ROLE OF THE NMDA RECEPTOR IN

CONSUMMATORY SUCCESSIVE NEGATIVE CONTRAST

by

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Introduction

Consummatory successive negative contrast (cSNC) is the abrupt disruption of consummatory behavior by an experimental group of animals after experiencing a downshift from a higher to a lower incentive value. Consummatory behavior in downshifted animals falls below the level of a control group that has received the lower incentive during a period of acquisition (Vogel, Mikulka, & Spear, 1968). More specifically, the term "contrast" refers to the difference in performance between downshifted animals and unshifted control animals, deriving its name from the perceptual contrast literature. In these experiments, the incentives are usually sucrose solutions of different concentrations (e. g., 32% and 4% solutions). The consummatory behavior of drinking during acquisition and the avoidance of the incentive after the downshift may be viewed as examples of Pavlovian conditioning. In this framework, approach and drinking are examples of appetitive conditioned and unconditioned responses, respectively, whereas the avoidance behavior is an example of an aversive conditioned response (Wasserman, Franklin, & Hearst, 1974; Papini & White, 1994). Using this simple perspective, one can ask seemingly complex questions about cSNC, breaking complicated phenomena into components easier to study. Previous research using this approach has focused primarily on behavioral and neurochemical processes occurring during reward downshift and subsequent recovery from cSNC (Flaherty, 1996; Papini, 2006). The goal of this research is to expand on these approaches by attempting to better characterize the memory processes involved in cSNC. Specifically, these experiments are designed to assess the role of the glutamate receptor *N*-methyl-D-aspartate (NMDA), crucial for Pavlovian learning and associative mnemonic processes, in the cSNC effect.

Pavlovian Conditioning and Extinction

Pavlovian conditioning is a basic associative process that forms the backbone of current research on the neuroscience of learning and memory. In Pavlovian or classical conditioning, an initially novel stimulus that elicits little more than an orienting response is paired with a stimulus that elicits a strong response (Pavlov, 1927). After several such pairings the novel stimulus begins to elicit a new response. The initially novel stimulus is called the conditioned stimulus (CS). The response that comes to be made to the CS as the result of the pairing process is called the conditioned response (CR). The stimulus that elicits the strong response with little or no explicit training is called the unconditioned stimulus (US) and the response that occurs to that stimulus is called the unconditioned response (UR).

Pavlov (1927) noted that salivation (UR) occurred after the presentation of food to the mouth of the dog (US). This demonstrates the unconditioned power of the food to induce salivation. Pavlov also presented the US shortly after presenting the initially novel stimulus of a metronome sound. After several trials of pairing the metronome sound with the presentation of food, the sound of the metronome elicited a similar amount of salivation as the food itself. The metronome came to serve as a CS capable of eliciting the CR of salivation from the dog.

In the same way that an animal shows learning about the signaling properties of the CS, an animal can also learn that a CS no longer signals the US. This is called extinction. The term “extinction” has three different meanings: as a procedure, as a result, and as an explanation (Rescorla, 2004). First, as a procedure, extinction refers to the post-acquisition technique of omitting the US after the presentation of the CS. The CS is presented alone so that it no longer signals the outcome. Second, as a result, extinction refers to the decrease in the CR that occurs when the US is omitted. Third, as an explanatory construct, extinction acts as an intervening

variable explaining the observed CR deterioration after a CS is presented alone. Another aspect of extinction is that the response decrement appears to reflect new learning, rather than simply the forgetting or *erasure* of the CS-US association (Bouton, 2004; Rescorla, 2004).

While the aforementioned training is appetitive, Pavlovian conditioning is not necessarily always appetitive. Subjects may undergo training with aversive stimuli, as in fear conditioning. Fear conditioning is a type of learning demonstrated after the repeated presentation of a CS, such as tone or light, followed by the presentation of the US, such as a physically painful stimulus induced by electric footshock. Over the course of repeated CS-US pairings, the subject's behavior changes from pain-elicited behaviors in reaction to the painful US, such as jumping, to fear-elicited behaviors, such as freezing to the CS. In essence, the CS (tone) elicits a CR (freezing), as the subject learns a CS-US association. Similar to appetitive conditioning, fear conditioning can also be extinguished if the CS is presented alone. Behaviorally, the CR decreases across CS-alone trials. In the case of fear conditioning, the fear-elicited behaviors, such as freezing, decrease as the animal is exposed to tone-only trials, indicating that learning is occurring. Presumably, the extinction experience is also encoded into memory in the brain.

Contemporary learning theorists have developed the concept of Pavlovian conditioning, utilizing theoretical frameworks that extend beyond the simplistic notion of conditioned reflexes (Rescorla, 1988). The Rescorla-Wagner model (Rescorla & Wagner, 1972) suggests that learning occurs where the US is surprising, whereas other models emphasize attentional processes directed at the CS (Mackintosh, 1975; Pearce & Hall, 1980). While each perspective provides an insight into these associative processes, their proliferation and specialized explanation for particular phenomena, restricts their general application. Even after more than a century, Pavlov's influential (1927) viewpoint remains firmly entrenched.

Allocentric and Egocentric Learning

Conditioning processes serve to track changes in the environment and respond appropriately (Papini, 2008). In appetitive scenarios, conditioning promotes behavior that helps the animal take advantage of available resources (e.g. food, water). In aversive scenarios, conditioning promotes behavior that aids the animal in responding to immediate physical threats. In both scenarios, animals learn to detect differences and update their representation of the physical environment accordingly. Such Pavlovian conditioning is called *allocentric learning*. Concurrent with allocentric learning, significant events can produce an emotional evaluation with hedonic content. Theoretically, this emotion-based evaluation can also undergo conditioning. This is called *egocentric learning*. Egocentric learning refers to the animal's ability to learn about its own emotional reaction in response to an environmental change (Papini, 2003). Together these two forms of learning allow the animal to construct a cognitive and emotive representation. Using fear conditioning as an example, allocentric learning involves the anticipation of pain, whereas egocentric learning involves the aversive tagging of such anticipation.

Memory for Fear Conditioning

Initially relegated to the realm of abstract construction, psychologists were puzzled as to how Pavlovian phenomena, such as fear conditioning, were encoded into memory in the brain. Over the course of acquisition training, how does the brain encode the CS-US associations? Over the course of extinction training, how does the brain encode the CS-no US association?

Associative Long-Term Potentiation

An early model of memory encoding was proposed by Hebb (1949). Hebb suggested that synaptic conduction is strengthened when a presynaptic neuron is activated simultaneously with

activation of the postsynaptic neuron. Hebb's idea can be expanded to various inputs acting in coordination into a coincidence detector to simulate Pavlovian conditioning. In abstract terms, when two weak inputs (A and B) and one strong input (C) are directed to a coincidence detector, and A and C are activated at the same time, A's connection to the coincidence detector is strengthened, but B's is not. However, when B is activated by itself, its strength is not modified. Subsequently, whenever input A is activated, a response occurs, that was once only elicited by the strong input from C. Applied to Pavlovian fear conditioning, the weak visual input (A) from the light and the strong input from the pain (C) occurring at about the same time strengthen the light´s ability to elicit a response similar to that elicited by the pain, without affecting the weak input from B, such as a tone, unpaired with C.

First described in the perforant pathways of the hippocampus (Bliss & Collingridge, 1993; Bliss & Lomo, 1973) and later discovered to be present in the amygdala (Chapman, Karriss, Keenan, & Brown, 1990; Rogan & LeDoux, 1995), a region critical for fear conditioning (Blanchard & Blanchard, 1967), long-term potentiation (LTP) was the first step toward the physiological confirmation of Hebb's idea. *In vitro* LTP can be induced in the following manner (Figure 1). In a pretest, a single pulse is presented to the presynaptic cell using a stimulating electrode. At the same time, a recording electrode located on the postsynaptic cell measures a baseline excitatory postsynaptic potential (EPSP). In the "training" phase, the stimulating electrode sends a train of pulses to the presynaptic cell (e.g., 100 pulses in one second). In a posttest, again the stimulating electrode sends a single pulse to the presynaptic cell. The recording electrode on the postsynaptic cell measures the EPSP. When LTP is induced, the amplitude of posttest EPSP is greater than the pretest EPSP. *In vivo* LTP requires a combination of a train of pulses produced by the presynaptic cell concurrent with activation in the postsynaptic cell.

While LTP serves as a useful model to understand how synapses are strengthened with repeated stimulation, its induction by a single input differentiates LTP from a typical associative learning situation. The findings of Bliss and Lomo (1973) were extended by McNaugthon, Douglas, and Goddard (1978) who showed that multiple inputs can produce an LTP-like effect on a postsynaptic system. In an associative LTP preparation, a single pulse is presented to presynaptic cell A (weak input) using a stimulating electrode during a pretest. At the same time, a recording electrode located on the postsynaptic cell measures a baseline EPSP, similar to LTP. However, in the training phase, cell A is active when presynaptic cell B (strong input) receives a train of pulses from a stimulating electrode (e.g., 100 pulses in 1 s). In a posttest, again presynaptic cell A receives a single pulse of stimulation. The recording electrode on the postsynaptic cell measures the EPSP. Similar to LTP, when associative LTP is induced, the amplitude of the posttest EPSP after stimulation of A is greater than the pretest EPSP.

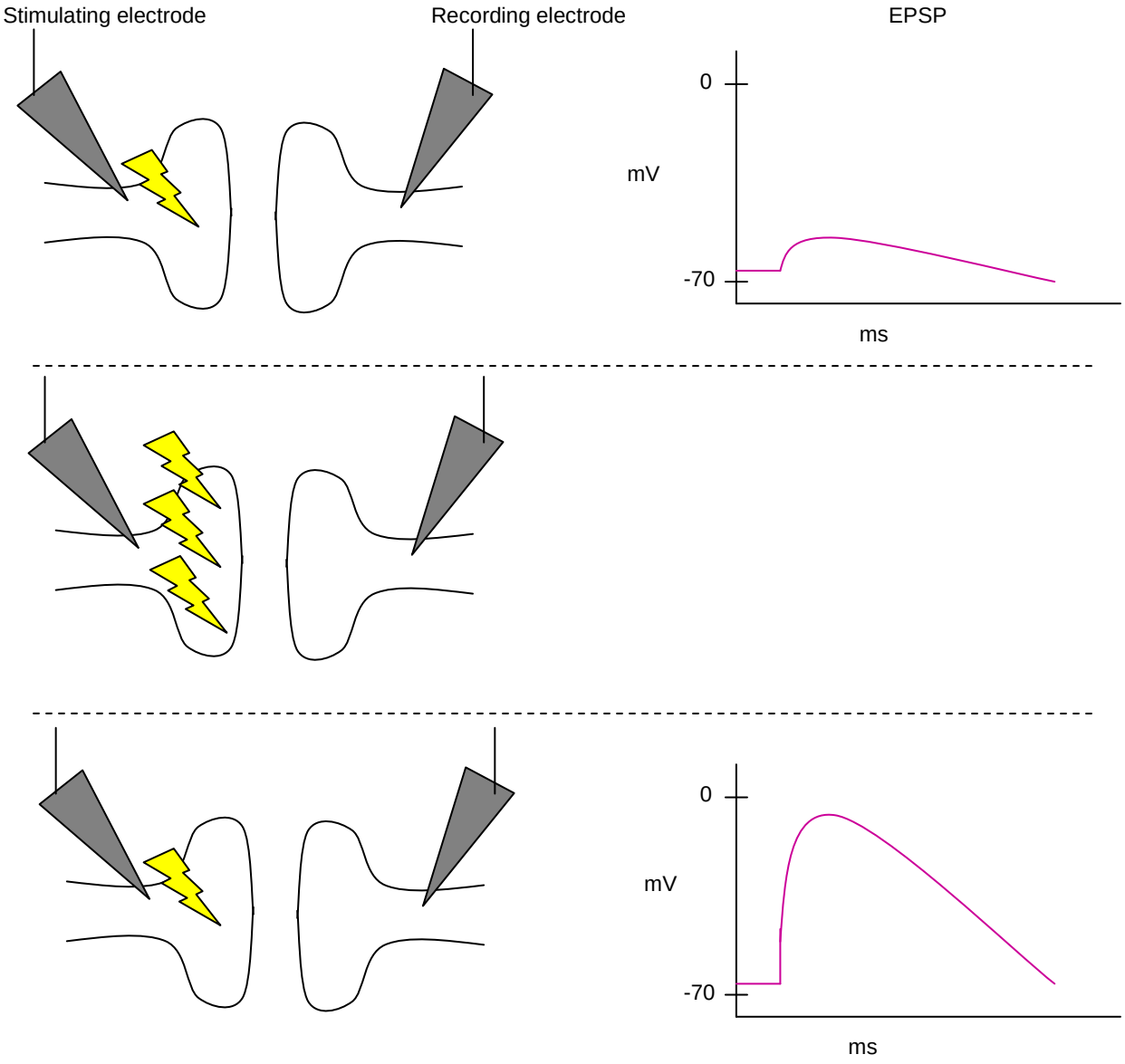


Figure 1. Schematic diagram of *in vitro* long-term potentiation (LTP). In a pretest (top portion), a stimulating electrode sends a single pulse onto the presynaptic cell. A recording electrode measures the resulting EPSP (represented on the right-hand portion). In training (middle portion), the stimulating electrode sends a train of pulses onto the presynaptic cell. In the posttest (lower portion), again the stimulating electrode sends a single pulse onto the presynaptic cell. The recording electrode measures the resulting EPSP. In the posttest, the EPSP is much greater, indicating a change in the postsynaptic cell (adapted from Gazzaniga, Ivry, & Mangun, 1998).

Associative LTP is attractive as a model of memory storage because it provides plasticity without sacrificing stability. It provides plasticity in the sense that it is associative. Thus, small, concurrent activations can result in relatively permanent synaptic changes in a network of neurons. Associative LTP provides stability in that synapses can be modified independently of each other. For example, unstimulated neurons and weakly stimulated neurons in isolation are not strengthened. This makes the neuron network system stable and resistant to random fluctuations in membrane potentiation (Lisman, 2007). While LTP's function is not universally agreed upon, as some LTP research is also consistent with nonmnemonic accounts (Shors & Matzel, 1997), it appears to be generally considered as a strong candidate for a cellular model of memory.

If an LTP-like process occurs during learning and memory, then it must be instantiated in the brain by neurotransmitter activity at synapses (which act to convey electrochemical activity between neurons). Various neurotransmitters have been shown to mediate memory, including serotonin (Meneses & Hong, 1997), acetylcholine (Markevich, Scorsa, Dawe, Stephenson, 1997; Warburton, 1972), and GABA (gamma-aminobutyric acid; Izquierdo, & Medina, 1991). Some neurotransmitters, such as acetylcholine (Auerbach, & Segal, 1994) and GABA (Platt, & Withington, 1998), are able to induce LTP. However, most LTP research has focused on glutamate. One type of glutamate receptor, the *N*-methyl-D-aspartate (NMDA) receptor, possesses the qualities necessary to depolarize the postsynaptic cell without causing an EPSP in the absence of further stimulation.

NMDA Receptors

NMDA receptors along with α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, and kainate receptors, are the major glutamate excitatory neurotransmitter

receptors in the brain. Unlike the AMPA receptor that provides the initial fast component responsible for excitatory transmission, the NMDA receptor's slower activation enables it to serve a crucial role as a coincidence detector, potentially encoding associations in the brain. The NMDA receptor is capable of this task because, unlike other receptors, it is voltage-gated and ligand-gated (see below), and modulated by a coagonist in order for NMDA receptors to allow calcium ion (Ca^{2+}) influx to occur. This has the net effect of inducing a series of changes in glutamate receptor activity as illustrated in Figure 2 (Cull-Candy, 2007).

Initially glutamate released from the presynaptic terminal will bind to AMPA receptors on the postsynaptic terminal. In turn, AMPA receptors will allow for sodium (Na^{+}) influx, which depolarizes the membrane. Membrane depolarization induces a series of processes leading to the release of a magnesium (Mg^{2+}) block that obstructs ion flow through the NMDA receptor channel. This is the reason why NMDA receptors are said to be voltage-gated, that is, their activation occurs only when the neuron is depolarized. If another release of glutamate occurs from the presynaptic terminal within a short time period (mirroring the strong train of tetanic input from the presynaptic neuron as modeled in LTP) glutamate will bind to the NMDA receptor site. This is why NMDA receptors are said to be also ligand-gated. While meeting these two requirements should provide for NMDA receptor activation, optimum activation is achieved through the additional binding of a co-agonist, such as glycine or D-serine to the glycine binding site of the NMDA receptor. While not entirely understood, it has been proposed that glycine is released from nerve terminals or glial cells in the presence of high levels of extracellular Ca^{2+}, as would be seen during LTP prior to the rapid influx of Ca^{2+} into the postsynaptic cell. The action of the co-agonist would therefore serve as another layer of coincidence detection.

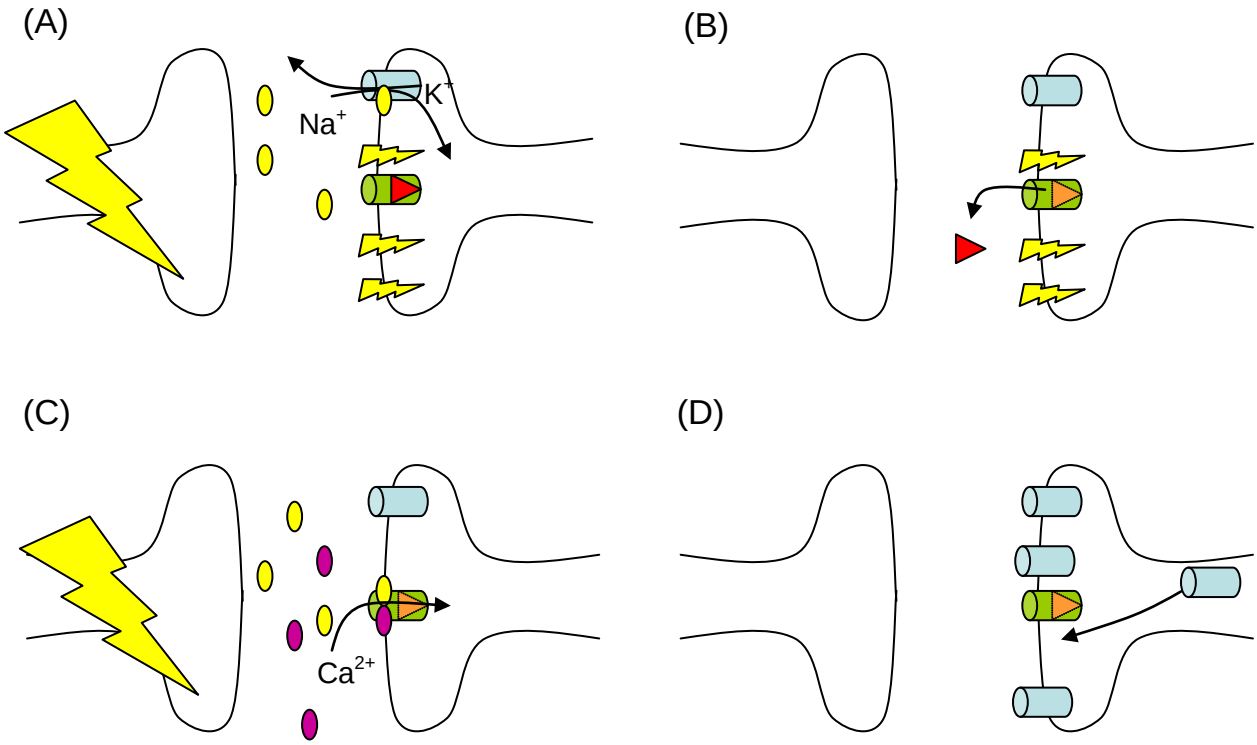


Figure 2. Schematic representation of the NMDA receptor activation and LTP-induction. (A) An action potential (big lightening bolt) from the presynaptic neuron (left) releases glutamate (yellow ovals) into the cleft, binding to an AMPA receptor (blue tube). In turn, the AMPA receptor allows Na^+ influx and K^+ efflux, depolarizing the membrane (small lightening bolts). (B) When the membrane is depolarized, the Mg^{2+} block (red triangle) is released from the pore domain of the NMDA receptor (green tube). (C) Another action potential from the presynaptic neuron releases glutamate into the cleft and there extracellular-glycine (violet ovals) is present. Glutamate and glycine bind to the NMDA receptors at the agonist binding domains on NR2 and NR1 subunits, respectively. This allows extracelluar Ca^{2+} influx, (D) inducing a cascade of changes that lead to increased numbers of AMPA receptors in the postsynaptic cell. These changes in receptor density make the synapse more excitable (adapted from Gazzaniga et al., 1998).

In order to turn the increased synaptic transmission into long-term changes, a within-cell mechanism must exist to solidify the increased synaptic sensitivity. Ca^{2+} influx activates a series of changes in gene expression that lead to a redistribution of AMPA receptors away from nonactive synapses and towards the activated synapse, phosphorylation of AMPA receptors and/or the insertion of additional AMPA receptors in the activated synapse. These changes produce the net effect of increasing the likelihood of an action potential in the postsynaptic cell. This strengthens the association between active neurons and, in theory, strengthens learned associations (Lisman, 2007).

NMDA Receptors and Fear Conditioning

Conclusions about the role of NMDA receptors in fear conditioning are made possible because of techniques based on *in vivo* models. Scientists administer pharmacological agents known to modify NMDA receptor activity *in vitro* to living animals and measure behavioral differences that would indicate memory alteration. Not surprisingly, in order to be activated, NMDA receptor channels must act in a concerted fashion, bound by agonists or co-agonists simultaneously. Therefore, pharmacological alterations (summarized in Figure 3) to any part of the NMDA receptor alter LTP's occurrence and induces behavioral changes, relative to controls.

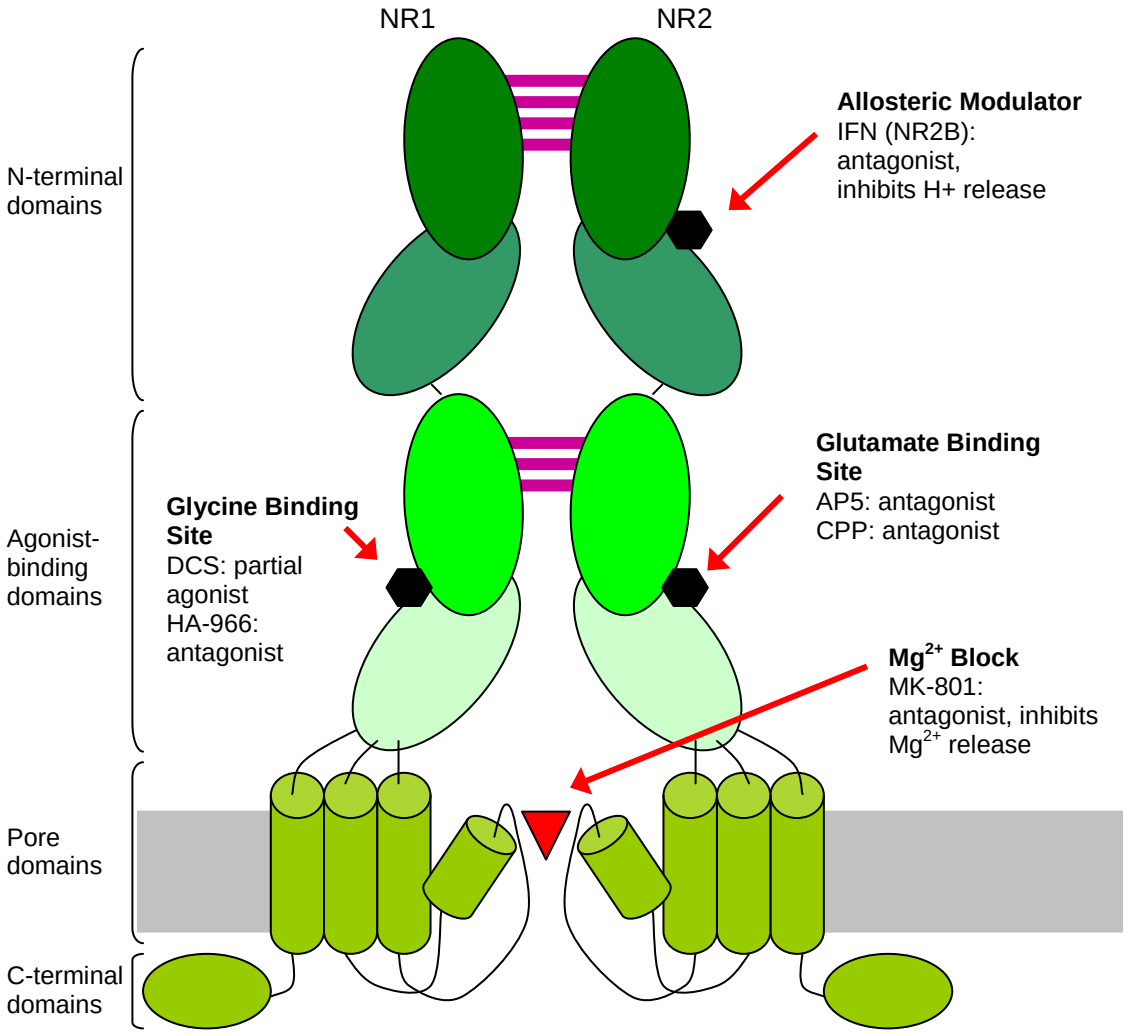


Figure 3. Schematic diagram of the NMDA receptor and sites of drug action. Various pharmacological manipulations have shown to affect fear conditioning, acting at various sites on the NMDA receptor. Generally, antagonists block the acquisition of fear and retard fear extinction. Agonists, generally, potentiate learning. IFN, an antagonist with an affinity for NR-2B containing NMDA receptors, blocks the release of H^+ which inhibits NMDA depolarization. DCS, a partial antagonist, and HA-966, an antagonist, bind to the glycine site on the NR1 subunit, common to all NMDA receptors. AP5 and CPP, both antagonists, bind to the glutamate site. MK-801, a partial antagonist, acts in the pore domain, preventing the release of the Mg^{2+} (adapted from Paoletti & Neyton, 2007; Cull-Candy, 2007).

For example, preventing the removal of the Mg^{2+} following initial membrane depolarization disrupts LTP. Systemically administering the NMDA receptor antagonist MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclo-hepten-5,10-imine maleate, prevents fear extinction and fear reinstatement when administered prior to extinction trials, using a light or an auditory clicker as CS (Baker & Azorlosa, 1996; Johnson, Baker, & Azorlosa, 2000; Lee, Milton, & Everitt, 2006). Blocking glutamate binding on the NR2 subunit of the NMDA receptor disrupts LTP (Cull-Candy, 2007). The NMDA receptor antagonist AP5, DL-2-amino-5-phosphonopentanoic acid, blocks acquisition and consolidation of fear, measured in the fear-potentiated startle preparation, when infused directly into the amygdala (Campeau, Miserendino, & Davis, 1992; Walker & Davis, 2000). The nonsubunit-selective NMDA receptor antagonist CPP, (7)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, administered systemically impairs extinction retention, but not acquisition (Santini, Muller, & Quirk, 2001; Suzuki, Josselyn, Frankland, Masushige, Silva, & Kida, 2004).

There are pharmacological manipulations that alter activity at the glycine binding site on the NR1 subunit (common to all NMDA receptors). HA-966, an antagonist, prevents fear extinction and blocks the enhancing effect of corticosterone on fear extinction (Yang, Chao, Ro, Wo, & Lu, 2007). D-cycloserine (DCS), a partial agonist at the glycine binding site of the NMDA receptor, facilitates extinction learning of a Pavlovian fear response when administered pretrial or immediately posttrial, systemically or infused into the amygdala (Ledgerwood, Richardson, & Cranney, 2003; Lee et al., 2006; Walker, Ressler, Lu, & Davis, 2002), reduces reinstatement following extinction (Ledgerwood, Richardson, & Cranney, 2004), and increases generalization of extinction to a second nonextinguished CS (Ledgerwood, Richardson, & Cranney, 2005). Generally, after receiving DCS, animals exhibited less freezing behavior compared to both saline and nonextinguished controls. DCS in subthreshold amounts acts

synergistically with subthreshold amounts of corticosterone to enhance fear extinction learning (Yang et al., 2007). However, in a preparation that replicated DCS's effect on extinction, DCS failed to prevent the contextual renewal of fear (Woods & Bouton, 2006). Because DCS increases generalization to a discrete CS, reduces reinstatement, but does not reduce fear renewal following a contextual shift, it has been proposed that DCS enhances the context→no US association presumably formed during extinction by enhancing NMDA receptor-mediated memory consolidation processes related to the role of context in extinction (see Vervliet, 2008). The effects of DCS cannot be readily explained by nonmnemonic factors, such as motor activity. For example, when administered prior to a single CS-alone trial, DCS enhances freezing on test trials 24 h and 72 h later, rather than reducing it (Lee et al., 2006).

In addition to disrupting activity at the Mg^{2+}, glutamate, or glycine binding sites, pharmacological manipulations can alter binding activity of modulatory endogenous ligands such as zinc ions, phenylethanolamines, polyamines, and protons (H^+) at an amino terminal domain of NMDA receptors (Huggins & Grant, 2005). For example, acting at amino terminal domains of NMDA receptors containing the NR2B subunit on either the NR1 or NR2 subunits, ifenprodil (IFN) facilitates the tonic inhibitory action of H^+ at the NMDA receptor (Mott, Doherty, Zhang, Washburn, Fendley, Lyuboslavsky, Traynelis, & Dingledine, 1998; Perin-Dureau, Rachline, Neyton, & Paoletti, 2002). At the NMDA receptor, tonic H^+ inhibition reduces the frequency of channel openings (Traynelis & Cull-Candy, 1990). Functionally, IFN acts as an NMDA receptor antagonist. When administered systemically or infused into the amygdala, IFN impairs both acquisition and retention of fear extinction, while avoiding possible motor effects, that may be more common with other compounds, such as CPP (Blair, Sotres-Bayon, Moita, & LeDoux, 2005; Sotres-Bayon, Bush, & LeDoux, 2007). DCS is of particular importance for the purposes

of this proposal, as it is the pharmacological agent used to investigate the role of the NMDA receptor in cSNC.

Consummatory Successive Negative Contrast

Not all aversive conditioning is physically painful. As noted in the introduction, the incentive downshift used to induce cSNC may act as an aversive incentive, prompting avoidance behavior. The cSNC effect is part of a larger set of behavioral phenomena called incentive contrast effects, a group of effects induced by shifts in the quantity or quality of incentives (Flaherty, 1996; Mackintosh, 1974; Williams, 1997). The SNC procedure was developed experimentally by Elliot (1928) and Crespi (1942), and applied to the consummatory situation by Vogel, Mikulka, and Spear (1968).

Procedure for cSNC

In the standard cSNC procedure, developed by Vogel et al. (1968) and standardized by Flaherty (1996), subjects are assigned to two groups, groups, a downshifted experimental group (Group 32-4) and an unshifted control group (Group 4-4). Subjects are placed into conditioning boxes and a sucrose solution is presented from a sipper tube that the subject can lick. The amount of time that a subject spends in contact with the sipper tube (goal-tracking time), the frequency of licking, or the amount of fluid intake are typical dependent variables. During the initial preshift phase, Group 32-4 receives a 32% sucrose solution and Group 4-4 receives a 4% sucrose solution. The preshift phase continues for a number of trials, usually between 10 and 20, each one lasting 5 min and conducted approximately 24 h apart. In the following postshift phase, lasting between 2-7 trials, a change is implemented for Group 32-4, but not for Group 4-4. For all postshift trials, Group 32-4 receives a 4% solution, instead of the 32% solution received during preshift trials.

Typically, this procedure produces the following results that illustrate basic properties of cSNC (see Figure 4). First, during the preshift trials (1-10), the subjects show a steady rate of acquisition; Group 32-4 typically (but not always) exhibits higher performance than Group 4-4. Second, on the first postshift trial, Trial 11, when Group 32-4 is downshifted from a 32% solution to a 4% solution, licking behavior declines sharply and falls below that of Group 4-4. Third, for each trial after the first postshift trial (12-15), Group 32-4 begins to recover normal levels of consummatory behavior until there is no discernible difference in the licking behavior of the experimental and control groups. Group 32-4's drop in performance below Group 4-4 on the first postshift trial and subsequent recovery, known as the cSNC effect, indicates that the change in behavior is not simply an adjustment to the level of 4% sucrose, but an active rejection of the downshifted solution (Flaherty 1996).

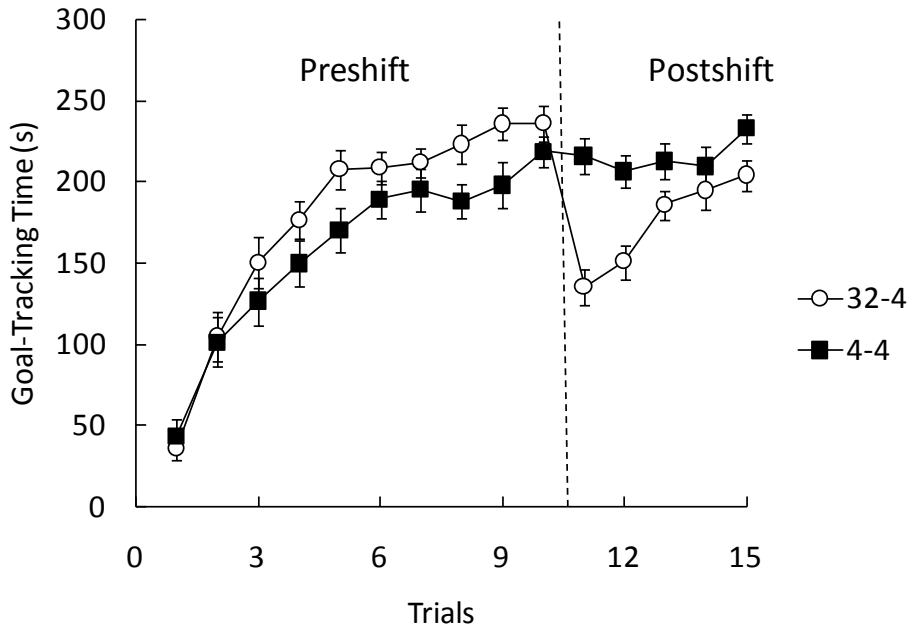


Figure 4. Illustration of the basic procedural characteristics of cSNC. During preshift trials, Group 32-4 receives 32% sucrose solution, while Group 4-4 receives 4% sucrose solution. During postshift trials both groups receive 4% sucrose solution.

Theories of the cSNC Effect

There have been several attempts to explain the cSNC effect, some based on search behavior (e.g., Elliot, 1928) and some on emotional reactions (Amsel, 1992; Tinklepaugh, 1928). These ideas were incorporated in two largely compatible hypotheses, Flaherty's (1996) multistage hypothesis and Wood, Daniel, and Papini's (2005) frustration hypothesis.

Flaherty's multistage hypothesis incorporates both search and emotional responses to explain cSNC. According to the multistage hypothesis, rats develop a representation of the 32% solution, established through a conditioning process. The initial taste of the sucrose functions to retrieve the representation of the solution concentration. It is not the training context that activates the representation because evidence indicates that altering the context during reward downshift does not eliminate contrast (Flaherty, Hrabinski, & Grigson, 1990). The 32-to-4 reward downshift starts a sequence of psychological processes, including detection, rejection, and search. First, the rat detects the discrepancy between actual 4% solution and the expected 32% sucrose solution. Next, the rat reacts to the change by rejecting the 4% solution and searching for the missing 32% solution. According to the multistage hypothesis, activation of this search process is the mechanism responsible for the initial consummatory suppression (i.e., during the first postshift trial).

Then there is the activation of an emotional process, characterized by a stress response. After the initial 5-min trial, rats exhibit evidence of increased stress levels in terms of an elevation of plasma corticosterone (Flaherty, Becker, & Pochorecky, 1985; Mitchell & Flaherty, 1998). This stress response occurs presumably in part because of an approach-avoidance conflict induced by the animal's inability to find the 32% solution and the forced acceptance of the 4% solution given the rat's state of food deprivation. Subsequently, a GABAergic circuit promotes recovery from cSNC. This is supported by evidence that only after at least 5 min of exposure to

the downshifted solution do GABA-dependent anxiolytics become effective in reducing contrast effects (Becker, 1986; Becker & Flaherty, 1983; Flaherty, 1990; Flaherty, Becker, Checke, Rowan, & Grigson, 1992). The activated GABAergic circuit inhibits a system that suppresses consummatory activity, having the net effect of promoting consummatory activity. For the remainder of the postshift trials, cSNC dissipates, as the 32% sucrose expectation is replaced by a 4% sucrose expectation, thus reducing the stress induced by reward downshift (Flaherty, 1996). These effects are interpreted as resulting from an aversive memory of the downshift event, which leads to anxiety-like behavioral and physiological outcomes.

Wood et al. (2005) proposed an alternative cSNC theory, suggesting a different consummatory suppression mechanism from Flaherty's search induction, called primary frustration. Similarly to Flaherty's multistage hypothesis, rats are conditioned to expect 32% solution. Again similar to Flaherty's hypothesis, the 32→4 reward downshift starts a sequence of psychological processes. Both Flaherty and Wood et al. propose that the initial processes are detection followed by rejection. However, Wood et al.'s hypothesis suggests that the violation of high incentive expectancy induces an unconditioned emotional response, called primary frustration that leads to both search and consummatory suppression (rejection).

The rationale behind Wood et al.'s (2005) argument is provided by Amsel's (1992) frustration theory. In frustrative conditioning procedures (of which cSNC is an example), there are two types of responses to reward downshift. Frustration theory defines the initial, unconditioned response to reward downshift as primary frustration, and the later, conditioned response to reward downshift as secondary frustration. When applied to cSNC, frustration theory implies that the subject's initial response to reward downshift may be predominantly dependent on primary frustration and the recovery from contrast that follows may depend largely upon the

approach-avoidance conflict induced by reward consumption (approach) and secondary frustration (avoidance).

Wood et al.'s (2005) hypothesis integrates emotional and search components to explain several behavioral properties of cSNC, paralleling Flaherty's (1996) multistage hypothesis, but providing more detail to explain the depression of consummatory behavior below that of the control group on the first postshift day. According to Wood et al.'s hypothesis, the mechanism responsible for consummatory suppression on Trial 11 is primary frustration. In primary frustration, the subject has its expectations violated when it detects the new 4% solution. This causes an aversive emotional reaction that inhibits consummatory responding and induces searching for the missing 32% solution. After the initial 5 min, secondary frustration builds to a critical level such that GABA-dependent drugs act to alleviate consummatory suppression. The opioid system also modulates both the initial reaction to the downshift and the recovery that follows (Pellegrini et al., 2005; Wood et al., 2005; Wood, Norris, Daniel, & Papini, 2008).

Allocentric and Egocentric Learning in cSNC

Both Flaherty's multistage hypothesis and Wood et al.'s (2005) frustration account of cSNC postulate similar psychological process in reaction to incentive downshift. Notably, both postulate a mnemonic process that encodes the aversive nature of reward downshift. Such memory can be recalled in subsequent trials. This mnemonic process is an example of egocentric learning (Papini, 2003). Applied to the cSNC preparation, an expectation of 32% or 4% sucrose would be viewed as examples of allocentric learning. During the preshift phase, animals in each group acquire such expectations about environmental events (allocentric learning), as well as an appetitive emotional memory of the sucrose incentive (egocentric learning). On Trial 11, when animals experience incentive downshift, memory processes are triggered. One involves the updating of the allocentric memory encoding information about the magnitude of the incentive.

This memory must be adjusted to reflect a downshift from 32% to 4% sucrose. The other involves the consolidation of the egocentric memory encoding of the frustrative experience triggered by the detection of the downshift incentive. The cSNC effect is thus viewed as arising from the unconditioned and conditioned emotional states driving the animal to reject and avoid, respectively, the sipper tube delivering the downshifted solution. Recovery from the downshift experience is driven by the update of the allocentric memory. The research reported here provides evidence consistent with the hypothesis that the downshift event consolidates an egocentric memory responsible in part for the suppression of consummatory behavior.

Parallels Between Fear Conditioning and cSNC

The cSNC procedure is analogous (although not identical) to the acquisition and extinction of fear conditioning. Similar to acquisition of fear conditioning, cSNC induces an aversive hedonic component. In addition, similar to extinction of fear conditioning, in the cSNC procedure the subject's initial aversive response diminishes (recovery). However, these similarities are preceded by a key difference. cSNC involves a preliminary training phase called preshift training, during which the animal comes to expect a highly preferred reward. The fear conditioning procedure has no such component. The reason for this asymmetry lies in the different conditions that induce frustration and fear. Whereas frustration requires the acquisition of reward expectancy before its violation causes emotional arousal (the US-like event in cSNC experiments), pain (the US in fear conditioning) is arousing without previous training.

Psychological Parallels

Fear and frustration paradigms yield similar behavioral effects. For example, they both require an aversive component. The fear component developed by tone-pain pairings and the frustration component developed by surprising nonreward are also rooted in similar brain

systems, as noted by Gray (1987). This became known as the fear = frustration hypothesis. In typical fear conditioning procedures, a signal predicts the onset of painful stimuli. Pain is unconditioned and fear is conditioned. Papini (2003) argued that the parallel between fear and frustration can extend to the unconditioned states that support them, namely, physical pain (since fear is usually generated by the administration of electric shocks) and primary frustration or psychological pain (generated by violation of reward expectancy).

There are similar behavioral effects using fear and frustration paradigms. For example, animals show similar escape behavior following both fear conditioning using mild electric shock and frustrative nonreward after having received continuous reinforcement (Bertsch & Leitenberg, 1970; Brooks, 1969; Daly, 1969). Frustrative nonreward and mild electric shock "summate" producing faster hurdle-jump escape responses, than either nonreward or shock-alone groups (Daly, 1970). Second, inescapable aversive challenges, whether physical or psychological, produce deficits in aggressive and sexual behavior (Friedin & Mustaca, 2004; Mustaca, Martinez, & Papini, 2000; Williams, 1982). Third, partial reinforcement attentuates both fear and frustration (Brown & Wagner, 1964; Pellegrini, Muzio, Mustaca, & Papini, 2004). Fourth, insufficient contextual processing produces deficits in fear and frustrative conditioning (Fanselow, 1986; Daniel, Wood, Pellegrini, Norris, & Papini, 2008).

Physiological Parallels

Lesions of the amygdala serve a central role in the acquisition of fear and the cSNC effect. Blanchard and Blanchard (1972) showed that the amygdala is important in the acquisition and expression of learned fear. Similarly, rats with lesions to the lateral amygdala showed a marked reduction in the size of cSNC. Whereas, lesions to the medial areas of the amygdala eliminated the cSNC effect in rats (Becker, Jarvis, Wagner, & Flaherty, 1984). These results

were supported by evidence reported by Pecoraro and Dallman (2005), that showed expression of c-Fos in the amygdala and anterior cingulate cortex, among other areas, following incentive downshift from 32% to 4%.

The analogy between cSNC and fear conditioning is also supported by pharmacological evidence (summarized in Table 1). For example, anxiolytics and opioid drugs alter both effects. Benzodiapezine tranquilizers, including chlordiazepoxide, flurazepam, and midazolam, reduced to cSNC when administered on the second postshift day (Becker, 1986; Becker & Flaherty, 1983; Flaherty, 1990; Flaherty et al., 1992). These results were analogous to those found using midazolam in fear conditioning situations. Midazolam reduced startle reactions in rats placed in a moderate fear conditioning situation and reduced freezing reactions in the testing chamber 24 h after contextual fear conditioning (Santos, Gárgaro, Oliveira, Masson, & Brandão, 2005; Szyndler, Sienkiewicz-Jarosz, Maciejak, Siemiatkowski, Rokicki, Członkowska, & Płaźnik, 2001). Diazepam eliminated contrast effects in mice (Mustaca, Bentosela, & Papini, 2000) and in rats when infused into the amygdala (Liao & Chuang, 2003). Strengthening the parallel, diazepam reduced the anticipatory fear-induced potentiated startle (Pietraszek, Sukhanov, Maciejak, Szyndler, Gravius, Wisłowska, Płaźnik, Bespalov, & Danysz, 2005). In cSNC and fear conditioning preparations, anxiolytics seem to be acting on anticipatory components, reducing the approach-avoidance conflict and fear-related behaviors, respectively.

Opioid agonists affect both cSNC and Pavlovian fear conditioning. Morphine reduced cSNC when administered before the first or second postshift trial (Rowan & Flaherty, 1987). Similarly, morphine reduced fear-potentiated startle when administered prior to training (Davis, 1979), and induced amnesia of contextual fear conditioning when administered after training (McNally & Westbrook, 2003a). The δ-opioid receptor agonist drug DPDPE ([D-Pen^2, D-Pen^5]enkephalin) effectively reduced cSNC during the first postshift trial, but not during the

second postshift trial (Wood et al., 2005). Similarly, Fanselow, Calcagnetti, and Helmstetter (1989) reported that DPDPE and two other selective δ-opioid receptor agonists produced high levels of conditioned analgesia compared to saline controls in a formalin test. Nonselective and δ-opioid receptor agonists appear to reduce pain-fear and frustration alike.

Similarly, opioid antagonists enhance both cSNC and fear conditioning. For example, the nonselective antagonist naloxone blocked morphine's cSNC-reducing effects at 1 mg/kg (Rowan & Flaherty, 1987) and enhanced cSNC on all postshift trials when administered at 2 mg/kg before trials 11 and 12. Naltrindole, a selective δ-opioid receptor antagonist, increased contrast on the first postshift trial (Pellegrini, Wood, Daniel, & Papini, 2005). Expectedly, the effect of naltrindole is the opposite to that of DPDPE on Trial 11. Likewise, in fear conditioning, naloxone reduced extinction from auditory fear conditioning (McNally & Westbrook, 2003a), contextual fear conditioning (Fanselow, 1981), and fear-potentiated startle (Davis, 1979). In both instances, opioid antagonists affect the subjects' recovery or extinction period. Studies using another nonselective opioid antagonist, naltrexone, had similar effects in contextual fear conditioning situations, reducing extinction (Helmstetter & Fanselow, 1987).

The stress hormone corticosterone has similar performance effects in both cSNC and fear conditioning. We already know that Sprague-Dawley rats showed elevated corticosterone levels on the second posthift day in cSNC. Similarily, Coover, Sutton, Welle, and Hart (1978) found that corticosterone levels were significantly elevated during classical fear conditioning or CS-alone trials, when the CS had previously signaled high shock intensity. In addition to subjects exhibiting elevated corticosterone levels during stressful events, administration of corticosterone seems to enhance learning. For example, corticosterone administration after tone–footshock pairings enhanced activity suppression during CS-alone trials administered a day later (Hui, Figuroa, Poytress, Roozendaal, McGaugh, Weinberger, 2004). Similarly, posttrial corticosterone

enhanced contextual fear conditioning when tested 1 and 7 days after acquisition (Cordero & Sandi, 1998). Posttraining administration of corticosterone also improves the retention of both T-maze active avoidance learning and punished drinking (Flood, Vidal, Bennett, Orme, Vasquez, & Jarvik, 1978; Kovács, Telegdy, & Lissák, 1977). In the cSNC situation, corticosterone enhances cSNC on Trial 12 when administered immediately following Trial 11, presumably by enhancing memory consolidation (Bentosela, Ruetti, Muzio, Mustaca, & Papini, 2006; Ruetti, Justel, Mustaca, & Papini, 2009). Taken together, these results show extensive parallels between cSNC and fear conditioning.

Memory for cSNC: A Dissimilarity?

While there are many similarities between fear conditioning and cSNC, there also appear to be some fundamental differences, especially as they relate to aversive memory. A great deal of evidence indicates aversive memory formation occurs during both fear acquisition and extinction. However, there is mixed evidence for aversive memory formation in the cSNC preparation. Behaviorally, if there was a strong aversive memory for cSNC, then suppression of drinking should be strongest at the onset of Trial 12 (due to the anticipatory effects of secondary frustration), analogous to strong freezing behavior at the onset of extinction test trials in fear conditioning. The animal should exhibit aversive response to the sipper tube based on an aversive memory and direct its behavior away from the sipper tube. However, this pattern does not appear to occur. Norris, Daniel, and Papini (2008) showed that consummatory suppression on Trials 12-15 is strongest not at the onset of the trial, but rather at the trial's end. This indicates that subjects may not encode into memory the aversive emotional component experienced on Trial 11, during incentive downshift. Instead, the animal may experience a reactivation of the preshift memory early in the trial, followed by rejection of the downshifted solution and by

learning of the new incentive value. At the onset of every trial, expectation for 32% sucrose is violated, thus inducing primary frustration, while simultaneously this experience updates to the new incentive conditions.

In addition, cSNC and fear conditioning differ with respect to reemergence following recovery and extinction, respectively. Fear conditioning readily demonstrates extinction phenomena, such as spontaneous recovery following extinction and following aversive-to-appetitive counterconditioning (Bouton & Peck, 1992; Quirk, 2003; Rescorla, 2004). Presumably spontaneous recovery of fear conditioning occurs because of memory processes. Bouton and Peck's (1992) memory retrieval account suggests that over time, extinction or counterconditioning memories degrade at a faster rate than fear-associated acquisition memories. Therefore, when subjects are returned to the testing environment, they recall fear-associated memory, as demonstrated by increased levels of fear behavior relative to extinction. However, spontaneous recovery of cSNC has failed to occur following full recovery, partial recovery, and administration of naloxone (shown to enhance cSNC; Norris et al., 2008). This discrepancy indicates a potential psychological difference in how these events are encoded.

In agreement with this idea, a variety of pharmacological agents shown to affect fear conditioning fail to affect cSNC when administered posttrial (summarized in Table 1). For example, opioid drugs, including DPDPE, naloxone, and U-50,488H fail to enhance memory consolidation following posttrial 11 administration (Daniel, Ortega, & Papini, in press; Wood et al., 2008) as do atropine (a cholinergic antagonist) and physostigmine (an acetylcholinesterase inhibitor; Bentosela, D'Ambros, Altamirano, Muzio, Baratti, & Mustaca, 2005). Data indicating the presence of an aversive memory in cSNC are indirect, as the effects of chlordiazepoxide (CDP) and corticosterone may be attributed to other processes (Becker & Flaherty, 1983; Bentosela et al., 2006; Ruetti et al., 2009). In both cases, it is plausible these manipulations

affected allocentric learning, rather than the egocentric learning. Pretrial administration of CDP may have enhanced allocentric learning about the 4% solution, not reducing expression of egocentric memory. Posttrial injections of corticosterone may have impaired allocentric learning producing the same behavioral result that would follow from an enhancement of aversive egocentric memory. Moreover, the few drugs known to act on the first postshift trial when administered pretrial 11, show a transient effect. DPDPE, naloxone, morphine, and cyproheptadine do not alter cSNC on subsequent, non-injection trials (Grigson & Flaherty, 1991; Pellegrini et al., 2005; Rowan & Flaherty, 1987; Wood et al., 2005). These results cast doubt on cSNC theories that postulate the formation of an aversive memory on Trial 11 (Flaherty, 1996; Wood et al., 2005).

Table 1

Summary of behavioral effects following pharmacological manipulations in fear conditioning and cSNC.

Pharmacological Manipulation (Drug)	Fear Conditioning (Reference)	cSNC (Reference)
Acetylcholinesterase inhibitor (i.e. physostigmine)	Posttraining Mice Enhances acquisition (Baratti, Huygens, Mino, Merlo, & Gardella, 1979)	Posttrial 11 Rats No effect (Bentosela, et al., 2005)
Benzodiapezine tranquilizers (i.e. diazepam)	Pretraining Rats Reduces acquisition (Pietraszek et al., 2005)	Pretrial Mice Reduces cSNC (Mustaca et al., 2000)
Corticosterone	Posttraining Rats Enhances acquisition (e.g., Cordero & Sandi, 1998; Hui, et al., 2004)	Posttrial Rats Enhances cSNC (Bentosela et al., 2006; Ruetti et al., 2009)
Opioid agonists (e.g., DPDPE, U-50, morphine)	Pretraining Rats Reduces acquisition (Davis, 1979; Fanselow et al., 1989) Posttraining Rats Reduces freezing (McNally & Westbrook, 2003b)	Pretrial Rats Reduces cSNC pretrial (Wood et al., 2005; Rowan & Flaherty, 1987; Wood et al., 2008)
Opioid antagonist (i.e. Naloxone, Naltrindole)	Pretraining Rats Enhances acquisition (Fanselow, 1981) Retards extinction (McNally & Westbrook, 2003a)	Pretrial 11 Rats Enhances cSNC (Daniel et al., submitted; Pellegrini et al., 2006)

Present Experiments

As noted earlier, there is extensive evidence that the NMDA receptor plays a crucial role in memory formation and consolidation in the fear conditioning preparation, but its potential role in cSNC remains unexplored. Given the role of NMDA receptors in fear conditioning and the extensive parallels between fear and frustration (as studied in cSNC), it is reasonable to ask whether NMDA receptors are involved in memory formation during cSNC. The current series of experiments have been designed to evaluate this possibility.

As reviewed earlier, D-cycloserine (DCS), a partial agonist at the glycine binding site of the NMDA receptor, facilitated extinction of a Pavlovian fear response. Additionally, DCS has a wide range of learning/memory enhancing effects. For example, DCS attenuated scopolamine-induced learning deficits in both the water maze and passive avoidance tasks (Sirvio, Ekonsalo, Riekkinen, Lahtinen, & Riekkinen, 1992; Zajaczkowski & Danysz, 1997). DCS facilitated LiCl-induced conditioned taste aversion (Nunnink, Davenport, Ortega, & Houpt, 2007) and conditioned place preference (Golden & Houpt, 2007). Thus, DCS affects several types of learning without affecting spontaneous motor activity or reward pathways (Herberg & Rose, 1990). These and similar results are summarized in Table 2. The current research experiments described here sought to extend these findings to the acquisition/consolidation of the aversive egocentric learning presumed to occur on Trial 11 of the cSNC preparation (Wood et al., 2005). The overall goal of these experiments was to assess whether DCS administered systemically affects cSNC. Animals received intraperitoneal (i.p.) injections of DCS either before or immediately following downshift exposure in different experiments. Theoretically, the goal of the experiments was to clarify the analogy between fear and frustration with respect to the NMDA receptor. Since various DCS administration fails to effect non-extinguished control

animals compared to extinguished DCS-treated animals in a fear extinction preparation it appears that DCS affects acquisition/consolidation processes, not retention of previously formed memories (Walker et al., 2002). Therefore, when administered in the cSNC situation it can have one of two results: It facilitates allocentric learning, in which case it should reduce cSNC, or it facilitates egocentric learning, in which case it should enhance cSNC. The overall hypothesis was that administration of DCS following reward downshift should enhance egocentric memory, thus retarding the recovery process cSNC during the postshift.

Table 2

Effects of DCS administration in various conditioning preparations.

Reference	Preparation	Behavioral Effect
Herberg & Rose (1990)	Spontaneous locomotor activity	No effect on motor behavior
Herberg & Rose (1990)	Variable-interval self-stimulation	No effect on self stimulation
Golden & Houpt (2007)	Conditioned place preference (CPP)	Enhanced CPP
Khanna et al. (1993)	Ethanol Tolerance	Decreased ethanol-induced motor impairement
Ledgerwood et al. (2003) Lee et al. (2006) Walker & Davis (2000) Walker et al. (2002)	Extinction of Pavlovian Fear	Reduced freezing
Ledgerwood et al. (2004)	Reinstatement of fear	Reduced reinstatement
Ledgerwood et al. (2005)	Generalization of fear extinction	Increased generalization
Lee et al. (2006)	Single CS-alone trial	Enhanced freezing
Mao et al. (2006)	Fear extinction/GluR1 expression	Decreased freezing Decreased GluR1 expression in the amygdala
Millescamps et al. (2007)	Place escape avoidance w/SNI-rats	Decreased escape Avoidance
Monahan et al. (1989)	Passive Avoidance	Increases Latency to Enter dark box
Monahan et al. (1989)	T-maze	Decreases trials to criteria after reversal
Nunnink et al. (2007)	Conditioned taste aversion (CTA)	Enhanced LiCl-induced CTA

Davenport & Houpt (2007)		Induced CTA at 30 mg/kg with saccharin solution
Quartermain et al. (1994)	Complex maze task	Decreased errors
Schuster & Schmidt (1992)	Radial arm maze w/HPC-lesioned rats	Reversed working memory impairments
Sirvio et al. (1992)	Water maze task w/scopolamine-treated rats	Decreased swim distance/latency
Thompson et al. (1992)	Eyeblink conditioning w/rabbits	Increases learning rate
Woods & Bouton (2006)	Renewal of fear: conditioned suppression	No effect on fear Reduced freezing SR
Yang et al. (2007)	Extinction of Pavlovian fear	Reverse metyrapone-blocked extinction Synergistic action with DEX
Zajaczkowski & Danysz (1997)	Passive avoidance w/scopolamine-treated rats	Reversed scopolamine-induced deficits

Experiment 1: DCS Administration Prior to 32-to-4 Downshift

Experiment 1 sought to extend the findings from fear conditioning to the acquisition/consolidation of the aversive learning presumed to occur on Trial 11 of the cSNC preparation. The goal of Experiment 1 was to assess whether DCS administered systemically affects cSNC. Animals received intraperitoneal (i.p.) injections of DCS (30 mg/kg) or saline (Sal) 30 min prior to Trials 11 and 12, a procedure similar to that used by Walker and Davis (2000). The following predictions were made: (1) DCS-treated animals will show prolonged cSNC relative to Sal controls and (2) unshifted DCS-treated controls will show no marked change in behavior relative to unshifted controls.

Method

The subjects were 40 male Long-Evans hooded rats from the TCU vivarium approximately 90 days old at the start of the experiment and experimentally naïve. Rats were housed in the TCU vivarium under a 12:12-h light:dark cycle (lights on at 07:00 h) and were deprived of food to an 81-84% of the free-feeding weight. Water was continuously available in each individual cage. Animals were trained during the light phase of the daily cycle.

Training was conducted in 4 conditioning boxes (MED Associates, St. Albans, VT) constructed of aluminum and Plexiglas, and measuring 29.4 cm in length, 28.9 cm in height, and 24.7 cm in width. The floor was made of steel rods 0.5 cm in diameter and 1.2 cm apart running perpendicular to the feeder wall. A bedding tray filled with corncob bedding was placed below the floor to collect fecal pellets and urine as needed. Against the feeder wall was an elliptical hole 1-cm wide, 2-cm high, and 3.5 cm from the floor. A sipper tube, 1 cm in diameter, was inserted through this hole. When fully inserted, the sipper tube was flush against the wall. A computer located in an adjacent room controlled the presentation and retraction of the sipper

tube, and detected contact with the sipper tube by way of a circuit involving the steel rods in the floor. Each conditioning box was placed in a sound-attenuating chamber that contained a houselight, a speaker to deliver white noise, and a fan for ventilation. Together, the speaker and fan produced noise with an intensity of 80.1 dB (SPL, Scale C).

Training lasted 15 daily Trials. Each rat was randomly assigned to one of the conditioning boxes and always trained in that box. The order of training of the 4-rat squads varied across days. After each trial, conditioning boxes were cleaned with a damp paper towel, feces removed, and bedding material replaced as needed. During trials, the houselight, white noise, and fan were on constantly. The 15 trials were divided into a preshift phase (10 trials) and a postshift phase (5 trials). Prior to trial 1, rats were matched by ad libitum weight and then randomly assigned to the downshifted or unshifted condition. After trial 10, downshifted rats were matched in terms of overall performance and randomly assigned to one of the two different drug conditions: Saline, or 30 mg/kg DCS (n = 10). The unshifted controls were assigned likewise (n = 10).

For the two 32-to-4 groups (32/Sal, 32/DCS), the 10 preshift trials involved access to a 32% sucrose solution (w/w, prepared by mixing 32 g of commercial sugar for every 68 g of distilled water); the 5 postshift trials involved access to a 4% solution (w/w, 4 g of sugar for every 96 g of distilled water). The two 4-to-4 groups (4/Sal, 4/DCS) received the 4% sucrose solution in all 15 trials. The experimental design is summarized in Table 3.

Each trial started with a variable pretrial interval of 30 s (range: 15–45 s). At the end of this interval, the sipper tube was automatically presented. A trial started when a rat contacted the sipper tube and lasted 5 min after the first contact. Retraction of the sipper tube was followed by a posttrial interval averaging 30 s (range: 15–45 s). The dependent variable was the cumulative

amount of time in contact with the sipper tube, measured in 0.05-s units and labeled goal-tracking time.

DCS was purchased from Sigma-Aldrich Chemicals (Saint Louis, MO). It was freshly dissolved in isotonic saline solution (30 mg/ml) and administered 30 min prior to training on Trials 11 and 12. Saline animals received an equal-volume injection of isotonic saline. It was assumed that DCS had no direct effects on Trials 13-15, as DCS's reported half-life is 20 min (Conzelman & Jones, 1956). Moreover, DCS at 30 mg/kg (i.p.) reduces spontaneous recovery, but not contextual renewal 24 h after administration before extinction training, indicating that DCS alters memory consolidation without affecting nonmemory processes (Woods & Bouton, 2006).

Goal-tracking times were subjected to conventional analysis of variance (ANOVA) and protected Fisher's LSD post hoc tests were used for pairwise comparisons as needed for between-trial analysis. The alpha value was set to $p \leq 0.0500$ for all statistical tests. Similar to previous work (Bentosela et al., 2006; Pellegrini et al., 2005; Ruetti et al., 2009; Wood et al., 2005; Wood et al, 2008) contrast effects were evaluated by comparing each downshift group to its unshifted control. This comparison has the advantage that it is consistent with the definition of incentive contrast based on the difference in performance between downshifted animals and unshifted control animals. It also has the advantage that animals are equated by drug condition and vary by behavioral treatment.

Table 3

Design of Experiment 1.

	Preshift	Postshift	
Group	Trials 1-10	Trials 11-12	Trials 13-15
32/Sal (n = 11)	Access to 32%	Sal→Access to 4%	Access to 4%
4/Sal (n =10)	Access to 4%	Sal→Access to 4%	Access to 4%
32/DCS (n =10)	Access to 32%	DCS→Access to 4%	Access to 4%
4/DCS (n =10)	Access to 4%	DCS→Access to 4%	Access to 4%

Results

The results are shown in Figure 5. There were no differences between the groups before assignment to drug condition. A Preshift x Drug x Sucrose ANOVA for trials 1-10 revealed a significant effect of trial, $F(9, 324) = 155.40$, $p < 0.001$, but no significant main effect of drug, sucrose, or their interaction, Fs < 1. A Postshift x Drug x Sucrose for Trials 11-15 revealed a significant main effect of trial, $F(4, 144) = 12.68$, $p < 0.001$, a significant main effect of sucrose, $F(1, 36) = 29.23$, $p < 0.001$, a trial by sucrose interaction, $F(4, 144) = 9.84$, $p < 0.001$. All other interactions were nonsignificant, Fs < 1.

To further analyze the postshift results, a series of one-way ANOVAs for Trials 11-15 were conducted, yielding a significant effect on Trials 11-14, Fs$(3, 36) > 2.88$, ps ≤ 0.05, but not on Trial 15, $F(3, 36) = 2.20$, $p > 0.10$. To identify the source of the effect on Trials 11-14, protected Fisher's LSD post-hoc tests were used to compare the downshifted groups to their unshifted drug control, as well as between Groups 4/Sal and 4/DCS. Groups 32/Sal and 4/Sal were significantly different on Trials 11-13, ps < 0.01, but not Trial 14, $p > 0.14$. Groups 32/DCS and 4/DCS were significantly different on Trials 11-14, ps < 0.03. Thus DCS extended cSNC by one trial. Finally, Groups 4/Sal and 4/DCS were not statistically different in any of the trials, ps > 0.63, showing that DCS affected only downshifted groups. These data were consistent with the prediction that DCS would enhance cSNC, but would not impact sucrose consumption in unshifted animals.

Norris et al. (2008) reported that downshifted animals did not suppress consummatory behavior at the onset of the second through terminal postshift trials. Instead, they reported that animals showed high levels of consummatory responding during the initial 100-s period. This was interpreted as evidence inconsistent with the consolidation of an egocentric memory. The

argument holds that if animals encode an aversive memory for the downshift event, then they should show suppression early in each remaining postshift trial. In the current experiment, if 32/DCS holds an enhanced aversive memory relative to 32/Sal, then 32/DCS should demonstrate consummatory suppression relative to 4/DCS in the initial 100-s, whereas 32/Sal should not show suppression relative to its 4/Sal control.

To evaluate the egocentric memory hypothesis, the initial 100 s of Trials 10-15 were analyzed using one way ANOVAs followed by protected Fisher's LSD pairwise comparisons for 32/Sal vs. 4/Sal and 32/DCS vs. 4/DCS (shown in Figure 6). These analyses revealed significant differences on trials 12 and 13, Fs(3, 36) > 2.94, ps < 0.05, but not on trials 10, 11, 14, and 15, Fs (3, 36) < 1.89, ps > 0.15. Fisher's LSD post hoc tests for trials 12 and 13 revealed 32/DCS was significantly lower than its unshifted control, 4/DCS, ps < 0.02. However, 32/Sal and 4/Sal were not different during the initial 100 s of Trials 12-13, ps > 0.33. Thus, DCS strengthened the aversive memory for the downshift event, as shown by early suppression of goal-tracking times. Notice that DCS did not significantly enhance cSNC on Trial 11, indicating that some experience must be necessary for the DCS to have an effect on behavior. These data indicate that DCS did not exert its effects via contextual/motivational processes, because the suppressive effects persisted on Trial 13, one trial beyond drug administration.

Discussion

These results provide support for the hypothesis postulating the encoding of an aversive memory of the downshift event. DCS administration enhanced cSNC during the postshift without altering the performance of unshifted drug controls. Furthermore, a within-trial analysis showed that DCS significantly suppressed behavior during the early portions of postshift trials 12 and 13, but not Trial 11. The fact that animals were under the influence of the drug on Trial

11, but failed to show alteration of behavior, and were not under the influence of DCS on Trial 13, but showed suppression, suggests DCS does not have unconditioned effects. Rather, these results are consistent with the hypothesis that DCS affected the consolidation and/or retrieval of an egocentric memory of the downshift experience.

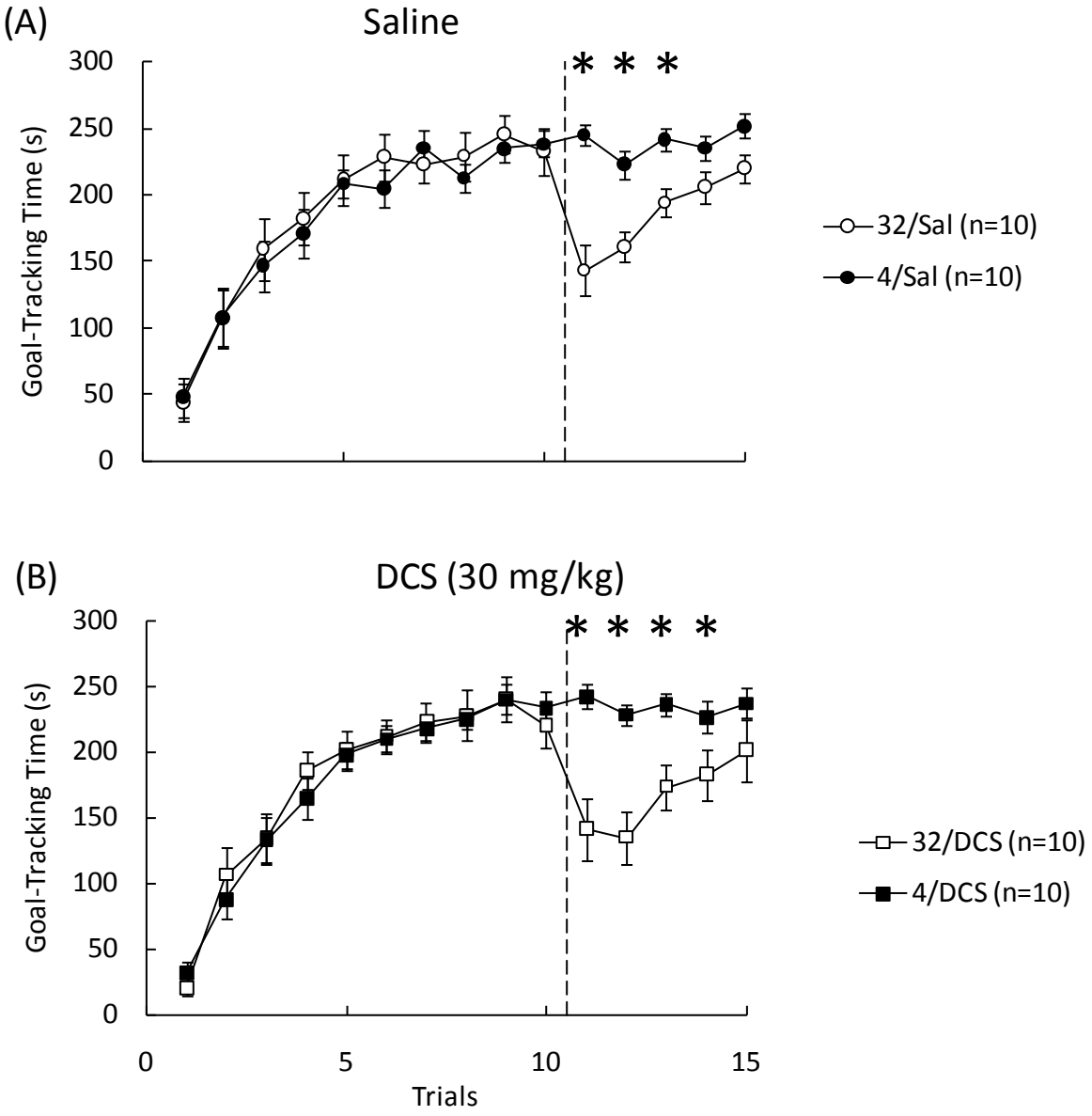


Figure 5. Overall results of Experiment 1. Mean goal-tracking time (±SEM) is plotted as a function of trial, contrast condition, and dug treatment. On Trials 1-10 32/Sal and 32/DCS received 32% sucrose solution. On Trials 11-15, 32/Sal and 32/DCS received 4% sucrose, similar to 4/Sal and 4/DCS. Animals were administered either Saline or DCS (30 mg/kg, i.p.) 30 min prior to trials 11 and 12. Protected Fisher's LSD post hoc tests revealed: (A) Saline-administered (32/Sal vs. 4/Sal) animals demonstrated cSNC on Trials 11-13, but recovered on Trial 14 and 15; (B) DCS-administered animals demonstrated cSNC on trials 11 through 14, recovering statistically on Trial 15.

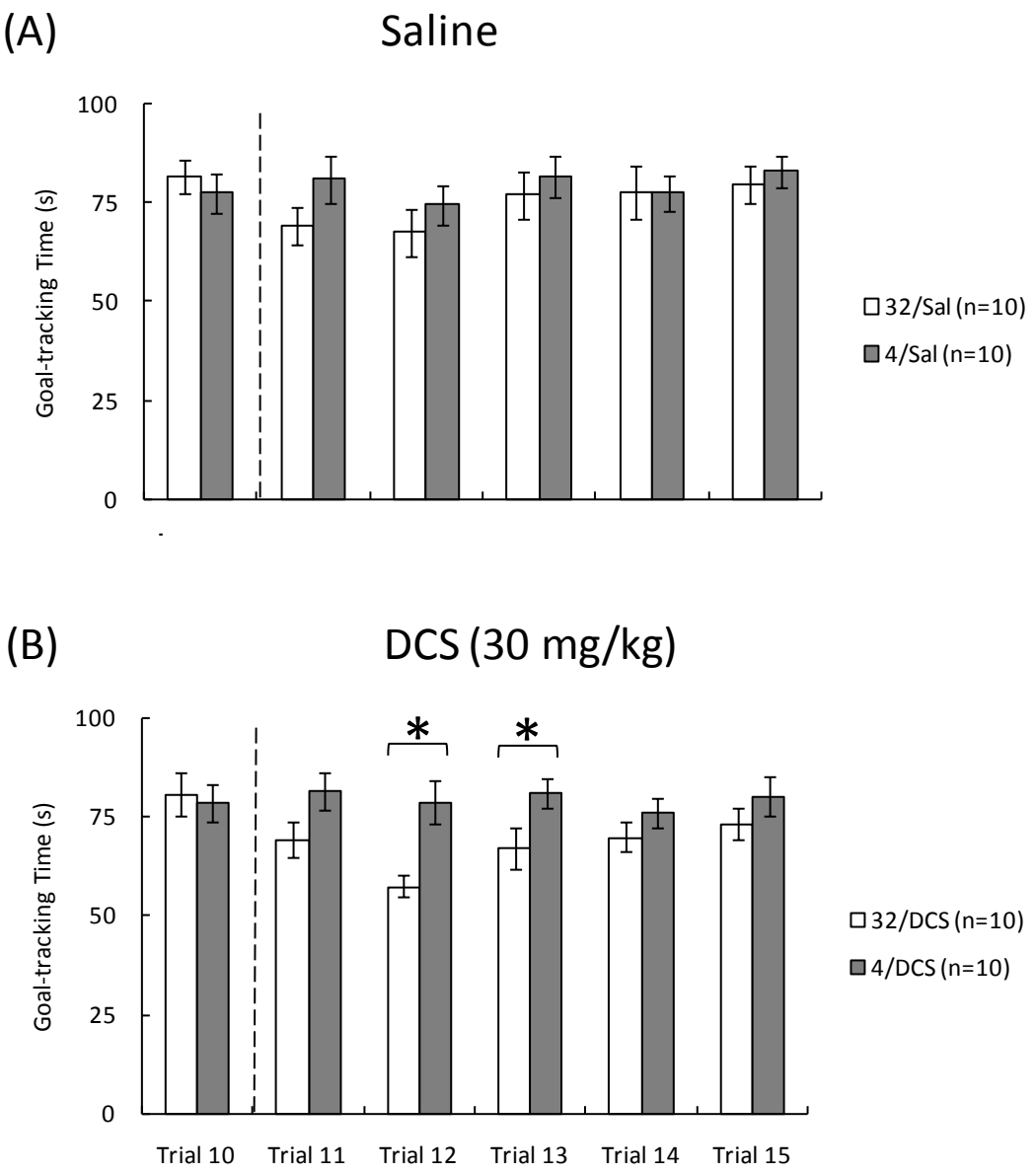


Figure 6. Initial 100 s of Trials 10-15 in Experiment 1. Mean goal-tracking time (±SEM) plotted as a function of trial. It was predicted that DCS-treated animals would show significant suppression of behavior early during postshift Trials 12-15, but not during Trials 10-11. Pairwise comparisons for the first 100 s of Trials 10-15 revealed significant suppression early in trials 12 and 13 in 32/DCS vs 4/DCS, but not 32/Sal vs. 4/Sal. These results indicate DCS enhanced cSNC during the early portions of postshift Trials 12-13, suggesting its effects are attributable to mnemonic process.

Experiment 2a: Administration of DCS Following 32-to-4 Downshift

As in many studies using DCS, in Experiment 1 the drug was administered prior to the trials, and the results were interpreted using a memory framework. However, pretrial administration confuses possible motor, motivational, or attentional of DCS effects with memory consolidation effects (McGaugh, 2003). In Experiment 2a, DCS (30 mg/kg) or saline was administered immediately following Trial 11, using a posttrial injection procedure similar to that developed to assess memory consolidation (Breen & McGaugh, 1961). Administering DCS following training allows researchers to examine memory consolidation effects on subsequent trials independently of performance effects because behavior is measured in drug-free animals. It was predicted that: (1) DCS-treated animals will show prolonged cSNC relative to saline controls on Trials 12-15, indexed by slower recovery to the level of unshifted drug controls; and (2) unshifted DCS-treated controls will show no marked change in behavior relative to unshifted saline controls.

Method

The subjects were 41 male Long-Evans hooded rats from the TCU vivarium approximately 90 days old at the start of the experiment and experimentally naïve. Group 32/Sal (n=11) had one more animal than all other groups (n=10). Housing, training procedure, apparatus, drug preparation, and route of administration were similar to Experiment 1. However, in Experiment 2a, drug was administered after the downshift. Immediately following Trial 11, animals were placed back into the transport rack, moved into the holding room and received the appropriate injection. Similar to Experiment 1, it was assumed that DCS was absent from the animal during Trials 12-15 (Conzelman & Jones, 1956; Woods & Bouton, 2006). The experimental design is summarized in Table 4.

For the two 32-to-4 groups (32/Sal, 32/DCS), the 10 preshift trials involved access to a 32% sucrose solution (w/w, prepared by mixing 32 g of commercial sugar for every 68 g of distilled water); the 5 postshift trials involved access to a 4% sucrose solution (w/w, 4 g of sugar for every 96 g of distilled water). The two 4-to-4 groups (4/Sal, 4/DCS) received the 4% sucrose solution in all 15 trials.

Results

The results are shown in Figure 7. There were no differences between the groups before assignment to drug condition. A Trial x Drug x Sucrose ANOVA for Trials 1-10 revealed a significant effect of trial, $F(9, 333) = 81.95$, $p < 0.001$, a main effect of sucrose, $F(1, 37) = 10.81$, $p < 0.01$, and a significant trial by sucrose interaction, $F(9, 333) = 3.18$, $p < 0.01$, but nonsignificant main effect of drug or of drug-related interactions, Fs < 1. A Trial x Drug x Sucrose ANOVA for Trials 11-15 revealed a significant main effect of trial, $F(4, 148) = 16.6$, $p < 0.001$, a significant trial by sucrose interaction, $F(4, 148) = 7.14$, $p < 0.001$, and a trial by sucrose by drug interaction, $F(4, 148) = 2.57$, $p < 0.001$. All other effects were nonsignificant, Fs < 1.

Table 4

Design of Experiment 2a.

	Preshift	Postshift	
Group	Trials 1-10	Trial 11	Trials 12-15
32/Sal (n = 11)	Access to 32%	Access to 4%→Sal	Access to 4%
4/Sal (n =10)	Access to 4%	Access to 4%→ Sal	Access to 4%
32/DCS (n =10)	Access to 32%	Access to 4%→DCS	Access to 4%
4/DCS (n =10)	Access to 4%	Access to 4%→DCS	Access to 4%

To further analyze the postshift results, a series of one-way ANOVAs for Trials 11-15 were conducted. The group effects were significant for Trials 11-13, *F*s(3, 37) > 7.79, *p*s < 0.001, but not for trials 14-15, *F*s(3, 37) < 2.20, *p*s > 0.10. To identify the source of the effect on Trials 11-13, protected Fisher's LSD post hoc tests comparing the downshifted animals to their unshifted drug control, as well as for 4/Sal and 4/DCS were conducted. Because the repeated-measure ANOVA for Trials 11-15 revealed a significant trial by sucrose by drug interaction, it was decided to perform planned comparisons for 32/Sal vs. 32/DCS. Groups 32/Sal and 4/Sal were significantly different on Trials 11-12, *p*s < 0.03, but not Trial 13, $p > 0.77$. Groups 32/DCS and 4/DCS were significantly different on Trials 11-13, $p < 0.001$. Thus DCS extended cSNC by one trial. Groups 4/Sal and 4/DCS were not different, *p*s > 0.12. Furthermore, groups 32/Sal and 32/DCS were significantly different on Trial 13, $p < 0.01$, but not Trials 11-12, *p*s > 0.07. These results indicate that the cSNC effect persisted to Trial 13 among DCS-treated animals, but to Trial 12 in saline-treated animals. The data were consistent with the prediction that DCS enhances cSNC, but does not affect behavior in unshifted animals.

The results of a within-trial analysis similar to that presented in Experiment 1 are presented Figure 8. If 32/DCS enhanced the consolidation of an aversive memory relative to 32/Sal, then 32/DCS should demonstrate consummatory suppression relative to 4/DCS in the initial 100-s, whereas 32/Sal should not show such suppression. Due to software malfunction, within-trial data points for the initial 100 s for some subjects were lost. Analysis was conducted with the remaining data points (group size for each trial is shown in the bottom of Figure 8).

Similar to Experiment 1, one way ANOVAs revealed significant effects on Trial 12, $F(3, 36) = 5.629$, $p < 0.01$, but not Trial 10, $F(3, 33) = 1.51$, $p > 0.22$; Trial 11, $F(3, 35) = 1.67$, $p > 0.19$; Trial 13, $F(3, 35) = 2.2$, $p > 0.10$; and Trial 14-15, *F*s < 1. Protected Fisher's LSD pairwise comparisons revealed the source of the difference on Trial 12 to be between Groups 32/DCS and

4/DCS, $p < 0.01$, but not between Groups 32/Sal and 4/Sal, $p > 0.14$. These results indicate that 32/DCS animals showed a significant degree of consummatory suppression during the onset of trial 12 relative to 4/DCS, whereas 32/Sal did not show significant suppression early in trial 12, relative to 4/Sal. This pattern of results, similar to those of Experiment 1, indicates that DCS enhanced suppression in the trial following its administration.

Discussion

The results of Experiments 1 and 2a provide support for the hypothesis that the downshift event consolidates an aversive egocentric memory. DCS administration enhanced cSNC during the postshift without altering the performance of unshifted drug controls. Experiment 2a has the advantage that DCS's suppressive effects were observed using a posttrial manipulation. Therefore, DCS's effects cannot be readily attributed to motor, attentional, sensory, or motivational consequences of the drug. These results were further clarified using a within-trial analysis. This analysis showed that posttrial 11 DCS significantly suppressed behavior during the early portions of Trial 12. Given that DCS animals were not under the influence of the drug during postshift trials and their behavior was not statistically different from that of saline animals prior to DCS administration, it is hypothesized that the effects of DCS were specific to the consolidation of a conditioning process.

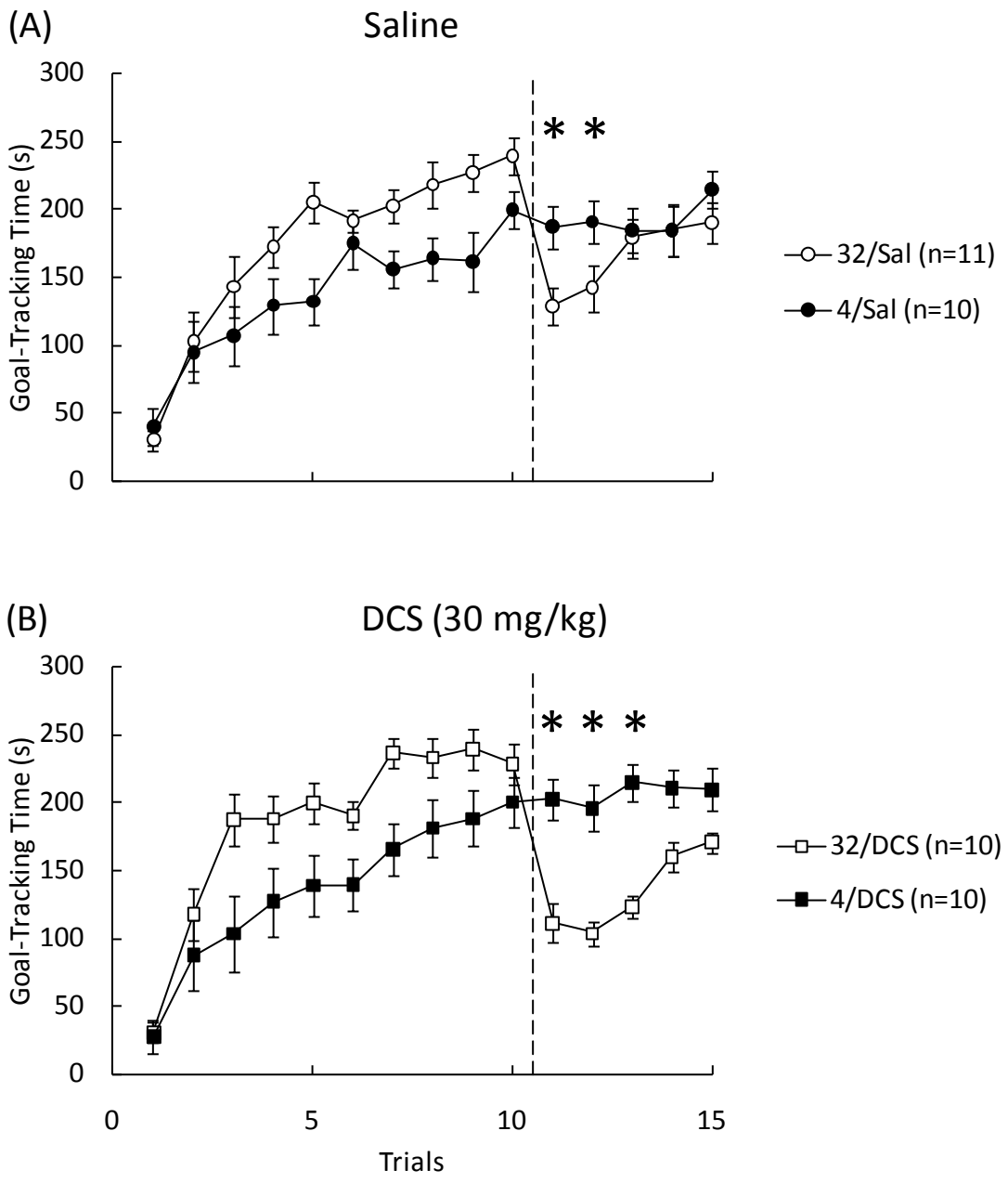
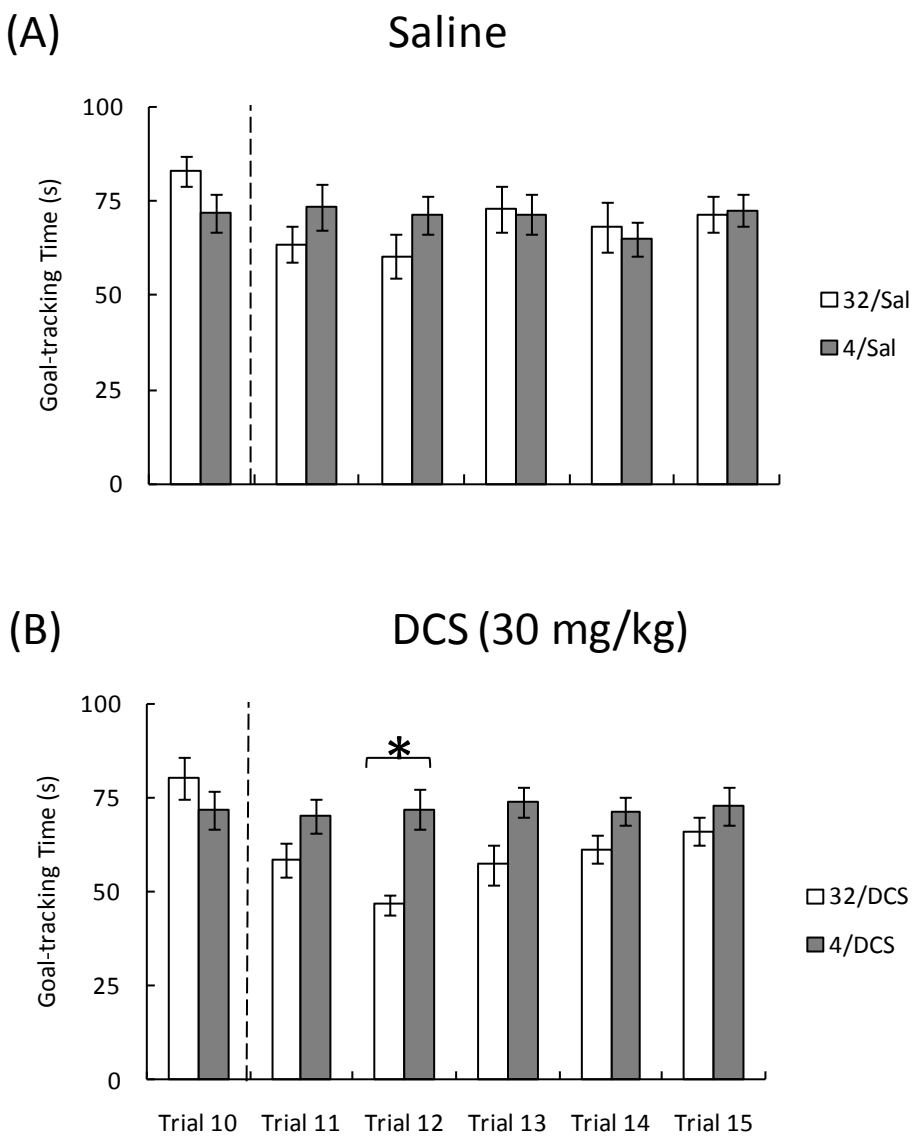


Figure 7. Overall results of Experiment 2a. Mean goal-tracking time (±SEM) is plotted as a function of trial, contast condition, and drug treatment. In Trials 1-10 32/Sal and 32/DCS received 32% sucrose solution. On Trials 11-15, 32/Sal and 32/DCS received 4% sucrose, similar to 4/Sal and 4/DCS. Animals were administered either Sal or DCS (30 mg/kg, i.p.) immediately following Trial 11. (A) Saline-administered (32/Sal vs. 4/Sal) animals demonstrated cSNC on Trials 11 and 12, but recovered on Trial 13 and all remaining postshift trials. (B) DCS-administered animals demonstrated cSNC on Trials 11 through 13, recovering statistically on Trials 14-15. On Trial 13, 32/DCS was significantly lower than 32/Sal, $p < 0.01$.



32/Sal	n=9	n=9	n=9	n=9	n=10	n=9
4/Sal	n=9	n=8	n=9	n=9	n=10	n=10
32/DCS	n=8	n=8	n=9	n=8	n=9	n=9
4/DCS	n=10	n=9	n=10	n=10	n=9	n=10

Figure 8. Initial 100 s of Trials 10-15 in Experiment 2a. Mean goal-tracking time (±SEM) plotted as a function of trial. Similar to Experiment 1, it was predicted that DCS-treated would show significant suppression of behavior early during postshift Trials 12-15, but not Trials 10-11. Pairwise comparisons for the first 100 s of Trials 10-15 revealed significant suppression early in Trials 12 in 32/DCS vs 4/DCS, but not 32/Sal vs. 4/Sal. These results are consistent with Experiment 1. The bottom portion shows the group size for each trial, as some data points were lost due to software malfunction.

Experiment 2b: Administration of DCS Following 32-to-6 Downshift

Experiments 1 and 2a demonstrated that DCS administration (30 mg/kg) both prior to and following the first downshift trial prolonged cSNC when using a 32-to-4. Pellegrini et al. (2005) showed that naloxone enhanced cSNC when administered prior to a 32-to-6 downshift. This less dramatic downshift allowed for researchers to evaluate the suppressive effects of the opioid antagonist by reducing the possibility of a floor effect. In Experiments 1 and 2a, it is possible that a floor effect may have reduced the detection of DCS's effects. Therefore in Experiment 2b, subjects received an injection of either saline or DCS (30 mg/kg) following a 32-to-6 downshift.

In addition to reducing the opportunity for floor effects, a 32-to-6 downshift has the advantage of clarifying a behavioral threshold. Ruetti et al. (2009) showed that posttrial administration of corticosterone enhanced cSNC following a 32-to-4 downshift, but not 8-to-4 downshift, concluding that corticosterone's enhancing effects were possible only when an animal experienced a severe reduction in sucrose concentration (corresponding to greater intensity of psychological pain). Similarly, Daniel et al. (in press) showed that naloxone administered prior to Trial 11 reduced consummatory behavior after a 32-to-6 downshift, but not after a 16-to-6 downshift, indicating that larger absolute downshifts result in greater psychological pain. Likewise, using a less intense downshift will help determine whether DCS's effects are dependent on a minimum incentive discrepancy (corresponding to lesser intensity of psychological pain). It was predicted: (1) DCS-administered animals will show prolonged cSNC relative to Sal controls on Trials 12-15; (2) unshifted DCS-administered controls will show no marked change in behavior relative to unshifted saline controls.

Method

The subjects were 37 male Long-Evans hooded rats from the TCU vivarium approximately 90 days old at the start of the experiment and experimentally naïve. Housing, training procedure, apparatus, drug preparation, and route of administration were similar to Experiment 2a. The experimental design is summarized in Table 5.

For the two 32-to-6 groups (32/Sal, 32/DCS), the 10 preshift trials involved access to a 32% sucrose solution (w/w, prepared by mixing 32 g of commercial sugar for every 68 g of distilled water); the 5 postshift trials involved access to a 6% sucrose solution (w/w, 6 g of sugar for every 94 g of distilled water). The two 6-to-6 groups (6/Sal, 6/DCS) received the 6% sucrose solution in all 15 trials.

Results

The results are shown in Figure 9. A preshift Trial x Drug x Sucrose ANOVA for trials 1-10 revealed a significant effect of trial, $F(9, 297) = 83.64$, $p < 0.001$, and main effect of sucrose, $F(1, 33) = 4.83$, $p < 0.04$, but nonsignificant main effect of drug or interactions, Fs (9, 297) < 1.56, ps > 0.12. A postshift Trial x Drug x Sucrose for Trials 11-15 revealed a significant main effect of trial, $F(4, 132) = 16.45$, $p < 0.001$, a significant main effect of sucrose, $F(1, 33) = 6.67$, $p < 0.02$, and a significant trial by sucrose interaction, $F(4, 132) = 18.4$, $p < 0.001$. All other effects were nonsignificant, Fs < 1.

Table 5

Design of Experiment 2b.

	Preshift	Postshift	
Group	Trials 1-10	Trial 11	Trials 12-15
32/Sal (n = 9)	Access to 32%	Access to 6%→Sal	Access to 6%
6/Sal (n = 9)	Access to 6%	Access to 6%→Sal	Access to 6%
32/DCS (n = 9)	Access to 32%	Access to 6%→DCS	Access to 6%
6/DCS (n =10)	Access to 6%	Access to 6%→DCS	Access to 6%

To further analyze the postshift results, a series of one-way ANOVAs for Trials 11-15 were conducted. The group effects were significant on Trials 11-12, Fs(3, 33) > 3.41, ps < 0.03, but not for Trials 13-15, Fs < 1. To identify the source of the effect in Trials 11-12, we conducted protected Fisher's LSD posthoc tests comparing the downshifted animals to their unshifted drug control. Groups 32/Sal vs. 6/Sal were significantly differenet on Trial 11, p < 0.01, but not Trial 12, p > 0.13. Groups 32/DCS vs. 6/DCS were significantly different on Trials 11-12, ps < 0.01. Thus, DCS extended cSNC by one trial. Furthermore, Groups 6/Sal vs. 6/DCS were not different, ps > 0.44. The data were consistent with the prediction that DCS enhances cSNC, but does not affect behavior in unshifted animals.

The results of within-trial analysis similar to Experiments 1 and 2a are presented in Figure 10. There were no significant effects in Trials 10-15, Fs(3, 36) < 2.27, ps > 0.09, indicating the DCS had no suppressive effects early in postshift trials.

Discussion

These results indicate that downshifted saline animals (32/Sal) recovered from cSNC one trial faster than downshifted DCS-treated animals. This is consistent with the results of the prior experiments. However, the within-trial analysis did not show the pattern of early suppression demonstrated in the previous experiments. When taken together, these results show that DCS has a greater effect after a 32-to-4 downshift (Experiment 2a) than after a 32-to-6 downshift (Experiment 2b). Like the effects of posttrial corticosterone on cSNC (Ruetti et al. 2009), DCS has detectable effects only with relatively large incentive downshifts.

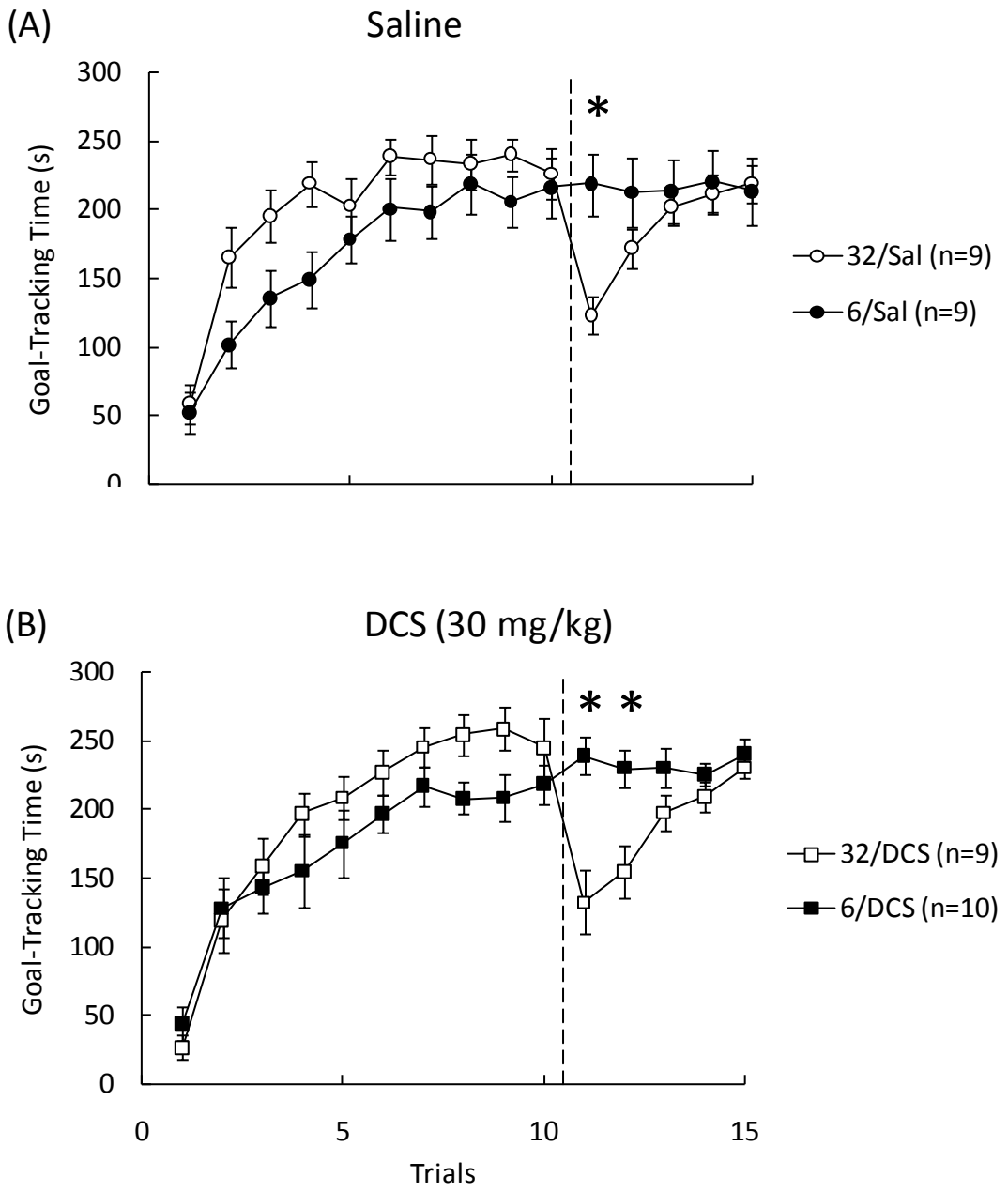


Figure 9. Overall results of Experiment 2b. Goal-tracking time (s) is plotted as a function of trial, contrast condition, and drug treatment. On Trials 1-10, 32/Sal and 32/DCS received 32% sucrose solution. On Trials 11-15, 32/Sal and 32/DCS received 6% sucrose, similar to 6/Sal and 6/DCS. Animals were administered either Sal or DCS (30 mg/kg, i.p.) immediately following Trial 11. (A) Sal-administered groups (32/Sal vs. 6/Sal) demonstrated cSNC on Trial11, but recovered on Trial 12 and all remaining postshift trials. (B) DCS-administered animals demonstrated cSNC on Trials 11-12, recovering statistically on Trials 13-15.

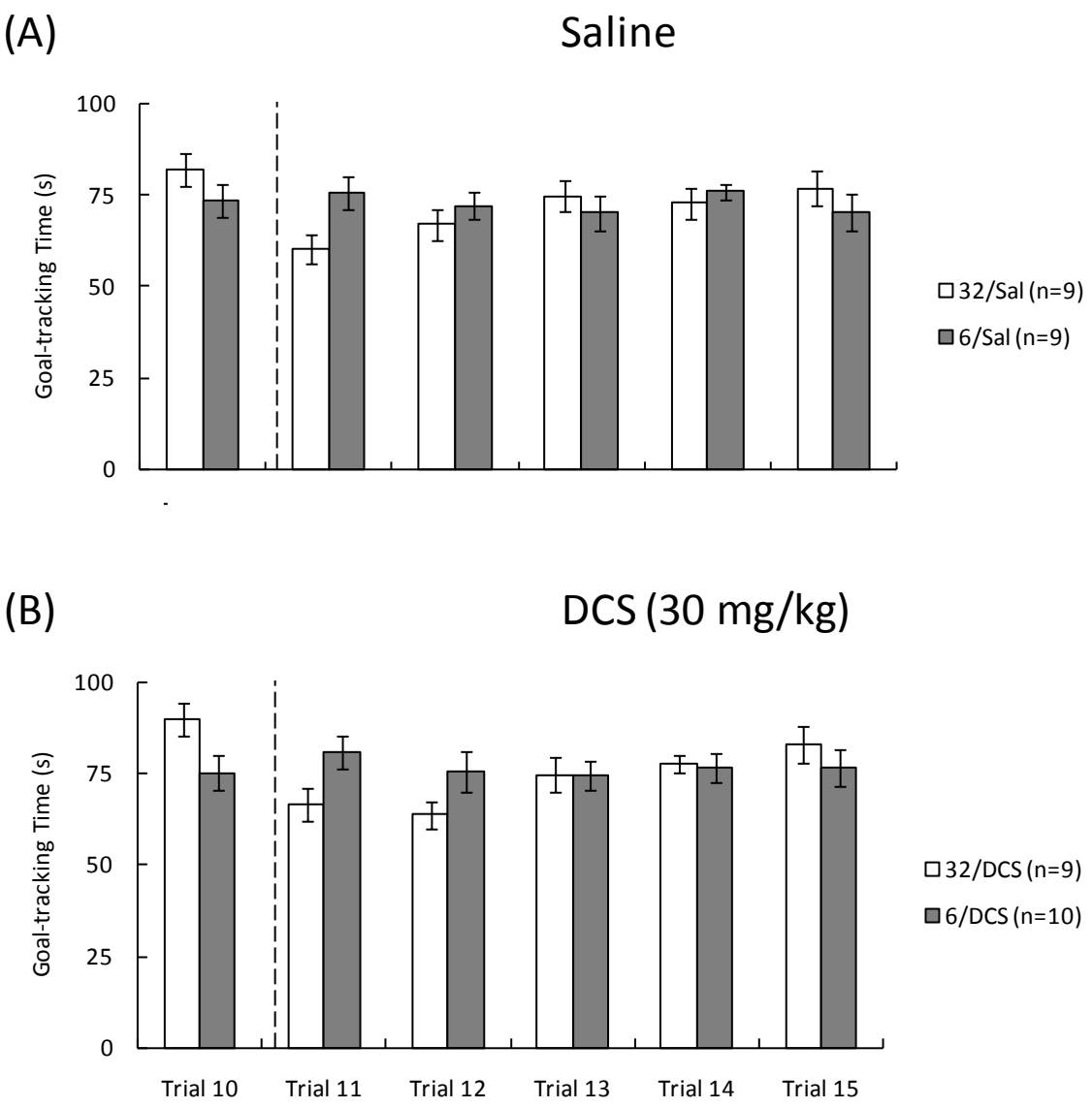


Figure 10. Initial 100 s of Trials10-15 in Experiment 2b. Mean goal-tracking time (±SEM) plotted as a function of trial, contrast condition, and drug treatment. One-way ANOVAs failed to reveal significant difference on Trials 10-15. DCS did not suppress behavior early in Trial 12.

Experiment 3: DCS at 15 and 60 mg/kg Following 32-to-4 Downshift

Experiments 1 and 2 demonstrated that DCS administration (30 mg/kg) both prior to and following downshift trials prolongs cSNC after a 32-to-4 downshift. DCS has been shown to decrease glycine binding in the rat forebrain functionally acting as antagonist rather than agonist when either the dose level is high or there is a high level of endogenous extracellular glycine (D'Souza, Charney, & Krystal, 1995; Hood et al., 1989). Given that DCS is a partial agonist rather than a full agonist, such as glycine or D-serine, it produces only 40-50% of the stimulation at NMDA receptors achievable by high levels of glycine (Hood et al. 1989). At lower doses, 3-20 mg/kg, enhancing effects have been reported, but at higher levels (e.g., higher than 40 mg/kg), antagonist-like effects have been reported to occur (D'Souza et al., 1995).

Other studies involving DCS in learning preparations have evaluated the drug's effect at doses ranging from 0.3 mg/kg to 80 mg/kg (Quartermain, Mower, Rafferty, Herting & Lanthorn, 1994; Sirvo, Ekonsalo, Riekkinen, Lahtinen & Riekkinen, 1992). Quartermain et al. (1994) found that posttraining administration of DCS tended to have similar effects on memory consolidation, irrespective of dose (3 mg/kg vs. 80 mg/kg) in a complex maze task. However, more recent studies (e.g., Woods & Bouton 2006) reported effects using 30 mg/kg, but not with 15 mg/kg in extinction of fear-induced conditioned suppression. Therefore, Experiment 3 was conducted to determine the effectiveness of two doses, half and twice the size of the dose used in Experiment 2a (i.e., 15 and 60 mg/kg) and using the same posttrial administration procedure. The following predictions were made: (1) all DCS-treated animals will show prolonged cSNC relative to Sal controls on Trials 12-15; (2) animals administered DCS at 60 mg/kg will exhibit prolonged cSNC relative to animals administered 15 mg/kg; (3) during the postshift, a significant Trial by Sucrose by Drug interaction will be caused by significantly less goal-tracking time in

the downshifted DCS-treated animals relative to the downshifted Sal-treated animals; (4) unshifted DCS-treated controls will show no marked change in behavior relative to unshifted Sal controls.

Method

The subjects were 56 male Long-Evans hooded rats from the TCU vivarium approximately 90 days old at the start of the experiment and experimentally naïve. Rats were housed, trained, and deprived as described in Experiment 2a. DCS was freshly dissolved in isotonic saline solution (15 mg/ml and 60 mg/ml) and administered appropriately following Trial 11.

Four groups had final size of $n = 9$ (32/Sal, 32/15, 4/15, and 32/60). Two groups had a final size of $n = 10$ (32/60 and 4/Sal). Within-trial data were not recorded in this experiment.

Results

The results are shown in Figure 11. There were no differences between the groups before assignment to drug condition. A Trial x Drug x Sucrose ANOVA for Trials 1-10 revealed a significant effect of trial, $F(9, 450) = 129.03$, $p < 0.001$, and main effect of sucrose, $F(1, 50) = 9.50$, $p < 0.01$, and trial by sucrose interaction, $F(9, 450) = 3.05$, $p < 0.01$. No other effects were significant, Fs (18, 450) < 1.1, ps > 0.44. A postshift Trial x Drug x Sucrose for Trials 11-15 revealed a significant main effect of trial, $F(4, 200) = 33.74$, $p < 0.001$, a significant main effect of sucrose, $F(1, 50) = 15.37$, $p < 0.001$, a trial by sucrose interaction, $F(4, 200) = 16.80$, $p < 0.001$. None of the interactions were significant, Fs < 1.

Table 6

Design of Experiment 3.

	Preshift	Postshift	
Group	Trials 1-10	Trial 11	Trials 12-15
32/Sal (n = 9)	Access to 32%	Access to 4%→Sal	Access to 4%
4/Sal (n = 10)	Access to 4%	Access to 4%→Sal	Access to 4%
32/15 (n = 9)	Access to 32%	Access to 4%→DCS (15mg/kg)	Access to 4%
4/15 (n = 9)	Access to 4%	Access to 4%→DCS (15 mg/kg)	Access to 4%
32/60 (n = 10)	Access to 32%	Access to 4%→DCS (60 mg/kg)	Access to 4%
4/60 (n = 9)	Access to 4%	Access to 4%→DCS (60 mg/kg)	Access to 4%

To further analyze the postshift results, a series of one-way ANOVAs for Trials 11-15 were conducted, yielding a significant effect on Trials 11-12, Fs(5, 50) > 3.32, ps < 0.02, but not Trials 13-15, Fs < 1. Protected Fisher's LSD posthoc tests comparing the downshifted animals to their unshifted drug control, as well as the each unshifted control to each other revealed the following: Groups 32/15 and 4/15 were significantly different on Trials 11-12, ps < 0.05, and Groups 32/60 and 4/60 were significantly different on Trials 11-12, ps < 0.01. However, 32/Sal and 4/Sal were significantly different on Trial 11, $p < 0.001$, but not Trial 12, $p > 0.07$. Furthermore, none of the unshifted controls were significantly different from each other on Trial 11-12, ps > 0.48. This pattern of results indicates that downshifted saline-treated animals (32/Sal) recovered from cSNC relative to their to unshifted counterparts by Trial 12, whereas downshifted DCS-treated animals persisted in showing cSNC on Trial 12 relative to their unshifted counterparts. The data were consistent with the prediction that DCS would enhance cSNC, but would not impact sucrose consumption in unshifted animals. However, the failure to yield a significant trial by sucrose by drug interaction and an accompanying significant difference between 32/Sal vs. either downshifted DCS-treated animals indicates that the optimal dose of DCS may approach 30 mg/kg, at least in the cSNC preparation.

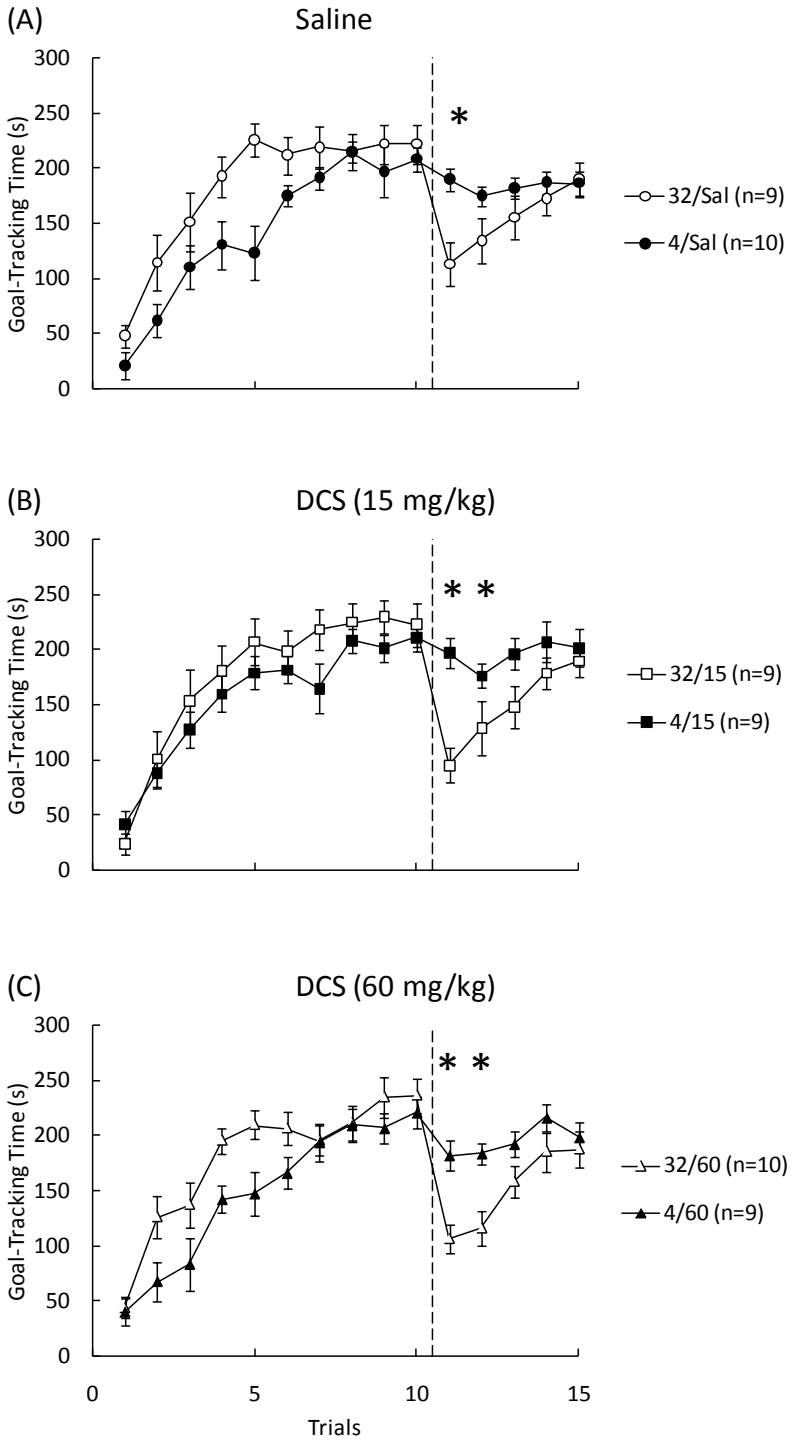


Figure 11. Overall results of Experiment 3. Mean goal-tracking time (±SEM) plotted as a function of trial. In trials 1-10 32/Sal, 32/15, and 32/60 received 32% sucrose solution. In Trials 11-15, 32/Sal, 32/15, and 32/60 received 4% sucrose, similar to 4/Sal, 4/15, and 4/60. Animals were administered either saline or DCS (15 or 60 mg/kg, i.p.) immediately following Trial 11. (A) Saline-administered (32/Sal vs. 4/Sal) animals demonstrated cSNC on trial11, but recovered on statistically on Trial 12 and all remaining postshift trials. (B)Animals administered 15 mg/kg DCS (32/15 vs. 4/15) demonstrated cSNC on Trial 11 and 12 recovering statistically on Trials 13-15. (C) Animals administered 60 mg/kg DCS (32/60 vs. 4/60) demonstrated cSNC on Trial 11 and Trial 12 recovering statistically on Trials 13-15.

Discussion

The results of Experiments 2a and 3 indicate that posttrial injections appeared to be most effective at 30 mg/kg, and only minimally (but still) effective at 15 mg/kg or 60 mg/kg. Consistent with the results of Experiments 1 and 2a all DCS-treated animals showed prolonged cSNC relative to saline controls on postshift trials. However, inconsistent with the predictions animals administered DCS at 60 mg/kg did not show prolonged cSNC relative to DCS-administered at 15 mg/kg. Moreover, no evidence was found of a significant trial by sucrose by drug interaction; downshifted DCS-treated animals at 15 mg/kg or 60 mg/kg did not show significantly less goal-tracking time relative to the downshifted saline-administered animals. This is consistent with data reported by Woods and Bouton (2006). In two follow-up experiments (using 30 mg/kg and 60 mg/kg) to the their conditioned suppression data described earlier, they reported that a 60 mg/kg dose of DCS failed to enhance extinction, whereas 30 mg/kg only had an effect when animals across all three experiments were collapsed into a single analysis. Together these results indicate that at higher doses, DCS's effects on memory consolidation may not necessarily increase linearly.

Experiment 4: DCS and Conditioned Taste Aversion

Experiments 1-3 showed that DCS prolongs cSNC. DCS administered pre- and posttrial enhanced cSNC at doses of 15, 30, and 60 mg/kg with the greatest effect seen at 30 mg/kg. While these experiments support the overall hypothesis that increasing activity at the NMDA receptor using DCS enhances cSNC by enhancing consolidation of aversive memory associated with surprising reward downshift, one alternative remains to be evaluated. Nunnink et al. (2007) reported that DCS at 30 mg/kg induced CTA when administered shortly before exposure to a novel saccharin solution using backwards conditioning, compared to a saline control. While this

experiment lacked an unpaired control it is plausible that DCS's effect on cSNC may be the result of a similar CTA. The 4% sucrose solution is relatively novel for animals experiencing the downshift and may act as a conditioned stimulus for the effects induced by DCS, which would act as the unconditioned stimulus. On subsequent presentations of the 4%, the animal would reject it, thus retarding recovery from cSNC. The kappa opioid agonist U50,488H administered at 3 mg/kg immediately following exposure to 4% sucrose induces such rejection whether in a cSNC or CTA paradigm (i.e., whether or not the rats are exposed to the incentive downshift; Wood et al., 2008). The relative novelty of the downshifted 4% sucrose is important because CTA is attenuated by nonreinforced preexposure to the conditioned stimulus (Davenport & Houpt, 2009). This would explain why CTA would be stronger in 32/DCS than in Group 4/DCS. Experiment 4 evaluated the CTA alternative explanation by pairing 4% sucrose to DCS in the absence of a downshift. The performance of this paired condition was compared to that of an unpaired control and a saline control. If DCS induces a CTA-like process on Trial 11 of the cSNC preparation, a similar suppression of consummatory behavior should be detected in the CTA preparation.

Method

The subjects were 36 male Long-Evans hooded rats from the TCU vivarium approximately 90 days old at the start of the experiment and experimentally naïve. Rats were housed, trained, and tested in the same apparatus as in Experiments 1-3. DCS (30 mg/kg) was prepared identically to Experiment 1.

Training lasted 3 daily trials, scheduled to be identical to trials in the cSNC experiments reported above. Subjects received 4% sucrose solution for all trials. Prior to Trial 1, rats were matched by ad libitum weight and then randomly assigned to one of three conditions: Paired (*n* =

12), Unpaired (n = 12), or Saline (n = 12). Subjects in the Unpaired and Saline conditions received an injection of saline immediately after Trial 1. Subjects in the Paired condition received an injection of DCS (30 mg/kg, ip). Three hours later, all subjects received a second injection. Animals in Paired and Saline conditions received a saline injection, whereas animals in the Unpaired condition received an injection of DCS (30 mg/kg). Thus, groups were matched in terms of the number and distribution of injections; moreover Groups Paired and Unpaired were also matched in terms of exposure to DCS. The design of the experiment is outlined Table 7.

Results and Discussion

The results are shown in Figure 12. A Trial x Group ANOVA for Trials 1-3 revealed a significant effect of trial, $F(2, 66) = 67.77$, $p < 0.001$. The Group effect was nonsignificant, $F(2, 33) = 1.14$, $p > 0.30$, as well as the Trial by Group interaction, $F < 1$. A series of one-way ANOVAs for Trials 1-3 were conducted, yielding a nonsignificant effect in Trials 1-3, Fs$(2, 35) < 1.57$, ps > 0.22. The nonsignificant effect on Trial 1, indicates that subjects were similar before injections took place. The Paired condition was not significantly different from either unpaired or saline controls. These results provide no evidence that DCS induced a CTA in the cSNC preparation.

Table 7

Design of Experiment 4.

		Drug		
Group	Trial 1	Immediate	3-h Delay	Trials 2-3
Paired (n = 12)	Access to 4%	DCS	Saline	Access to 4%
Unpaired (n =12)	Access to 4%	Saline	DCS	Access to 4%
Saline (n =12)	Access to 4%	Saline	Saline	Access to 4%

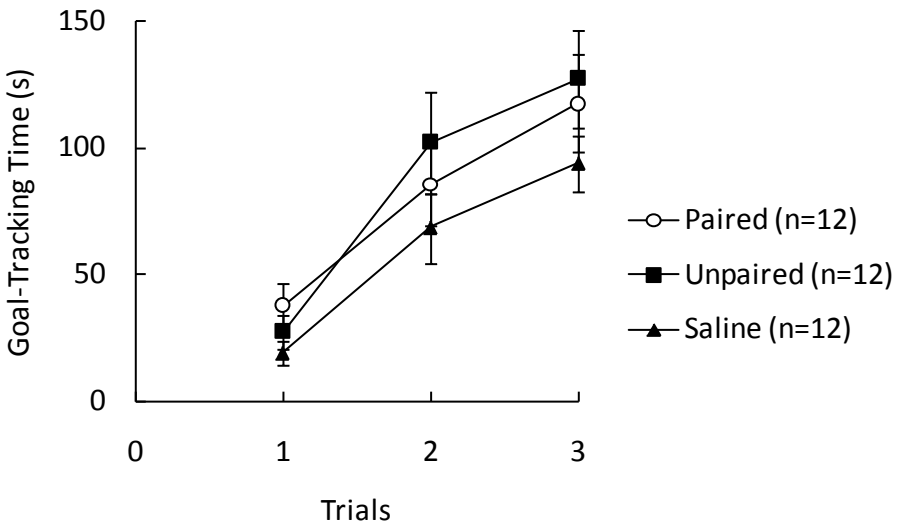


Figure 12. Overall results of Experiment 4. Goal-tracking time (s) is plotted as a function of trial, and drug treatment. On Trials 1-3 Paired, Unpaired, and Saline groups received 4% sucrose solution. Animals were administered either saline (Unpaired and Saline) or DCS at 30 mg/kg (Paired) immediately following trial 1. Animals were injected a second time 3 h later. Animals were administered either saline (Paired and Saline) or DCS (Unpaired). Paired condition did not develop CTA on remaining trials.

General Discussion

The current studies demonstrated that the partial NMDA receptor agonist DCS prolonged recovery from cSNC when administered either pre- or posttrial following a 32-to-4 and a 32-to-6 reward downshift. DCS failed to induce CTA, suggesting its suppressive effects on consummatory behavior were not the result of conditioned rejection of the relatively novel 4% sucrose solution independently of the downshift event. Moreover, DCS had no detectable effect on the consummatory performance of unshifted, 4-to-4 or 6-to-6 groups. These results were interpreted to support the overall hypothesis that NMDA receptor activation via the glycine-binding site enhances the egocentric aversive memory induced by the unexpected reward loss event. What follows is a critical evaluation of extent to which the data reported here support the egocentric memory hypothesis. Such evaluation is first approached at the physiological level and then at the psychological level. The implications support a modified version of the cSNC model proposed by Wood et al. (2005).

Physiological Factors

The current results conform to the main hypothesis that DCS enhances aversive egocentric learning in the cSNC situation via memory consolidation, increasing NMDA receptor activity possibly in areas of the brain known to be involved in aversive learning. Moreover, it was assumed that DCS leads to an increase in LTP-like processes in these areas of the brain, increasing synaptic connectivity. However, because these processes were not directly assessed careful consideration of the literature and the evidence from the present studies is warranted. The following factors are considered below: the pharmacokinetics of DCS, DCS-induced postsynaptic alterations, the possibility that DCS acted as an antagonist, and possible sites of drug action.

Pharmacokinetics. The effects of posttrial DCS treatment administration were interpreted as acting on mnemonic processes that presumably occur after the injection of DCS, but conclude prior to next day's trial. However, one might argue two points with respect to this issue. First, DCS administered after Trial 11 may not have been evacuated from the body to ineffective levels within 24 h and may therefore have impacted behavior on subsequent trials. It was assumed that DCS had no direct effects on Trials 12-15, as DCS's reported half-life is 23 min (Conzelman & Jones, 1956). Therefore a single 30 mg/kg dose of DCS should be metabolized to ineffective levels within 24-h. Additionally, behavioral evidence indicates that a single dose of DCS administered at 30 mg/kg will not necessarily affect behavior 24-h later (Woods & Bouton, 2006). Prior to a single fear extinction session, animals were administered either saline of DCS (30 mg/kg). 24-h later, half of the animals underwent spontaneous recovery testing, while the other half underwent contextual renewal testing. DCS impaired spontaneous recovery of fear, but not contextual renewal. This differential outcome, despite identical pharmacological treatment suggests that DCS's effects depend on the conditioning treatment. Moreover, Experiment 1 indicates that DCS does not have direct effects on behavior, even in the presence of reward downshift, as DCS was administered prior to Trial 11 in that study, and Experiment 4 suggests that posttrial DCS may not affect consummatory behavior in the absence of an incentive. Therefore, even if DCS failed to be metabolized from the body within 24 h, there is no evidence that DCS would affect consummatory behavior.

Second, physiological processes associated with memory consolidation may not have concluded prior to testing 24 h later. With respect to this critique we must rely upon previous studies investigating memory consolidation for aversive learning in the cSNC paradigm. Bentosela et al. (2006) found that posttrial administration of corticosterone immediately, but not 3 h later enhanced consummatory suppression. In broader terms, Wood et al. (2008) found CTA

to 4% sucrose solution when U50,488H was administered immediately, but not 3 h later. This consumption evidence suggests that any mnemonic processing associated with cSNC or sucrose solution diminishes well before the next day's testing.

DCS-induced postsynaptic alternations. The current studies assumed that DCS enhanced an LTP-like process in areas of the brain associated with aversive learning. Recent evidence from fear conditioning studies may contradict this assumption (Mao, Hsiao, & Gean, 2006). An alternative explanation for the DCS's effects in many of the fear extinction studies may be that DCS enhanced the *erasure* of fear conditioning, rather than the extinction of fear learning, a presumably different physiological process. Mao et al. (2006) found that DCS enhanced the reversal of postsynaptic changes caused as a result of fear extinction. This was indexed by decreased surface expression of GluR1, a subunit of the AMPA receptor (a type of receptor shown to increase following LTP) in the amygdala.

In their first study, Mao and colleagues showed that presentation of the conditioned stimulus alone 1 h following fear conditioning, but not 24 h after conditioning, reversed increases in GluR1 expression on the cell surface. In another study, animals underwent extinction 24 h following fear conditioning. A portion of the animals received DCS either before or after the extinction trials. Other animals in control conditions received vehicle, vehicle without extinction, the antagonist HA-966 plus DCS, or HA-966 alone. Behaviorally, when administered either before or after extinction trials, DCS decreased the startle response on a test trial 24-h following extinction. After the test trial, animals were decapitated and tissue samples from the lateral and basolateral portions of the amygdala were collected. Expression of GluR1 in the amygdala was assessed using Western Blots (or immunoblot, a semi-quantitative assay for detecting specific proteins in a tissue sample). DCS-treated animals showed a reduction in GluR1expression relative to controls. A follow-up experiment tested in vitro slices of amygdala

exposed first to tetanic stimulation, and then to low frequency stimulation (LFS), a process shown to reverse LTP and reduce EPSPs. The slices exposed to DCS had reduced EPSPs relative to samples that received only LFS. Moreover, Western Blot analysis revealed that the DCS plus LFS samples showed a reduction in GluR1expression, relative to other conditions. Together from these results, Mao and colleagues (2006) concluded that DCS may act to facilitate NMDA-mediated endocytosis of AMPA receptors, effectively erasing learning.

Based on these results, one might argue that DCS did not enhance aversive egocentric learning, but instead erased previous learning. However, if this argument is extrapolated from fear conditioning to cSNC, then aversive egocentric memory and/or previous allocentric memory for the 4% should be impaired following administration of DCS, as DCS would have reversed GluR1 expression occurring as a result of learning on Trial 11. In either case, DCS-treated animals should have shown a pattern of behavior on Trial 12 similar to that shown on Trial 11. For example, DCS-treated subjects should not have shown early suppression in Trials 12-13 (Experiment 1) and Trial 12 (Experiment 2a). However, in the current studies, DCS-treated animals showed suppression of behavior during the initial 100 s on trials following drug treatment, casting doubt on the memory-erasure explanation of DCS's effects on cSNC. Moreover, this explanation cannot adequately explain DCS's effects in maze learning, conditioned place preference, or conditioned taste aversion (Golden & Houpt, 2007; Nunnink et al., 2007; Quartermain et al., 1994). Thus while remaining an interesting proposition, the memory erasure hypothesis does not provide a comphrensive account DCS's effects on cSNC.

Pharmacological function of DCS. A third possible physiological concern lies in the assumption that DCS acts as agonist in the cSNC preparation. DCS acts to enhance activity at the glycine modulatory site on the NR1 subunit of the NMDA receptor (see Figure 3). Functionally, it was assumed that DCS increased NMDA receptor activity, thus enhancing learning about the

downshift event and increasing the strength of the associated aversive memory. Some research has shown that DCS can act as antagonist (e.g., Hood et al., 1989), while other research showed consistent results irrespective of dose (e.g., Quartermain et al., 1994). The pharmacological role of DCS as either an agonist or an antagonist in cSNC needs to be evaluated with respect to the dose and the extracellular glycine concentration in the brain.

The first factor, dose level, can be addressed by results in Experiment 2b and 3. In Experiment 3, if DCS acted as an antagonist, then 60 mg/kg should have yielded stronger suppressive effects than either 15 or 30 mg/kg (Experiment 2a). Generally, antagonists should act in a cumulative fashion, higher doses producing greater effects than lower doses. In some cases however, higher doses will not produce correspondingly greater effects, mostly due to saturation of receptors. In such cases, the higher dose should still continue to yield a magnitude of effect consistent with the lower, maximally effective dose. In our case, 60 mg/kg did not produce greater suppression than 15 mg/kg. In fact, 30 mg/kg was the only dose that yielded a trial by drug by sucrose interaction. Although these results are compared across experiments, this leads one to the conclusion that 30 mg/kg is an optimal dose in the cSNC and acted as an agonist.

Furthermore, the effectiveness of DCS at 30 mg/kg scaled to the magnitude of the downshift. DCS enhanced cSNC to a greater extent following a 32-to-4 downshift than following a 32-to-6 downshift. If 30 mg/kg DCS acted as an antagonist, then in Experiment 2b, the administration of 30 mg/kg DCS following 32-to-6 reward downshift should have yielded results similar to those seen after a 32-to-4 downshift. But, if DCS acted as an agonist at 30 mg/kg following 32-to-6 downshift, the consummatory suppression should likewise be mild, as aversive learning and its resultant memory should scale to the degree of incentive downshift. The failure to yield a significant trial by sucrose by drug interaction together with a significant difference

between 32/Sal vs. 32/DCS indicates that DCS at 30 mg/kg did not function as an antagonist in our preparation.

With respect to extracellular glycine levels, one possibility is that DCS acts as an antagonist, because the levels of extracellular glycine in the relevant portions of the brain where DCS might act upon learning/consolidation were sufficiently elevated, thus reducing glycine's ability to stimulate the NMDA receptor. In addition to the arguments given with respect to dose levels, there is evidence suggesting that glycine levels within the brain are below the level of maximal stimulation, indicating that DCS did not act as an antagonist. Martina, Gorfinkel, Halman, Lowe, Periyalwar, Schmidt, and Bergeron (2004) reported extracellular glycine levels in hippocampal slices from juvenile male Sprague-Dawley rats. In these samples, the glycine transporter GlyT1 kept extracellular glycine below the level needed to induce LTP. Administration of the GlyT1 antagonist CP-802,79 increased the amplitude of NMDA receptor currents and LTP. Applying reverse microdialysis (a procedure similar to microinjection, except that it maintains a constant level of drug throughout testing) in free moving rats showed that this drug elevated only the extracellular concentrations of glycine in the hippocampus. These results are consistent with previous work suggesting that extracellular glycine concentrations are around 150 nM; well below maximum levels (Attwell, Barbour, & Szatkowski, 1993; Roux & Supplisson, 2000). This is well below the 0.6 μM glycine levels used for early *in vitro* experiments (e.g., Hood et al., 1989).

However, Saul'skaya and Solov'eva (2005) reported that glycine levels in the nucleus accumbens are transiently elevated following correction learning. Animals that received tone-shock pairings in Phase 1 were presented with tone-food pairings in Phase 2. During these tone-food presentations, subjects showed increased glycine levels (increasing from a mean baseline of 29 nM to approximately 49 nM). Authors interpreted these results to mean that glycine facilitates

processes associated with forced correction learning, also known as counterconditioning, a preparation with procedural similarities to cSNC. This is especially relevant to the current studies as the nucleus accumbens shows increased c-Fos transcription (a transcription factor for immediate early gene expression correlated with synaptic changes such as growth and protein kinase activity) and reduced dopamine efflux following 32-to-4 reward downshift (Genn, Ahns, & Phillips, 2004; Pecoraro & Dallman, 2005). This leads us to the final physiological concern: possible neuroanatomical sites of action.

Possible sites of drug action. Many studies evaluating DCS have focused on the amygdala's role in fear conditioning (Vervliet, 2008). Accordingly, many studies have shown similar effects when DCS was infused directly into the basolateral amygdala to those shown by systemic administration, as in the current studies. Despite such results, it could be argued that in these studies DCS did not act solely upon associative processes occurring in the amygdala. For example, even though lesion studies have failed to implicate the hippocampus as a site of action for the occurrence of cSNC (Flaherty, 1996), more recent studies using c-Fos expression have shown hippocampal activity during reward downshift (Pecoraro & Dallman, 2005). Behaviorally, Daniel et al. (2008) reported a mild within-subject cSNC effect when allowing subjects a 90-s pretrial interval to process distinct contextual features, a process likely to be hippocampally dependent. DCS is known to reverse impairments resulting from hippocampal lesions and to facilitate hippocampal-dependent learning (Schuster & Schmidt, 1992; Thompson, Moskal, & Disterhoft, 1992). This makes intuitive sense, given that NMDA hippocampal neurons were among the first to be used to study LTP and the reported studies of extracellular glycine utilized hippocampal slices from rats (Martina et al., 2004).

Moreover, Pecoraro and Dallman (2005) reported elevated c-Fos transcription in a wide array of brain regions, including the medial prefrontal cortex (mPFC), a region responsive to

both chronic and acute DCS-administration in rats with spared nerve injury (SNI), a model of neuropathic pain (Millescamps, Centeno, Berra, Rudick, Lavarello, Tkatch, & Apkarian, 2007). DCS-treated animals showed increased tactile thresholds following repeated oral administration and a single infusion of DCS into the mPFC and the amygdala-effects similar to NMDA and glycine treatments. Among the antinociceptive effects shown, Millescamps and colleagues (2007) reported SNI rats receiving infusions of DCS into the mPFC, did not shift preference in a place escape avoidance task, relative to saline-infused SNI controls. In the place escape avoidance task (Labuda & Fuchs, 2001), subjects were placed into a partitioned chamber (half black/half white) with a wire mesh floor after undergoing SNI. Subjects received stimulation of the injured hindpaw in the presence of the black portion (the preferred side) and the uninjured paw when in the white portion. Over the trial, Millescamps et al. (2007) found that saline-infused SNI rats shifted preference from the black portion to the white portion, whereas DCS-infused did not shift preference. However, these results were complicated by the finding that saline-infused SNI rats tested 24 h, but not 1 h after infusion also failed to shift preference. Interpreting their results from an incidental learning perspective, they concluded that DCS facilitated extinction of incidental aversive learning that occurred as a result of SNI.

In summary, in addition to the amygdala, evidence suggests that DCS may have acted at multiple sites, each potentially affecting cSNC. Moreover, evidence from the current experiments and prior research indicates that DCS generally acts as an agonist in the amygdala, hippocampus, and mPFC. DCS may have retarded forced correction learning in the nucleus accumbens, as elevated glycine levels following reward downshift would have been blocked by DCS. Whereas in the amygdala, hippocampus, and mPFC such blockade by DCS would have diminished learning about the aversive downshift, blockade in the nucleus accumbens actually

may have retarded adjustment to 4% sucrose. Physiologically, the net effect of DCS acting on various sites independently may have led to an increase in the salience of the downshift event.

Psychological factors

Physiologically, the results were consistent with the main hypothesis: DCS-administration enhances the egocentric memory of the reward downshift experience. Confirmation of this hypothesis is important as it helps to disambiguate the psychological processes underlying cSNC. A cursory evaluation shows that these results were consistent with an account of cSNC that postulates an aversive egocentric memory for the downshift event and contradicts accounts that postulate only primary frustration and cognitive processes involving allocentric updating of the presented sucrose solutions. However, one must carefully consider the implications this hypothesis of DCS's effects holds for SNC for an understanding of incentive downshift.

The simplest argument is that DCS acted upon cognitive-based allocentric conditioning. According to the frustration hypothesis of cSNC (Wood et al., 2005) two allocentric memories or expectations play a role on downshift trials: expectation of 32% ($e_{32\%}$) and expectation of 4% ($e_{4\%}$). Theoretically, the expectation of sweetness is adjusted from the old $e_{32\%}$ to the newer $e_{4\%}$. Consummatory suppression is the result of unconditioned primary frustration, resulting from violation of $e_{32\%}$ by the current 4% sucrose outcome ($O_{4\%}$). One possible argument is that DCS acted as an antagonist to retard the update of $e_{32\%}$ into $e_{4\%}$. However, data from physiological studies appear to make this explanation unlikely. For example, DCS infused into in the amygdala augments fear extinction (Ledgerwood et al., 2003), which implies that DCS tends to facilitate, rather than retard, the update of old memories to new conditions (allocentric learning).

Alternatively, given that procedurally, a single 5-min trial is sufficiently brief to mirror a single fear-extinction trial, it may be argued that DCS facilitated memory reconsolidation for the $e_{32\%}$. Lee et al. (2006) reported that DCS's effects are dependent upon the number of fear-extinction trials. More specifically, when administered prior to a single extinction trial, DCS enhanced freezing behavior 24 h and 72 h later, relative to animals administered DCS prior to several extinction trials. Lee and colleagues (2006) argued that small number of fear-extinction trials evoked the expectation of pain, making the memory labile and subject to change, or reconsolidation (for a review of memory reconsolidation, see Tronson & Taylor, 2007). Similarly, in the cSNC preparation the effects of DCS in the present study might be the result of memory reconsolidation, a process also shown to involve NMDA receptor activation (Lee et al., 2006; Torras-Garcia, Lelong, Tronel, & Sara, 2005). According to this account, the brief presentation of the sipper tube on Trial 11 would be analogous to a single CS-alone trial, making the already present $e_{32\%}$ stronger. Relative to saline-treated animals, $e_{32\%}$ would be strengthened following DCS administration. Behaviorally, this account would predict that downshifted DCS-treated animals show similar levels of consummatory suppression early on Trial 12 relative to saline controls. At variance with this prediction, within-trial data from Experiments 1 and 2a indicates that downshifted DCS-treated animals showed increased suppression on Trial 12, relative to the downshifted saline animals. The saline results are consistent with within-trial analyses of cSNC from Norris et al. (2008). Therefore the reconsolidation hypothesis cannot explain the present results.

It may still be argued that DCS reduced spontaneous recovery associated with $e_{32\%}$. Norris et al. (2008) and Experiment 2a indicate that noninjected and saline downshifted animals show little consummatory suppression at the onset of Trials 12 and 13. The majority of consummatory suppression under those conditions occurs towards of the end of the trial in the

last 200 s (see Norris et al., 2008, Figure 4). These animals appear to show spontaneous recovery of $e_{32\%}$-associated consummatory behavior. Similarly, in fear extinction experiments, DCS has been shown to reduce spontaneous recovery of conditioned fear behavior 24 h after administration (Lee et al, 2006; Woods & Bouton, 2006). Based on this perspective, one might argue that DCS's suppressive effects are not the result of the enhancement of an aversive egocentric memory of the downshift event, but rather a reduction in spontaneous recovery of $e_{32\%}$-associated consummatory behavior. The results of the within-trial analysis of Experiments 1 and 2a can be interpreted in these terms. Within the first 100 s, 32/DCS showed less spontaneous recovery at the onset of trials 12 and 13. However, differences between 32/Sal vs. 32/DCS cannot be attributed entirely to a reduction in spontaneous recovery on trials 12 and 13. In Experiment 2b, 32/DCS did not show enhanced suppression early on Trial 12 (Figure 10). If DCS reduced allocentric learning then suppression of consummatory behavior should have occurred irrespective of the downshift magnitude. DCS should have reduced spontaneous recovery independent of the downshift event. Therefore, the spontaneous recovery hypothesis also fails to account for the present results of DCS on cSNC.

Despite this conclusion, a better understanding of spontaneous recovery in the consummatory behavior situation will allow for a better understanding of the associative processes underlying cSNC. In turn, this might elucidate the psychological mechanisms, engaged by DCS administration. Using a discrete trial procedure (see Norris et al., 2008, Experiment 5), unpublished data from our lab (shown in Figure 13) indicates that unlike in many extinction preparations, the extinction of consummatory behavior does not result in higher levels of spontaneous recovery following longer resting periods (168 h) rather than shorter (24 h) resting periods after a downshift from 32% sucrose to an empty sipper tube. This would suggest a mechanism of spontaneous recovery for consummatory behavior consistent with Skinner's

(1938) account of spontaneous recovery. Skinner suggested that stimuli at the onset of the session have more in common with stimuli present during acquisition and therefore maintain some value as excitatory stimuli. This mechanism does not appear to regulate spontaneous recovery in some cases (Robbins, 1990; Thomas & Sherman, 1986), as it can not account for the growth of spontaneous recovery with time. When applied to consummatory behavior, Skinner's (1938) account is also preferable to one based on memory retrieval (Bouton, 1993), which wrongly predicts the spontaneous recovery of cSNC (Norris et al., 2008).

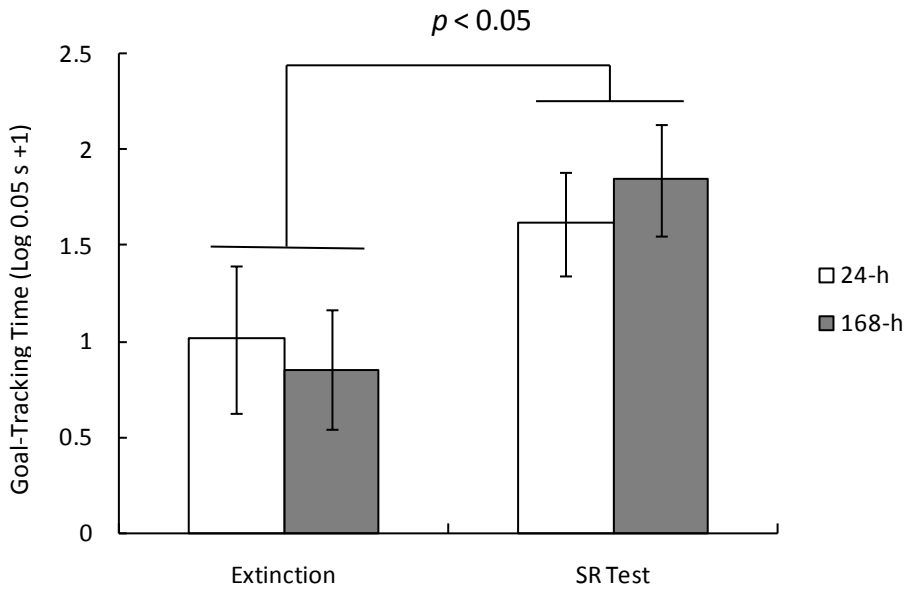


Figure 13. Extinction data from unpublished experiment. Goal-tracking time (log 0.05s +1) is plotted as a function of trial. During acquisition (not shown) animals received 6 trials of 50-s access to 32% solution for 10 sessions. During extinction and SR (spontaneous recovery) test trials animals received similar access to an empty sipper tube. Following extinction, animals were matched by terminal performance and assigned to either 24-h or 168-h rest periods, after which they underwent testing for SR. Both conditions showed SR, $F(1,15) = 4.79$, $p < 0.05$. However, there was no main effect of rest period, or a rest period x SR test, Fs <1.

A Modified Model of cSNC

Based on the behavioral and neurobiological data on cSNC that has been accumulating over the past three decades (e.g., Flaherty, 1996; Papini et al., 2006; Pecoraro & Dallman, 2005), it is possible to propose some modifications to the model suggested by Wood et al. (2005). This model is based on the following assumption:

(1) Incentive contrast requires a comparison between a current incentive outcome and a remembered incentive outcome (Papini & Pellegrini, 2006).

(2) Licking behavior is controlled by antecedent stimuli (S→R) and expectations (S→S) in a manner akin to that proposed by two-factor learning theory (Mowrer, 1960).

(3) This model distinguishes between allocentric learning (i.e, learning about environmental changes) and egocentric learning (i.e., learning about the emotional reaction to environmental changes), but assumes that the same Pavlovian mechanisms underlies both types of acquisition process (Papini, 2003).

(4) The goal of this model is to provide a guide to interpret the behavioral effects of neurobiological manipulations such as drug administration and lesions of selected brain areas.

Figure 14 presents the modified model of cSNC by representing behavioral and associative changes in four key timepoints: 10, 11, 12, and 13-15. Furthermore, the events corresponding to the early vs. late portions of the Trial 11 are separated into different panels to facilitate the understanding of within-trial changes in information processing.

(1) During the preshift phase (panel A) stimuli predict the presence of 32% sucrose solution. Such stimuli (S) may involve some elements of the context, tactile

stimulation from the initial licking of the sipper tube, fluid stimulating the oral cavity, and even sensory feedback from the licking response. Irrespective of the identity of the element (or elements) that acquire control, this stimulus directs licking behavior via stimulus→response (S→R) learning. In turn, this yields early signs of 32% sucrose, which signals the presence of more 32% solution (stimulus→stimulus or S→S learning), which motivates further licking. Because both S→S and S→R motivate licking, consummatory behavior at the onset of each trial occurs at a near maximum level. The relationship between the stimuli and sucrose solution could be viewed as a Pavlovian pairing (P_1).

(2) During the early portions of Trial 11 (panel B) the following processes occur. Early trial cues signal reward and evoke $e_{32\%}$; the animal engages the sipper tube accordingly; within approximately 100 s, the animal detects the discrepancy between the signal and the downshifted outcome ($O_{4\%}$). The detection of this $e_{32\%}$-$O_{4\%}$ discrepancy elicits primary frustration (Ψ), an emotional state of psychological pain; the unconditioned response to Ψ suppresses licking behavior.

(3) This has the net effect that in the late portions of Trial 11 (panel C) in addition to the unconditioned response (R_o) a Pavlovian pairing occurs between $O_{4\%}$ and Ψ (P_2), forming the expectation of psychological pain (e_Ψ), or secondary frustration.

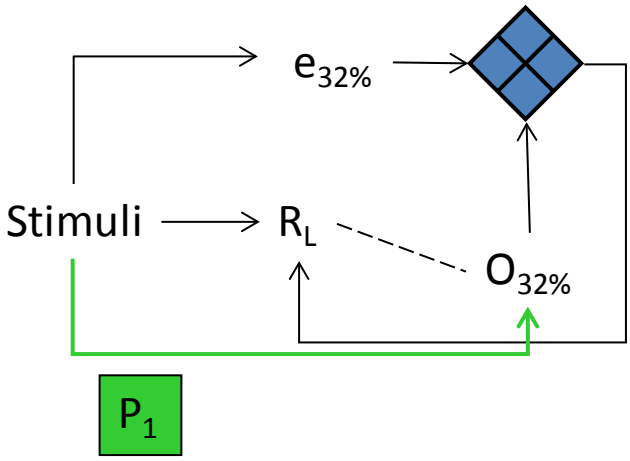
(4) On Trial 12 (panel D) the following process occurs: early trial cues signal reward (the expectation is greater than 4%, but less than 32%); the animal engages the sipper tube; detects the discrepancy and the $O_{4\%}$, the $O_{4\%}$ is paired with Ψ, evoking the e_Ψ, and the animal engages in a conditioned response that further suppresses behavior.

(5) By Trials 13-15 (panel E) recovery from cSNC is driven by two processes: early trial cues become more predictive of 4% solution, which reduces the discrepancy between signal and $O_{4\%}$, and hedonic need for caloric intake. Similar to the proposed Pavlovian pairing that occurred during the preshift phase (P_1), $e_{4\%}$ is the result of a third Pavlovian pairing, this time involving the new incentive value (P_3). This model of contrast has the advantage that it contains the memory related processes necessary to explain posttrial effects, trial selective drug effects, corticosterone data, and frustration-induced psychogenic fever, while incorporating behavioral evidence showing high initial licking behavior (used as evidence against frustration-based egocentric memories).

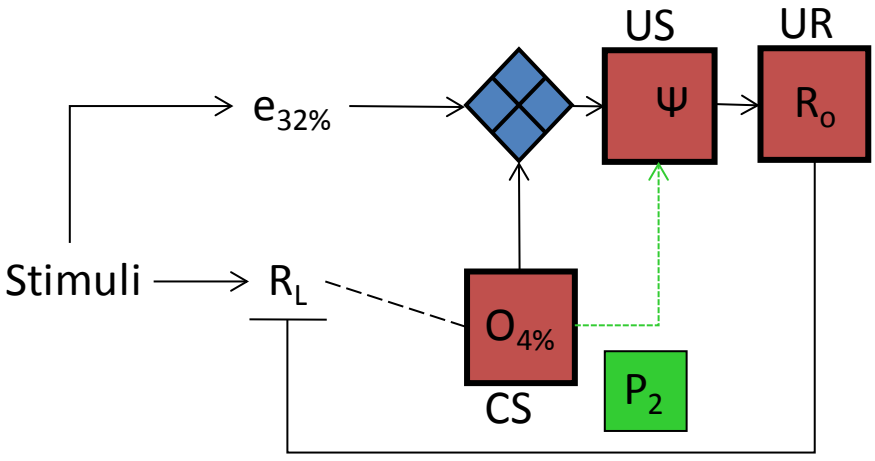
According to this account, DCS's effects acted to enhance aversive egocentric learning (P_2 association) leading to anticipatory psychological pain (e_{Ψ}). The enhanced learning readily guides behavior directed away from the 4% solution (R_{Avoid}) earlier in the trial than in the saline-treated animals. Alternatively, if one leans towards generalities across learning preparations, one might argue that DCS enhances some aspect of contextual learning while not affecting the associative learning. In this account, based on evidence from renewal and spontaneous recovery literature reviewed by Vervliet (2008), DCS enhances the context→no 32% solution association. While plausible, the current experiments made no use of context manipulations as these manipulations have resulted in small cSNC effects and under conditions different from those used in the present experiments (Daniel et al., 2008). Administration of posttrial corticosterone, should act in a similar fashion, enhancing the aversive egocentric learning, possibly by modulating NMDA receptor activity. This would be similar to what has been indicated in fear extinction procedures following the co-administration of subthreshold levels of synthetic glucocorticoid agonist dexamethasone and DCS, resulting in a synergistic effect on extinction

(Yang et al., 2007). The effects of opioid drug manipulations may act in two locations, influencing the value of the new incentive alternative, 4% sucrose, that is present following the downshift event (Norris, Ortega, Perez-Acosta, & Papini, submitted). Alternatively, opioid drug manipulations may enhance the unconditioned emotional state of psychological pain (Ψ). In this model, benzodiazepine drug effects would act similar to the mechanism proposed by Flaherty (1992), acting on the expression of conditioned responses to reward loss, reducing search behaviors, but not affecting memory consolidation processes.

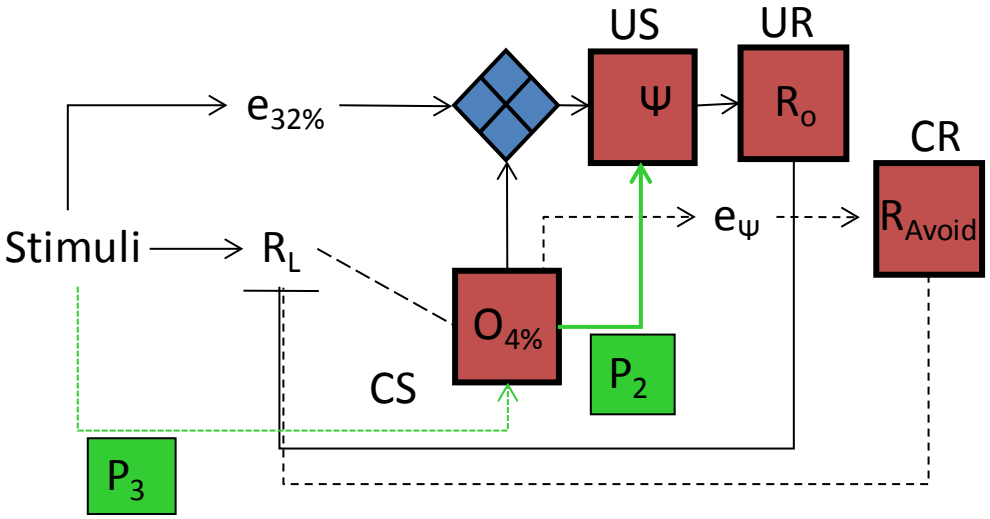
(A) Trial 10



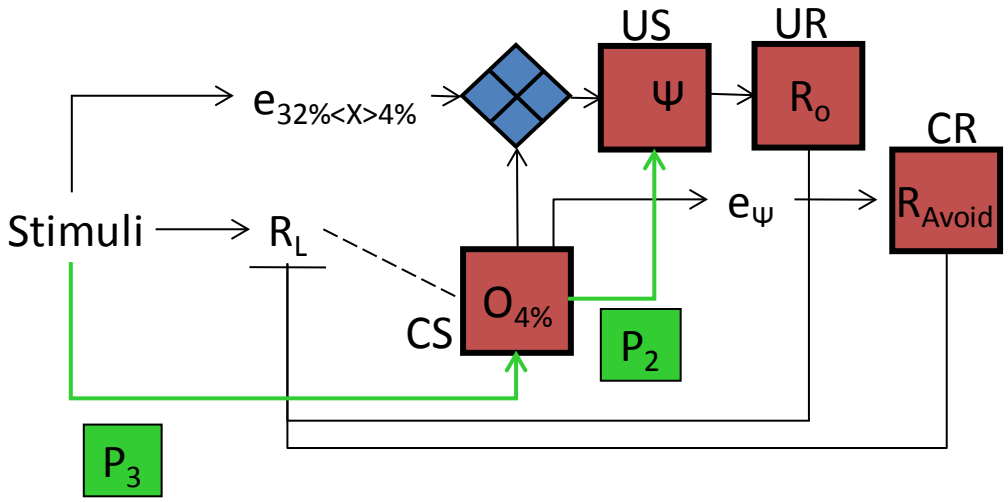
(B) Early Trial 11



(C) Late Trial 11



(D) Trial 12



(E) Trials 13-15

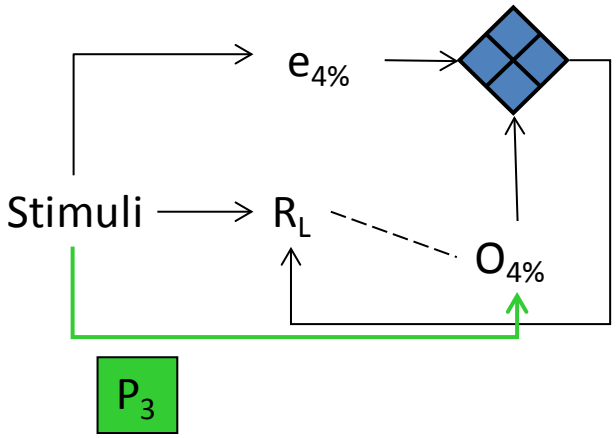


Figure 14. A modified model of cSNC. (A) In Trial 10, compound stimuli elicit consummatory behavior (R_L) and animals consume fluid, receiving feedback that enhances the consummatory behavior. P_1 refers to the Pavlovian association between stimuli and 32% sucrose. (B) In the early portions of Trial 11, stimuli elicit R_L that results in receipt of 4% solution ($O_{4\%}$). The discrepancy between the $e_{32\%}$ and $O_{4\%}$ results in the formation of an aversive US containing allocentric information about the discrepancy (blue circle with X) and psychological pain (Ψ). This elicits an UR (R_o) consisting of search behavior, rearing, grooming, and other unspecified behavior, causing consummatory suppression. (C) In the late portions of Trial 11, the CS-US pairings (P_2) between $O_{4\%}$ and Ψ form an expectation of Ψ (e_Ψ). The e_Ψ elicits an avoidance response (R_{Avoid}), which in additional to R_o suppresses consummatory behavior. (D) In Trial 12, the animal holds an expectation less than 32%, but greater than 4%. Similar to late portions of Trial 11, behavior is suppressed via conditioned and conditioned mechanisms. Throughout the course of postshift trials, the $O_{4\%}$ updates the expectation associated with the compound stimuli. As the expectation approaches $e_{4\%}$, the discrepancy between expectation and outcome is reduced, eliminating, or gradually, "turning down" the aversive US. With increasingly less US activation, the CS's ability to elicit the CR is reduced. With less aversive US and CR, consummatory behavior is no longer suppressed. (E) By Trials 13-15 the expectation and outcome are in agreement, the compound stimuli accurately predict the presence of 4% solution, supporting a robust level of consummatory behavior. This leads to the solidification of a Pavlovian association between stimuli and 4% sucrose (P_3) that had started forming late in Trial 11. It is likely that following recovery there is no measurable memory for frustration as research has failed to find extinction phenomena (Norris et al., 2008). In the cSNC preparation, memory for frustration may be transient, similar to the cSNC phenomenon.

Limitations and Future Directions

The current experiments contained several weaknesses that will be outlined along with possible future directions of such research. First, unlike early memory studies using a posttrial manipulation or previous work using posttrial manipulations in the cSNC preparation (Bentosela et al., 2006; Breen & McGaugh, 1961; Ruetti et al., 2009; Wood et al., 2008), the current experiments did not include a control condition in which the downshift event and DCS were separated by several hours. In previous studies, animals were administered the pharmacological manipulation either immediately or several hours later (e.g., 3 h later), presumably after the memory consolidation processes had concluded. The main purpose of this condition is to show that the drug effect is time dependent relative to the downshift experience (i.e., there must be temporal contiguity between training and the drug effect). Whereas the pharmacokinetics and behavioral consequences of DCS are well documented (Conzelman & Jones, 1953; Hood et al. 1989; Vervliet, 2008), the time course of egocentric memory consolidation in the cSNC situation is not well understood. DCS could be used to characterize the time-course of memory consolidation (McGaugh, 2003). Future research investigations should address this theoretical shortcoming, now that parameters showing robust effects have been established.

Second, the current experiments used only DCS to explore the role of the NMDA receptor on cSNC. As shown in Figure 3, the NMDA receptor has several sites of drug action which need to be explored in the cSNC preparation. Some pharmacological manipulations that could be administered systemically include MK-801 and ifenprodil. Both these compounds have been shown to influence fear conditioning, typically blocking fear extinction (Baker & Azorlosa, 1996; Johnson, Baker, & Azorlosa, 2000; Blair, Sotres-Bayon, Moita, & LeDoux, 2005; Lee, Milton, & Everitt, 2006; Sotres-Bayon, Bush, & LeDoux, 2007). As these compounds act on

other regions of the NMDA receptor their effects would lead to a better understanding of the role of these receptors in cSNC. In this vein, an experiment currently underway is assessing the effects of HA-966, an antagonist at the glycine modulatory site, alone and in combination with DCS, on cSNC. Past research has shown that HA-966 blocks the fear reducing effect of DCS without itself having an effect (Mao, Hsiao, & Gean, 2006). This compound is of special importance because while DCS administration shows that the NMDA receptor *can* be involved, agonist administration does not necessarily show that the NMDA receptor *must* be involved. A demonstration of the necessity of a biological component for a given function, is generally shown by inactivating such component. HA-966 administration would allow us to determine whether the NMDA receptor must be involved to encode the egocentric memory of the downshift.

Third, the current experiments only used systemic injections. While this technique has proved useful in establishing the neurochemical profile of cSNC (Flaherty, 1996), it does not identify the locus of drug action due to the diffuse representation of receptors in the brain. Moreover, DCS may have caused effects that counteracted or synergized each other. For example, DCS administration may have had opposite effects in the nucleus accumbens and the amygdala, retarding learning in one area while enhancing it in the other. This limitation can be overcome with a microinjection procedure targeting specific brain areas. Whereas systemic drug administration may limit the experimental search for underlying mechanism, it has the positive feature that it shows potential for the clinical use of DCS in the treatment of anxiety disorders (see next section).

Fourth, no additional measures were used. For example, previous research shows that reward loss potentiates a number of behaviors such as escape, rearing, defecation, search activity, and submissive behavior (Papini & Dudley, 1997). These behaviors could have been

recorded using video-taping equipment (Pellegrini & Mustaca, 2000) or utilizing an escape from frustration apparatus similar to that used by Daly (1969, 1970).

Therapeutic Implications and Conclusions

DCS has been shown to alter behavior in fear conditioning situations. The present experiments extended these findings to cSNC, to better understand psychological pain induced by unexpected incentive loss. Based on animal work in fear conditioning, DCS has been applied to human populations in clinical settings. In small clinical trials, DCS has been used to enhance exposure-based psychotherapy for the treatment of specific phobias, social anxiety disorder, obsessive-compulsive disorder, and panic disorder with mild or moderate agoraphobia (Hofmann, 2007). In these settings, DCS appears to have small but consistent effects under some conditions. For example, utilizing nonphobic college-aged participants with a self-reported fear of spiders, DCS had no significant effects on fear reduction, relative to saline controls (Guastella, Dadds, Lovibond, Mitchell, & Richardson, 2007). However, in clinically phobic individuals, as classified by the DSM-IV, participants with social phobia and acrophobia showed reduced anxiety following a behavioral manipulation accompanying oral DCS administration (Guastella, Richardson, Lovibond, Rapee, Gaston, Mitchell, & Dadds, 2008; Ressler, Rothbaum, Tannenbaum, Anderson, Graap, Zimand, Hodges, & Davis, 2004). Overall, the results from these human studies suggest that DCS administration in regulated clinical settings with diagnosable psychiatric conditions is beneficial. This is consistent with the results of the Experiment 2b. DCS had limited effects on cSNC following a less aversive 32-to-6 downshift.

In the same way, a better understanding of the role of NMDA receptors in cSNC may allow for a better utilization of DCS in clinical settings in two possible ways. First, data from Experiments 1-3 suggest that DCS enhanced conditioned negative emotional states, thus

suggesting that its use maybe counterproductive in some situations. These may include acute treatment with DCS immediately following sudden traumatic loss. Proximity to the stressful loss event may interact with drug therapy to enhance aversive memories for such event, thus impairing recovery. This is consistent with fear conditioning work using human participants showing that DCS enhanced fear memory when administered after CS→electric shock pairings (Kalisch, Holt, Petrovic, De Martino, Kloppel, Buchel, & Dolan, 2009).

Second, under certain conditions, the enhancement of aversive memories promoted by DCS may be desirable. For example, DCS may be useful as an adjunct to resilience or perseverance training. The purpose of resilience training is to build a person's "inner stoic" in order to teach better emotional regulation and increase the sense of self-mastery. Similarly to the effects of partial reinforcement training on extinction behavior (Nation & Woods, 1980), resilience training would mildly frustrate participants in settings similar to exposure-based therapy. However, unlike exposure-based therapy, the goal during frustration trials would be to teach participants to recognize emotional arousal during mildly stressful tasks, persist through such tasks, and incorporate such memories in their self-schemas; a cognitive framework consisting of organized information about one's self with respect to roles and actions in specific areas of experience (*APA concise dictionary of psychology*, 2009). Rather than attempting to eliminate negative emotion, the thrust of resilience training would be to "inoculate" individuals against the disruptive expression of negative emotion, instructing them to recall difficult situations that they overcame. In such training, DCS administration may serve to enhance memories of difficult situations. In properly controlled settings this would hypothetically have the net effect of increasing hope and reducing negative self-schema.

This therapeutic approach is consistent with Snyder's (1994) hope theory, in which the therapeutic aim is to generate a greater sense of agency and defining clearer pathways to goal-

achievement. According to hope theory, therapists should be able to increase agency (ability to succeed in the future) by reviewing perceived gains or reflecting on past successes (Cheavens, Feldman, Woodward, & Snyder, 2006). The driving theoretical point is the idea that hope decreases depressive symptoms and reduces vulnerability to anxiety, acting as a balance against negative psychological forces (Snyder, 2004). For example, a pilot study using hope therapy showed that increased agency was associated with increased self-esteem and decreased depressive and anxiety symptomology (Cheavens, Feldman, Gum, Michael, & Snyder, 2006). Conversely, the number of negative self-schema predicted early-onset alcohol drinking in teenagers (Corte & Zucker, 2008), indicating that psychotherapy directed at reducing negative self-schema may reduce risky behavior. The effect of negative self-schema may be related to the occurrence of a wide range of risky or maladaptive behaviors. DCS may alleviate these problems, in conjunction with such therapeutic techniques. However, at this point the generality of these findings to other frustration-based preparations such as partial reinforcement has not been determined. The effects of DCS on behavioral preparations, such as partial reinforcement training and escape from frustration has yet to be explored.

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ABSTRACT

ROLE OF THE NMDA RECEPTOR IN CONSUMMATORY SUCCESSIVE NEGATIVE CONTRAST

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Four experiments using the partial agonist D-cycloserine (DCS) were conducted to investigate the role of the *N*-methyl D-aspartate (NMDA) receptor in consummatory successive negative contrast (cSNC), an animal model of loss-induced anxiety. In Experiment 1, pretrial 11 and 12 administration of DCS (30 mg/kg) enhanced cSNC following the reward downshift from 32%-to-4% sucrose solution. In Experiment 2, posttrial 11 administration of DCS enhanced cSNC greatest at 30 mg/kg, following 32%-to-4%, and mildly following 32%-to-6%. In Experiment 3, DCS at 15 mg/kg and 60 mg/kg prolonged cSNC mildly (32%-to-4%). Experiment 4 suggested enhancement was not the result of conditioned taste aversion (CTA) to DCS (30 mg/kg); CTA to novel 4% solution failed to develop, compared to unpaired and saline controls. Results were interpreted to mean that activation of NMDA receptors via the glycine-modulatory site enhances the aversive memory associated with unexpected reward loss. Possible applications for therapeutic settings are discussed.