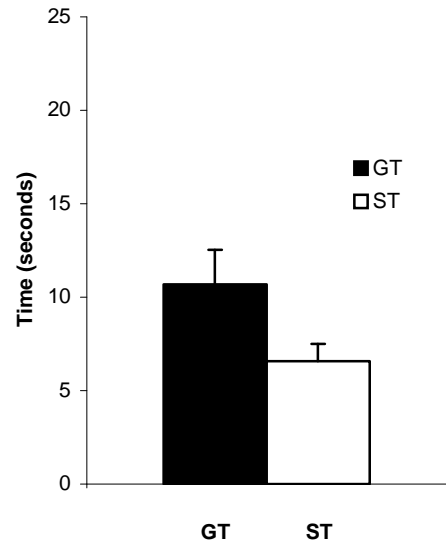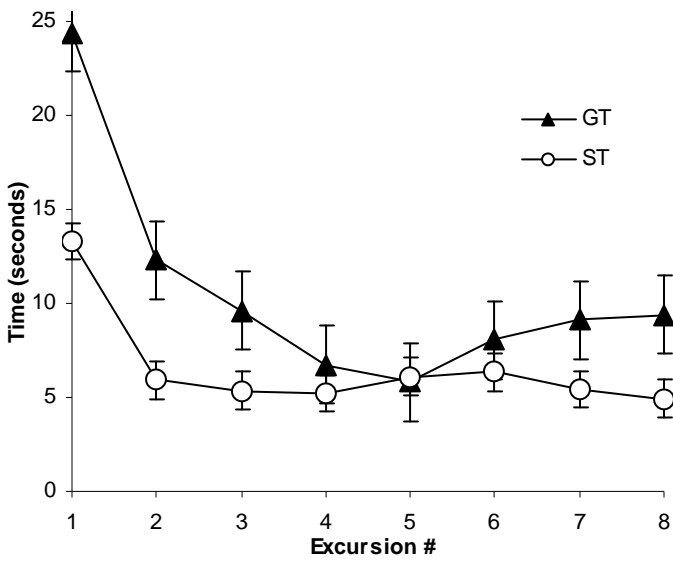
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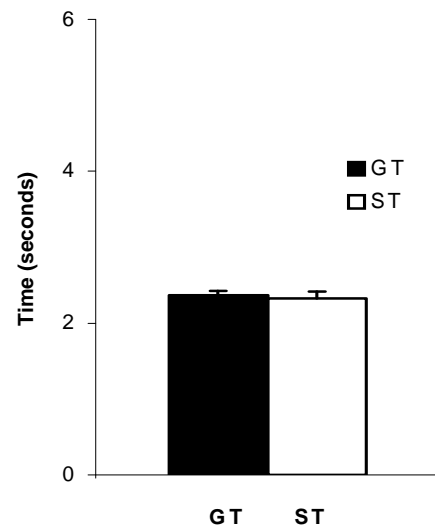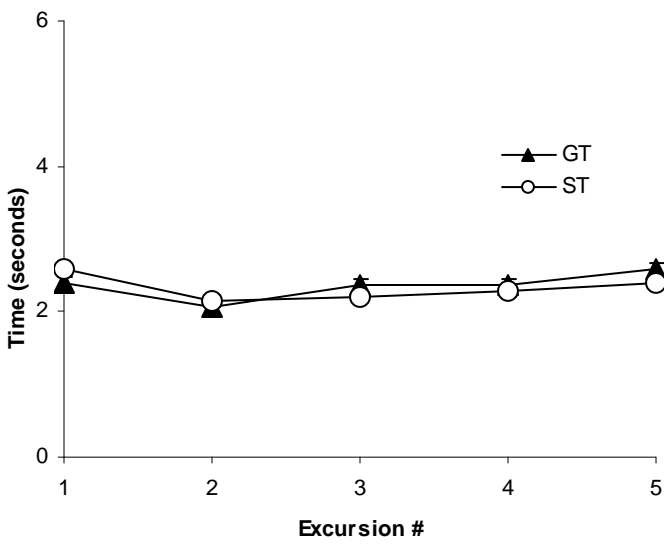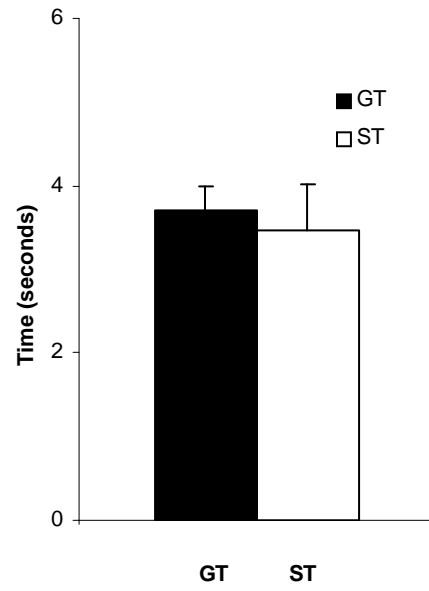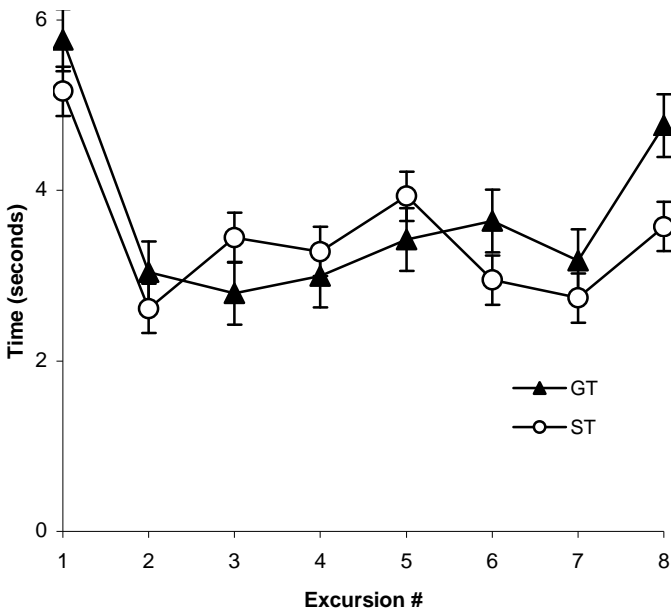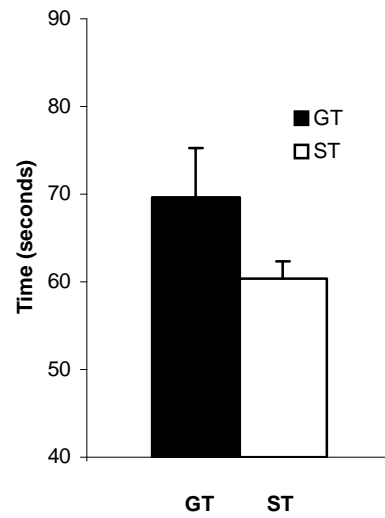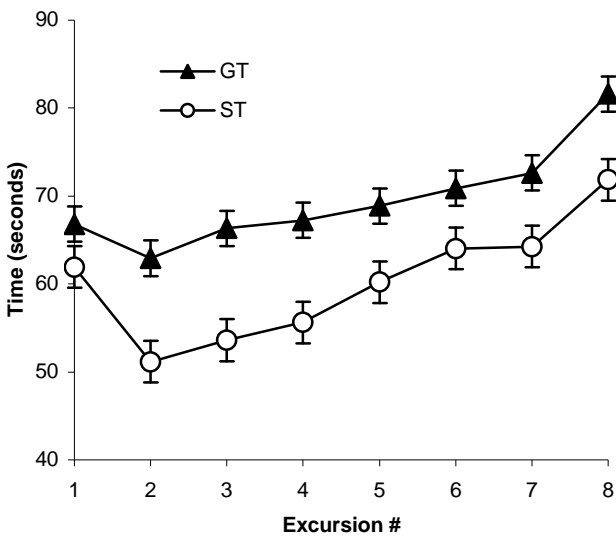
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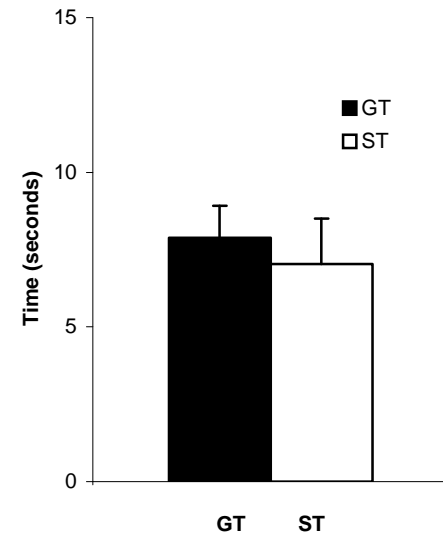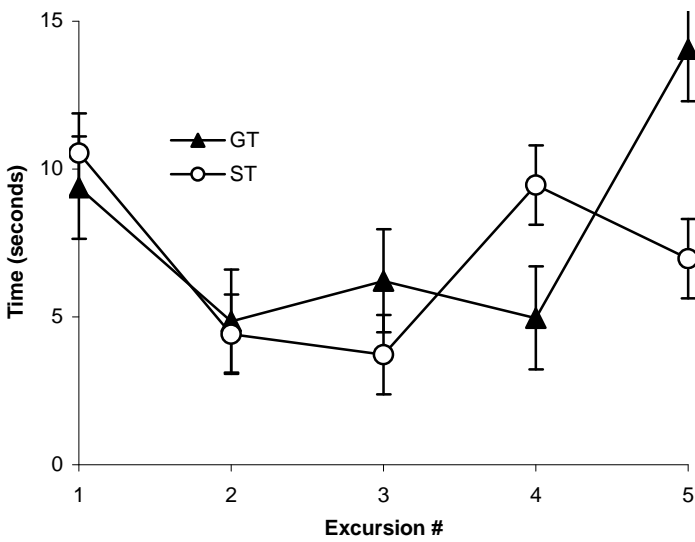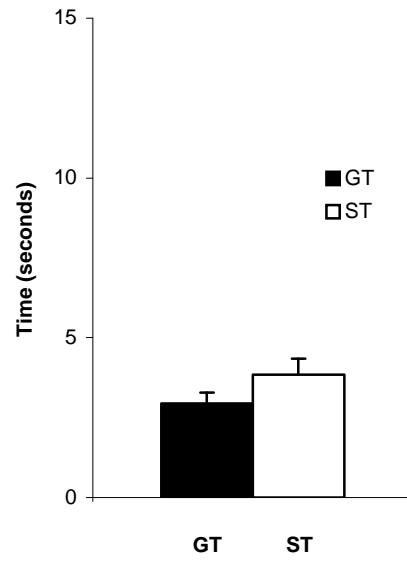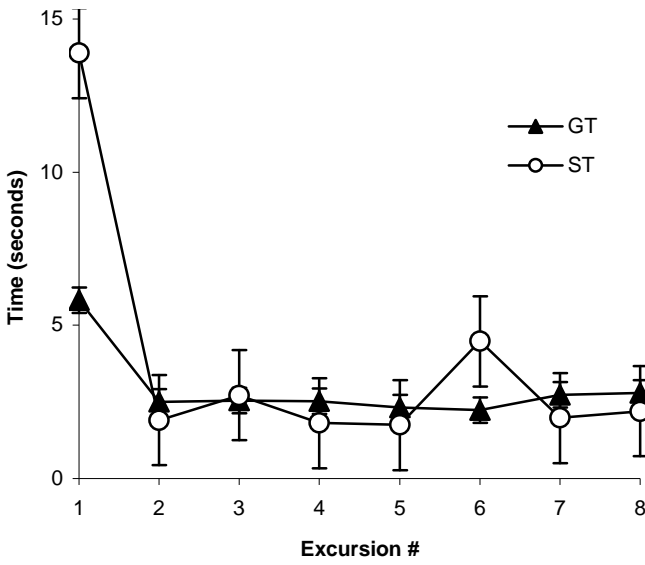
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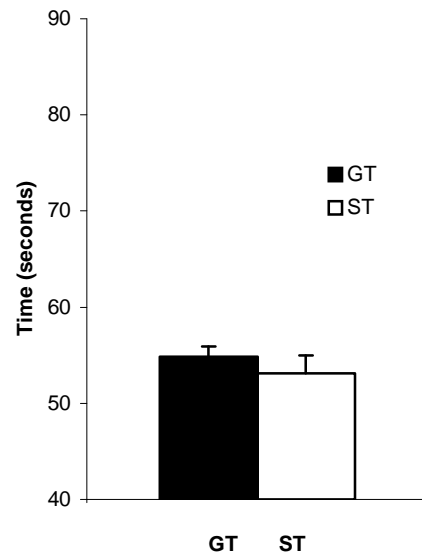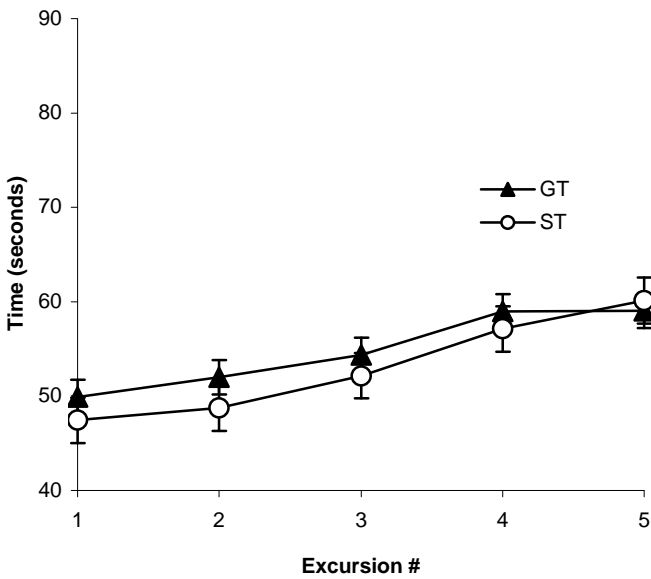
Figure 9 (a) and (b)

Figure 10 (a) and (b)