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Learning with Sucrose and Sucrose-Ethanol Reinforcement

David H. Root

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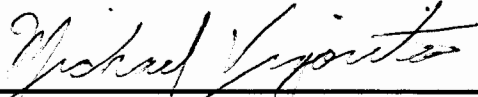
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Abstract

While much research has focused on the acquisition of habitual responding, few studies have examined the transition from flexible, goal-directed responding, to stimulus-bound, habitual responding, and its subsequent maintenance. Lesion studies have provided evidence demonstrating the dorsolateral striatum (DLS) to be involved in the acquisition of habitual responding. The present study investigates if the DLS is involved in the maintenance of habitual responding by allowing habitual responding to develop through extensive training on a variable ratio 5 schedule of reinforcement and by inactivating the DLS with an intracranial injection of lidocaine after initial training, extended training, and outcome devaluation. Moreover, the hypothesis that responding for ethanol (EtOH) may be more prone to becoming habitual than responding for sucrose was examined along with the contribution of the DLS to each. Differences in baseline responding for EtOH or sucrose responding were evident after initial training but allowing for extensive training abolished all baseline differences for the remainder of the study. DLS inactivation reduced responding for sucrose and EtOH both early and late in training. This effect, however, was more pronounced after extensive training. In addition, after extensive training, responding for EtOH was slightly more attenuated by DLS inactivation than responding for sucrose. These data suggests that the DLS has a role in responding for EtOH and sucrose after initial training, extensive training, and outcome devaluation. The DLS, however, may play a larger role in the maintenance of habitual responding that develops after extensive training. Two additional hypotheses stem from the results of the present experiment. First, DLS inactivation reduced performance for EtOH more than for sucrose only when outcomes were present and never under

extinction. Thus, odor, hedonic, physiological, or other cues provided by EtOH may be stimuli that activate the DLS and maintain habitual responding for EtOH. Second, in contrast, DLS inactivated responding for EtOH was attenuated more so after extended training than after initial training. Thus, responding for EtOH may not be more prone to habitual responding than sucrose *per se*, but more dependent on habitual responding neural regions such as the DLS when habitual responding has already been instated.

Keywords: habit, striatum, alcohol.

The Role of the Dorsolateral Striatum in the Maintenance of Stimulus-Response
Learning with Sucrose and Sucrose-Ethanol Reinforcement

The study of animal behavior is a cornerstone of experimental psychology and behavioral neuroscience. The experimental analysis of animal (and human) behavior has been extensively studied by use of two traditional learning and conditioning paradigms. These paradigms allow for the opportunity to examine how behavior manifests changes in the brain and nervous system.

The classical (or Pavlovian) conditioning paradigm was developed by the Russian physiologist Ivan Pavlov working with canines. Pavlov (1927) found that by repeatedly sounding a bell contiguously with the presentation of food, the sound of the bell alone, which originally could not elicit salivary secretion, gradually developed this ability. The food was deemed an *unconditioned stimulus*, in that food naturally produced salivation, an *unconditioned response*. The ability of food to elicit salivation did not depend on any previous training; therefore the stimulus and response are unconditioned. Pavlov repeatedly paired the unconditioned stimulus (food) with a novel, but *neutral stimulus* (a bell; e.g. that did not produce salivation). After many pairings of the neutral stimulus with the unconditioned stimulus, the neutral stimulus became a *conditioned stimulus* able to elicit a *conditioned response* of salivation. The response is regarded as conditioned because the originally neutral stimulus has been trained to elicit a response similar to the unconditioned response.

Learning theorists assume that an association is formed between the pairing of unconditioned and conditioned stimuli that is reflected in measurable behavior. An

example of classical conditioning is fear conditioning. In this procedure, an animal, such as a rat, is able to move freely throughout a chamber with a metal grid floor connected to a shocking device. A neutral stimulus (such as a light or tone) is paired with the onset of a foot shock, the unconditioned stimulus, which causes the unconditioned response of freezing in rats. Freezing is an innate reaction of rats to fearful and aggressive stimuli that is marked by complete immobilization except for respiration (Bolles, 1970). The pairing of an unconditioned stimulus with another stimulus is at the core of classical conditioning. A neutral stimulus is conditioned to elicit a conditioned response similar to the unconditioned response. In fear conditioning, an extinction test is later given to measure the presumed association of the conditioned stimulus to elicit the conditioned response. An extinction test is given because it can test responding to a CS in the absence of the US. If the unconditioned stimulus of foot shock has been associated with the conditioned stimulus, the rat will perform the conditioned response of freezing when in the presence of the conditioned stimulus. The magnitude of the unconditioned stimulus is a critical factor of classical conditioning (Domjan & Burkhard, 1986). In fear conditioning, if the rat is shocked by a high intensity of electricity, high amounts of freezing (conditioned response) will occur in the presence of the conditioned stimulus. If the rat is shocked by a low intensity of electricity, low amounts of freezing will occur in the presence of the conditioned stimulus.

The adaptive contribution of classical conditioning to living organisms is extremely significant. For example, taste aversion is a procedure that pairs a neutral flavor with illness. This classical conditioning procedure can show learning in one pairing (Domjan & Burkhard, 1986). Humans may have learned long ago that a certain

food will cause internal distress. The ability to associate the taste, smell, sight, or even name of the food to illness gives a distinct advantage of promoting overall well-being and survival.

Operant (or Skinnerian) conditioning was developed and brought to the forefront of experimental psychology by the classic research of Burrhus Frederic Skinner (1938). When a hungry rat is placed inside a chamber where small pellets of food are dropped for consumption, the rat will come to associate the sound of the pellet dropping with receiving a food pellet by classical conditioning. Through exploration of the box, the rat presses a lever (or bumps into it by accident), which causes the food pellet to be presented. The rat hears the sound of the pellet dropping and consumes the pellet. After consumption, the rat once again presses the lever for a food pellet presentation. It was the outcome of the lever press (presentation of a food pellet) that influenced future responding or lever pressing by the rat. While there are similarities between the two learning paradigms, they are in fact quite different. In classical conditioning, an unconditioned stimulus such as a puff of air to the eye (causing the unconditioned response of an eye blink) can be paired with a neutral stimulus such as a tone. With repeated pairings, the neutral stimulus becomes a conditioned stimulus able to elicit a conditioned response of an eye blink. Conditioned behavior is therefore controlled by its antecedents because conditioned stimuli in the environment elicit a response.

Classical conditioning can be described as a passive type of learning based on the relationships between environmental stimuli (Pavlov, 1927; Skinner, 1938; Hull, 1943; Dickinson, 1980, 1989; Rescorla, 1988; Kirsch, Lynn, Vigorito, & Miller, 2004). The classical conditioning paradigm is passive because subjects attend to correlated stimuli or

events in the environment and learn to respond so as to adapt to these environmental events. In operant conditioning, however, learning is a more active process because behavior is controlled by its consequences or outcomes. Thus, the probability of an emitted response can be increased with reinforcement or decreased with punishment (Domjan & Burkhard, 1986).

Skinner's influence on the study of conditioning changed the methods of studying learning and behavior. Prior to Skinner, the study of instrumental conditioning involved discrete trial procedures whereby animals were trained trial-by-trial in puzzle boxes (Thorndike, 1898) or mazes (Hull, 1930, 1931; Tolman, 1933). Skinner changed the procedure when he invented the operant chamber (or Skinner box). In the operant chamber subjects are not tested trial-by-trial, but are free to respond whenever they desire by pressing on a lever that is available. Skinner named this new dependent variable *operant behavior* because the behavior operates on the environment (Skinner, 1938). Skinner also manipulated the type of consequences and called them reinforcers or punishers. Unlike other learning theorists at the time, Skinner viewed responses changed by their consequences (i.e., operant responding) as distinctly different from classically conditioned responding (he called classically conditioned responses *respondents*). Skinner defined operant conditioning as any procedure involving rewards and punishers that increased or decreased, respectively, the probability of responding in the presence of discriminative stimuli. Although Skinner had a tremendous influence on the study of instrumental and operant conditioning his approach was atheoretical. Much of the work discussed in this paper is based on theories of associative learning (e.g., Thorndike (1898, 1911), Hull (1930, 1931, 1943), Dickinson (1985), and Rescorla (1985a, 1988)).

Operant responding has been proposed to be controlled by two separate processes (Dickinson, 1980, 1985, 2002). The first is the classic stimulus-response (S-R or habitual responding) process which has been a focus of study in our laboratory (Vigorito, 1994, 2001; Kirsch, Lynn, Vigorito, & Miller, 2004). The S-R process is based on the research of Thorndike (1898, 1911) and Hull (1930, 1931, 1943). Thorndike's early research of animal intelligence introduced the idea that the consequences of behavior can lead to an increase or decrease of that behavior. His law of effect influenced the development of S-R learning. The law of effect states that if a response in the presence of a stimulus is followed by a satisfying event, the association between the stimulus and the response is strengthened. If the response is followed by an annoying event, the association is weakened (Thorndike, 1911). Animals learn an association between the response and stimuli present at the time of the response; the consequence of the response is not part of the association (Domjan & Burkhard, 1986). While Thorndike's S-R theory is no longer dominant in the field of experimental psychology, it provided a basis for much future experimental research and theorization.

Hull (1943) was the first to introduce the idea that a drive, such as hunger drive, can motivate behavior. When deprived of food, an animal is more likely to use food as a reinforcer in an effort to return to homeostasis (Hull, 1943). In this case, the animal is motivated to return to a physiological homeostasis of a normal body weight and to not be hungry. Hull's (1943) drive reduction theory contended that in order for learning to occur, there must be a drive, stimuli and responses must be detected, responses must be made to learn, and learning will only happen when responding satisfies a drive. Further, Hull (1930, 1931) originally added a classical conditioning component to instrumental

behavior, initially proposed in Thorndike's law of effect (1911). Hull suggested that this classical conditioning component encourages or motivates the instrumental behavior (Domjan & Burkhard, 1986). In the S-R process, when an operant response is made, associations between the response and stimuli present at the time of the response are conditioned, such as a light, presence of a lever, tone, context or odor. With further conditioning, these stimuli in turn acquire the capacity to elicit or modulate the operant response without respect to its consequence. This S-R process is characterized by habitual instrumental responding controlled by conditioned stimuli, and without regard to the outcome or current value of the reinforcer. Note that the term reinforcer can be used to refer to reward or outcome. For the purpose of this study, these terms will be designated as an outcome.

The second process used by learning theorists to explain operant conditioning is the response-outcome (R-O) process based on the research of Edward Tolman (1933, 1948). Unsatisfied with the view that all behavior could be explained with the S-R process (Hull, 1943), Tolman argued that learning consists not in S-R associations but in the building up of cognitive maps and the expectation of specific outcomes (Tolman, 1948). An example of this process is Blodgett's (1929) latent learning experiment. Rats were tested in a maze with one group was reinforced at the completion of the maze and another group that was not. Without reinforcement, the rats did not appear to learn as indicated by errors in navigating the maze. When the nonreinforced group began receiving food reinforcement at the completion of the maze, however, a dramatic drop in errors was observed. Therefore, the rats in the nonreinforced group may have actually been learning to navigate the maze even though this did not show in their behavior.

Tolman (1948) argued that regardless of reinforcement, the rats had been building up a cognitive map of the maze and only utilized this map when they expected to find food in the goal box. That is, the rats used their knowledge of the maze when they expected that running to the goal box would produce a specific outcome, food outcome. An association between the operant response and its consequences or outcome is formed using the R-O process (Adams & Dickinson, 1981). Since the R-O process is based on the expectation of a specific outcome, it is characterized by cognitive actions that are flexible and goal-directed. If a hungry rat expects to be shocked when pressing a lever, the rat will not respond, however if the animal expects food, it will respond. Since operant conditioning has been shown to involve both the R-O and S-R processes (Dickinson, 1985; Dickinson et al, 1995), a determination of which process is controlling behavior must be made distinct.

Devaluation procedures have been adapted from classical conditioning for operant conditioning procedures to experimentally separate the S-R and R-O processes (Adams & Dickinson, 1981a; Kirsch, Lynn, Vigorito, & Miller, 2004). This is possible because outcome devaluation can modify the performance of an operant response that is based on the expectation of specific outcomes (Colwill & Rescorla, 1985a). The procedure involves the use of animals that are trained to perform an operant response for an outcome, such as lever pressing for sucrose. Then, the outcome (sucrose) is devalued by one or more pairings with sickness, induced by a drug, such as lithium chloride (LiCl). Finally, an extinction test is given to determine the nature of the association (Adams, 1982). Recall that extinction tests measure responding to the CS and not the US. Therefore, an extinction test is necessary to study solely the expectation of an outcome

and not a reaction to other stimuli that may facilitate responding such as the taste or smell of the outcome. A reduction of responding in the extinction test is diagnostic of the goal-directed R-O process (Dickinson et al, 2002). In the R-O process, the subject expects a certain desired outcome to lever pressing as a result of original training. With devaluation, the outcome's value is lowered considerably, which causes the subject to subsequently respond less (if at all) for the outcome. The outcome is less desirable to the subject, marked by the sensitivity to devaluation. Conversely, the S-R process is controlled by its antecedents. The operant response is presumed to be automatically caused by the conditioned stimuli in the environment that were associated with responding. The outcome of the response is not included in the S-R association and as a result, devaluing the outcome will show no change in operant responding. Because the S-R process lacks an expectation of an outcome, the insensitivity to outcome devaluation suggests habitual control of the behavior.

The acquisition and maintenance of actions such as lever pressing for outcomes has been shown to involve both R-O and S-R processes (Dickinson et al, 1995). Whether operant responding is under the influence of S-R or R-O processes is influenced by several factors such as the schedule of reinforcement and duration of training. Research in this laboratory and Dickinson's has found that the sensitivity of operant lever-pressing maintained by simple schedules of reinforcement to changes in the outcome value depends on the schedule of reinforcement (Dickinson et al, 1983; Vigorito et al, 1994). In a simple schedule, one lever is used in operant conditioning for an animal to press for an outcome under either a ratio or interval reinforcement schedule.

Certain schedules of reinforcement have been shown to produce habitual responding better than others. In ratio schedules, reinforcement is contingent on the number of responses made while in interval schedules, reinforcement is given only when a response occurs after a specific time has elapsed since the last reinforced response (Domjan & Burkhard, 1986). Initially, simple interval schedules produce insensitivity to outcome devaluation (i.e. habitual responding) while simple ratio schedules do not (Dickinson et al, 1983). This may be due to the relationship between an animal's responding and its associated outcome. Variable ratio schedules generate high rates of responding and high rates of outcome delivery (Domjan & Burkhard, 1986; Vigorito et al, 1994). In interval schedules, the relationship between response rate and reinforcement rate is weak because the outcome does not depend only on the number of responses made but also on the passage of time (Dickinson, 1989). Therefore, the correlation between response rate and outcomes received is very high in ratio schedules, but much lower in interval schedules. Dickinson argues that in the absence of a strong response-reinforcement correlation, instrumental performance is maintained by an S-R mechanistic process and is autonomous of the consequent outcomes (Dickinson, 1989; Kirsch, Lynn, Vigorito, & Miller, 2004).

Alternative to simple schedules, Rescorla and his colleagues have used concurrent schedules of reinforcement to study the effects of devaluation on operant responding. Unlike simple schedules, in concurrent schedules rats are trained to press a lever for one type of outcome, such as food pellets, and a second lever for another outcome, such as sucrose (Colwill & Rescorla, 1985a). When devaluation experiments are conducted with concurrent schedules, one outcome is devalued and both levers are simultaneously tested

in an extinction test. If lever press responding is controlled by R-O associations, then the rat will have an expectation of a devalued outcome when pressing one lever. The rat should also have the expectation of a non-devalued outcome if it presses the other lever. The rat therefore, should choose to press the lever that produces the non-devalued outcome, and not press the lever that leads to the devalued outcome. However, if the association that controls responding is S-R, responding should continue for both the devalued and non-devalued outcomes because conditioned stimuli in the chamber paired with responding control responding autonomous of an expectation of an outcome. Colwill and Rescorla (1985a) found evidence of R-O associations similar to simple schedules, in that there was a decreased responding for a devalued outcome. However, these experimenters found contradictory results to Dickinson's (1983) simple interval schedule. Colwill and Rescorla (1985) successfully devalued an outcome using a concurrent interval schedule while Dickinson (1983) only found devaluation effects in a simple ratio schedule, and not a simple interval schedule. Why would an interval schedule of reinforcement result in S-R learning when a single lever is used (simple schedule) but result in R-O learning when two levers are used (concurrent schedule)? Dickinson (1989) explains that the concurrent schedule of reinforcement allows for the opportunity to learn strong R-O correlations that do not exist in simple schedules. During training in concurrent schedules of reinforcement the subject is free to change back and forth between the response alternatives. Because of this "freedom" concurrent schedules are popular for studying choice behavior (Domjan & Burkhard, 1986). Allowing for choice is a cognitive task indicative of the R-O process, because the animal can compare each expected outcome of the operant responses. The opportunity for choice in concurrent

schedules appears to encourage the development of R-O associations. When a simple interval schedule of reinforcement is used, however, a weak response-outcome correlation is experienced and there is no other opportunity for learning relationships between behavior and outcomes. Therefore, the S-R process controls responding and insensitivity to outcome devaluation is observed. Is there any time when even choice behavior results in S-R or habitual responding? Recent evidence suggests yes.

Dickinson, Wood, and Smith (2002) used a concurrent schedule of reinforcement by training rats to press one lever for food pellets and a separate lever for an EtOH solution (5% sucrose/10% EtOH mixture). Either the EtOH solution or a food outcome was subsequently devalued with an injection of LiCl. Test performance on the pellet lever replicated sensitivity to devaluation, exhibiting mediation by the R-O process. However, test performance on the EtOH lever showed a resistance to devaluation, displaying mediation by the S-R process. This result suggests that reinforced behaviors under choice conditions can come under the control of S-R processes under some circumstances. The results from this experiment also suggest that alcohol seeking behavior is especially prone to a habitual S-R habit process. Understanding the transition from goal-driven actions to stimulus-bound habits will be central to our understanding of voluntary behavior generally, and of certain disorders, specifically drug, alcohol, and other addictions (Olmstead et al, 2001; Everitt & Wolf, 2002). This transition from goal directed actions to habitual responding has been shown experimentally in rats.

Habitual responding has been shown to be at least partly based on the amount of training the animal has received (Adams, 1982; Dickinson, 1985). During the initial training of simple and concurrent schedules, performance is flexible and goal-directed,

representative of the R-O process. With extended training, performance of simple schedules shifts to become autonomous or habitual, illustrative of the S-R process. Extensively trained varieties of behavior such as over learned motor skills (lever pressing) can be qualified as habitual (Kimble & Perlmutter, 1970). Adams (1982) and Dickinson (1985) provided evidence suggesting that extended operant training in simple schedules can lead to the onset of habitual responding. These experimenters showed that rats not receiving extended training became sensitive to devaluation (rats respond less for the devalued outcome, representative of the R-O process) whereas rats that did receive extended training became insensitive to devaluation (rats still respond for the devalued outcome, representative of the S-R process). The neural basis of goal directed and habitual responding in operant conditioning has long been an interest to experimental psychologists and behavioral neuroscientists. Recent studies have pointed to the involvement of the basal ganglia in habitual responding in particular.

The basal ganglia are part of a collection of subcortical nuclei in the forebrain, which lie beneath the anterior portion of the lateral ventricles (Carlson, 2004). The basal ganglia are critical for motor control. Damage to the basal ganglia will cause severe motor deficits such as weakness, tremors, rigidity of the limbs, poor balance, and difficulty in initiating movements (Carlson, 2004). In addition, patients who suffer from basal ganglion disorders such as Parkinson's disorder, Huntington's chorea, Gilles de la Tourette syndrome show various skill or habitual learning and performance impairments (Salmon & Butters, 1995; Poldrack & Packard, 2003; Marsh et al, 2005). The rich sources of dopamine input from the ventral tegmental area and substantia nigra to the striatum suggest a system supporting a role in learning and memory (Graybiel, 1998;

Berke & Hyman, 2000). Dopaminergic neurons in this brain region have been shown to be involved in animal and human learning (Kimura, 1995; Suri et al, 2001; Delgado et al, 2005). The focus of much research in basal ganglion learning has come from two structures within this brain area, the caudate nucleus and putamen, which together are called the striatum. Dopamine D1 and D2 receptors are highly expressed on medium spiny neurons in the striatum, which are output neurons that constitute 95% of the neurons in this brain area (Genova, Berke, & Hyman, 1997). These neurons and cholinergic interneurons of the striatum have been suggested to have a central role in mediating S-R learning (Berlanga et al, 2003), which is an important process underlying the development and maintenance of habits.

The progression from actions to habits in learning may have its neural basis within the circuitry of the striatum (Everitt et al, 2001). The dorsolateral striatum (DLS) in particular, may be part of a larger anatomical system that is essential for S-R learning (Featherstone & McDonald, 2004). The extensive connections of the DLS with the sensorimotor neocortex and substantia nigra pars compacta presents a neural circuitry well suited for S-R learning (Kimura, 1995; Packard & McGaugh, 1996; Graybiel, 1998; Devan et al, 1999; Gerdeman, 2003). The acquisition of habitual responding has been proposed to be induced by plasticity involving the DLS. Lesions of this area have been shown to impair the acquisition of S-R learning in operant conditioning (McDonald & White, 1993; McDonald & Hong, 2004; Compton, 2004; Featherstone & McDonald, 2004). Featherstone and McDonald (2004) investigated the effects of DLS lesions on two conditioning discrimination tasks. Rats were trained to operant lever press or chain pull under a simple interval schedule of reinforcement when a light or sound stimulus was

present. If the rat responded without the stimulus present no reinforcement occurred. Animals in the DLS lesion group did not differ in operant simple interval training on the first two days of training. This is consistent with the notion that during the initial training of operant responding, performance is representative of the R-O process and that the DLS is not necessary for R-O representations. Further, DLS lesions impaired the acquisition of operant conditional tasks across training sessions while sham rats had no such impairment. This is also consistent with the theory that the DLS is crucial for the development of S-R learning and associations. Moreover, animals in the DLS lesion group did not increase their response rate across training sessions while sham groups did. The increased response rate across training sessions of sham rats is possibly due to increasing S-R associations and animals with a destroyed DLS never increased responding beyond baseline levels (Featherstone & McDonald, 2004). This is consistent with the notion that, with extended training, behavior can be mediated by S-R associations and that plasticity of the DLS is crucial for the development of habitual S-R responding. If indeed there are two separate processes involved in operant conditioning, without the acquisition of S-R learning, R-O associations will have control of the learned behavior, which has been hypothesized as “switching systems”.

Featherstone and McDonald (2004) also trained rats in a conditioned place preference (CPP) apparatus. The purpose of using task was to eliminate the alternative explanation that the DLS may be involved in encoding outcome information, which is not part of the S-R process. This apparatus consisted of a triangular black chamber connected to a square white chamber. Training consisted of pairings of one chamber with food for the rat to consume and the other chamber paired with the absence of food. Thus, the CPP

task is typically noted with stimulus-outcome learning and not S-R learning because there is no required response to earn an outcome. Results from this task showed no difference in sham and DLS lesioned animals in CPP learning which further supports that the DLS is not involved in associations involving an outcome or stimulus-outcome relationships. The fact that DLS lesions impair operant S-R learning but spare CPP stimulus-outcome learning suggests the DLS to be involved solely in S-R learning. Moreover, research has shown that when the DLS is destroyed, an increased sensitivity to outcome devaluation occurs that is not seen with an intact DLS (Yin et al, 2004). Taken together, the DLS may be critical for S-R learning and not involved in learning associations that involve an outcome.

While evidence exists that the DLS is involved in acquisition, it is unclear if the DLS is involved in the maintenance of habitual operant conditioning. All of the operant responding studies mentioned previously have destroyed the dorsal striatum. No study has allowed for the development of habitual responding through plasticity of the DLS to examine the role of this brain structure in the maintenance of habitual responding. Furthermore, the DLS has not been explicitly tested to be involved in responding for alcohol, which may be more prone to habitual responding (Dickinson, Wood, & Smith, 2002). The present study will be the first to explicitly test the DLS in the maintenance of habitual responding based solely upon the length of training (habitual responding develops with extensive training; Adams, 1982, Dickinson, 1985). Further, this is the first study to explicitly test whether the DLS is involved in alcohol-seeking behavior.

Although the present study is not overtly investigating addiction, understanding the synaptic changes mediated by behavior and drugs of abuse hold the promise for

elucidating the neurological underpinnings of addiction (Robbins & Everitt, 2002; Gerdeman et al, 2003). A certain drug might enhance plasticity processes in the dorsal striatum that may mediate S-R habits (Packard et al, 1989). Recent evidence has shown that human type 1 alcoholics have a high correlation of dopamine D1 receptors between the nucleus accumbens and dorsal striatum that controls do not, which suggests pathology related to addiction (Tupala & Tiihonen, 2004). In addition, repeated administration of cocaine or amphetamine appears to render dopaminergic systems hypersensitive to drugs, stimuli associated with drugs by Pavlovian conditioning, and to stressors (Genova, Burke, & Hyman, 2000; Canales, 2005). This enhanced sensitivity to Pavlovian conditioning has been shown to be involved through mechanisms of the dorsal striatum (Gerdeman, 2003; Canales, 2005). Whether or not enhanced sensitivity to Pavlovian conditioning will be shown with a depressant such as alcohol is currently unknown. Addiction involves the execution of habitual actions, and the apparently equivalent homolog form of learning in experimental animals is S-R learning (Canales, 2005). The results from the present study may give new information to the study of addiction.

The present experiment poses the following questions and goals. Is the DLS involved in the maintenance of S-R learning? The first primary goal is to look for evidence that the DLS is involved in habitual (S-R) responding by allowing plasticity of the DLS to acquire an S-R habit association (Featherstone & McDonald, 2004; Yin et al, 2004), and test if the DLS maintains responding after extended training but not after initial training. Therefore, the second primary goal is to determine if the role of DLS depends on the extent of training. Will an EtOH outcome contribute to more habitual responding than a sucrose outcome? The secondary goal is to compare EtOH and sucrose

outcomes by testing if the DLS maintains responding for EtOH after initial and extended training while the DLS maintains responding for sucrose after extended training.

In order to determine the role of the DLS after initial and extended training of instrumental conditioning, a method of probe testing was used based on the research of Packard and McGaugh (1996). These probe tests consisted of bilateral intracerebral injections of 2% lidocaine to functionally inactivate the DLS. Inactivation through sodium channel blockers such as lidocaine has the advantage over permanent lesion techniques of being temporary, reversible, and not subject to the compensatory plasticity that may result from permanent lesions (Boehnke & Rasmusson, 2001). The volume of 2% lidocaine solution was chosen on the basis of previous evidence indicating that this volume produces functional inactivation of the striatum sufficient to cause memory impairment (Perez-Ruiz & Prado-Alcala, 1989; Packard & McGaugh, 1996). Using a method of reversible lesions, the role of the DLS was examined after the distinct learning phases of initial and extensive training. This method allowed for a within-subjects comparison in that each animal is its own experimental and control group and calls for fewer subjects than a between-groups design.

The main focus of this experiment is to examine the gradual development from goal-directed behavior to stimulus-bound habitual responding. A simple ratio schedule of reinforcement was used in the present study because previous research has established that ratio schedules result in early R-O responding but transitions late in training to S-R habitual responding (Adams, 1982; Dickinson, 1985). Since interval schedules have been shown to produce habitual control of behavior even very early in training (Dickinson, 1983) and ratio schedules allow for the opportunity to explore a transition of responding

(Dickinson, 1985), a ratio schedule was used rather than an interval schedule. If the DLS is indeed critical for habitual responding, then inactivation of this brain region should only show impaired performance after extended training when habitual responding is likely to occur.

A simple (rather than concurrent) ratio schedule was used in the present experiment to investigate the development of habitual responding with either sucrose or EtOH outcomes. Although Dickinson, Wood, and Smith (2002) showed a concurrent schedule was able to produce habitual responding, a concurrent schedule of reinforcement allows for the opportunity to learn strong R-O correlations that do not exist in simple schedules (Dickinson, 1989). Unlike Dickinson, Wood, and Smith's (2002) experiment, rats that were trained with a food outcome (in this experiment, sucrose) never had an opportunity to respond for an EtOH outcome, and vice versa, to avoid possible conflicts of choice, preference, and responding for one outcome affecting the other. Therefore a simple rather than concurrent schedule was used.

If the initial training for EtOH or sucrose outcomes is not an S-R process, then the temporary deactivation of the DLS should have no effect on operant performance. In the early probe test after initial training, the experimenter expects to find results similar to Featherstone and McDonald (2004), in that rats will perform similar operant responding regardless of the current activation state of the DLS for each outcome.

If extensive training for EtOH or sucrose outcomes renders behavior an S-R process, then the temporary deactivation of the DLS should impair operant performance. In the late probe test after extended training, the experimenter expects to find impairment in operant responding by DLS inactivated rats. Although lesions of the DLS prevent the

acquisition of habit formation in operant conditioning (Featherstone & McDonald, 2004; Yin et al, 2004), it is unclear if the DLS will be involved in the maintenance of habitual behavior when rats have had extended training. It is also uncertain if there will be differences between EtOH and sucrose outcomes.

It is possible for animals responding for an EtOH outcome to be more prone habitual responding than a sucrose outcome. As indicated by Dickinson et al's (2002) concurrent schedule EtOH devaluation showing a resistance to outcome devaluation (and sensitivity to outcome devaluation for sucrose), it may be possible to find DLS inactivation to affect rats' operant responses for EtOH earlier than in rats' responding for sucrose.

Several researchers have suggested that during instrumental learning both S-R and R-O associations are formed, however under conditions where responding becomes habitual it is the S-R process that dominates and takes control over behavior. According to this view, if the S-R control was to be disabled then the previously over-powered R-O process should be able to take over control of responding. Evidence consistent with this view comes from lesion studies. For example, Yin et al (2004) trained DLS-lesioned rats and sham controls on a simple interval schedule to establish habitual responding and then devalued the outcomes. The sham control animals showed resistance to outcome devaluation as usual, however the DLS-lesioned rats showed reduced responding for the devalued outcome. This result suggests that when the S-R habit system is disrupted, control is taken over by the R-O action system. If this interpretation is correct, then when the DLS is inactivated in the proposed experiment it is possible that no effect on responding will be observed because although the S-R system has been disrupted, the

system that controls the R-O process (which is still intact because it does not involve the DLS) may take over responding. That is, although a reduction in operant responding following the temporary inactivation of the DLS supports the role of the DLS in habitual responding, failure to find an effect on responding does not rule it out. Therefore, in the proposed experiment another phase was added in an attempt to evaluate a “switching systems” explanation.

Following behavioral training, the outcome was devalued through taste aversion by pairing the sucrose and EtOH outcomes with LiCl. The purpose of this devaluation was to eliminate the goal-directed action that may be maintaining operant responding during the DLS inactivation probe tests. By devaluing the outcome the animals still have an R-O association, but because the outcome has been devalued the animals should not be motivated to respond to obtain the devalued outcome. The observation that DLS inactivation reduces responding following outcome devaluation when it did not have an effect prior to outcome devaluation would support the “switching systems” explanation. If no effect of DLS inactivation is observed, then the “switching systems” explanation can not account for the failure of DLS-inactivation to affect operant responding.

In summary, to investigate if the DLS is involved in habitual S-R responding, rats were trained daily in an operant task using a simple ratio schedule and sucrose or EtOH outcome performances (response rates, total session responses, and session lengths) were probed at various times during training while the DLS was active (saline) and inactive (lidocaine). The experiment was carried out in two experimental phases. In phase one, the performance of rats after initial and extensive training while the DLS is active and inactive under reinforced responding conditions is compared. Lesser responding of the

inactivated DLS group supports the notion that the DLS maintains habitual S-R responding. Impaired responding on the late probe test but not the early probe test supports the notion that the amount of training affects plasticity in the DLS which comes to maintain habitual S-R responding. To compare EtOH and sucrose outcomes, early and late probe test differences are compared. Impaired responding on the early and late probe test of the EtOH group but only impaired responding on the late probe test of the sucrose group supports the notion that alcohol is more prone to the S-R process. Further, this supports the notion that alcohol increases plasticity of the DLS that develops and maintains habitual S-R responding. In phase two, the performance of rats after extensive training under extinction conditions while the DLS was active and inactive is compared. This allows a comparison of performance with extinction in the next phase after outcome devaluation. The performance of DLS inactivated rats after extensive training and outcome devaluation under extinction and outcome reinstatement conditions was also examined in phase two.

Method

Subjects

The subjects were twelve male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN), 7-8 weeks old and 200-224 grams in weight when received from the supplier. The animals were individually housed in clear plastic cages and maintained on a 12:12 hour light-dark cycle with lights on at 8:00am and lights off at 8:00pm. Animals were fed with Techlad LM485 mouse/rat diet and their cages were bedded with Harlan Techlad corncob bedding. The vivarium is maintained at constant temperature and humidity conditions. All procedures were in accordance with the Seton Hall University

Institutional Animal Care and Use Committee.

Apparatus

Two standard operant chambers with a grid floor were used for operant training and testing. The operant chambers dimensions are 9.5" length x 11.5" width x 8" height. The right and left walls of the chamber are metal while all other sides (except the floor) are Plexiglas. All of the walls of the chambers are empty except for the right wall. The right wall consists of 3 lights, 2 levers, and a feeding hole. The feeding hole measures 1.5" length x 2" height x 1" deep and is in the center of the right wall .25" above the grid floor. A dipper (Gerbrands Corporation Model #G5600B-LH, Arlington, MA) is directly behind the right wall that protrudes into the feeding hole with a .1ml outcome. The two lever dimensions are 2" x .75". The center of the right lever is 1" above and 1.5" to the right of the feeding hole. The center of the left lever is 1" above and 1.5" to the left of the feeding hole. The house light is 3.5" directly above the feeding hole. The other two lights are not used in this experiment but are 1.5" to the right of the right lever and 1.5" to the left of the left lever. The operant chambers are controlled by an IBM NetVista computer using MED-Associates instrumentation and MED-PC software (MED Associates, St. Albans, VT).

Drugs and Solutions

All sucrose solutions were made weight to volume by using standard cane sugar and tap water. All EtOH solutions (200 proof, Pharmco Inc, Brookfield, CT) were made volume to volume with tap water. LiCl (Sigma, St. Louis, MO) was prepared by dissolving in sterile saline immediately before injections. Lidocaine hydrochloride (2%) was acquired from Western Medical Supply, Inc, Los Angeles, CA.

Surgery

After 6 days of adaptation to the vivarium and 3 days of handling, rats were given surgery. Rats were anesthetized with ketamine (60-80 mg/kg, i.p.) and xylazine (.3 mg/kg, i.p.) and placed in a standard stereotaxic instrument. Small holes were drilled into the skull bilaterally, and 22-gauge guide cannulae (extending 3.5mm beyond the pedestal; Plastics One, Roanoke, VA) were inserted into the brain at the following coordinates: 0.7 mm anterior to bregma and ± 3.6 mm lateral to midline; and 3.5 mm below the skull surface based on the atlas of Paxinos and Watson (2005). Four jeweler screws were also introduced into the skull to help fasten dental cement to the skull and cannulae. Projection cannulae (23-gauge that extended 1mm beyond the guide cannulae; Plastics One, Roanoke, VA) were used to infuse all solutions into the DLS.

Procedure

Sucrose Fading Procedure. One week after surgery, a modified version of 'Samson's sucrose fading procedure' (1985, 1986, 2003) for the home cage was used to induce a stable pattern of EtOH drinking. Animals do not readily accept higher percentages of EtOH when presented at first. This initiation procedure makes use of the innate taste preference of the animals for sweet solutions, with gradual introduction and increasing of EtOH in the solution and slow "fading out" of the adulterant (Czachowski et al, 1999). Until this point the animals had been food and water access ad libitum. All fluids during the sucrose fading procedure were presented 24 hours a day with animals having no access to other fluids, such as water. Food was also restricted to promote ingestion of fluids. Pilot and experimental data indicated that rats maintained their free feeding body

weight while food was restricted. This suggests that the rats had substituted calories from food with the calories in the fluid provided. In order to avoid a potential confound of outcome pre-exposure, all rats were placed on the sucrose fading procedure. The amount of fluid ingested was determined by the difference between the weight of the solution before placing in the home cage and 24 hours later. If spillage occurred, data was discarded for that day only.

The complete schedule of the sucrose fading procedure can be viewed in Table 1. During days one and two of the sucrose fading procedure, the rats are presented with a 10% EtOH solution. On days three and four, EtOH is introduced in a 2% EtOH/10% sucrose solution. On days five and six, the concentration of EtOH is increased to 5% EtOH/10% sucrose and on days seven and eight, increased to 10% EtOH/10% sucrose. On days nine and ten, the amount of sucrose is begun to fade out with a solution of 10% EtOH/5% sucrose, days eleven and twelve a solution of 10% EtOH/2% sucrose, and on days thirteen and fourteen a solution of 10% EtOH. On days fifteen and sixteen, sucrose is returned into the solution to induce a greater amount of EtOH ingestion with a 10% EtOH/2% sucrose solution. Finally, on day seventeen, sucrose is increased in the solution to 10% EtOH/5% sucrose.

The use of an EtOH/sucrose solution in operant conditioning presents several advantages over using EtOH alone. Humans do not drink EtOH alone; therefore an EtOH solution with a palatable vehicle is more appropriate for comparison to humans. These solutions have been shown to produce large intakes (Files et al, 1995; Heyman, 1993). Moreover, the addition of sucrose has been shown to not interfere with the early metabolism or absorption of EtOH (Czachowski et al, 1999).

Table 1

<i>Sucrose Fading Procedure</i>									
Days	1 - 2	3 - 4	5 - 6	7 - 8	9-10	11-12	13-14	15-16	17
% ethanol	10%	2%	5%	10%	10%	10%	10%	10%	10%
% sucrose	0%	10%	10%	10%	5%	2%	0%	2%	5%

Rats were separated into two groups by the results of the first day of exposure to 10% EtOH. The six rats that consumed the most EtOH were separated into the EtOH group while the remaining six rats were separated into the sucrose group. Both groups completed the entire sucrose fading procedure and group differences began with operant training.

Lever Press Training. Following the sucrose fading procedure, water was available at all times in the home cage and food was restricted to deprive animals to 85% of their free feeding weight over 4 days. During lever press training and all subsequent operant training sessions, animals in the EtOH group were presented with a solution of 10% EtOH/10% sucrose as the outcome while animals in the sucrose groups were presented with a solution of 10% sucrose as the outcome.

In all operant training and probe testing sessions, a daily 30 minute session started with the illumination of the house light and ended with the turning off of the house light. The rats began lever press training with one session of magazine training. In this session, the dipper presented the appropriate outcome for each group on a Variable Time 40 second schedule (0-80 second intervals with an average of 40). In the second lever press training day, rats were shaped by the experimenter. Some of the EtOH group rats needed additional shaping sessions with a 10% sucrose outcome. The experimenter reinforced the rats for each gradual approximation towards the lever until the animal was pressing the lever on its own under a continuous reinforcement (CRF) schedule. Examples of gradual approximations towards the lever were facing the lever, rearing to the lever, touching the lever, and so on. Once the rat had been shaped to press the lever, the session ended either with 50 lever-press outcome presentations or after 30 minutes had elapsed.

On the next lever press training day, the rats were run under a CRF schedule and testing ended after 50 lever-press outcome presentations or after 30 minutes had elapsed.

Phase 1: Variable Ratio Training and DLS deactivation on Probe Tests. Interval schedules produce less sensitivity to outcome devaluation than ratio schedules even very early in training (Dickinson, 1983). The main focus of this experiment was to examine the gradual development of habitual responding; therefore a ratio schedule was chosen to be used rather than an interval schedule. The development of habitual responding has been shown to occur by extensively training rats to lever press on a ratio schedule (Adams, 1982; Dickinson, 1985). Although the acquisition of S-R learning has been shown to be impaired with lesions of the DLS (McDonald & White, 1993; McDonald & Hong, 2004; Compton, 2004; Featherstone & McDonald, 2004), no study has yet to determine if the DLS is involved in the maintenance of S-R learning. To assess if the DLS is maintaining S-R responding, a method of probe testing (Packard & McGaugh, 1996) was used.

Probe testing allows for the determination of the role of the DLS in the initial and extended training of stimulus-response learning and alcohol-seeking behavior. By functionally inactivating the DLS, the role of this brain structure after distinct learning phases can be examined. The probe tests were conducted early and late into training to examine the role of the DLS after extended training. Thus, the present study utilized a 2 x 2 x 2 mixed subjects design with outcome between-groups (EtOH, sucrose), infusion within-groups (lidocaine, saline), and training test within-groups (initial, extensive). The dependent variables collected were response rate, total session responses, and session length, monitored by a custom programmed MED-PC program (MED Associates, St.

Albans, VT). A total session response was calculated by the number of lever presses on the active lever for the entire session without respect to session length. A session length was calculated by the amount of time it took animals to finish a session. A session was terminated after 100 outcomes had been earned or 30 minutes had elapsed. Thus, the animal had control of when the session was terminated. Response rate was calculated by the number of lever presses on the active lever per minute with respect to session length. Response rate and session length are therefore highly correlated but the session length dependent variable is contingent on the number of outcomes earned whereas response rate is contingent on the number of responses per minute.

On probe test days, either lidocaine or saline were infused into the DLS. A 2% lidocaine hydrochloride solution was used to produce reversible inactivation of the DLS. Bilateral injections (0.5 μ l) were administered intracerebrally using 23-gauge projection cannulae inserted into the guide cannulae. The projection cannulae were connected by polyethylene tubing to 10 μ l Hamilton micro syringes (Hamilton Co., Reno, NV). The injections were delivered over a period of 37 seconds using a syringe pump (Sage Instruments), and the projection cannulae (extending 1 mm from the end of the guide cannulae) were left in place an additional 60 seconds to allow for sufficient diffusion of the solution away from the cannula tip. Lidocaine or saline injections were administered approximately 2–3 minutes prior to all probe tests.

One day after the CRF training day, rats began Variable Ratio (VR) training. In the first day of training, rats were trained in a VR 3 schedule while on the second day the rats were trained on a VR 5 schedule. On these and further VR sessions, testing began with the illumination of the house light and ended after 100 lever-press outcome

presentations or after 30 minutes had elapsed. On the third day of operant training the two-day early probe test began. On the first day of the early probe test, rats in the EtOH and sucrose groups were infused with lidocaine while on the second day of the early probe test, rats were infused with saline. This method allows for a within-subjects comparison in that each animal is its own experimental and control group. One day after the early probe test, the rats underwent thirteen days of extended training under a VR 5 schedule of reinforcement. After thirteen days of extended training the two-day late probe test began. On the first day of the late probe test, rats in EtOH and sucrose groups were infused with saline while on the second day of the late probe test rats in EtOH and sucrose groups were infused with lidocaine. The complete schedule of operant training and probe test sessions can be viewed in Table 2.

Phase 2: Extinction Tests, Outcome Devaluation, and Reinstatement. The animals continued VR 5 operant responding sessions for two days following the late probe test. One day after these sessions, the rats were tested on a 15 minute extinction test in the operant chamber after saline was infused into the DLS. The animals then continued VR 5 operant responding sessions for two more days. One day following these sessions, the rats were tested on a second 15 minute extinction test in the operant chamber while the DLS was inactivated via lidocaine infusion.

Similar to the procedures of Dickinson, Wood, and Smith (2002) and Yin et al (2004), the outcome of EtOH and sucrose groups after operant training was devalued. One day after completion of the second extinction test, the sucrose solution in sucrose groups and EtOH solution in EtOH groups was devalued for three days. For 30 minutes during each of the three daily sessions, the rats were presented in their home cages the

Table 2

Operant Training and Probe Test Schedule

Day	Schedule	Infusion Treatment	Probe Test Label
Phase 1: VR Training and DLS inactivation Probe Tests			
1	Magazine	-	
2	Shaping	-	
3	CRF	-	
4	VR3	-	
5	VR3	-	
6	VR5	-	
7	VR5	Lidocaine	Early
8	VR5	Saline	Early
9-21	VR5	-	
22	VR5	Saline	Late
23	VR5	Lidocaine	Late
Phase 2: Extinction, Outcome Devaluation, and Reinstatement			
24	VR5	-	
25	VR5	-	
26	Extinction	Saline	Extinction-Saline
27	VR5	-	
28	VR5	-	
29	Extinction	Lidocaine	Extinction-Lidocaine
30	VR5	-	
31	VR5	-	
32	VR5	-	
33-35	Devalue	-	
36	Extinction	Lidocaine	Devalued Extinction
37	VR5	Lidocaine	Devalued Reinstatement

Note: The devaluation procedure during days 33-35 took place in the home cages, tests on all other days were in the operant chambers

proper outcome for their group from a plastic graduated cylinder. Immediately following outcome consumption for 30 minutes, the outcome was removed and the rats were immediately injected with LiCl (0.15 M, 20 mL/kg, i.p.). The animals were not tested in the operant chambers during these devaluation days.

One day after the third outcome devaluation session, the rats were tested on a third 15 minute extinction test in the operant chamber while the DLS was inactivated via lidocaine infusion. The following day, the outcome was reinstated by testing the rats on a VR 5 schedule with the outcome present while the DLS was inactivated via infusion of lidocaine.

Histology. Following all behavioral tests, the rats were euthanized (pentobarbital 1.0-ml, I.P.) and perfused transcardially with 0.9% saline followed by a 10% buffered formaldehyde solution. The brains were stored in a 10% formalin solution for at least 72 hours before they were stored in a 30% sucrose solution for 24 hours. Brains were mounted on a standard table microtome with a stage connected to a CO₂ gas tank for freezing. 35- μ m sections were cut through the cannulae tract regions and stained with Cresyl violet. Cannulae placements were examined for verification of cannula tip location using the atlas of Paxinos and Watson (2005).

Results

Sucrose Fading Procedure

Experimental groups were separated by the amount of 10% EtOH consumed after the first presentation, displayed in Figure 1. The mean intake of the first presentation of EtOH for the EtOH-designated group ($M = 38.1$) was significantly greater than the sucrose-designated group ($M = 18.6$), $t(10) = 5.07$, $p < .000$. Both groups then received the same sucrose fading procedure in which sucrose was faded in, out, and back in to EtOH solutions. Figure 2 displays the ingested amount of each solution per group, averaged across two day presentations for each solution (except the last presentation of 10% EtOH/5% sucrose, which was presented once). A Groups (2) x Days (8) mixed subjects ANOVA yielded main effects for groups, $F(1, 10) = 5.407$, $P < .05$, and days, $F(7, 70) = 212.906$, $P < .000$, but no groups x days interaction, $F(7, 70) = 1.033$, $p > .1$. Thus, intakes decreased as the fading procedure progressed but the EtOH group consistently drank more of the solutions than the sucrose group. Although intakes decreased, animals still consumed EtOH, therefore it should be able to serve as an outcome in the present experiment. Experimental groups were treated exactly the same until the both groups completed the sucrose fading procedure. For the remainder of the experiment, the EtOH group received 10% EtOH/10% sucrose outcomes in the operant chamber and the sucrose group never received EtOH again, but received 10% sucrose outcomes in the operant chamber.

Baseline responding

A baseline measure of responding was considered the group performance (response rate, total session responses, and session length) averaged over the two sessions

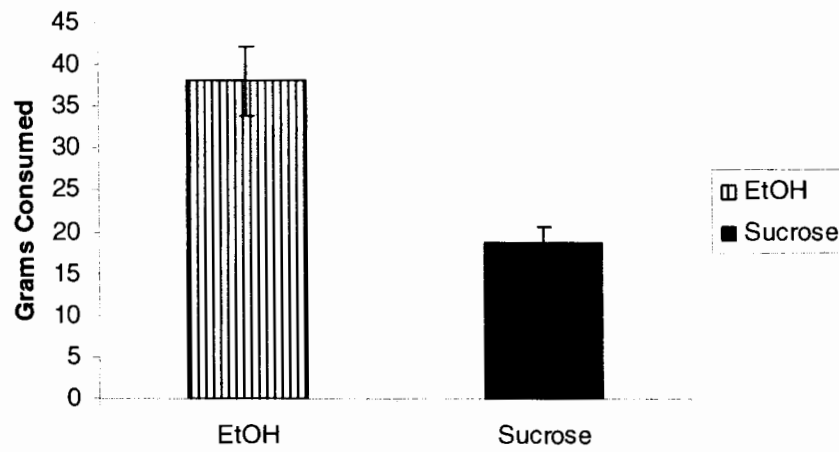


Figure 1. Mean ingested amounts of 10% EtOH (+SE) on the first presentation. Rats consuming the most (EtOH group) and the least (sucrose group).

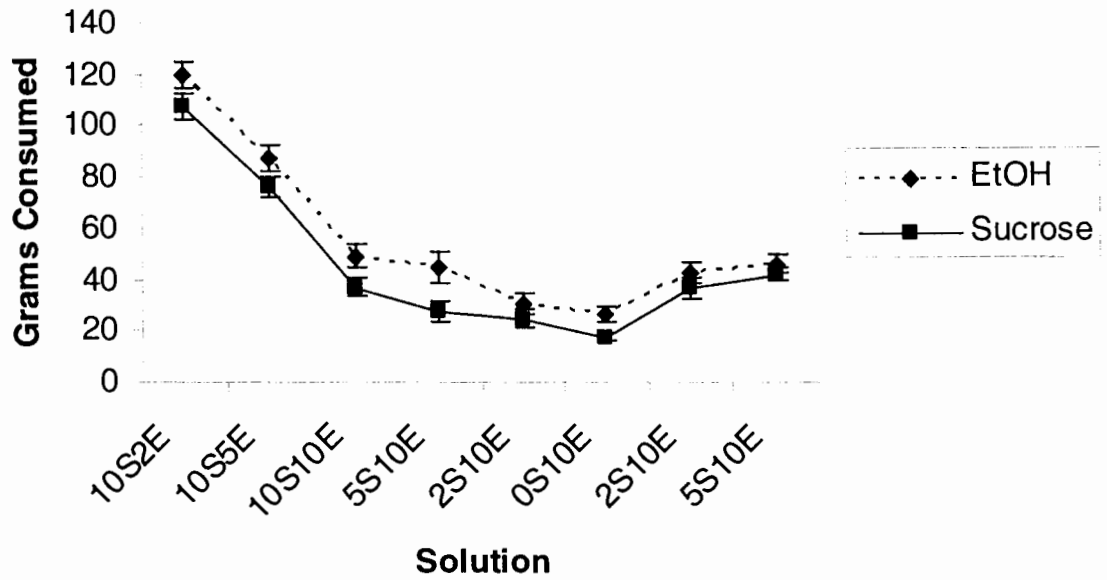


Figure 2. Mean ingested amount of solutions per group (+SE) in the sucrose fading procedure. Each solution consisted of an EtOH and sucrose mixture. The number in front of the solution designation indicates the percent concentration of the solution (e.g. 10S2E is a mixture of 10% sucrose and 2% EtOH).

before probe tests. Differences were first examined between baseline responding and saline DLS infused responding. This analysis allows the opportunity to determine if the restraint provided in the DLS infusion procedure alone can disrupt operant performance. Before the early probe test, a groups (2) x infusion (2) mixed subjects ANOVA yielded nonsignificant main effects for infusion on response rate, $F(1,10) = .158, P > .1$, total session responses, $F(1,10) = .158, P > .1$, but significant for total session responses, $F(1,10) = 6.889, P < .05$. No infusion x groups interaction was found for any dependent variable. Thus, while saline infusion had no effect on response rate or the time it took to finish the session, total session responses *increased* with saline administration (data not shown). This effect is most likely due to the fact that the animals were still learning the task (initial training) and needed additional training to acquire habitual responding. The most important fact of this information, however, is that the stress imposed by infusion of saline into the DLS, did not hinder performance as compared to a noninvasive baseline condition. On the contrary, total session responses improved with additional training days. Therefore, saline was compared with lidocaine infusion into the DLS on the subsequent early, late, and extinction probe tests.

The early effect of saline infusion into the DLS increasing total session responses was not found when comparing late baseline responding to late saline infused responding (data not shown). A groups (2) x infusion (2) mixed subjects ANOVA yielded a nonsignificant main effect for infusion and a nonsignificant infusion x groups interaction for all dependent variables. Therefore, while animals were still acquiring VR 5 schedule of reinforcement lever press skill in the early test, animals had acquired skill by extensive training before the late test. In fact, when comparing baseline performance, only after

initial training did differences between outcome groups in performance occur. Figure 3 displays the mean baseline performances of EtOH and sucrose group rats for response rate (A), total session responses (B), and session length (C), on the five different baseline conditions: early probe test, late probe test, extinction saline probe test, extinction lidocaine probe test, and extinction lidocaine devalued probe test. In order to distinguish a difference between responding after initial and extended training, a groups (2) x phase (2) mixed subjects ANOVA was used. The main effect of phase did not include all 5 testing conditions because extinction sessions and outcome devaluation procedures were used following the late probe test. The ANOVA yielded significant main effects of phase, for response rate, $F(1, 10) = 77.241, P < .000$, total responses, $F(1, 10) = 58.561, P < .000$, and session length, $F(1, 10) = 63.965, P < .000$, and significant main effect of groups for response rate, $F(1, 10) = 10.474, P < .01$, total responses, $F(1, 10) = 8.202, P < .05$, and session length, $F(1, 10) = 25.028, P < .001$. A significant phase x groups interaction was yielded for total responses, $F(1, 10) = 20.362, P < .001$, and session length, $F(1, 10) = 5.398, P < .05$, but not for response rate, $F(1, 10) = .911, P > .1$. Therefore, evidence indicates that after initial training, responding is higher for sucrose than EtOH, but this effect is faded out with extended training. Since the phase x groups interaction was not significant for all dependent variables, individual t-tests were used to indicate if there were any differences in responding between groups at all baselines. The sucrose group outperformed the EtOH group after initial training under baseline conditions, for response rate, $t(10) = 3.742, p < .005$, total session responses, $t(10) = 5.102, p < .000$, and session length, $t(10) = 4.892, p < .001$. This effect was not seen for any dependent variable for any other baseline test. For the late test, response rate, $t(10) = 1.57, P > .1$, total responses,

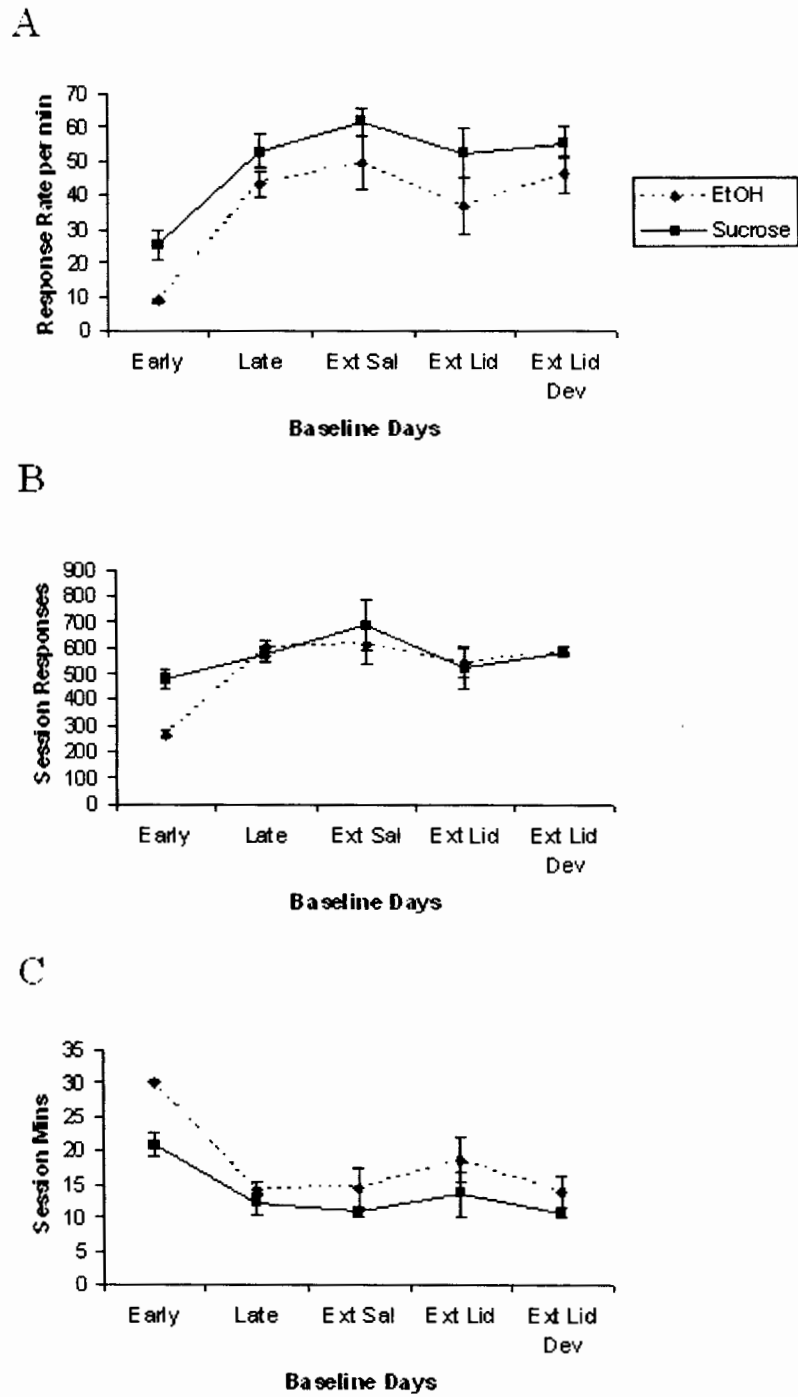


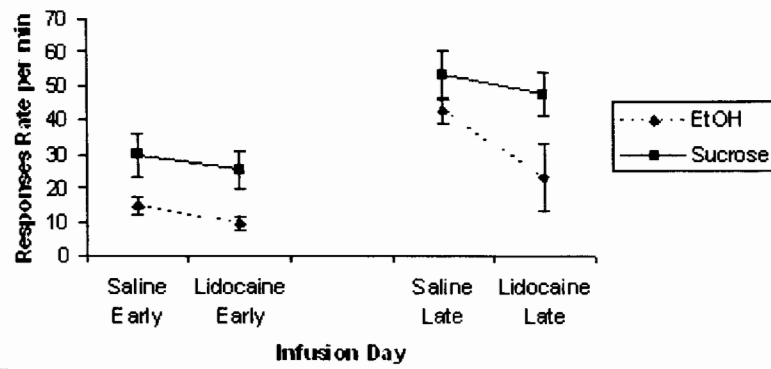
Figure 3. Mean baseline operant measures of performance (+SE) before early, late, extinction saline, extinction lidocaine, and devaluation tests for: A. Response rate B. Total session responses C. Session length.

$t(10) = .491$, $P > .1$, and session length, $t(10) = 1.08$, $P > .1$ were nonsignificant. For the extinction saline test, response rate, $t(10) = 1.362$, $P > .1$, total responses, $t(10) = .663$, $P > .1$, and session length, $t(10) = 1.1$, $P > .1$ were nonsignificant. For the extinction lidocaine test, response rate, $t(10) = 1.441$, $P > .1$, total responses, $t(10) = .204$, $P > .1$, and session length, $t(10) = 1.08$, $P > .1$ were nonsignificant. For the extinction lidocaine devalued test, response rate, $t(10) = 1.256$, $P > .1$, total responses, $t(10) = .064$, $P > .1$, and session length, $t(10) = 1.382$, $P > .1$ were nonsignificant. Thus, while group differences appeared after initial training, performance in the EtOH group significantly improved after extensive training and group baseline differences were abolished throughout the remainder of the study.

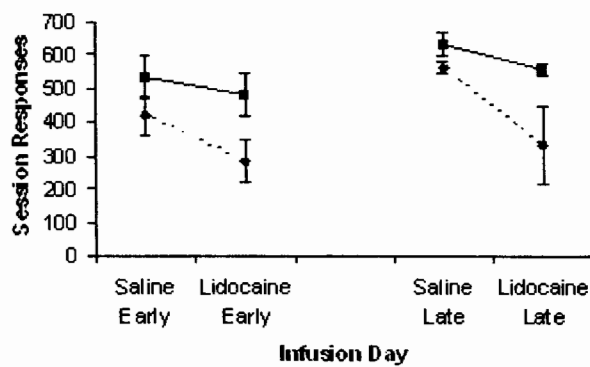
Phase 1: Variable Ratio Training and DLS deactivation on Probe Tests.

The role of the DLS after initial and extensive training on a VR 5 schedule was examined. Due to the possible light restraint stress of the infusion, statistics were calculated based on saline infused and lidocaine infused performances. Figure 4 displays the mean performances of EtOH and sucrose group rats for response rate (A), total session responses (B), and session length (C), for the early and late probe tests. A groups (2) x infusion (2) x training (2) mixed subjects ANOVA yielding a main effect of training for response rate, $F(1,10) = 28.541$, $P < .000$ and session length, $F(1,10) = 36.017$, $P < .000$, but not for total session responses, $F(1,10) = 3.187$, $P > .1$. This suggests that while rats can finish the entire experimental session (100 outcomes in a VR 5 schedule of reinforcement under 30 minutes) early or late into training, extensive training improves performance/skill as noted by higher response rates and shorter session length late in training rather than early. A significant main effect of groups was revealed for response

A



B



C

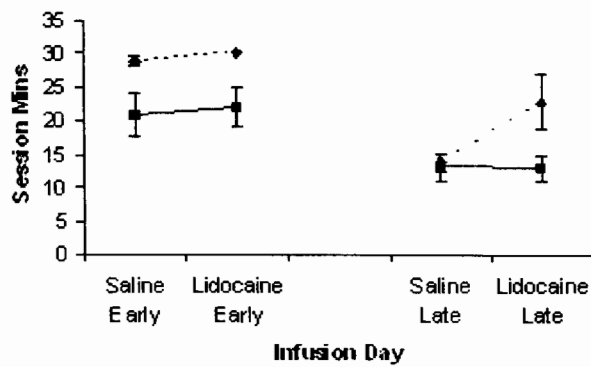


Figure 4. Phase 1 - Mean operant measures of performance (+SE) during initial (early) and extended (late) training after saline and deactivation of the DLS with lidocaine infusion for: A. Response rate B. Total session responses C. Session length.

rate, $F(1, 10) = 6.466$, $P < .05$, total session responses, $F(1, 10) = 19.192$, $P < .001$, and session length, $F(1, 10) = 6.99$, $p < .05$. This suggests that the sucrose group rats outperformed EtOH group rats in the early and late probe tests. A significant main effect of infusion was found for response rate, $F(1, 10) = 28.541$, $P < .000$, total session responses, $F(1,10) = 7.223$, $P < .05$, and session length, $F(1,10) = 7.011$, $P < .05$ but no training x infusion interaction was found for any dependent variable. Therefore, while DLS inactivation decreased maintenance of responding, this effect was seen early *and* late into training, contrary to what was predicted. A training x groups interaction was nonsignificant for all dependent variables and infusion x groups interaction was nonsignificant for response rate, $F(1,10) = 1.148$, $P > .1$, and total session responses, $F(1,10) = 1.843$, $P > .1$, but just missed significance for session length, $F(1,10) = 4.662$, $P < .056$. A three-way interaction of groups x training x infusion in the session length dependent variable was seen, whereby the late lidocaine infusion significantly increased EtOH group session length, $F(1,10) = 7.453$, $P < .05$, but did not affect sucrose group dependent variables. Although extensively trained EtOH inactivated DLS rats had significantly longer session length durations, this effect was not seen for response rate or total session responses. The DLS may have a role in responding for EtOH after extensive training slightly more so than for sucrose. Response rate data (Figure 3A) suggests that our predicted result of DLS inactivation affecting response rate late in training more than after initial training is consistent with session length data. A significant difference may have been seen with a larger N, nonetheless the results are encouraging.

Each probe test was then inspected separately. In the early test only, a groups (2) x infusion (2) mixed subjects ANOVA yielded a near significant main effect of infusion

for response rate, $F(1,10) = 4.836$, $p < .053$, but nonsignificant total session responses, $F(1,10) = 2.332$, $P > .1$, and session length $F(1,10) = 3.658$, $P > .08$. A main effect of groups was revealed for response rate, $F(1, 10) = 6.673$, $p < .05$, total session responses, $F(1, 10) = 6.581$, $p < .05$, and session length, $F(1, 10) = 7.455$, $p < .05$ but no significant groups x infusion interactions were noted for any dependent variable. Therefore, while sucrose group rats outperformed EtOH group rats, evidence of DLS inactivation attenuating performance was only found in response rate and had no effect for total session responses or session length.

In the late test only, a groups (2) x infusion (2) mixed subjects ANOVA yielded a significant main effect of infusion for response rate, $F(1,10) = 5.994$, $p < .05$, total session responses, $F(1,10) = 6.536$, $P < .05$, and session length, $F(1,10) = 5.83$, $P < .05$. A main effect of groups was found for total session responses, $F(1, 10) = 5.253$, $p < .05$, but not response rate, $F(1, 10) = 3.748$, $p > .08$, or session length, $F(1, 10) = 3.038$, $p > .1$. A groups x infusion interaction was noted for session length, $F(1, 10) = 6.424$, $p < .05$, but not for response rate, $F(1, 10) = .1.802$, $P > .1$, or total session responses, $F(1,10) = 1.861$, $P > .1$. This suggests that DLS inactivation significantly attenuated performance in the late test. Attenuated performance was more pronounced in the late probe test because all dependent variables were significant whereas in the early probe test only response rate was affected by DLS inactivation. Some group differences were seen in the early probe test, such as total session responses significantly lower in the EtOH than sucrose group, however this effect was not seen in response rate or session length. During the late probe test, the EtOH group session length significantly increased, an effect not seen in the early probe test. This is not only in contrast to the sucrose group session length, which

remained stable across DLS activation, but indicates that extensive training with EtOH outcome may be more utilized by the DLS than sucrose outcome.

Phase 2: Extinction Tests, Outcome Devaluation, and Reinstatement

Following the late probe test, the animals were run for two sessions under reinforced responding conditions. The following day, saline was infused into the DLS and the animals were run under extinction conditions. Following the saline extinction test, responding was reinstated by allowing animals to lever press under reinforced conditions for two days/sessions. The following day, lidocaine was infused into the DLS and the animals were run under extinction conditions. These extinction sessions were 15 minutes in duration and lever presses had no consequence. Thus, the animals had no opportunity to finish the session and session length was not a dependent variable in this phase. In addition, when placing the dummy cannulae back into guide cannulae, one EtOH group rat's dental cement fell off. The rat was immediately sacrificed, perfused, and the brain was removed (see histology procedure). Thus, after the saline extinction test, EtOH group N was reduced from 6 to 5.

Figure 5 displays the mean performances of EtOH and sucrose group rats for response rate (A) and total session responses (B) for the extinction saline and lidocaine tests. A groups (2) x infusion (2) mixed subjects ANOVA yielded a main effect for infusion in response rate, $F(1, 9) = 5.345, p < .05$, and total session responses, $F(1, 9) = 5.355, p < .05$, suggesting that DLS inactivation decreases response rate under extinction conditions. There was no main effect for groups in response rate or total session responses, $F(1, 9) = .139, p > .1$, nor an infusion x groups interaction in response rate or total session responses, $F(1, 9) = .000, p > .1$. Recall that under reinforced responding

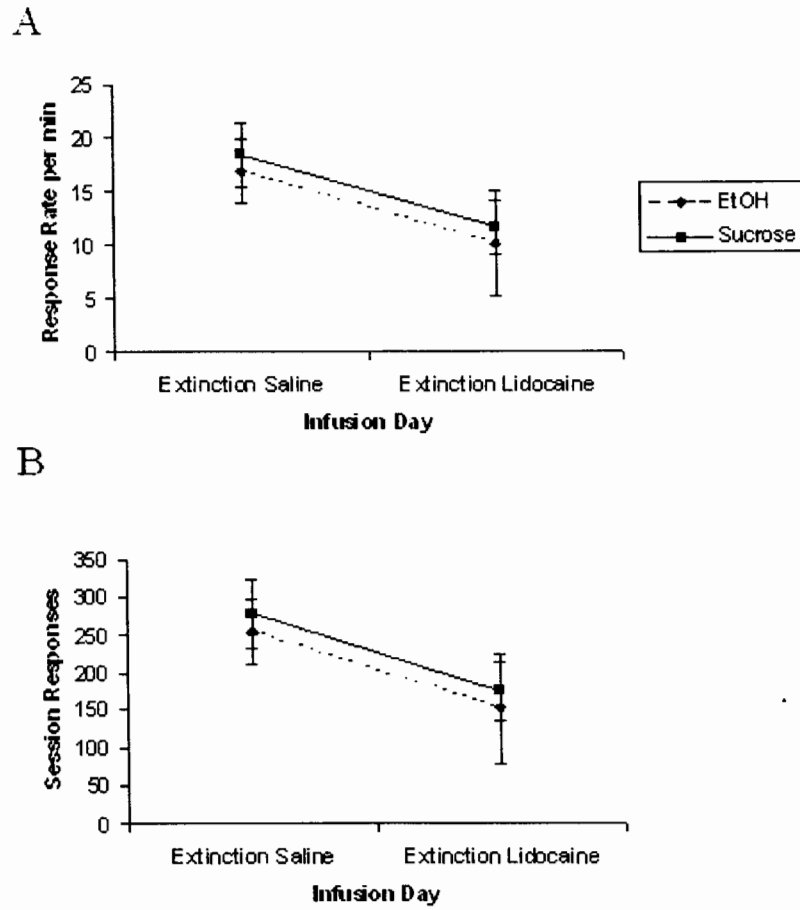


Figure 5. Phase 2 – After extended training, mean operant measures of performance (+SE) in extinction after saline and deactivation of the DLS with lidocaine infusion for: A. Response rate B. Total session responses.

during the early and late probe tests, responding for EtOH was less than responding for sucrose when the DLS was inactivated by lidocaine administration. The absence of any group difference in extinction suggests that EtOH consumption may have been producing a response decreasing effect in the EtOH group.

Devaluation Intake:

Outcome devaluation is used to separate the outcome dependent R-O process from the habitual S-R process by pairing the outcome with sickness. Rats respond for an outcome paired with sickness considerably less under the R-O process compared to responding under the S-R process because conditioned stimuli, and not the outcome, control responding in the S-R process. Following the second extinction test, both groups received 3 daily pairings of their respective outcome with LiCl administration. Figure 6 displays the ingested amount of each solution per group before and after three days of LiCl-outcome pairings. A groups (2) x devaluation (2) mixed subjects ANOVA yielded a significant main effect for groups, $F(1, 9) = 5.9$, $P < .05$, and devaluation, $F(1,9) = 71.328$, $p < .000$, while the devaluation x groups interaction was nonsignificant, $F(1,9) = 26.021$, $p > .1$. Therefore, although the sucrose group significantly consumed a greater amount of their respective outcome before and after devaluation, outcome devaluation was successful because LiCl administration significantly suppressed outcome consumption to 20% of baseline consumption.

One day after the third outcome-LiCl home cage pairing in the outcome devaluation procedure, the animals were infused with lidocaine and tested under extinction conditions once more. Similar to phase 2, the extinction session was 15 minutes in duration and it was not possible to collect data on the session length dependent

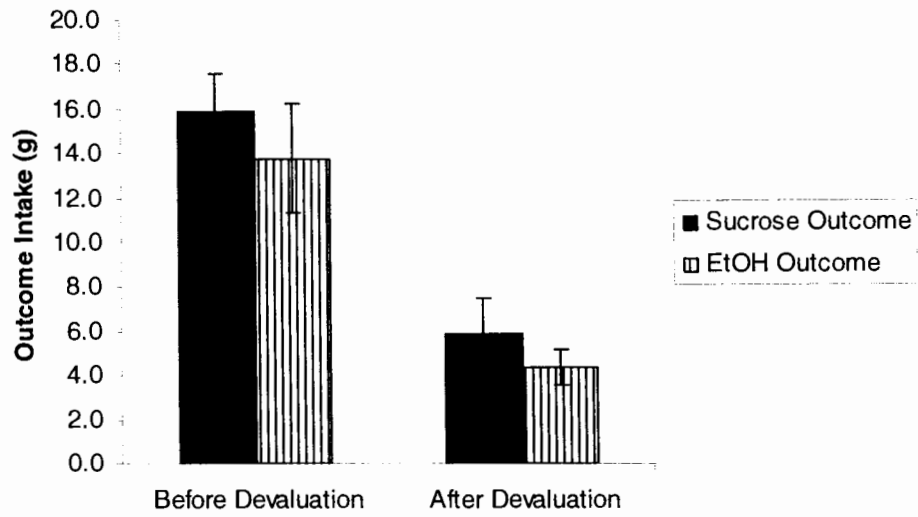
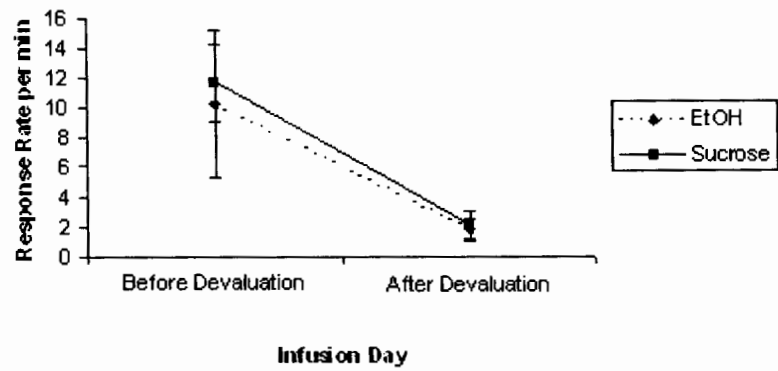


Figure 6. Ingested amount of solutions per group (+SE) in the outcome devaluation procedure. Rats consuming the most (before outcome devaluation) and the least (after outcome devaluation).

variable. Data from this extinction session was compared with the animals' last session performance in the lidocaine extinction test. The purpose of this test was to look for differences in responding due to outcome devaluation under extinction conditions. Figure 7 displays the mean performances of EtOH and sucrose group rats for response rate (A) and total session responses before and after outcome devaluation extinction lidocaine tests. A groups (2) x devaluation (2) mixed subjects ANOVA yielded a main effect for devaluation in response rate, $F(1, 9) = 16.414$, $P < .01$, and total session responses, $F(1, 9) = 11.768$, $p < .01$, but nonsignificant main effect for groups for all dependent variables. This suggests that devaluation significantly decreases response rate while the DLS is inactivated under extinction conditions. In addition, group differences that were seen when the outcome was present were again absent under extinction conditions. Response rate and total session responses were nearly identical in the extinction lidocaine devaluation tests, thus no devaluation x groups interaction for response rate, $F(1,9) = .298$, $P > .1$, or total session responses, $F(1, 9) = .123$, $p > .1$, was found. Therefore, while the DLS is inactive under extinction conditions, devaluation significantly reduced operant performance and no differences were found between responding for sucrose or EtOH outcomes.

One day after the extinction lidocaine devaluation test, the animals were infused with lidocaine and the animals were allowed to lever press in the final operant session. Since the appropriate group outcome was present on this session, this was called the lidocaine devalued reinstatement test. In addition, animals were allowed to finish the session by accumulating 100 outcomes or after 30 minutes had elapsed, therefore session length data was recorded. Data from this session was compared with the animals last

A



B

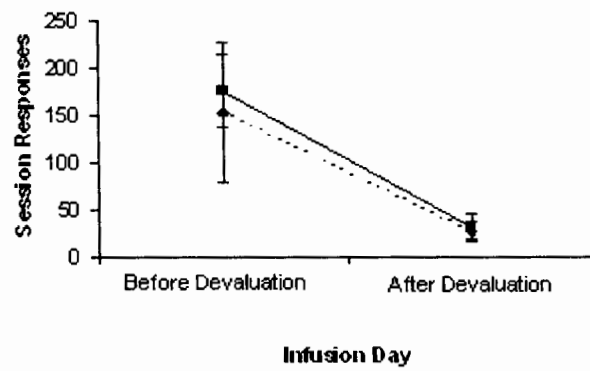


Figure 7. After extended training, mean operant measures of performance (+SE) for the effect of DLS inactivation with lidocaine infusions before and after outcome devaluation in extinction (when the outcome was not present) for: A. Response rate B. Total session responses.

lidocaine session under reinforced responding conditions, the late lidocaine test (phase 1). The purpose of this test was to look for differences in responding due to outcome devaluation under reinforced responding conditions. Figure 8 displays the mean performances of EtOH and sucrose group rats for response rate (A), total session responses (B), and session length (C), for the lidocaine devalued reinstatement tests. A groups (2) x devaluation (2) mixed subjects ANOVA yielded a main effect of devaluation for response rate, $F(1,9) = 13.187$, $p < .005$, and session length, $F(1,9) = 15.627$, $p < .005$, but not for total session responses, $F(1,9) = 1.207$, $p > .1$. This suggests that while the DLS is inactive, response rate and the time it took animals to finish the session is significantly modified by devaluation, whereby response rate decreases and session length increases, but the total number of responses across the entire session is unaffected under reinforced responding conditions. Group differences just missed significance for response rate, $F(1, 9) = 4.128$, $p > .07$, and session length, $F(1, 9) = 4.417$, $p < .064$, but was significant for total session responses, $F(1, 9) = 7.293$, $p < .05$, with sucrose group rats responding more than EtOH group rats. There was no devaluation x groups interaction for any dependent variable. Therefore, while the DLS is inactive under reinforced responding conditions, devaluation significantly reduced operant performance but did not alter the total number of session responses of rats. It is important to note that some of the group differences (and the other dependent variables were close to significance) that were absent in the previous extinction tests, reappeared when the outcome was reinstated. In addition, we would likely see group differences in response rate had we had a larger N or the one previous EtOH rat had not had to have been

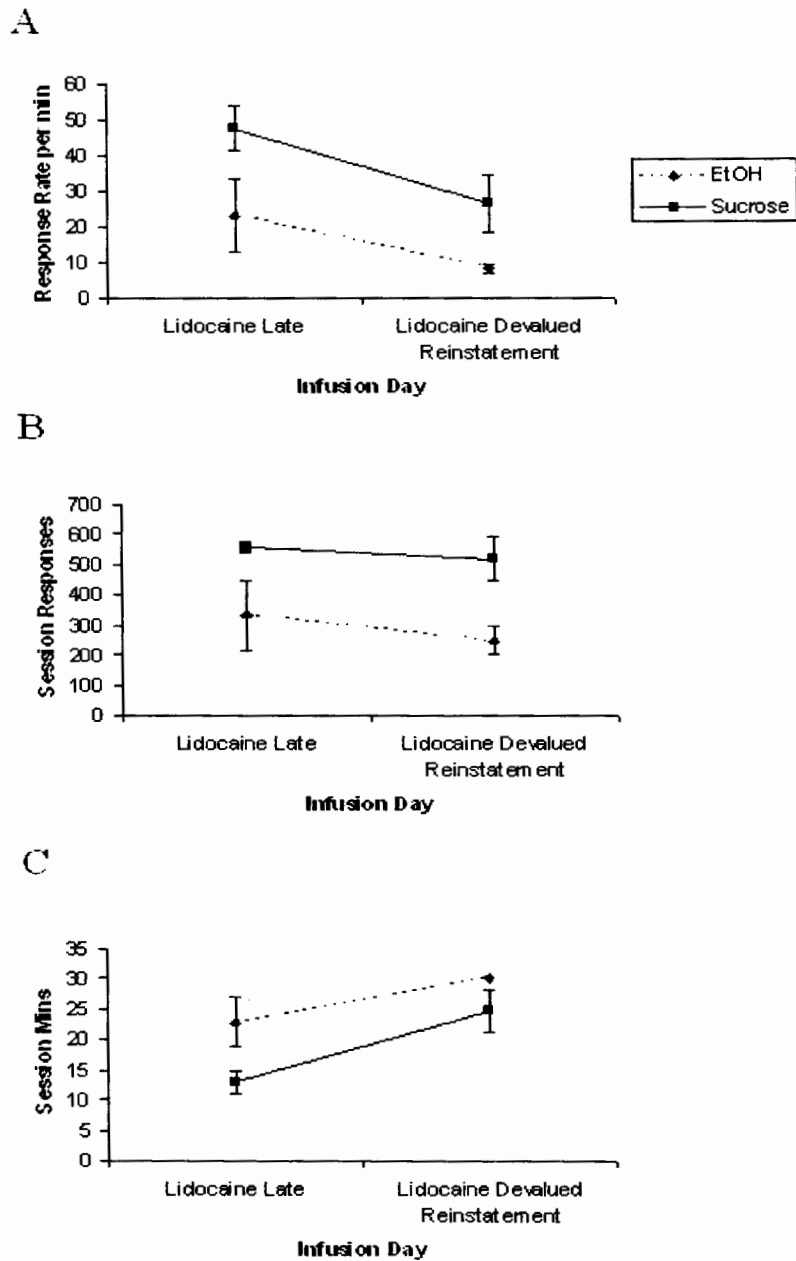


Figure 8. After extended training, mean operant measures of performance (+SE) for the effect of DLS inactivation with lidocaine infusions before and after outcome devaluation when the outcome was present for: A. Response rate B. Total session responses C. Session length. Note that the means for the late lidocaine infusion day are the same means plotted in Figure 4.

sacrificed. Therefore, responding for EtOH seems to induce an attenuated performance not seen with responding for sucrose.

One method to distinguish the role of the outcome in performance is to investigate differences in responding when the outcome is present and when the outcome is not present (in extinction). For these analyses, groups (2) x phase (2) ANOVAs were used to compare the response rates when the outcome was present or absent and the DLS is active, inactive, and inactive after outcome devaluation, also represented in Figure 9. While the DLS was active (saline), a main effect of phase was found, $F(1, 10) = 59.476$, $P < .000$, while a main effect of groups, $F(1, 10) = 1.336$, $P > .1$, and groups x phase, $F(1, 10) = 1.336$, $P > .1$, interaction was nonsignificant. While the DLS was inactive (lidocaine), a main effect of phase was also found, $F(1, 9) = 11.056$, $P < .01$, while a main effect of groups, $F(1, 9) = 3.655$, $P > .8$, and groups x phase, $F(1, 9) = 1.552$, $P > .1$, interaction was again nonsignificant. While the DLS was inactive after devaluation, a main effect of phase was found, $F(1, 9) = 13.294$, $P < .005$, and groups were nonsignificant, $F(1, 9) = 3.726$, $P > .8$, however, a phase x groups interaction just missed significance, $F(1, 9) = 4.47$, $P < .064$. Thus, extinction was successful in decreasing responding by removing the outcome, but group differences were not statistically found until after outcome devaluation, which decreased EtOH group responding more than sucrose group responding. While not statistically significant, this effect was found in the lidocaine comparison and much less so in the saline comparison. These effects may have been statistically significant if our N was larger, or if the single rat in the EtOH group was not sacrificed during the experiment.

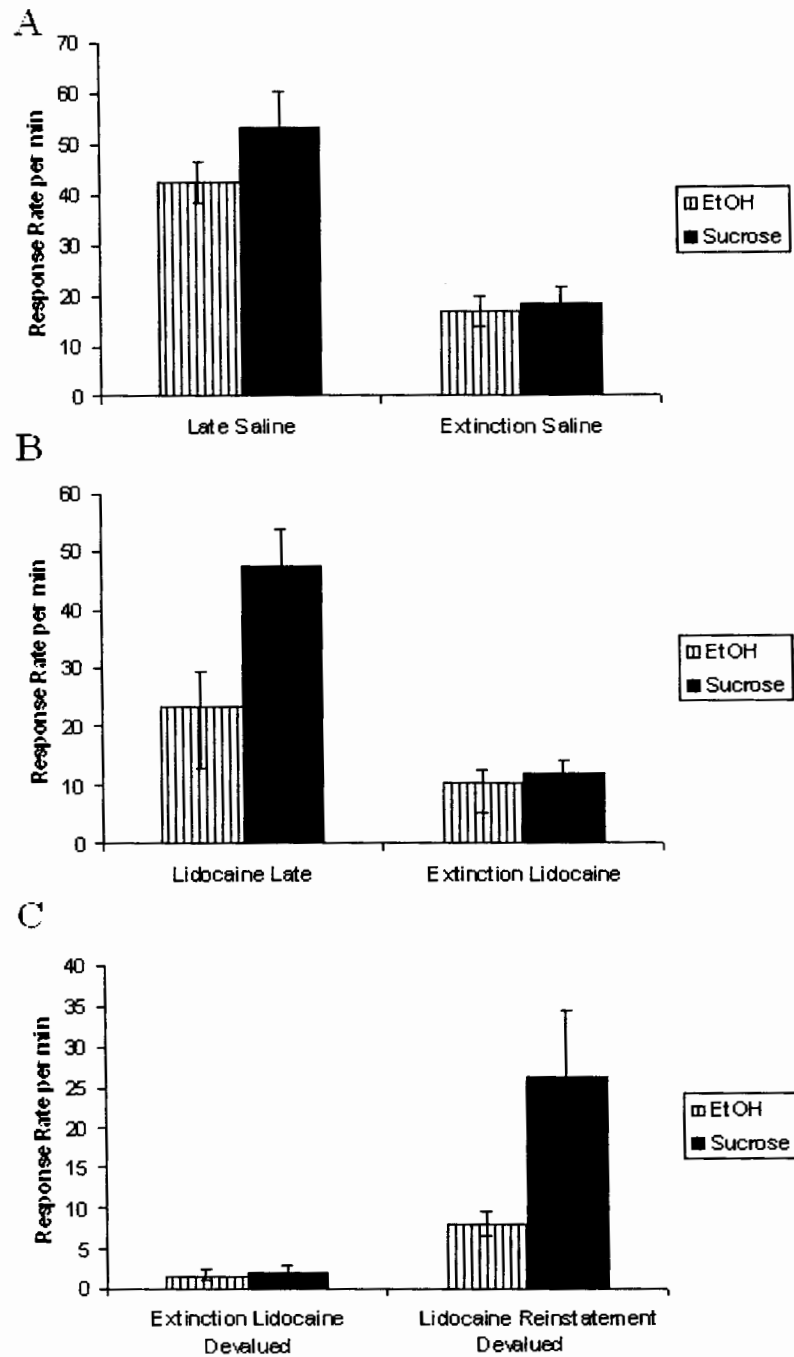


Figure 9. After extended training, mean operant measures of performance (+SE) comparing responding with and without (extinction) outcomes for: A. Late saline vs. Extinction saline B. Late Lidocaine vs. Extinction lidocaine C. Extinction lidocaine devalued vs. Extinction lidocaine reinstated.

Histology:

Figure 10 displays the location of projection cannulae that were 1mm ventral of guide cannulae. Although a few subjects were slightly more anterior than the target coordinates, all subjects cannulae were documented in the dorsolateral striatum.

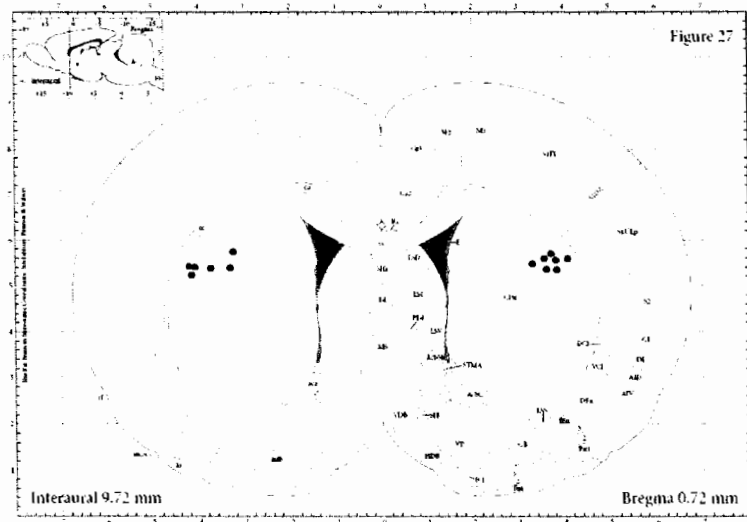
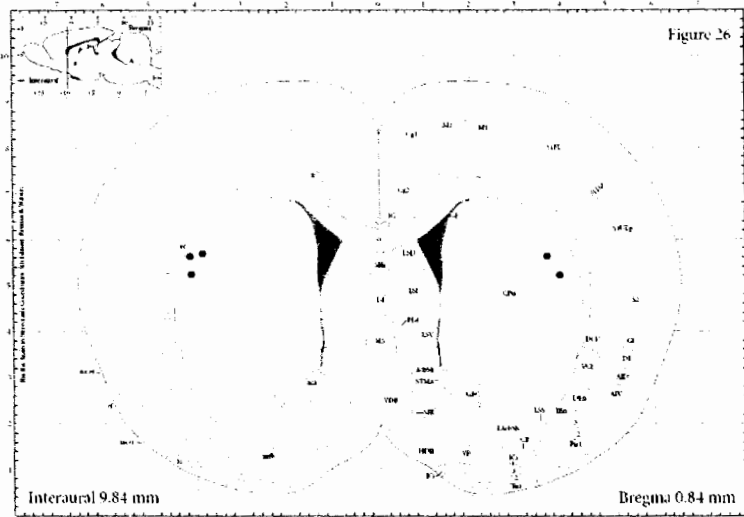
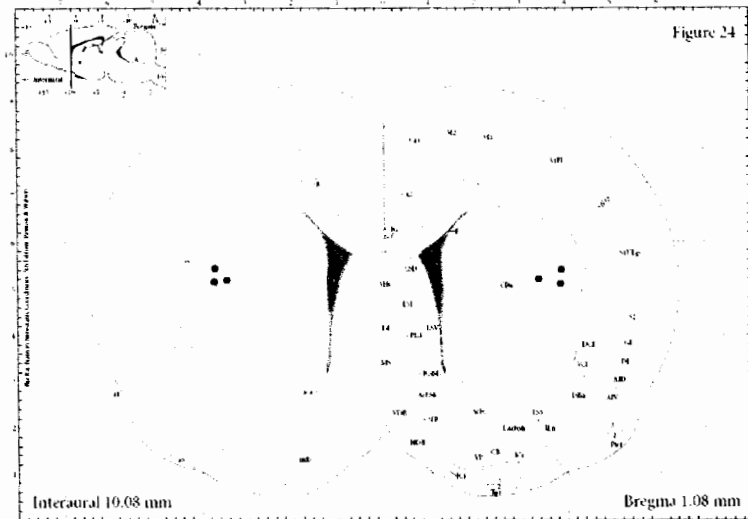


Figure 10. DLS projection cannulae placements of all subjects.

Discussion

Experimental groups were separated by presenting 10% EtOH to rats in the home cage. Half of the rats were designated to the EtOH group by their significantly higher consumption while the other half of the rats were designated to the sucrose group by their lower consumption of EtOH. This propensity by the EtOH group to consume EtOH solutions remained significantly higher than the sucrose group across the entire sucrose fading procedure. This suggests that there are natural select populations of rats that are more and less accepting of EtOH. This is in accordance to human research whereby some humans may be genetically predisposed for alcohol acceptance while others may more readily reject the substance (Prescott & Kendler, 1999).

It is important to note that many sucrose fading procedures do not proceed in the home cage (or when they do, offer a choice between the EtOH solution and water) and train rats to eventually respond for pure EtOH without sucrose (Samson, 1986; Files, Samson, & Denning, 1997; Samson, Files, & Denning, 1999). However, the fact that animals did respond at high rates for either sucrose or EtOH outcomes suggests that the modified sucrose fading procedure used in the present experiment can be successfully used in the home cage before operant training. On the other hand, there was no control group that did not receive the modified sucrose fading procedure. Therefore, we cannot state conclusively that the 10% EtOH/10% sucrose outcome used in this experiment required the modified sucrose fading procedure for animals to respond for the solution. The goals of the present experiment were to examine the gradual development of habitual responding. The standard Samson (1986) sucrose fading procedure uses numerous operant training sessions in which sucrose is faded out of an EtOH solution. As training

proceeds in the operant chamber to be extensive, responding has been shown to shift from outcome mediated responding to stimulus-bound habitual responding (Adams, 1982; Dickinson, 1985). Thus, the experimental goal of examining the transition between these responding mechanisms would be impossible to achieve if the sucrose fading procedure was in the operant chamber following the research of Samson (1986).

The possibility that previous EtOH exposure changed response rates in operant training and testing conditions cannot be discounted. In fact, acute and chronic EtOH exposure has indeed been shown to alter cognition by changing both hippocampus and striatum neurophysiology (for reviews and experiments, see Fadda & Rosetti, 1998; Mathews & Silver, 2004; Rothblat, Rubin, & Schneider, 2001). Therefore, it is possible that the differences in responding between EtOH and sucrose groups were due to altered striatum neurophysiology, but not likely. While the EtOH group did have more experience with EtOH as a result of training in the operant chamber, the maximum EtOH a rat could earn in a session (10ml) was considerably less than what was consumed in the sucrose fading procedure (37-49ml of the same solution, 10% EtOH/10% sucrose). The fact that both groups completed the sucrose fading procedure allows us to rule out the possibility of differences presented in the responding between experimental outcome groups. The sucrose fading procedure completed what it was intended to do, have an impact on future EtOH drinking patterns in the operant chamber (Files, Samson, & Denning, 1997). In addition, baseline results suggest that differences in responding may be more related to the qualities of the outcome for each group rather than the previous amount of EtOH exposure.

Baseline responding for EtOH was significantly lower than responding for sucrose after initial training. However, after extensive training, this difference was abolished and no differences in baseline responding were found throughout the remainder of the study. This was a basic assumption of the methodology chosen. Previous research has shown that after initial ratio schedule training, performance is flexible and goal-directed (Dickinson, 1985). The initial lower performance of EtOH group rats may reflect odor, hedonic, or caloric differences between outcomes that affect the R-O process, but not the S-R process. Indeed, extensive training abolished these group differences and likely reflects the induction of habitual responding (the S-R process, which has no outcome component). Extensive training with simple ratio schedules has been shown to be resistant to outcome devaluation and become habitual (Dickinson, 1985). Featherstone and McDonald (2004) found that DLS lesioned rats do not increase in response rate across training sessions in an S-R operant responding task. Moreover, Yin et al (2004) found that DLS lesioned rats are sensitive to outcome devaluation while sham controls are resistant after extensive training using an interval schedule. Thus, plasticity of the DLS is critical to the development of habitual responding over training sessions. Therefore, although not explicitly tested at this point, the disappearance of group differences by extensive training throughout the remainder of the study was assumably due to the instatement of habitual responding.

In phase 1, DLS inactivation reduced performance after initial and extensive training. However, performance was attenuated more so after extensive training (more dependent variables were affected by DLS inactivation late rather than early in training). Some labs have posited the DLS to be involved in habitual responding (Packard &

McGaugh, 1996; Featherstone & McDonald, 2004, 2005; Yin et al 2004) while other labs have posited the DLS to be involved in sensorimotor integration and not involved with habitual responding (Carelli, Wolske, & West, 1997). To aid in distinguishing the role of the DLS in habitual responding, this study offers information that partially agrees with both hypotheses.

DLS inactivation reduced performance after initial training, suggesting that the DLS is involved with sensorimotor integration learning. Yet, DLS inactivation reduced performance more so after extensive training, suggesting the DLS is involved with habitual responding as well. Classic habitual responding involves the gradual development of contextual and/or discriminative stimuli gaining control over responding. The fact that DLS inactivation reduced performance before but more so after extensive training, suggests that the DLS is involved in the sensorimotor (stimuli and motor response, S-R) integration learning and maintenance that becomes increasingly strengthened in habitual responding over training sessions.

Much research has used sucrose outcomes in operant conditioning studies. This is the first study to compare the role of the DLS in maintaining responding for sucrose or EtOH outcomes. Previous research suggested that rats responding for EtOH may be more prone to habitual responding than food (sucrose in the present experiment; Dickinson, Wood, & Smith, 2002). The present study's results, for the most part, are in accordance with this hypothesis. If responding for EtOH was more prone to habitual responding, DLS inactivation would have reduced EtOH group performance more than sucrose group performance after initial training. This effect was not found in the present experiment. Instead, performance of the EtOH group was reduced more so after extensive training

than after initial training. This attenuated performance in the EtOH group was also larger than the reduced performance of the sucrose group. This suggests that the maintenance of habitual responding instated after extensive training for an EtOH outcome may involve the DLS (shown here to be a critical factor in the maintenance of habitual responding) slightly more than sucrose outcome, although both were dependent. Dickinson, Wood, and Smith's (2002) hypothesis that responding for EtOH may be more prone to habitual responding cannot be discounted by the present experiment because it is possible that the early probe test was too early in training to find a similar effect as the late test. Multiple probe tests may be needed to increase the chance of observing the EtOH group transition from an action to habit. According to the present experiments results, however, responding for EtOH may not be more prone to habitual responding *per se*, but more dependent on habitual responding neural regions such as the DLS when habitual responding has already been instated.

The present study allowed for the development and plasticity of the DLS through extensive training and then inactivated the DLS during operant performance. This is because a functional DLS is critical in the acquisition of habitual responding (Featherstone & McDonald, 2004; Yin et al, 2004). In this sense, lesion studies that have confirmed the DLS to be involved in the *acquisition* of habitual responding (Featherstone & McDonald, 2004; Yin et al, 2004) can be compared with the present study that examined if the DLS is involved in the *maintenance* of habitual responding. The present study extends lesion literature in that the maintenance of habitual responding was affected by DLS inactivation after extensive training under reinforced (Phase 1) and

extinction conditions (Phase 2). Behaviorally however, only outcome devaluation can separate goal-directed from habitual responding.

Outcome devaluation has been the standard procedure for behaviorally separating the R-O or S-R processes in rats for decades (Adams & Dickinson, 1981a; Adams, 1982; Dickinson, 1985). Outcome devaluation significantly suppressed outcome consumption by both groups to 20% of baseline consumption. While devaluation has been shown to occur with sucrose outcomes (Adams, 1982; Dickinson, 1985; Yin et al, 2004), mixed results have been found when using EtOH outcomes. After devaluing an EtOH solution, Dickinson, Wood, and Smith (2002) found a resistance to devaluation while Samson and colleagues (2004) found sensitivity. Samson explains that major procedural differences such as the amount of EtOH ingested and availability to self-administer EtOH during devaluation training may explain this discrepancy. Samson and colleagues (2004) have been successful in training their animals to respond for free access to 20% EtOH for 20 minutes while Dickinson, Wood, and Smith (2002) trained animals to respond for multiple small quantities of 10% EtOH/sucrose mixture, similar to the present study (although a simple schedule of reinforcement was used and not concurrent).

If the DLS is involved in maintaining habitual responding, then functionally inactivating the DLS with lidocaine infusion should show a reduced response for outcomes paired with LiCl because the R-O process has been minimized and will not maintain responding. Ideally, an unpaired control group not receiving devaluation would be included in the design of the experiment to show that differences in responding are attributed to the expectation of a specific devalued outcome rather than to the induced sickness by LiCl in the outcome devaluation procedure. Given the small number of

subjects however, this control was not possible. Previous experiments have shown outcome devaluation to reduce operant performance in DLS lesioned rats under extinction conditions but not in sham lesioned rats (Yin et al, 2004). In the present experiment, devaluation significantly reduced DLS inactivated performance under extinction (phase 2) and reinforced responding (phase 2 reinstatement) conditions, supporting and extending DLS lesion literature to include the maintenance of habitual responding, respectively. Prior to the outcome devaluation procedure, the rats were extensively trained in operant responding that likely produced habitual responding. Outcome devaluation was used because group responding differences may appear if the R-O system controls responding after DLS inactivation. These differences between outcome groups were not expected because both groups received extensive training, when habitual responding is likely to occur. A difference would suggest that habitual responding is different between the two outcomes. No differences were found, suggesting a similar habitual responding system, possibly mediated by the DLS controls responding after extended training for both sucrose and EtOH. Yet, because this study lacked a devaluation control, a complete theory of habitual responding and devaluation cannot be made.

A “switching systems” hypothesis maintains that either the R-O or habitual S-R process can control responding. Thus, if one process is controlling behavior, the other cannot. If a specific region of the brain controls responding and is temporarily deactivated, then the other process, presumably controlled by a disparate region, should take over responding. To minimize any possible “switching”, outcome devaluation was used in the present study. In accordance, a “switching systems” explanation of results

(from S-R to R-O) may have been observed because DLS inactivation reduced responding following extensive training and outcome devaluation. It must be noted though, that no saline condition tested the corollary that a functional DLS will be resistant to outcome devaluation. However, previous research has shown that extensively trained rats responding for sucrose (Yin et al, 2004) or EtOH (Dickinson, Wood, & Smith, 2002) with a functional DLS, are resistant to outcome devaluation. Thus, while a “switching systems” hypothesis cannot be fully evaluated, the present results are encouraging.

Evidence that DLS inactivation affected EtOH more than sucrose habitual responding was slight (phase 1 late test session length and phase 2 reinstatement test for total session responses). It is possible that the DLS may not have any more of a role in EtOH habitual responding maintenance than sucrose, although not likely. Interestingly, differences between outcome groups were only seen when the animals were free to respond when the outcome was present (phases 1 and 2 (reinstatement)) and not under extinction (phase 2) where no outcome was present. Since extinction sessions do not offer the opportunity to earn outcomes and no differences were found in these sessions, motivational or incentive explanations of responding cannot be attributed. In addition, VR schedules of reinforcement, as used in the present experiment, are a reliable quantitative measure of the hedonic strength of an outcome (Vigorito, Kruse, & Caretta, 1994). This suggests that differences in responding may be attributed to the odor, hedonic, drug, and/or caloric differences between sucrose and EtOH. This explanation cannot be discounted and there is additional evidence in support of this explanation.

Although goal-dependent explanations of alcohol-seeking behavior in humans have been hypothesized (Sheeran et al, 2005), Pavlovian cues have been shown to modulate alcohol-seeking behavior (Glasner, Overmier, & Balleine, 2005). These authors explain that these cues elicit an excitatory influence on the general motivational arousal to respond for EtOH. In addition, performance did not depend on the consequences of responding actions, a hallmark of habitual responding. This effect has also been shown by a resistance to outcome devaluation after extensively training alcohol-seeking behavior in rats (Dickinson, Wood, & Smith, 2002). Therefore, stimulus control of responding without regard to the outcome may be critical for maintaining alcohol-seeking habitual behavior. Since differences between outcome groups were only seen when the outcome was present, it is plausible that odor, hedonic, drug, and/or caloric cues provided by EtOH are the stimuli that control responding in habitual responding for EtOH. These stimuli may activate the DLS which in turn may increase habitual responding. Therefore, results of the present experiment support a role for the acquisition and maintenance of alcohol-seeking and habitual behavior by the DLS.

In accordance with this hypothesis, EtOH cues may be considered more effective stimuli that control habitual responding than sucrose cues. EtOH intake causes physiological, caloric, drug, and hedonic effects that are not present in sucrose intake. Additionally, the fact that Dickinson, Wood, and Smith (2002) found resistance to EtOH devaluation while Samson and colleagues (2004) found sensitivity may suggest that low levels of EtOH self-administration, rather than Samson's high levels, or the use of EtOH/sucrose mixture, is sufficient for the establishment of habitual responding. The present experiment utilized this hypothesis and found sensitivity to outcome devaluation

while the DLS was inactivated. Therefore, the DLS may be more responsive to EtOH odor, hedonic, caloric, and/or drug cues that motivate habitual responding. Since there was no manipulated discriminative stimulus in the present experiment, environmental cues, such as the inherent qualities of EtOH, likely controlled habitual responding. This hypothesis seems ideal after the extensive EtOH exposure in the sucrose fading procedure. As a result of the sucrose fading procedure, the animals responding for EtOH may have been sensitized to the characteristics of EtOH such as odor, taste, and its effects on the body, inciting an excitatory influence on the general motivational arousal to respond for EtOH that existed in habitual responding (Glasner, Overmier, & Balleine, 2005). This may be similar to animal (Pickering & Liljequist, 2003) or human addicts (Petrakis et al, 2001) that relapse in the presence of Pavlovian cues from the substance (e.g. scent of alcohol; Robinson & Berridge, 2003). However, the corollary of resistance to outcome devaluation while the DLS was active was not tested and a complete theory cannot be made at the present time. On the other hand, it seems clear that future neurological investigations of alcohol-seeking behavior will need to test subjects under normal conditions, as opposed to extinction. If the odor, hedonic, caloric, and/or drug qualities of EtOH are the stimuli that motivate habitual responding, then extinction tests are simply not examining habitual responding.

The idea that the striatum is involved in the maintenance of habits is not new, as when post-learning striatum lesions are made, animals have difficulty remembering how to execute a task (Graybiel, 1995). However, it is important to note that Carelli, Wolske, and West (1997) found electrophysiological data that seems to contradict the present study's findings. For example, dorsolateral striatum movement-associated neurons of the

responding contralateral forepaw were found to be responsive after initial training but not after extensive training. Although not explicitly tested with outcome devaluation, behavioral performance significantly improved over training sessions similar to the present study. Therefore, the West lab posited that the dorsolateral striatum is involved in the acquisition of habitual learning through sensorimotor integration, but not in its maintenance. Differences between the West lab's and the present study's results were likely due to the environmental contingencies and methodologies chosen. The West lab trained rats to place their forepaw on a white piece of tape for a stable measure of neural activity. Subsequently, a discriminative stimulus tone was activated and the rat was required to press the lever once for the final outcome. This chain of events required for outcomes is different from the present study's contingency. First, the West lab's use of a chain of events with a discriminative stimulus to acquire an outcome may require different neural activation than the simple VR 5 schedule with no discriminative stimulus used in the present experiment. Second, the present study compared sucrose and EtOH outcome responding in food-deprived rats while the West lab used water outcome with water-deprived rats. Third, dopamine release in the striatum, believed to be critical for the maintenance and persistence of habits (Faure, Haberland, Conde, & Massioui, 2005), has been shown to be dependent on the schedule of reinforcement (Neill, Fenton, & Justice, Jr., 2002). Rats responding for intracranial self-stimulation outcome with a FR10 schedule of reinforcement show higher dopamine and dopamine metabolite concentrations in the striatum than rats using a CRF schedule of reinforcement. Finally, the CRF schedule of reinforcement used by the West lab has a one-to-one response-outcome correlation. Extensive training using ratio schedules has been shown to produce

habitual responding because animals no longer experience the correlation between variations in performance and variations in the associated consequences during extensive training (Dickinson, 1985). Although extensive training using a CRF schedule can show a resistance to devaluation, extensive training on a VR schedule produces more resistance to devaluation than a CRF schedule (Adams, 1982). A simple habitual responding task may involve the DLS more than a more complicated habitual responding task. Thus, the decreased responsiveness of DLS neurons may reflect that the West lab may have had other brain regions possibly aiding in habitual responding. A testimony to how complex habitual responding can be.

Colwill and Triola (2002) also found behavioral evidence contradicting with the results of the present study. In their study, extensive training of instrumental responses did not become habitual with extensive training. Procedural differences between the experiments may explain these differences. Colwill and Triola (2002) used a concurrent schedule of reinforcement with two distinct discriminative stimuli, two distinct responses, and two distinct outcomes. One or both of these responses were extensively trained and transfer tests were conducted to test the discriminative stimuli with respective responses. Dickinson (1989) has explained that the concurrent schedule of reinforcement allows for the opportunity to learn strong R-O correlations that do not exist in simple schedules (used in the present study). The subject is free to change back and forth between response alternatives; hence, concurrent schedules are popular for studying choice behavior (Domjan & Burkhard, 1986). The present study allowed rats to earn only one outcome and thus, choice was nonexistent. In addition, Holland (2004) provided direct support for the assumption that multiple outcomes in concurrent schedules control instrumental

responding differently than simple schedules that utilize one outcome. Moreover, Colwill and Triola (2002) also discovered that the transfer test may not be able to distinguish R-O strength because it was affected by thirst modulation rather than training parameters. The fact that Dickinson, Wood, and Smith (2002) found resistance to outcome devaluation for EtOH in a concurrent schedule makes their observations more impressive.

The striatum has been implicated to be involved in habitual learning and responding because Parkinson patients that have degenerated dopaminergic input to the striatum show impairments in habitual learning, memory, and motor tasks (Graybiel, 1998). Evidence, however, suggests the striatum is also involved in habitual responding of cognitive disorders such as obsessive-compulsive disorder, which involves cognitive habits (e.g. obsessions) as well as motor habits (e.g. compulsions) (Baxter et al, 1992; Graybiel & Rauch, 2000), and Gilles de la Tourette's syndrome (Saka & Graybiel, 2003). Thus, the striatum seems to be part of a cognitive and motor habitual responding system. Graybiel (2000), Packard and Teather (1998), and Coutureau and Killcross (2003) suggest that the loops interconnecting the neocortex, striatum, amygdala, prefrontal cortex, and dopaminergic cells in the midbrain may be part of this learning and memory system. The contribution of the dorsal striatum in habitual responding has become a hot topic in recent years and the present study suggests that more research is needed to understand habitual behavior.

The present study has implications to the study of habitual behavior in general. Habitual behavior is a hallmark of most psychological illnesses and drug abuse. This is the first study to examine the role of the DLS, a structure strongly associated with habitual behavior, with EtOH. The results suggest that alcohol-seeking behavior in rats

may involve a common mechanism with standard habitual behavior. In addition, the combination of reduced DLS activity with outcome devaluation may constitute a novel treatment for habitual behavior, especially alcohol-seeking habitual behavior. Future research is needed to explore these possibilities.

Conclusion

The present study allowed the development of habitual responding through extensive training on a VR schedule of reinforcement (Adams, 1982). The DLS was inactivated with an intracranial injection of lidocaine after initial training, extended training, and outcome devaluation. Additionally, differences between responding for EtOH and sucrose were examined. The DLS was found to be important for responding in general, after both initial and extensive training, but more so after extensive training. This suggests that the role of the DLS is sensorimotor integration, as previously theorized (Carelli, Wolke, & West, 1997), but function of the DLS may become strengthened over training sessions to possibly maintain habitual responding (integrating stimuli with the response, S-R habitual responding). In addition, responding for EtOH was attenuated more than responding for sucrose when the DLS was inactivated after extended training. The fact that this effect was only found when the outcomes were present and never under extinction suggests that future research of the neural basis of alcohol-seeking behavior will need to investigate responding with EtOH present. Further, the present experiment's results provide information on Dickinson and colleagues' (2002) hypothesis that responding for EtOH may be more prone to habitual responding than responding for sucrose. First, in support of this hypothesis, odor, hedonic, physiological, and/or caloric cues provided by EtOH may be the stimuli that activate the DLS and maintain habitual responding for EtOH after extensive training. Second, in contrast, responding for EtOH may not be more prone to habitual responding than sucrose *per se*, but more dependent on habitual responding neural regions such as the DLS when habitual responding has already been instated. However, an encompassing theory of the role of the DLS in

habitual responding cannot be made at this time due to the present studies lack of outcome devaluation and saline condition control groups after outcome devaluation. Although DLS inactivation produced sensitivity to outcome devaluation after extensive training and previous research demonstrates an active DLS produces resistance to outcome devaluation after extensive training with sucrose (Yin et al, 2004) and EtOH (Dickinson, Wood, & Smith, 2002) outcomes, the lack of the aforementioned control groups does not allow the chance to test if the corollary, a functional DLS will be resistant to outcome devaluation, would be found in the present study.

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