ROLE OF OPIOIDS IN MEMORY CONSOLIDATION DURING CONSUMMATORY SUCCESSIVE NEGATIVE CONTRAST

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The word "pain" has two meanings; it can refer to physical pain, such as a skin cut, or to psychological pain, such as bereavement. Scientific research on pain has focused primarily on the former. In the current state of this literature, the underlying mechanisms of the affective components of the two types of pain are indistinguishable. As shown below, recent studies have revealed that the opioid system, which is renowned for its role in physical pain, has a similar involvement in situations that evoke psychological pain from reward loss. However, *how* does the opioid system modulate the organism's adjustment to reward loss? Evidence presented here will suggest that the opioid system influences both the acquisition and consolidation of memories for aversive events. The present experiments will explore the role of the opioid system in memory consolidation of a loss event. The next three sections review available information about the reward loss situation, memory consolidation, and potential roles of the opioid system.

Incentive Relativity

The study of how animals adjust to new situations is a fundamental aspect of psychology. The first major theories of learning articulated in the early 20th century were created to explain such behavioral adjustments. Psychologists such as E. L. Thorndike and J. B. Watson formulated purely reflexive, S-R learning theories, in which learning is the result of associations between a stimulus (S) and a response (R) (Watson, 1917). A reward (S^R) for producing the response serves to strengthen the S-R connection, but the reward itself is not encoded. Thorndike's (1911) experiments with cats, dogs, and chicks led him to note that animals learned over time to reduce behaviors that did not deliver reinforcement (i.e., food), and to increase behaviors that led to the reinforcer. To explain these findings theoretically, the Law of Effect states that different amounts of reward

(positive outcomes) lead to different "habit strengths." These habit strengths represent the force of the associative bond between a stimulus and its corresponding response (i.e., the S-R association). Larger rewards create stronger habit strength than do smaller rewards, therefore facilitating learning. Smaller rewards or nonreward weaken associative strength, resulting in fewer occurrences of the behavior in question.

The Law of Effect and S-R learning theory provided a theoretical basis for the study of behavior founded upon empirical data. They explain why acquisition rates differ with different magnitudes of reward and why animals stop pressing a lever when lever pressing is no longer followed by food delivery. Acquisition and extinction can be explained without assuming the development of expectancies about the goal event. The animal does not need to expect food reward from a lever press; it only needs to know that when it encounters a lever, it should press it. Thorndike (1911) was unable to prove the need for expectations of a particular outcome, though he suspected that such information was encoded based on some inconclusive, informal experiments. The lack of evidence for expectancies allowed early learning theories to take the more parsimonious view that such intervening variables were not needed. However, behavioral effects that could not be explained by simple S-R associative theory, such as the effects of transitions in reward magnitude, soon became apparent. These cases require additional components, such as the acquisition of reward expectancies.

Evidence for Expectation

The evidence for expectancies began to accumulate within two decades after Thorndike's (1911) S-R theory was published. Tinklepaugh (1928) conducted an experiment to show that monkeys can develop expectations. A monkey was trained to

look for a piece of either a banana or lettuce under one of two cups. Monkeys prefer bananas to lettuce, but will eat the lettuce if they are hungry. In a typical trial, the monkey saw the banana (or lettuce) placed under one of two cups and then a blind was lowered during a retention interval lasting a few seconds. Finally, the screen was lifted and the monkey could make a choice between cups and consume the reward. In some occasional trials, the monkey was shown the banana, the blind was lowered, and the banana was then replaced with a piece of lettuce while the monkey could not see it. Once the blind was lifted, the monkey was allowed to choose a cup. Upon looking under the cup and finding lettuce instead of the expected banana, the monkey examined the cup carefully and looked around as if searching for the missing banana. She occasionally turned toward the observers in the room and shrieked in apparent anger. In these trials, the lettuce was usually left uneaten. The rejection of the lettuce may be understood by assuming that an expectation of "banana" had been formed, such that the finding of lettuce violated this expectation. Associated with the violation of the expectation of a banana with a less desirable food is an apparent emotional response: frustration.

In the same lab, around the same time, Elliott (1928) conducted a similar study with rats in an instrumental conditioning situation. Rats were trained to navigate a complex maze for bran mash and then shifted to a sunflower seed reward. Shifted animals ran more slowly for the sunflower seed than rats trained always with sunflower seeds. They also entered more blind alleys, suggesting that animals were searching for the missing reward. Again, the rats appeared to not only develop an expectation for the reward, but the shift to a less desirable reward elicited significant changes in behavior. To Tinklepaugh and Elliott, "incentive contrast" involved not only the strengthening of S-R

connections via a reward, but also the development of a representation of that particular reward. Tinklepaugh (1928, p. 234) referred to these “representative factors” as “ideational in function,” and also standing for “qualitative and quantitative aspects of those [reward] objects.” Elliott (1928, p. 29) attributed the errors made by his rats in the maze to “searching for the accustomed (and more desirable) food.” He concluded, “Rats running the maze…were learning to expect a specific reward rather than mere satisfaction of hunger.”

The experiments of both Tinklepaugh and Elliott involved qualitative reward shifts, but the phenomenon was later shown in a situation involving a quantitative shift in reward. Crespi (1942) reported that rats in a runway showed a sudden decrement in running speed when shifted from a large to small reward of the same type, and the decrement was to a level below that of unshifted controls. When shifted from a small to a large reward, rats increased their speed above and beyond the terminal level of responding before the upshift. Crespi termed these effects "depression" and "elation," respectively, and assumed that they were due to emotional responses (frustration and joy). Clearly, in its simplest version lacking an “expectation” component, S-R theory was not capable of accounting for these behavioral changes.

These findings prompted a move away from the idea that reward magnitude directly affects learning, and toward the notion that reward magnitude influences incentive motivation, which in turn, affects the behavior independently of learning. As Crespi (1942, p. 513) stated, "Incentive-value is profitably viewed as proportional to the distance between *level of expectation* (both of quality and quantity) and *level of*

attainment." Thus, the emotional component is a change in motivation based on the difference between expectancy and the actual reward.

Zeaman (1949) coined the terms “positive contrast” and “negative contrast” in his experiments with rats and runways, using cheese as a reinforcer. Rats shifted from a 2.4 g to 0.6 g cheese reinforcer for traversing a runway increased their latency to the goal significantly above rats only given the 0.6 g reinforcer (negative contrast). He also found that rats shifted from the small to the large reinforcer decreased runway latency below unshifted large reward controls (positive contrast). The term “contrast” refers to the difference between the preshift and postshift rewards, and “positive” or “negative” refer to the direction of the shift. Later, the terms “successive” and “simultaneous” were also introduced; the former describes instances where the differing rewards are given one after another (as in all the studies cited so far), while the latter describes situations in which the experimental group receives both rewards throughout the experiment but in different contexts (Bower, 1961). Bower trained rats to expect a large reward in a black alley and a small reward in a white alley. These subjects were then compared to two groups which received only a large reward or only a small reward in both alleys. Rats taught to discriminate between the alleys ran slower for the small reward than rats that only received the smaller reward, and faster for the large reward than rats given only large rewards. These effects are known, respectively, as simultaneous negative and positive contrast.

Vogel, Mikulka, and Spear (1968) devised an animal model for frustration based on consummatory behavior. Rats were given a 32% sucrose solution for 5 min daily for 11 trials, then shifted to a 4% solution for 6 trials. The shifted rats produced significantly

fewer licking responses than did controls that always received the 4% solution. Typically, the initial reduction in drinking behavior on the first day after the shift is very acute, but over subsequent trials the shifted group recovers to the same level of responding as the unshifted controls. This is called consummatory successive negative contrast (cSNC), to distinguish it from the experiments described by Elliott (1928) and Crespi (1942), in which the relevant behavior is instrumental (iSNC). The cSNC procedure is unique because of its ability to measure behavior during the downshift event (i.e., while the reinforcer is present). Only anticipatory behavior (i.e., behavior that occurs before reaching the goal), after the downshift, can be measured in the iSNC situation.

There are other types of contrast that must be distinguished from successive and simultaneous contrast. Behavioral contrast involves relative rates of responding and refers to the notion that performance of an instrumental response varies based on alternative rewards available in the same session, but under different stimulus conditions. Thus, the rate of responding to one component of a multiple schedule can increase or decrease as the reinforcement schedule on the other component decreases or increases, respectively (Reynolds, 1961). Anticipatory contrast refers to another form of behavioral suppression. Rats are given access to a less preferred reward and then given access to a more preferred reward immediately afterward. The lesser reward becomes a cue for predicting the greater reward. This anticipation of the greater reward leads to suppression of responding to the smaller reward. Since the reward change is not surprising in anticipatory and behavioral contrast, it is unlikely that emotional responses analogous to those of cSNC play a part in such response suppression. Chlordiazepoxide, a benzodiazepine anxiolytic, does not affect anticipatory contrast (Flaherty & Rowan,

1988), indicating that the behavioral suppression in anticipatory contrast does not rely upon an emotional response.

Appetitive extinction is another situation that involves reward loss. First, a reward US is paired with a CS in an acquisition phase. Then, in an extinction phase, the CS is presented without the US, and any appetitive behavior (e.g., approach, lever pressing, latency to traverse a runway) acquired in acquisition reduces. Since the problem at hand involves emotional reactions to unpredictable loss events, the present studies will focus on situations involving successive downshifts of incentive magnitude, including cSNC and appetitive extinction. This will also allow for a distinction between effects influencing acquisition, consolidation, or retention that would not be possible with iSNC. In iSNC, behavior is measured *after* the downshift, whereas during cSNC, behavior is measured *during* the downshift—that is, during the acquisition of the aversive memory of the downshift event.

Theories of cSNC

Flaherty (1996) characterized cSNC using a multistage hypothesis consisting of two distinct stages. First, there is an initial reaction to the reward downshift. During the initial reaction, the discrepancy between received and expected rewards must first be detected. Detection of the change is followed by rejection of the new reward, and instigates a search for the missing reward. Failure to locate the missing reward initiates a second stage, recovery, during which conflict might be involved. The rewarding value of the small reward facilitates approach, while the discrepancy between expected and received rewards facilitates searching for the missing reward. Based primarily upon pharmacological data, Flaherty (1996) described the first stage in the multistage

hypothesis as purely cognitive, while the second stage involved an emotional reaction of frustration.

The multistage hypothesis has since been adapted to Amsel's (1992) frustration theory, in order to make some of the components more explicit. Amsel (1992) developed a theory of frustration attributing the emotional reaction during reward loss to the violation of an incentive expectancy by the presentation of a smaller reward than expected. His qualitative model allows ordinal predictions to be made about processes that will arise before and after the reward downshift. First, experience with a relatively large reward creates an expectation of that reward. In the consummatory contrast setting, this is achieved through access to the large reward during preshift trials, where an association between some stimulus in the context (S) and the large reward allows S to control an approach response (R) and an expectation of the large reward (e_{Large}; Figure 1a). Then, the subject unexpectedly receives a smaller reward during the postshift, generating a discrepancy between the received and expected rewards. The comparison of received and expected rewards is analogous to the detection phase of the multistage hypothesis, but unlike Flaherty's (1996) predictions, frustration theory describes the output of this comparison as an emotional reaction (Figure 1b). The emotional reaction is an unconditioned aversive internal state, termed primary frustration, which is analogous to the rejection component described by Flaherty (1996) and it initiates search. Primary frustration is especially strong in the first shifted trial, when the animal has had no prior experience with the smaller reward. An association develops between the external cues (S) and the internal state of primary frustration paired with them, through Pavlovian conditioning. This association induces an aversive anticipatory state, or an expectation of

frustration, called secondary frustration (Figure 1c). At this point, therefore, S has the ability to control R_{App}, e_{Large}, and $e_{Frustration}$. At the behavioral level, this multiple control of competing expectations can be seen as an approach-avoidance conflict (Miller, 1944). During postshift trials, the expectation of frustration also becomes counterconditioned to the smaller reward. In addition, a new expectation develops for the smaller reward, and the expectation for the large reward weakens (Figure 1d). Both of these factors contribute to the recovery of consummatory behavior to a level appropriate for the postshift incentive magnitude.

According to Amsel (1992), primary frustration has several properties. It invigorates behavior (e.g., lever pressing, Papini & Dudley, 1995), dramatically increasing response rates in animals when they encounter a surprising reward reduction (akin to pressing the button on a vending machine several times in rapid succession when it fails to deliver a beverage). Primary frustration is hedonically aversive, as animals will learn to escape from it if given the opportunity (Daly, 1974). As noted in the earliest reward shift experiments (see above), it initiates search behavior (e.g. Flaherty, Troncoso, & Deschu 1979). Primary frustration also maintains some stimulus properties on its own, retaining the ability to cue, for example, aggressive behaviors (Gallup, 1965), vocalizations, and increased locomotor activity (Papini & Dudley, 1997).

There are other additional consequences of surprising nonreward that occur in SNC. For example, associated with reward downshift is the emission of an odor, which can actually serve as a signal to other rats (McHose & Ludvigson, 1966; Spear & Spitzner, 1966). Alone, this odor appears to have aversive properties because it can

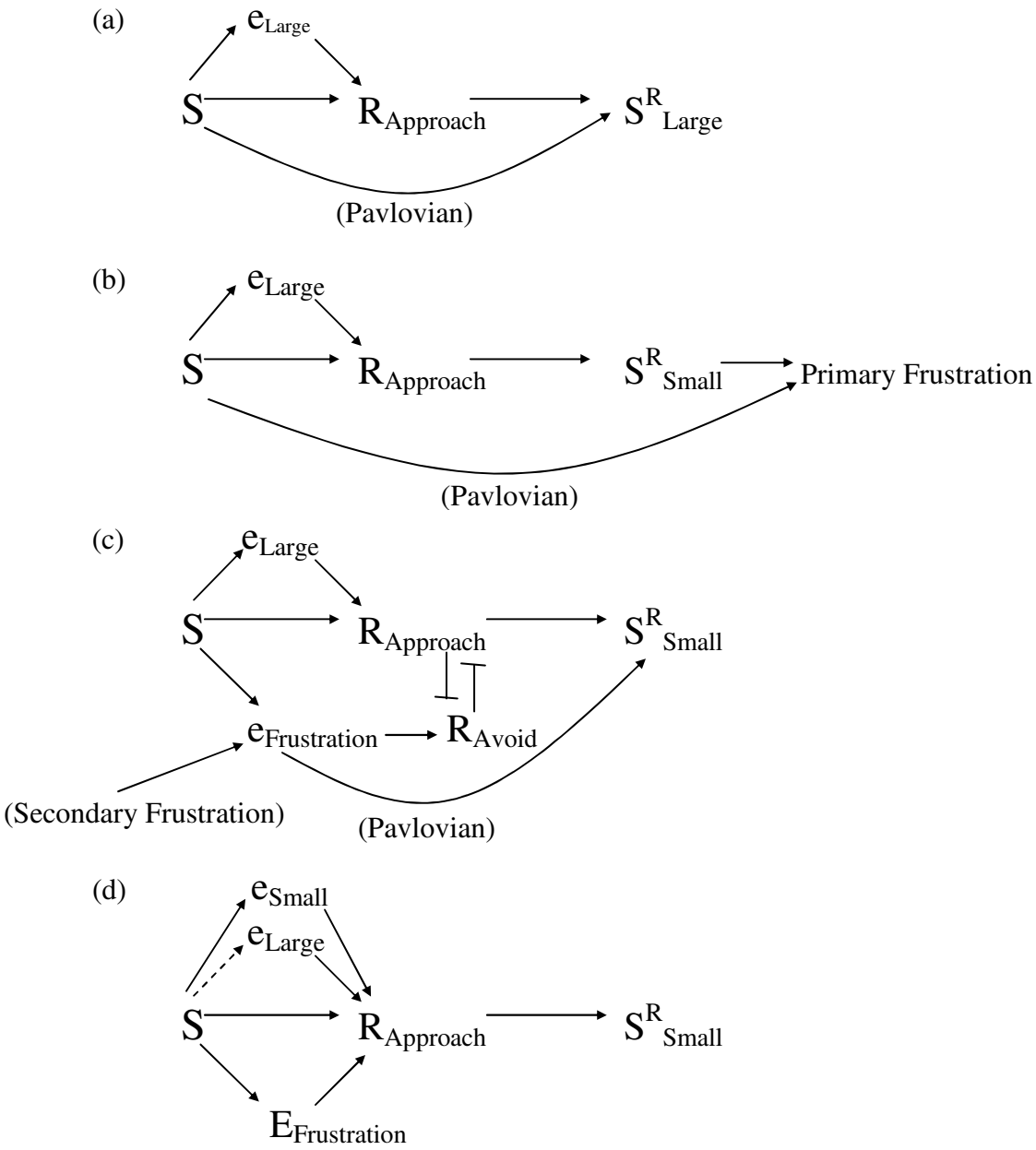

Figure 1. Amsel's (1992) frustration theory applied to cSNC: (a) preshift trials, when an expectation for the large reward arises from pavlovian pairings between the test chamber and 32% sucrose; (b) trial 11, in which the expectation of 32% sucrose is violated by receiving the 4% sucrose which generates primary frustration, which in turn is paired with the contextual stimuli; (c) trial 12, in which the contextual cues now arouse competing expectations for reward and frustration, and in which an expectation of frustration is paired with reward; and (d) trial 15, in which the aversive aspect of the expectation of frustration has been counterconditioned, and the expectation for the small reward has become stronger and the expectation for the large reward has diminished.

induce escape responses (Collerain & Ludvigson, 1972; Mellgren, Fouts, & Martin, 1973; Wasserman & Jensen, 1969). However, the aversive value can be counterconditioned or extinguished under appropriate conditions (Collerain & Ludvigson, 1977).

Secondary frustration also has a distinct set of properties. Secondary frustration invigorates behavior, as seen in animals partially reinforced during acquisition. These animals perform the instrumental response more vigorously than do continuously reinforced animals. Goodrich (1959) trained rats to run an alley to receive rewards, either continuously or intermittently. Partially reinforced animals ran faster than did continuously reinforced animals for the same reward. This partial reinforcement acquisition effect was most evident at the beginning of the alley and less evident nearer the goal. Secondary frustration is also aversive, and animals will terminate a conditioned stimulus for nonreward if given the opportunity (Terrace, 1971). Flaherty, Becker, and Pohorecky (1985) reported elevated corticosterone release, an indicator of stress, in the second trial after shifting rats from 32% to 4% sucrose. In the first postshift trial, no corticosterone release was detected. This finding was replicated by Mitchell and Flaherty (1998). Secondary frustration also generates withdrawal from goal stimuli (Jones, 1970; Papini & White, 1994).

Based on Amsel's (1992) theory, it could be argued that in cSNC, the suppression of consummatory performance in the first shifted trial is controlled predominantly by primary frustration, whereas the suppression that occurs in the following trials (e.g., during the recovery of performance) is controlled by a mixture of primary and secondary frustration.

Factors Affecting SNC

Many factors affect the size of cSNC. For instance, level of food deprivation can profoundly change the contrast effect. Nondeprived animals shifted from 32% sucrose to 4% recover from contrast very slowly (Grigson, Spector, & Norgren 1992; Riley & Dunlap, 1979). Presumably, nondeprived animals have less need to consume any reward at all and can avoid the smaller reward altogether. Highly deprived animals have more necessity in consuming the calories in the smaller reward, even though it may be less desired, and will therefore recover more quickly. However, experiments attempting to show the effect of deprivation levels side-by-side have indicated that lower deprivation levels make contrast less likely to occur. Several studies have revealed situations in which a high deprivation group exhibited contrast (iSNC and cSNC), whereas a low deprivation group showed no contrast (e.g., Cleland, Williams, & DiLollo, 1969; Flaherty & Kelly, 1973). It is possible that deprivation may serve as a catalyst in situations that normally yield very little contrast.

Another factor influencing contrast size is the disparity between rewards. In the iSNC situation, as the differences between the large and small reward increases, the size of contrast also increases (e.g., DiLollo & Beez, 1966; Gonzalez, Gleitman, & Bitterman, 1962). Crespi (1942) reported that rats shifted from 256 to 16 units of reward showed greater disruption of behavior than those shifted from 64 to 16. In fact, the size of the behavioral suppression after a downshift in the cSNC situation is a constant proportion of the ratio between the concentration of the pre- and postshift rewards (Papini & Pellegrini, 2006). For example, animals shifted from 32% to 8% sucrose exhibit a similar magnitude

of behavioral suppression as animals shifted from 16% to 4%—a 4:1 ratio holds in both cases.

The intertrial interval (ITI) can also affect the SNC effect. Contrast effects achieved with an ITI of just a few minutes are much larger than contrast effects with an ITI of 24 h (Capaldi, 1972). Similarly, the retention interval between the subject's last experience with the preshift reward and the introduction of the postshift reward can reduce contrast. Vogel, Mikulka, and Spear (1968) reported clear contrast when shifting subjects to 4% sucrose up to 10 days after the last 32% preshift trial. At 17 days, the effect waned to marginal significance, and after 32 days the effect had completely disappeared. Flaherty and Lombardi (1977) gave rats a chance to discriminate between the preshift and postshift solutions, and then gave 10 days access to the 32% solution before a 10-day retention interval. This prior experience enhanced the degree of contrast, compared to the animals that did not receive prior discriminative training.

Prior experience can affect contrast in a variety of ways. Experience with the smaller reward before administration of the contrast training can reduce or eliminate contrast (Capaldi, 1972). Partial reinforcement during preshift training produces less contrast than continuous reinforcement in both iSNC and cSNC procedures (Mikulka, Lehr, & Pavlik, 1967; Pellegrini, Muzio, Mustaca, & Papini, 2004). With regard to Amsel's theory, this reduction of cSNC could be viewed as a consequence of the counterconditioning of frustration prior to the shift, induced by partial reinforcement training (Figure 1d). Each nonrewarded trial is like a shift to extinction, creating a level of frustration that is paired with reward on the next reinforced trial. Eventually,

secondary frustration becomes a signal for reward, encouraging the instrumental or consummatory response, thus attenuating the SNC effect.

Pharmacological studies of SNC have produced some interesting findings. If primary frustration and secondary frustration are controlled by different brain mechanisms, they should be dissociable. Furthermore, on the previously mentioned assumption that consummatory suppression is mainly dependent on primary frustration in the first postshift trial, but on secondary frustration in subsequent postshift trials, one would predict differential effects of drugs on these trials. For example, it has been shown that anxiolytic drugs can reduce the amount of contrast on the second, but not the first, postshift trial (e.g., Becker, 1986; Flaherty, Grigson, Demetrikopoulos, Weaver, Krauss, & Rowan, 1990; Flaherty, & Rowan, 1989). The benzodiazepine chlordiazepoxide (CDP) and midazolam both reduce contrast only on the second postshift trial (Becker, 1986; Flaherty, Lombardi, Wrightson, & Deptula, 1980). Several experiments demonstrate that benzodiazepines do affect behavior on the first trial when the trial is longer (Flaherty, Grigson, & Rowan, 1986; Mustaca, Bentosela, & Papini, 2000) or when the animal is repeatedly shifted (Flaherty, Clarke, & Coppotelli, 1996). Anxiolytics affect behavior only after some experience with the new, downgraded solution. Secondary frustration is a likely candidate on which benzodiazepines may be selectively taking effect.

The Opioid System

The opioid system is widely distributed throughout the mammalian central nervous system. Opioid receptors belong to the group of G-protein coupled receptors and include three main receptor subtypes: μ, δ, and κ (Uhl, Childers, & Pasternak, 1994). In addition, there is a fourth receptor that exhibits a high structural homology with respect to

the other opioid receptors, typically designated as opioid receptor-like (ORL) receptors. Despite its homology with the other members of the opioid family, selective and nonselective opioid ligands with high affinity for the other receptors bind with low affinity to ORL receptors (Meng et al., 1996). The μ, δ, and κ receptors are differentially distributed in the brain, but mRNA expression patterns show high concentration for all three receptor types in the amygdala (Mansour et al., 1995), a structure notable for its role in both fear and frustration (Becker, Jarvis, Wagner, & Flaherty, 1984).

Endogenous opioid compounds dynorphins, enkephalins, and endorphins are derived from their respective precursors pro-enkephalin, pro-dynorphin, and pro-opiomelanocortin. Genes ultimately code for both the opioid receptors and the precursors to endogenous neuropeptides, leaving considerable opportunity for genetic differences to influence opioid function. Allelic variations have been identified in both humans and rats, some of which influence opioid function. For example, a single nucleotide polymorphism (SNP) at codon 40 of the human μ opioid receptor in which adenine at base position 118 is replaced with guanine (A118G) changes the resulting amino acid at position 40 from asparagine to aspartate (Asn40Asp), which in turn reduces the potential glycosylation sites on the extracellular face of the receptor (Mayer & Höllt, 2001). This mutant has been shown to exhibit an increased affinity for β-endorphin compared to the wild type. In the case of the rat, an isoform of the rat μ opioid receptor exhibits higher resistance to agonist-induced desensitization compared to the wild type (Zimprich, Simon, & Höllt, 1995). Opioid-related genetic variability is a potential factor involved in the individual differences in cSNC to be described below (Pellegrini et al., 2005). Because of its role in

pain and fear conditioning, the opioid system is a candidate regulatory system for situations involving reward loss.

Opioids and cSNC

Frustration shares similarities with another form of anxiety: fear. Gray (1987) suggested that fear and frustration are analogous, since they share many behavioral effects, depend upon the same brain structures, and are affected by the same drugs in similar ways. For example, the partial punishment extinction effect (PPEE) is similar to the partial reinforcement extinction effect (PREE; Brown & Wagner, 1964). The PREE refers to partially reinforced animals showing greater persistence when shifted to extinction than do continuously reinforced counterparts, presumably due to counterconditioning of an expectation of nonreward (Amsel, 1992). In the PPEE, animals are trained to collect a reward, but in some of the trials, an aversive shock is administered together with the reward. The shock creates conditioned fear, which, as training continues, is in some trials paired with the reward. This type of pairing tends to reduce the disrupting effects of fear (in the PPEE situation), just as it tends to reduce the disrupting effects of anticipatory frustration (in the PREE situation). As a result, when shifted to extinction, these animals are more persistent in performing the conditioned behavior than are those that received only food in acquisition. The shock itself becomes a cue for reward, and is therefore counterconditioned in a similar manner to what happens in the PREE and in the SNC after partial reinforcement. Anxiolytic drugs decrease suppression of the conditioned behavior induced by signals of pain or reward loss. Administration of CDP has been shown to increase persistence during extinction in continuously reinforced animals (Fowler, 1974), while resulting in faster extinction for

partially reinforced rats (Demarest & MacKinnon, 1978). CDP, therefore, appears to be reducing the secondary frustration generated by reward loss. The faster extinction in partially reinforced animals treated with CDP is very much like the faster recovery seen when secondary frustration in cSNC is reduced by anxiolytics.

Gray's (1987) comparisons were between fear, a conditioned expectation of pain, and secondary frustration, a conditioned expectation of primary frustration. The idea can be taken a step further. In the experiments reviewed by Gray (1987) as background for his fear=frustration hypothesis, fear was generally induced by the administration of electric shock to the animal's feet, a stimulus that causes peripheral pain. The extension, then, takes the form of relating the two unconditioned events that support both fear and secondary frustration. Thus, if fear=secondary frustration, then pain=primary frustration (Papini, 2003). If these relationships are correct, they explain why some opioids, which are notorious for their effects on pain (for a review, see McNally & Akil, 2002), also affect cSNC.

Rowan and Flaherty (1987) conducted a series of experiments in which deprived rats were given access to either 32% or 4% sucrose for 5 min per trial for 10 trials; then, all rats were given 4% sucrose in 3 postshift trials and the number of licks recorded. An injection of morphine sulfate was given 20 min prior to testing on the second (Experiment 1) or first (Experiment 2) postshift trial. In both experiments, the contrast was significantly reduced for both 4.0 and 8.0 mg/kg morphine doses. Morphine is a nonselective opioid agonist, with an affinity for the μ, κ, and δ-opioid receptors. It is therefore unclear exactly how morphine acts to reduce contrast.

The effects of morphine were antagonized by pretreatment with naloxone, a nonselective opioid antagonist. Naloxone (0.5 mg/kg) was given before administration of an ineffective (1.0 mg/kg) or effective (4.0 mg/kg) dose of morphine on the second postshift day. Naloxone had no effect on the ineffective morphine dose, but it reversed the effects of the 4.0 mg/kg dose of morphine on cSNC. Naloxone alone, however, did not produce a significant effect on cSNC at doses of 0.25, 0.5, and 1.0 mg/kg. Since no effect of naloxone was found, these experiments failed to support the idea that the opioid system is normally engaged in cSNC. However, the consummatory contrast procedure is susceptible to floor effects, given that response suppression is the measure of contrast. To avoid the masking of a possible opioid antagonist-induced increase in contrast size by a floor effect, conditions that normally produce small contrast (i.e., lead to little suppression) may be used.

Pellegrini, Wood, Daniel, and Papini (2005) explored this hypothesis, and showed that rats shifted from 32% to 6% sucrose exhibited an enhanced cSNC and slower recovery when naloxone (2.0 mg/kg) was administered before trials 11 and 12 (i.e., the first and second postshift trials, respectively). In another experiment, naloxone was administered before each of the five postshift trials in a more traditional 32-4 downshift. Naloxone-treated rats showed greater suppression than shifted saline controls. In these two experiments, the effects of naloxone on cSNC were assessed directly by testing rats that were under the influence of this opioid antagonist. The role of the opioid system in cSNC can also be studied indirectly. This has been done following two different procedures. In one experiment, Pellegrini et al. (2005) trained rats with a typical 32-4 downshift and recovery. Rats were required to show at least a 10% reduction in goal

tracking time on trial 11 relative to trial 10, then were matched into quadruplets based on trial 11 performance. A difference score was computed by subtracting the performance on trial 12 from the performance on trial 11, resulting in the absolute recovery value between those trials for each rat. These two trials were chosen because most of the recovery occurs in the transition from trial 11 to trial 12. Then, rats were classified, in pairs, into either fast or slow recovery groups, determined by the difference score. The two rats with the highest difference scores from each quadruplet were classified as fast recovery, whereas the two rats with the lowest difference scores were classified as slow recovery. Then, in preparation for an activity test independent of the reward downshift experience, one rat in a pair of fast or slow recovery rats was randomly assigned to the naloxone or saline group and the other rat was assigned to the alternative group.

In the activity test, slow recovery animals showed less habituation of activity over the 15 min test than did fast recovery rats. Administration of naloxone before the test had no effect on the fast recovery group, but the slow recovery group given naloxone habituated significantly more than did the slow recovery saline group. Thus, animals that recover more slowly from a reward downshift are more sensitive to naloxone as assessed in an independent test of activity. Since naloxone is an opioid antagonist, behavioral effects are the result of blocking endogenous opioids released after the conditioning treatment. As a whole, these three experiments demonstrated that the opioid system normally plays a role in recovery from a reward downshift experience.

Another way of indirectly assessing the role of the opioid system in cSNC is to assess pain sensitivity immediately after a 32-4 downshift. For example, Mustaca and Papini (2005) subjected rats to a typical downshift, and then administered a hot plate test

to determine pain sensitivity after either trial 11 or trial 12. In a hot plate test, rats are placed on a heated metallic plate and the latency to remove a paw from the surface is recorded. Previous research implicated the involvement of the opioid system in the hot plate test. Morphine was shown to increase withdrawal latency in rats tested with the hot plate (Hunskaar, Odd-Geir, & Hole, 1986). Downshifted rats had longer latencies to withdraw a paw in the hot plate test, presumably because of the analgesic effects of endogenous opioids released during the downshift. The effect was observed on trial 12, but not on trial 11, suggesting that the release of endogenous opioids coincides more closely to secondary frustration than to primary frustration.

As previously discussed, the opioid system consists of three major receptor subtypes, μ, κ, and δ, differentially distributed throughout the nervous system (Lester & Traynor, 2006; Mansour et al. 1995). Morphine and naloxone are nonselective and bind to all three receptors, and affect both trial 11 and 12. Thus, to better understand where and which specific part of the opioid system is involved in cSNC, compounds that are selective for specific receptor subtypes have been explored. Wood, Daniel, and Papini (2005) found that D-Ala2-,N-Me-Phe4,Gly-ol enkephalin (DPDPE), a δ-receptor agonist, reduces contrast on the first, but not the second postshift trial. After 10 trials (one trial per day) of preshift training with either 32% or 4% sucrose, all rats received access to 4% sucrose for five postshift trials. An injection of either DPDPE (24 μg/kg) or vehicle was administered 6 min prior to testing on either the first or second postshift trial. Shifted animals given vehicle on the first shifted trial showed significant contrast, whereas those given DPDPE did not. However, subjects that received DPDPE on the second shifted trial were not different from animals given vehicle. DPDPE had a selective effect on cSNC on

trial 11. DPDPE was the first compound discovered to reduce contrast selectively on the first postshift day, completing the dissociation of primary and secondary frustration with drugs such as the benzodiazepines and DPDPE. Furthermore, the selectivity of the δ-receptor is preserved when antagonists are administered. Pellegrini et al. (2005) demonstrated that naltrindole, a δ-selective antagonist, enhances contrast when administered before trial 11, but not before trial 12 for animals shifted from 32% to 6% sucrose.

The role of the κ -opioid subsystem in cSNC has also been explored, to a degree. The κ agonist *trans*-(±)-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl] benzeneacetamide methanesulfonate (U-50) has been shown to have a selective effect on trial 12 when administered before either trial 11 or trial 12. Furthermore, its effects are bidirectional, depending on the dose, with enhanced cSNC at 3.0 mg/kg and attenuated cSNC at 1.0 mg/kg (Wood, 2006). Unpublished data suggests that U-50 under the same dose and training parameters is capable of producing conditioned taste aversion (CTA), but this does not fully account for the effects of U-50 on cSNC because U-50 administered before the first downshift trial (i.e., the first 4% sucrose trial, after 10 trials of exposure to 32% sucrose) has no effect on consummatory behavior.

Analysis of the factors affecting cSNC has brought about new distinctions and allowed new parallels to be made between other aversive conditioning situations, such as fear. Yet, incentive contrast research is far from exhaustive. Many aspects of incentive contrast are yet to be fully explored, such as the role of the memory of the downshift event, and how the opioid system may modulate this memory, the topic of the present studies.

Memory Consolidation

To understand the effect opioids have in modulating the conditioned form of frustration (i.e., secondary frustration), the memory of the downshift event must be investigated. Memory formation involves at least two distinct phases (McGaugh, 2000). First, the memory is acquired. Then, the memory is consolidated and stored more permanently. Consolidation takes time, because the cellular machinery must produce the proteins that allow for synaptic plasticity. The initial evidence that consolidation happens over time arose from the finding that learning new information was disrupted by learning other new information immediately afterward (Muller & Pilzecker, 1900). The consolidation idea provided an explanation for retrograde amnesia in humans with head traumas. This spurred studies using electroconvulsive shock to create retrograde amnesia in rats (Duncan, 1949). Each day, the rats would receive a trial of one-way active avoidance training, learning to jump from an electrically charged grid into a safe compartment. After the trial, some of the animals received an electroconvulsive shock. Rats that received electroconvulsive shock immediately after the trial did not learn to jump into the safe box as quickly as the control rats, but rats that received the electroconvulsive shock after a delay showed a reduced learning deficit. Longer delays between the end of the trial and the electroconvulsive shock tended to reduce the learning deficits; at a delay of 1 h, rats did not differ from nontreated controls. Duncan concluded that there must be a consolidation period following each trial, during which the memory was stored.

Further evidence for consolidation over time came from studies with goldfish trained in a 2-way, active avoidance shuttle apparatus (Agranoff, Davis, & Brink, 1966).

Goldfish learned to swim over a barrier to avoid an electric shock, but when the protein synthesis inhibitor puromycin was administered immediately after training, their memory was impaired when tested 3 days later. However, goldfish that received puromycin 2 h after initial training did not exhibit a deficit on the test trials. Thus, goldfish were able to learn the task, but were not able to consolidate it into a long term memory when protein synthesis was inhibited.

Consolidation can also be enhanced, such as when stimulant drugs are administered shortly after training (McGaugh, 1973). For example, rats trained to navigate a complex maze for food show enhanced learning when picrotoxin (1.0, 1.25, but not 0.75 mg/kg) was administered after each daily training trial (Breen & McGaugh, 1961). With studies such as these, posttrial injections after the training trials became a standard method of influencing memory consolidation and retrieval under testing conditions when animals were not directly affected by the drug (McGaugh, 1973, 2000).

It should be noted that posttrial drug treatments may affect behavior by disrupting memory consolidation and/or by inducing retrieval failure (for a review, see Miller, Kasprow, & Shachtman, 1986). For example, the effects of amnesic drugs or treatments administered posttraining can sometimes be reversed by presenting the CS, the US, or another stimulus associated to training before the test trial. Miller and Springer (1972) accomplished this by training rats in a one-trial passive avoidance situation, in which the rats received a footshock after crossing a gate between two compartments in the conditioning box. Amnesia was then induced with posttrial electroconvulsive shock. Exposing rats to the footshock again 2 h after the initial training resulted in a recovery of the memory in a training task 24 h later—indicating that the memory of the training was

consolidated and stored, and could be reinstated. The present studies will use the term "consolidation" to describe the effects of posttrial opioid administration on cSNC, given that the proposed experiments were not designed to distinguish between consolidation and retrieval failure.

Interestingly, the amygdala, which plays a role in emotional processing, including the cSNC effect (Becker et al., 1984), also plays a role in memory consolidation. Goddard (1964) implanted electrodes into the amygdalas of rats, such that he could stimulate it directly. He then trained four groups of rats in a 2-way avoidance task: The experimental group, a surgical control, a group matched for stimulation, and an unaltered control. Following training, the experimental group received stimulation in the amygdala. After 200 trials, all of the control animals had reached a criterion for avoidance learning, but posttrial stimulation of the amygdala disrupted consolidation of avoidance learning. Posttrial stimulation of the amygdala also disrupted consolidation of one-way avoidance learning.

The amygdala's modulatory influence on consolidation is thought to work through its projections to other areas of the brain. For example, the stria terminalis contains both afferent and efferent projections connecting the amygdala to the thalamus, hypothalamus, and septum (Davis, 1998). Liang and McGaugh (1983) showed that lesions of the stria terminalis attenuate the amnestic effects of amygdaloid stimulation. This finding suggests that the stimulated amygdala modulates consolidation through its efferents to other processing areas in the brain. Liang, Juler, and McGaugh (1986) infused norepinephrine directly into the amygdala after training rats on a one-trial step-through passive avoidance task. Infusions immediately after the trial enhanced

consolidation, whereas infusions 3 h after training did not affect consolidation. Furthermore, propranolol, a β-noradrenergic antagonist, blocks the enhancing effect of norepinephrine and impairs retention when administered on its own. Cahill and McGaugh (1991) discovered that lesioning the amygdala can block the effects of β-noradrenergic drugs on consolidation. McGaugh (2000) suggested that activation of the amygdala allows for plasticity in other brain areas, as illustrated in Figure 4. It should be noted that μ, κ, and δ opioid receptors are all densely distributed in the basolateral amygdala (Figure 2).

Opioid compounds, which have a modulatory effect on cSNC (see p. 18), also influence consolidation. The μ-selective agonist levorphanol creates time- and dose-dependent disruptions of consolidation when administered into the amygdala after a passive avoidance conditioning task (Gallagher & Kapp, 1978). In contrast, the antagonist naloxone enhances consolidation when administered the same way. It should be noted that though opioids influence nociception and many of these tasks involve conditioned avoidance of shock, naloxone also affects consolidation on a food-rewarded radial maze task in a time-dependent matter (Gallagher, Bostock, & King, 1985). However, these opioid effects on memory are not sufficient to account for opioid effects on cSNC, because at least the δ system is engaged on the first postshift trial, before the memory of the reward loss event is consolidated. It is possible that the opioid system acts partially upon the US representation, thus indirectly influencing conditioning, and partially upon the CS-US association directly. Pretrial injection effects provide evidence for the former, and posttrial injection effects suggest the latter situation.

Posttrial manipulations of iSNC have been explored to a degree. Salinas et al. (1997) trained rats to run an alley for ten 45-mg food pellets for 60 trials and then downshifted to one pellet per trial. In addition to contrast effects relative to unshifted one-pellet controls, administration of the muscarinic agonist oxotremorine enhanced iSNC, causing longer latencies to enter the goal during postshift trials. In opposition to these results, the non-selective beta-adrenergic antagonist propranolol reduced iSNC, leading to faster recovery. These results are consistent with the assumption that the effects on memory seen in shock avoidance paradigms are common to situations involving incentive downshifts.

Memory and cSNC

There are several ways of exploring the role of memory in the cSNC situation. First, a retention interval can be inserted to study the longevity of the memory. In the iSNC situation, the memory of 18 or 20 pellets endures after a 24-h retention interval (RI) between the last preshift trial and a downshift to 2 pellets. At longer RIs the contrast diminishes, and at 68 days has completely disappeared (Gleitman & Steinman, 1964; Gonzalez, Fernhoff, & David, 1973). Similarly, a RI interpolated before the downshift in the cSNC situation reduces suppression in an orderly manner (Gordon, Flaherty, & Riley, 1973). In addition, mature rats (14–16 months old) recover from cSNC more quickly than young rats (3 months old) when a 5-day RI is interpolated between the last preshift and first downshift trial, but not after a 1-day RI (Bentosela, D'Ambros, et al., 2006).

A second way to look at the role of memory in cSNC is to study the nature of the memory itself. Normally, contextual cues do not seem to affect consummatory behavior in the cSNC situation (Flaherty, Hrabinski, & Grigson, 1990). Daniel et al. (2007) trained

animals with 16% sucrose in one context and 2% in another distinct context to produce a within-subject contrast effect. When the context becomes a reliable cue for predicting which solution will be presented *and* there is sufficient context processing time before the solution is presented, contextual control can be obtained. Papini and Pellegrini (2006) proposed that cSNC relies upon recognition memory, in which the cue that evokes the prior memory for comparison is the downshifted solution itself. Contextual discrimination training can turn consummatory behavior into a cued-recall situation, in which the context paired with the solution evokes the memory of the reward (Daniel et al., 2007).

In the cSNC situation, the aversive memory of the downshift experience is initially acquired on the first downshifted trial. It is plausible that manipulations introduced immediately after the downshift event can influence consolidation of the memory of the loss event. An example of such a manipulation would be the posttrial injection procedure described above. Three such studies have followed this type of design. First, Bentosela et al. (2005) tested the effects of posttrial 11 administration (trial 11 was the first postshift trial) of a cholinergic antagonist, atropine, as well as an acetylcholinesterase inhibitor, physostigmine. They found that the cholinergic system does not influence memory of the downshift. Second, Bentosela, Ruetti, et al. (2006) showed that posttrial 11 administration of corticosterone enhanced cSNC when the injection followed the trial immediately, but not when given 3 h after trial 11. Finally, Wood (2006) observed that the kappa-selective agonist U-50 administered immediately after trial 11 produced increased suppression of drinking on subsequent trials.

As mentioned above, unpublished data suggests that the effects of U-50 administered immediately after trial 11 may be attributed to conditioned taste aversion (CTA) to the relatively novel 4% sucrose solution. However, additional data has discounted this possibility with posttrial 11 corticosterone administration. The enhancing effects of U-50 were interpreted as involving an enhancement of the consolidation of the aversive memory of the downshift event of trial 11. However, it is possible that either enhancement or depreciation of the trial 11 memory could result in greater suppression on trial 12, but for different reasons. On the one hand, posttrial enhancement of the trial 11 memory of primary frustration should enhance secondary frustration on trial 12 and suppress drinking. On the other hand, posttrial 11 depreciation of the primary frustration should result in greater primary frustration on trial 12, which, in turn, should suppress drinking. In both cases, frustration theory predicts suppressed drinking on trial 12, but because of different mechanisms. There are thus several questions that must be answered: Do nonselective agonists have similar effects? Is the opioid effect with posttrial injections time sensitive? How do other selective opioid agonists and antagonists that affect cSNC influence memory? Are the opioid effects restricted to cSNC, or can they be generalized to other situations involving surprising reward loss?

Wood, Daniel, and Papini (2005) narrowed the potential mechanism of DPDPE on contrast to two possibilities derived from frustration theory: DPDPE either decreases the intensity of primary frustration or it interferes with the acquisition of secondary frustration (Figure 2). A similar distinction has been suggested for fear conditioning in terms of the strength of the US representation versus the strength of the CS-US association (Delamater, 2004). It may be possible to distinguish these two possibilities by

using posttrial injection procedures, which selectively look at the consolidation of the memory of primary frustration (necessary for secondary frustration). A third possibility not examined by Wood et al. (2005) suggests that DPDPE affects the comparison between the expected solution (32%) and the received solution (4%). To explore this possibility, a paradigm that investigates the relationship between the two solutions must be used. For example, the scaling property of cSNC shows that solution shifts with the same ratio result in similar amounts of suppression (Papini & Pellegrini, 2006). Do opioids affect the comparison between solutions, or do they modulate consummatory behavior without affecting constant proportionality?

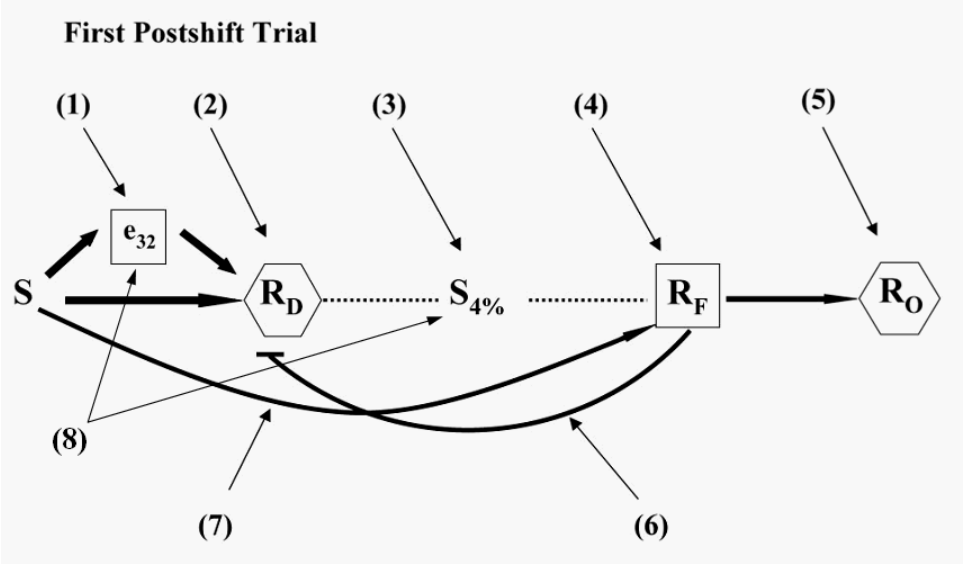

Figure 2. Seven potential mechanisms postulated to account for the effects of DPDPE on cSNC, as described by Wood et al. (2005, Figure 2 and text). Control groups in the original experiment allowed dismissal of five of the original seven possibilities, leaving as potential mechanisms (4) the intensity of primary frustration and (7) acquisition of secondary frustration. An additional mechanism is suggested here, (8): the comparison mechanism that relates actual and expected rewards during the process of detection. It is suggested that this comparator mechanism might be one locus of action of the opioid system.

The goal of the present experiments is to examine the role of opioids in the three potential mechanisms described above:

- Scaling property: modulating the comparison between expected and received rewards (detection)
- Emotional activation: modulating the intensity of primary frustration (rejection)
- Memory consolidation: modulating the acquisition of secondary frustration (recovery)

Ultimately, these experiments will determine which component(s) in frustration theory are influenced by opioid compounds.

Experiment 1: Posttrial Naloxone

The connection between the opioid system and memory of the loss event must first be established. Pretrial injection procedures may influence memory either indirectly, through performance factors such as perceptual or motor effects, through changes in motivational states that affect acquisition, or directly by modulating the acquisition of new information. Posttrial procedures have the advantage that the acquisition phase, (i.e., the trial) is complete when the injection is given, so changes resulting from the drug treatment cannot be attributed to performance factors or influences on acquisition. Instead, the posttrial injection procedures target consolidation of the new information or retrieval of the consolidated memory. Wood (2006) first explored the effect of posttrial opioid administration with the κ-selective agonist U-50. By administering the nonselective opioid antagonist naloxone, the present experiment will explore a more general effect of the opioid system that is normally engaged after a reward downshift.

It should be noted that the design of this experiment resembles experiments exploring CTA, in which exposure to a novel food is paired with an emetic or other

compound which produces an aversive state, such as lithium chloride (LiCl) (Garcia & Koelling, 1966). Subsequently, groups which received this novel food-LiCl pairing tend to avoid eating the food, relative to unpaired controls. One possible alternative interpretation of the enhancing effects of naloxone administered posttrial 11 is that the presentation of the relatively novel 4% sucrose is paired with an aversive state induced by the drug, which results in CTA to the 4% on subsequent trials. CTA is not expected to be a major problem for the unshifted 4-4 groups because by the time U-50 is injected, they have received 10 trials of preexposure. Such latent inhibition effect has been demonstrated in CTA induced by other drugs (Elkins & Hobbs, 1979). Naloxone has been shown to induce mild or no CTA at doses similar to the 2.0 mg/kg dose used in the present experiment after multiple food-naloxone pairings. At a dose of 10.0 mg/kg, naloxone can induce CTA with only a single pairing (Frenk & Rogers, 1979; Hutchinson et al., 2000; Lett, 1985; Stollerman, Pilcher, & D'Mello, 1978). Unpublished data from our laboratory has shown that Naloxone at 2.0 mg/kg does not produce evidence for CTA under the training parameters of the present experiments, but can promote CTA at a dose of 10.0 mg/kg. Thus, CTA can be dismissed as a potential explanation for any effects of naloxone in the present experiment.

Method

Subjects. Seventy-one male (n = 34) and female (n = 37) Long-Evans hooded rats, 90 days old at the start of training, were used in this experiment. The average ad libitum weight was 421 g for males and 253 g for females. Rats were bred and 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 81-84% of their free-food weight. Nondeprived rats also exhibit

cSNC, but the level of consummatory behavior tends to be quite low. Because naloxone was predicted to suppress consummatory behavior, a higher level for the saline controls was desirable. In addition, most experiments on cSNC use levels of deprivation between 80% and 85% of ad libitum levels. Downshifted rats that are not food deprived do not show recovery from the cSNC within the 5 postshift trials typically administered (Grigson, Spector, & Norgren 1992; Riley & Dunlap, 1979). Water was continuously available in each individual wire-mesh cage. Animals were trained during the light phase of the daily cycle.

Apparatus. Training was conducted in four conditioning boxes (MED Associates, Vermont) constructed of aluminum and Plexiglas (29.3 cm×21.3 cm×26.8 cm, *L*×*H*×*W*). The floors were made of steel rods, 0.4 cm in diameter and 1.6 cm apart, running parallel to the feeder wall. A bedding tray filled with corncob bedding was placed below the floor to collect fecal pellets and urine. Against the feeder wall was an elliptical opening 1 cm wide, 2 cm high, and 4 cm from the floor, through which a sipper tube, 1 cm in diameter, was inserted. When fully inserted, the sipper tube was flush against the wall of the box. A house light (GE 1820) located in the center of the box's ceiling provided diffuse light. A computer located in an adjacent room controlled the presentation and retraction of the sipper tube. When rats contacted the sipper tube, a circuit involving the steel rods in the floor and the sipper tube was closed and the signal was recorded by the computer. Each conditioning box was placed in a sound-attenuating chamber that contained 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).

Procedure. Training lasted 15 daily trials. The first 10 trials were the preshift trials, and the last 5 trials are the postshift trials. For shifted groups, the preshift consisted of 5 min daily access to a 32% sucrose solution (w/w, prepared by mixing 32 g of commercial sugar for every 68 g of distilled water). The postshift consisted of 5 min daily access to 4% sucrose. For unshifted groups, 4% sucrose was presented for all preshift and postshift trials. The experiment was run in three replications. For each replication, rats were divided into two groups and matched by sex and then by weight. For cSNC to occur there must be a decrement in behavior on trial 11, which can be defined as a drop of more than 90% goal-tracking time relative to trial 10 for all shifted animals (trial 11/trial 10 < 0.90). A greater proportion of rats were assigned to the shifted groups in anticipation of the possibility that some rats would not meet this criterion. One group received 32% sucrose during the preshift (n = 41), and the other received 4% sucrose (n = 30). After trial 10, the groups were further divided into a total of six groups, matched by preshift responding: 32/V/V (n = 12), 32/NAL/V (n = 14), 32/V/NAL (n = 15), 4/V/V (n = 10), 4/NAL/V (n = 10), and 4/V/NAL (n = 10). Rats were trained in squads of four that remained constant throughout the experiment. The order in which the squads were tested was varied across days.

Drug Treatments. All drugs were dissolved into physiological saline as a vehicle to a concentration such that each subject received a 1 ml/kg injection. The dose of naloxone in the present experiment (2.0 mg/kg, all injections were i.p., all drugs from Sigma-Aldrich, MO) has been previously shown to enhance cSNC when administered 15 min before postshift trials (Pellegrini et al., 2005). Each rat received two injections: one immediately after the end of trial 11 and another 3 h later. For the 32/NAL/V and

4/NAL/V groups, naloxone was administered immediately after the trial and an equal volume saline injection was administered 3 h after the trial. Groups 32/V/NAL and 4/V/NAL received immediate saline and 3-h naloxone. For the vehicle groups, 32/V/V and 4/V/V, both the immediate and the 3-h injections were saline. The two-injection procedure allows the saline groups to serve as controls for both the immediate and the 3-h drug groups, reducing the number of necessary groups from eight to six.

Results and Discussion

The data from six female and four male rats that did not meet the contrast criterion on trial 11 were discarded, leaving five males and five females in each group (n = 10), with one additional female and one fewer male in Group 32/NAL/V (n = 10) and one additional male in Group 32/V/NAL (n = 11). Because the injections were scheduled immediately after trial 11, the groups could not be balanced for responding on trial 11. Chance individual differences in consummatory suppression on Trial 11 caused the groups to be unequal. Group 32/V/NAL was higher than the other groups, obfuscating any interpretations made by analyses including the 3-h posttrial condition. A one-way analysis of variance (ANOVA) with LSD pairwise posthoc comparisons on trial 11 revealed that while Groups 32/NAL/V and 32/V/V showed significant contrast relative to their unshifted controls, Group 32/V/NAL did not differ from Group 4/V/NAL. For this reason, the 3-h condition was excluded from further statistical analysis. The overall results are presented in Figure 3.

The preshift phase is marked by the acquisition of drinking behavior, with no measurable difference in consumption between groups receiving 32% and 4% sucrose. Males tended to drink more than females, which has been previously linked to differences

in body size rather than a factor specifically related to sex (Flaherty, 1996). The groups did not differ with regard to drug conditions during the preshift, indicating that the matching procedure was successful. A 2 X 2 X 2 X 10 (Contrast X Drug X Sex X Trial) ANOVA confirmed this portrayal, with a main effect of trial ($F(9, 39)= 8.44$, $p < 0.01$) which reflected acquisition of drinking behavior, and a significant main effect of sex ($F(1, 39)= 5.19$, $p < 0.04$). All other effects and interactions were nonsignificant (Fs < 3.75, ps > 0.05).

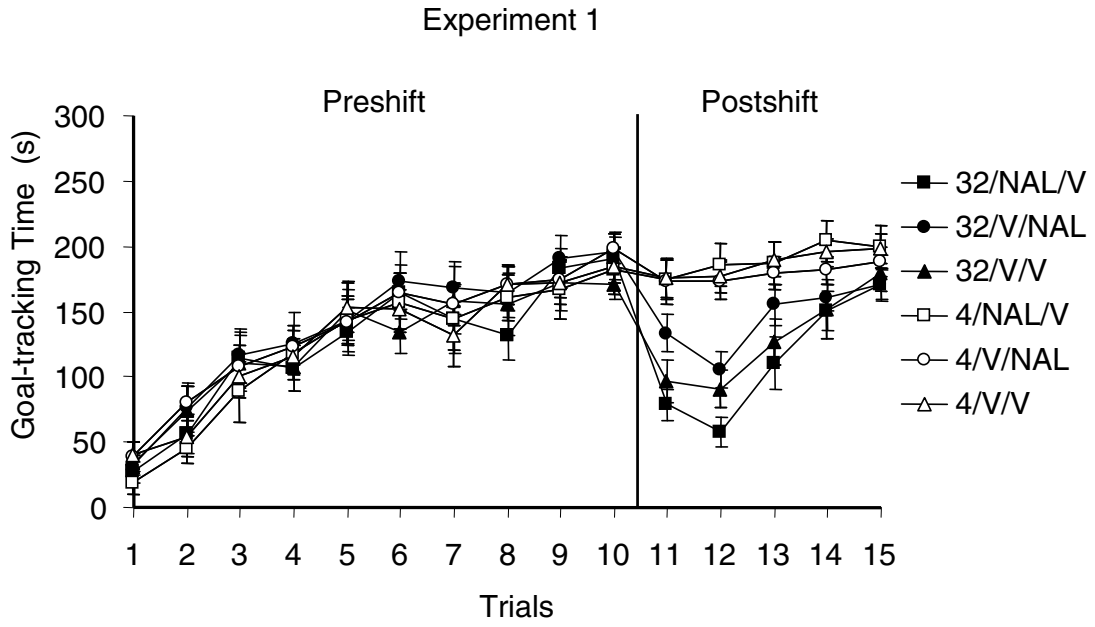

Figure 3. Mean (± SEM) goal-tracking time for Experiment 1. Groups 32/NAL/V and 4/NAL/V received naloxone (2.0 mg/kg, i.p.) immediately following trial 11, while Groups 32/V/V, 32/V/NAL, 4/V/V, and 4/V/NAL received equal volume saline injections. All groups received a second injection 3 h after training of either naloxone (Groups 32/V/NAL and 4/V/NAL) or vehicle (Groups 32/V/V, 32/NAL/V, 4/V/V, and 32/NAL/V).

During the postshift phase, Group 32/NAL/V remained similar to Group 32/V/V, and both showed significant cSNC relative to Groups 4/NAL/V and 4/V/V, respectively. Groups 4/NAL/V and 4/V/V did not differ from each other. A 2 X 2 X 2 X 4 (Contrast X Drug X Sex X Trial) ANOVA for trials 12-15 supported this description, with significant

main effects of trial ($F(3, 39) = 30.38$, $p < 0.01$), contrast ($F(1, 36) = 22.03$, $p < 0.01$), and a trial by contrast interaction ($F(3, 39) = 14.26$, $p < 0.01$). All other main effects and interactions were nonsignificant (Fs < 2.29, ps > 0.13). Notably, all effects and interactions involving drug were nonsignificant (Fs < 1).

There was a nonsignificant trend for Group 32/NAL/V to respond below that of the Group 32/V/V. Because of the inability to balance for individual differences in responding on trial 11, Groups 32/NAL/V and 32/V/V were subjected to an additional 2 X 2 X 4 (Drug X Sex X Trial) ANCOVA with goal-tracking time on trial 11 as a covariate. The ANCOVA results supported the initial ANOVA, with a significant main effect of trial ($F(3, 19) = 8.36$, $p < 0.01$). The main effects of drug, sex, and all interactions were nonsignificant (Fs < 2.89, ps > 0.10).

Previous experiments showed that the dose of naloxone was effective in enhancing cSNC (Pellegrini et al. 2005). The results of Experiment 1 indicate that the effects of naloxone on cSNC appear to be limited to pretrial administration, thus culling the consolidation of the aversive downshift memory from the list of possible mechanisms of cSNC modulation. This narrows the action of naloxone to two possibilities: acting on the intensity of primary frustration or the comparison between preshift and postshift solutions.

Experiment 2: Posttrial DPDPE and Naltrindole

Wood et al. (2005) showed that DPDPE attenuated contrast on trial 11 selectively (i.e., DPDPE had no effect when administered before trial 12). It was unclear whether DPDPE had its effects on the intensity of primary frustration or whether it interfered with the acquisition of secondary frustration. Administration of DPDPE immediately after trial

11 should have no influence on primary frustration during the trial, thereby allowing examination of its potentially selective effects on the acquisition of secondary frustration (i.e., development of the expectation of frustration). In addition, Pellegrini et al. (2006) showed that the δ-selective antagonist naltrindole selectively enhanced cSNC on trial 11, mirroring the effects of DPDPE. The present experiment will determine if DPDPE and naltrindole, both of which act on δ opioid receptors, influence memory immediately after the downshift (i.e., after trial 11).

Method

Subjects. Fifty male Long-Evans rats were used, following the same procedure described in Experiment 1.

Apparatus. The apparatus was the same as described in Experiment 1.

Procedure. The procedure was the same as in Experiment 1. Rats were divided into two groups of 24 balanced by weight, shifted and unshifted. After trial 10, the groups were subdivided into six groups balanced by preshift performance: 32/V (n = 8), 32/D (n = 9), 32/NTI (n = 9), 4/V (n = 8), 4/D (n = 8), and 4/NTI (n = 8).

Drug intervention. All injections were administered immediately after trial 11. Groups 32/D and 4/D received an injection of DPDPE (24 μg/kg; i.p.), 32/NTI and 4/NTI received naltrindole (1.0 mg/kg; i.p.), and 32/V and 4/V received equal-volume saline injections. Doses were chosen based upon previous positive results in the cSNC situation with these compounds.

Results and Discussion

The data from six shifted rats (one from 32/NTI, two from 32/D and three from 32/V) failed to exhibit a decrement in consummatory behavior on trial 11 and were

therefore discarded from statistical analyses. A data recording error affected trials 11 and 12 for one subject in group 4/D; the missing values were replaced with group averages, following the procedure described by Kirk (1968). The results are shown in Figure 4. A 2 X 3 X 10 (Contrast X Drug X Trial) ANOVA conducted on the preshift phase revealed a main effect of trial ($F(9, 43) = 92.3$, $p < 0.01$), but no other significant main effects or interactions (Fs < 1.5, ps > 0.18). A 2 X 3 (Contrast X Drug) ANOVA on trial 11 revealed a main effect of contrast ($F(1, 43) = 38.0$, $p < 0.01$). A 2 X 3 X 4 (Contrast X Drug X Trial) ANOVA conducted on the remaining four postshift trials revealed significant main effects of trial ($F(3, 43) = 30.6$, $p < 0.01$), contrast ($F(1, 43) = 16.7$, $p < 0.01$), and a trial by contrast interaction ($F(3, 43) = 6.7$, $p < 0.01$). There were no other significant effects (Fs < 1.7, ps > 0.14), indicating that neither of the drugs had a significant effect when given posttrial 11.

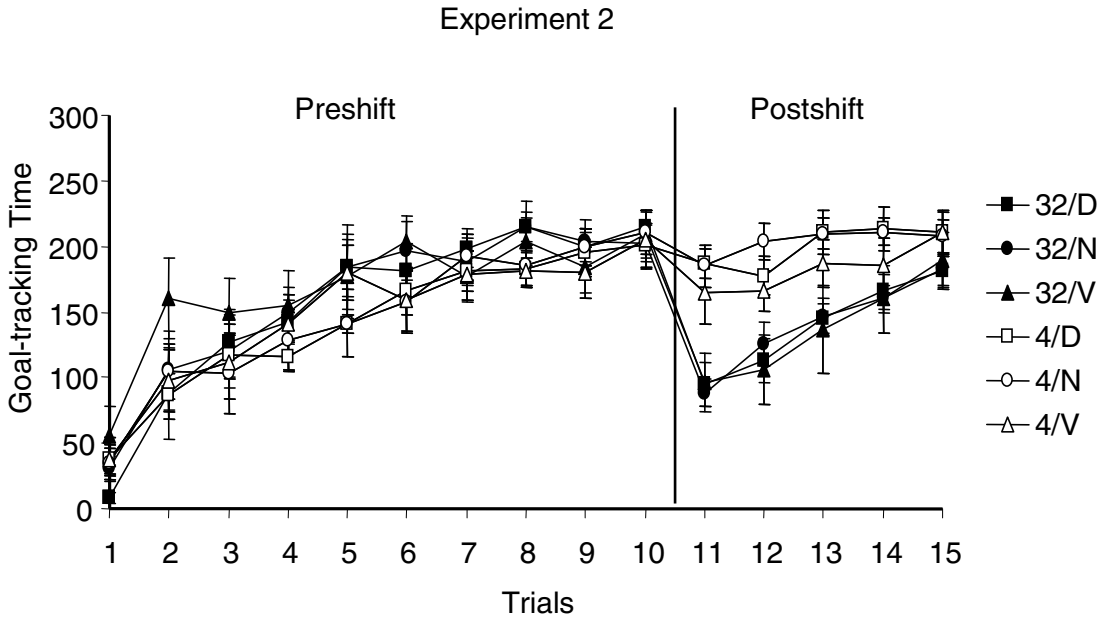

Figure 4. Mean (± SEM) goal-tracking time for Experiment 2. Immediately after trial 11, Groups 32/D and 4/D received injections of DPDPE (24 μg/kg, i.p.), Groups 32/N and 4/N received naltrindole (1.0 mg/kg, i.p.), and Groups 32/V and 32/D received equal volume vehicle injections.

As was the case in Experiment 1, the inability to balance for individual differences in responding on trial 11 made it necessary to subject Groups 32/N, 32/D, and 32/V to an additional 3 X 4 (Drug X Trial) ANCOVA with goal-tracking time on trial 11 as a covariate. The ANCOVA results supported the initial ANOVA, with a significant main effect of trial ($F(3, 18) = 11.29$, $p < 0.01$). The main effect of drug and all interactions were nonsignificant (Fs < 2.34, ps > 0.08).

Neither DPDPE nor Naltrindole administered immediately after trial 11 measurably affected consolidation of the downshift memory. Combined with previous data showing its selectivity for effects on trial 11, this experiment demonstrates that the δ-opioid system appears to play a role in modulating the direct impact of primary frustration, but not processing of its trace or consolidation of the aversive downshift memory. It is important to note that the doses used in this experiment have been previously established as effective in modulating cSNC on trial 11. As with naloxone in Experiment 1, the action of naltrindole and DPDPE can be narrowed to two possibilities: acting on the intensity of primary frustration or on the comparison between preshift and postshift solutions. Together, the results of Experiments 1 and 2 suggest that the opioid system might not be involved in consolidation of the downshift memory in the cSNC situation. The role of opioids in memory for other situations involving surprising reward loss is yet to be determined.

Experiment 3: Naloxone and Autoshaping Extinction

Would opioids affect other situations involving surprising reward loss? To explore the generality of the previous results, the effects of opioids on Pavlovian appetitive extinction were investigated in Experiment 3. Appetitive extinction can be

viewed as a special case of contrast, in which the subject is shifted from reinforcement to nonreinforcement, instead of being shifted from a large to a small reinforcer. The question arises as to whether the opioid system is involved in memory consolidation in appetitive extinction.

Autoshaping was chosen to study appetitive extinction. In autoshaping with rats, a lever (conditioned stimulus, CS) is presented for a fixed time period and its retraction is paired with the response-independent delivery of a food pellet (unconditioned stimulus, US). Although rats are not required to press the lever to obtain food, they nonetheless approach and contact the lever in anticipation of food delivery. The autoshaping procedure has the benefit that the dependent variable is not consummatory behavior. Autoshaping also exhibits sensitivity to manipulations involving surprising reward loss. For example, Papini et al. (2001) demonstrated that rats preexposed to 10% sucrose US but autoshaped with a food pellet US in a second phase showed higher rates of acquisition than did rats preexposed to and conditioned with food pellets. Likewise, rats preexposed to food pellets but autoshaped with 10% sucrose showed slower acquisition than did sucrose-only controls. In other words, rats undergoing a qualitative shift in reinforcer between preexposure and conditioning phases exhibited a pattern of responding that resembled positive (10% sucrose→pellet) and negative (pellet→10% sucrose) contrast, with the pellet as the more preferred reward over the 10% sucrose. Similar results were found with quantitative downshifts from 12-1 pellets (although no evidence of positive contrast was obtained with 1-12 upshifts).

Other related phenomena have also been found. The frustration effect has been demonstrated in the autoshaping procedure (Papini & Dudley, 1995). Rats received

light→food pairings followed by lever→food pairings. The food was omitted after the light on 10% of the trials. Rats pressed the lever more following surprising reward omissions than following rewarded trials, and also more relative to a control condition matched for US delivery. Such response invigoration was presumably due to frustration resulting from the surprising reward omission. The magnitude of reinforcement extinction effect (MREE) has also been reported with autoshaping procedures. The MREE describes a phenomenon in which rats undergoing extinction after a shift from acquisition with large rewards show a faster response decrement than do rats shifted from acquisition with small rewards, seemingly because larger downshifts result in greater frustration. Papini et al. (2001) autoshaped two groups of rats with either 1 or 12 pellets, then shifted both groups to extinction. The group trained with 12 pellets extinguished lever pressing significantly faster than the group trained with 1 pellet, demonstrating the MREE.

The PREE is another related phenomenon that can be demonstrated with autoshaping procedures. Boughner and Papini (2006) trained rats in either a continuously reinforced or partially reinforced acquisition phase. When shifted to extinction, partially reinforced rats exhibited greater persistence of lever pressing than did continuously reinforced rats. Overall, this evidence indicates that autoshaping procedures have the ability to induce frustration effects in terms of anticipatory behavior (i.e., behavior occurring before the rat comes into consummatory contact with the US).

The question posed in the present experiment was whether opioids have an influence on the aversive memory of appetitive extinction in an autoshaping procedure.

Would an injection of naloxone after each extinction session in an autoshaping situation influence subsequent extinction?

Method

Subjects. Twenty-five female Long-Evans rats were used in this experiment. Housing and maintenance conditions were as described in Experiment 1.

Apparatus. Four standard conditioning chambers were used, each enclosed in a sound-attenuating cubicle. The internal dimensions of each chamber were 20.1 cm wide, 28 cm long, and 20.5 cm high. The floor of each chamber was made of stainless steel bars 0.4 cm in diameter and spaced 1.6 cm apart, center to center. Located in the center of the front wall was a recessed magazine, 2 cm from the floor, into which the pellets (45-mg Noyes rat formula A/I) were delivered automatically. An aluminum retractable lever (4.8 cm wide, 1.9 cm deep, and 7 cm above the floor) was located 2 cm to the left of the magazine. Insertion (or retraction) of the lever took 0.2 s. A light bulb (GE 1820) attached to the ceiling of the chamber provided diffuse illumination and was positioned opposite the magazine. A speaker and fan provided background noise (75 dB, SPL scale C, measured in front of the magazine) and ventilation, respectively.

Procedure. Rats were trained in two phases, acquisition and extinction. In the first phase (acquisition), each of the 10 sessions started with the onset of the house light and ended when the house light was turned off. There were 10 training trials per session separated by a variable intertrial interval (ITI) with a mean of 50.1 s (range: 33-64 s). Before the first trial in each session, there was an interval of duration and range equal to that of the ITI. Each trial started with the insertion of the retractable lever for 10 s (the CS). A computer recorded lever-contact responses while the lever was inserted in the

chamber. At the end of the 10 s, the lever was retracted and five pellets were delivered on the magazine cup at a rate of one pellet per 0.2 s (the US). Each rat consumed 50 45-mg pellets per session. In the second phase (extinction), the training conditions were the same for 5 additional sessions, except that all food delivery was withheld. After the final acquisition session, the rats were divided into three groups, matched for responding in acquisition: 0NAL (n = 8), 3NAL (n = 7), and V (n = 7). Three rats failed to emit at least 10 responses per minute during the acquisition phase and were therefore not tested in extinction, nor were they assigned to drug conditions. The data from these three rats were eliminated from statistical analyses.

Drug Treatment. Immediately after the each extinction session, rats in Group 0NAL received an injection of naloxone (2.0 mg/kg, i.p.), whereas rats in Groups 3NAL and V received an equal-volume saline injection. Conversely, 3 h after each extinction trial, rats in Group 3NAL received an injection of naloxone (2.0 mg/kg, i.p.), whereas rats in Groups 0NAL and V received an equal-volume saline injection.

Results and Discussion

Due to an experimenter oversight, one rat in group 0/Nal received a 48 h ITI between trials 14 and 15 instead of the usual 24 h, but this did not significantly affect the outcome when included in the statistical analyses. During acquisition, all groups were similar, which is not surprising due to the matching procedure. During the extinction phase, there was a significant extinction spike followed by significant extinction, although there were no differences between drug conditions. These data are presented in Figure 5.

A 3 X 10 (Drug X Session) ANOVA on the acquisition phase revealed significant acquisition as evidenced by a main effect of session ($F(9, 21) = 23.01$, $p < 0.01$), but drug groups did not differ, nor was there a significant interaction (Fs < 1, $p > 0.40$). Similarly, during the extinction phase, a 3 X 5 (Groups X Session) ANOVA indicated that significant extinction occurred, with a significant effect of session ($F(4, 21) = 11.78$, $p < 0.01$), but drug groups did not differ, nor was there a significant interaction (Fs < 1.17, $p > 0.34$). Finally, a 3 X 2 (Drug X Session) ANOVA on trials 10 and 11 revealed a significant extinction spike, ($F(1, 21) = 5.36$, $p < 0.04$), but again drug groups were not different, nor was there a significant interaction (Fs < 1, $p > 0.59$).

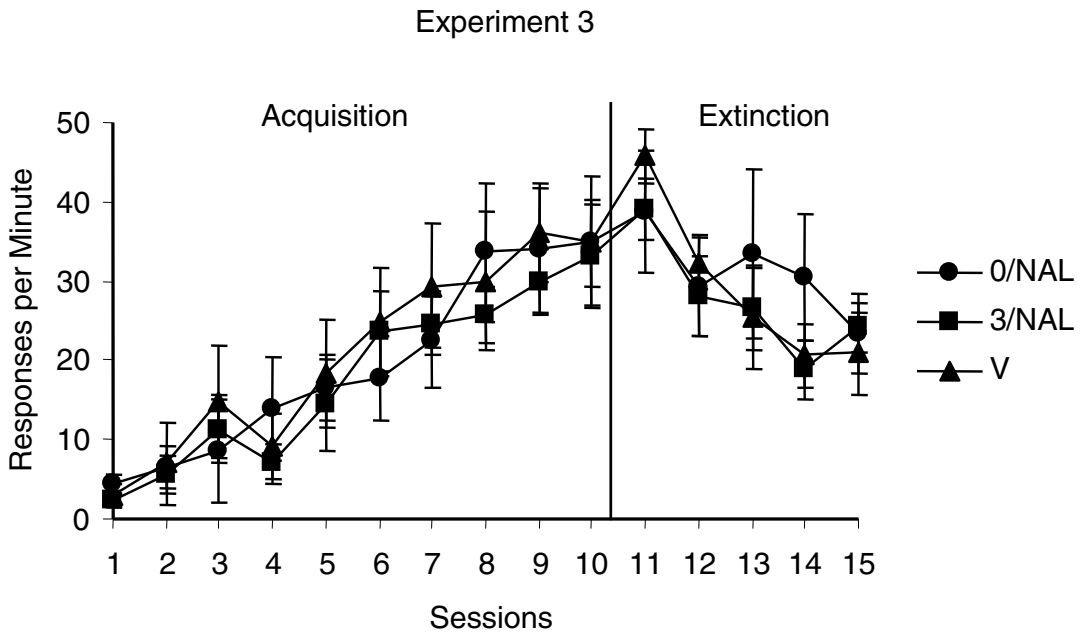

Figure 5. Mean (± SEM) lever presses per minute for Experiment 3. Group 0/NAL received naloxone (2.0 mg/kg, i.p.) immediately after each extinction session, and Groups 3/NAL and V received equal volume vehicle injections. Three hours after each extinction session, Groups 0/NAL and V received vehicle, and Group 3/NAL received naloxone.

The lack of an effect of naloxone administered after each extinction session in the present experiment provides further and more general evidence that the opioid system

does not play an important role in consolidation of the downshift memory in situations involving surprising reward loss. The present experiment was designed to mimic the training parameters used in the cSNC preparation as closely as possible, with regard to session length and number of sessions. It is possible that the five extinction sessions were not sufficient for differences to emerge, and a longer extinction phase might be sensitive to such differences. In addition, any effects of naloxone early in extinction interact with the extinction spike, resulting in increases in responding rather than in faster extinction analogous to enhanced cSNC. These issues are addressed in Experiment 4.

Experiment 4: Naloxone in Early vs. Late Extinction

To ensure that the lack of an effect of posttrial naloxone in Experiment 4 was not due to insufficient extinction or influence of the extinction spike, a second autoshaping experiment was run. The design was similar, except that the extinction phase was extended to 10 sessions, and groups received naloxone injections either early or late in extinction, the latter of which to minimize the influence of the extinction spike.

Method

Subjects. Thirty-two male (n = 16) and female (n = 16) Long-Evans rats were used in this experiment. Housing and maintenance conditions were as described in Experiment 1.

Apparatus. The same apparatus described in Experiment 3 was used in this experiment.

Procedure. The same daily procedure was used in this experiment as in Experiment 3. Both the acquisition and extinction phases of the experiment consisted of 10 daily trials. After the final acquisition session, the rats were divided into three groups,

matched for responding in acquisition: NAL/V (n = 9), V/NAL (n = 10), and V/V (n = 9). Three female and one male rat failed to emit at least 10 responses per minute during the acquisition phase and were discarded from further testing. The final groups consisted of four females and five males each, except for group V/NAL which contained five males and five females.

Drug Treatment. Immediately after sessions 11 and 12, rats in Group NAL/V received an injection of naloxone (2.0 mg/kg, i.p.), whereas Groups V/NAL and V/V received vehicle. This condition replicated the early extinction injections given in Experiment 3. After trial 13, all subjects received vehicle injections to maintain the same daily training routine. Immediately after trials 14-19, Groups NAL/V and V/V received vehicle, and Group V/NAL received naloxone. This condition was to assess the effects of naloxone on late extinction without the interference of the extinction spike.

Results and Discussion

The results of Experiment 4 are presented in Figure 6. The acquisition phase was marked by acquisition of lever pressing, without differences between groups. Females reached a lower overall rate of lever pressing on the terminal acquisition trial than males. On sessions 11-20, all groups exhibited similar rates of extinction. Males tended to extinguish more than females, but the difference is likely due to the higher rate at which they responded in acquisition. Naloxone had no measurable effect on extinction of lever pressing whether administered early or late in the extinction phase.

The description above of the acquisition phase was supported statistically by a 3 X 2 X 10 (Drug X Sex X Session) ANOVA on sessions 1-10, which revealed a significant effect of session ($F(9, 27) = 33.34$, $p < 0.01$) and of sex ($F(1, 27) = 5.67$, $p <$

0.03), and a significant sex by session interaction ($F(9, 27) = 4.12$, $p < 0.01$). All main effects and interactions involving drug were nonsignificant (Fs < 1, ps > 0.67). Similarly, an overall 3 X 2 X 10 (Drug X Sex X Session) ANOVA on extinction sessions 11-20 revealed a significant extinction effect of session ($F(9, 27) = 30.38$, $p < 0.01$) and an effect of sex ($F(1, 27) = 9.07$, $p < 0.01$), and a significant sex by session interaction ($F(9, 27) = 1.98$, $p < 0.05$). Again, all effects and interactions involving drug were nonsignificant, (Fs < 1.14, ps > 0.33).

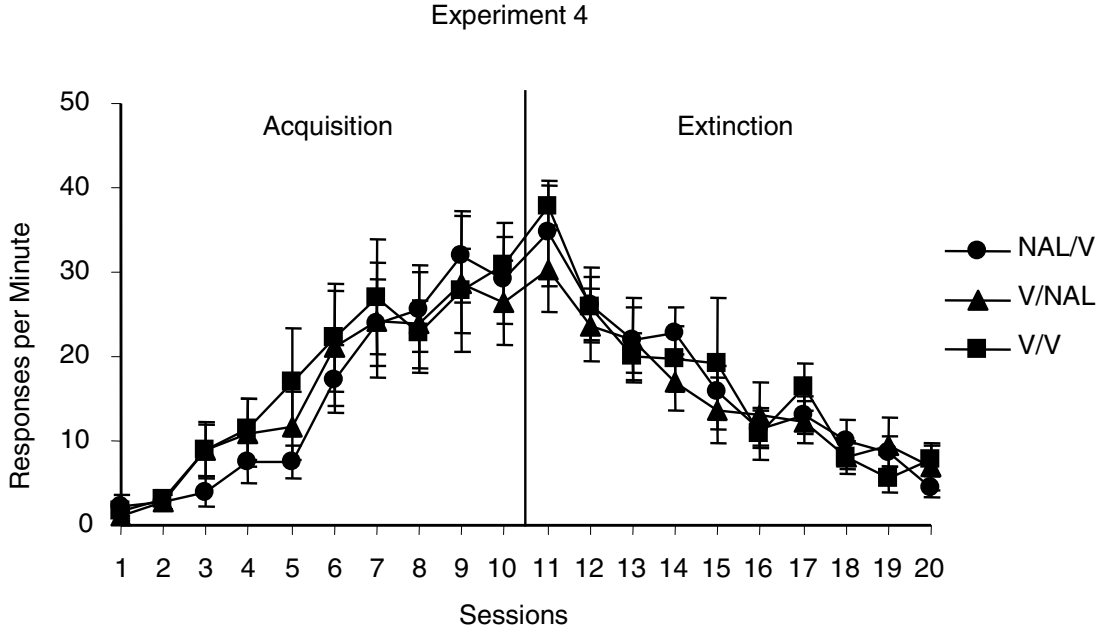

Figure 6. Mean (± SEM) lever presses per minute for Experiment 4. Group NAL/V received naloxone (2.0 mg/kg, i.p.) early in extinction, immediately following sessions 11 and 12, and vehicle injections after all subsequent sessions. Group V/NAL received vehicle following sessions 11-13, and naloxone after all subsequent sessions. Group V received vehicle after each extinction session.

All three groups exhibited an extinction spike, verified by a 3 X 2 X 2 (Drug X Sex X Session) ANOVA for sessions 10 and 11. Main effects of session ($F(1, 27) = 5.29$, $p < 0.03$) and sex ($F(1, 27) = 6.38$, $p < 0.02$) were significant, while all other effects and interactions were nonsignificant (Fs < 3.00, ps > 0.07), indicating that although all groups

showed a significant spike, groups did not differ in the degree to which they showed the extinction spike. Males tended to show higher responding than females, but similar extinction spikes.

Further analyses were conducted on the sessions immediately following naloxone administration, early and late, to ensure that any local effects of the drugs were detected. For early extinction, a 3 X 2 X 2 (Drug X Sex X Session) ANOVA of sessions 12 and 13 revealed a main effect of sex ($F(1, 27) = 13.51$, $p < 0.01$), but the difference between sessions was nonsignificant ($F(1, 27) = 3.86$, $p > 0.06$). All other effects and interactions were nonsignificant (Fs < 1.67, ps > 0.21). For late extinction, a 3 X 2 X 6 (Drug X Sex X Session) ANOVA of sessions 15-20 indicated a main effect of session ($F(5, 27) = 5.83$, $p < 0.01$), but sex was nonsignificant ($F(1, 27) = 4.06$, $p > 0.05$). All drug effects and interactions were nonsignificant, (Fs < 1, ps > 0.61).

The present experiment replicated the lack of naloxone effects on autoshaping extinction reported in Experiment 3. Furthermore, the present results extend those of the previous experiment, suggesting that those results were not due to an extinction phase with insufficient sessions, nor were the effects masked by an interaction with the extinction spike early in extinction. This result is consistent with the hypothesis that naloxone has no measurable influence on memory consolidation in situations involving surprising reward loss. One important distinction about the autoshaping procedure that must be made is that although the behavior measured is instrumental, the procedure is pavlovian (i.e., the response is not required to obtain the reward), and therefore it might be different from other instrumental appetitive extinction procedures, such as runway extinction. Thus, any conclusions about the influence of naloxone on memory for reward

downshifts or omissions must be reserved to Pavlovian procedures until instrumental procedures can be tested.

Experiment 5: Naloxone and Scaling

Overall, the results of Experiments 1-4 suggest that the posttrial administration of the nonselective opioid receptor antagonist naloxone plays little or no role in the consolidation of the aversive memory of the downshift event. This was the case whether the effects of reward loss were assessed in terms of consummatory or anticipatory behavior, whether the reinforcer was sucrose solution or food pellets, whether experience with the reinforcer occurred in a single trial or in several trials spread during a session, and whether the downshift was to a smaller reward or to no reward. A similar picture emerged from the results of the posttrial administration of δ-opioid receptor opioids, including the agonist DPDPE and the antagonist naltrindole. The latter results are restricted to the cSNC situation. As a working framework to understand the role of the opioid system in situations involving surprising reward loss, it is suggested here that the memory hypothesis outlined previously is possibly inadequate. Thus, Experiment 5 shifts attention to the possibility that the opioid system intervenes in the process of incentive comparison known to underlie downshifts in incentive value in a variety of situations (Papini & Pellegrini 2006; Pellegrini & Papini, 2007).

Papini and Pellegrini (2006) demonstrated that shifts of different magnitudes but with the same ratio of discrepancy between solutions resulted in similar amounts of consummatory suppression. If opioids influence the comparison between the solutions, then administration of naloxone will distort this scaling property. For example, if naloxone enhances the disparity in comparison between preshift and postshift solutions,

then naloxone administration should cause the groups with greater absolute disparities between solutions to show enhanced contrast compared to the groups with the same ratio but smaller disparities. The goals of the present experiment were to replicate the scaling effect reported by Papini and Pellegrini 2006 in the cSNC situation and to determine the extent to which naloxone disrupts such scaling.

Method

Subjects. Sixty-six male Long-Evans rats housed and deprived as in Experiment 1 were used in this experiment.

Apparatus. The conditioning chambers described in Experiment 1 were used in this experiment.

Procedure. The procedure was the same as in Experiment 1, except that five different sucrose solutions were used: 32%, 16%, 12%, 6%, and 3%. These concentrations were chosen so as to generate postshift/preshift ratios of specific values, as described below. Since naloxone enhances contrast, smaller downshifts than the usual 32-4 one were used to reduce the possibility of floor effects (Pellegrini et al., 2005). During the preshift phase, rats were divided into two groups that received either 32% or 16% sucrose. At the end of the preshift, groups were each divided into two subgroups matched for preshift responding. The four groups received one of the following shift magnitudes: 32-6, 16-3, 32-12, or 16-6. Groups 32-6 and 16-3 share the same ratio of disparity between solutions (postshift/preshift ratio equal to 0.1875), which is different from the ratio of groups 32-12 and 16-6 groups (postshift/preshift ratio equal to 0.375). Training continued for five postshift trials.

Drug Treatment. The four groups were further subdivided into naloxone or saline, for a total of eight groups (n = 8, n = 9 for Groups 32-6/N and 16-3/N). Groups 32-6/N, 16-3/N, 16-6/N, and 32-12/N receive injections of naloxone (2.0 mg/kg, i.p.) 15 min before trial 11. Groups 32-6/V, 16-3/V, 16-6/V, and 32-12/V receive equal-volume saline injections 15 min before trial 11.

Results and Discussion

The critical comparisons for this experiment must accomplish the following goals:

(1) Determine the existence of a special case of contrast between two groups downshifted to the same solution from different preshift solutions (i.e., Groups 32-6 and 16-6).

(2) Replicate the scaling property of cSNC described by Papini and Pellegrini (2006).

(3) Determine the possible influence of naloxone on cSNC.

Due to a computer malfunction, data were lost for two rats in group 16-6/V on trial 13, and were replaced with the group average (Kirk, 1968). The overall results of the experiment are presented in Figure 7, with the groups exposed to a ratio of 0.375 in the top panel and the groups exposed to a ratio of 0.1875 in the bottom panel. As expected based on previous results (Papini & Pellegrini, 2006), the groups with access to 16% sucrose displayed higher goal-tracking times than the groups with access to 32% sucrose. However, only the change in goal-tracking time across preshift trials was significant. A 2 X 2 X 2 X 10 (Preshift Solution X Ratio X Drug X Trial) ANOVA revealed a main effect of trial ($F(9, 65) = 149.63$, $p < 0.01$), and no other significant effects or interactions (Fs < 3.31, ps > 0.07).

During the postshift, Groups 32-12/V and 16-6/V showed similar levels of consummatory suppression, but both showed less suppression than did Groups 32-6/V and 16-3/V, which were also similar to each other. These results replicate two aspects of

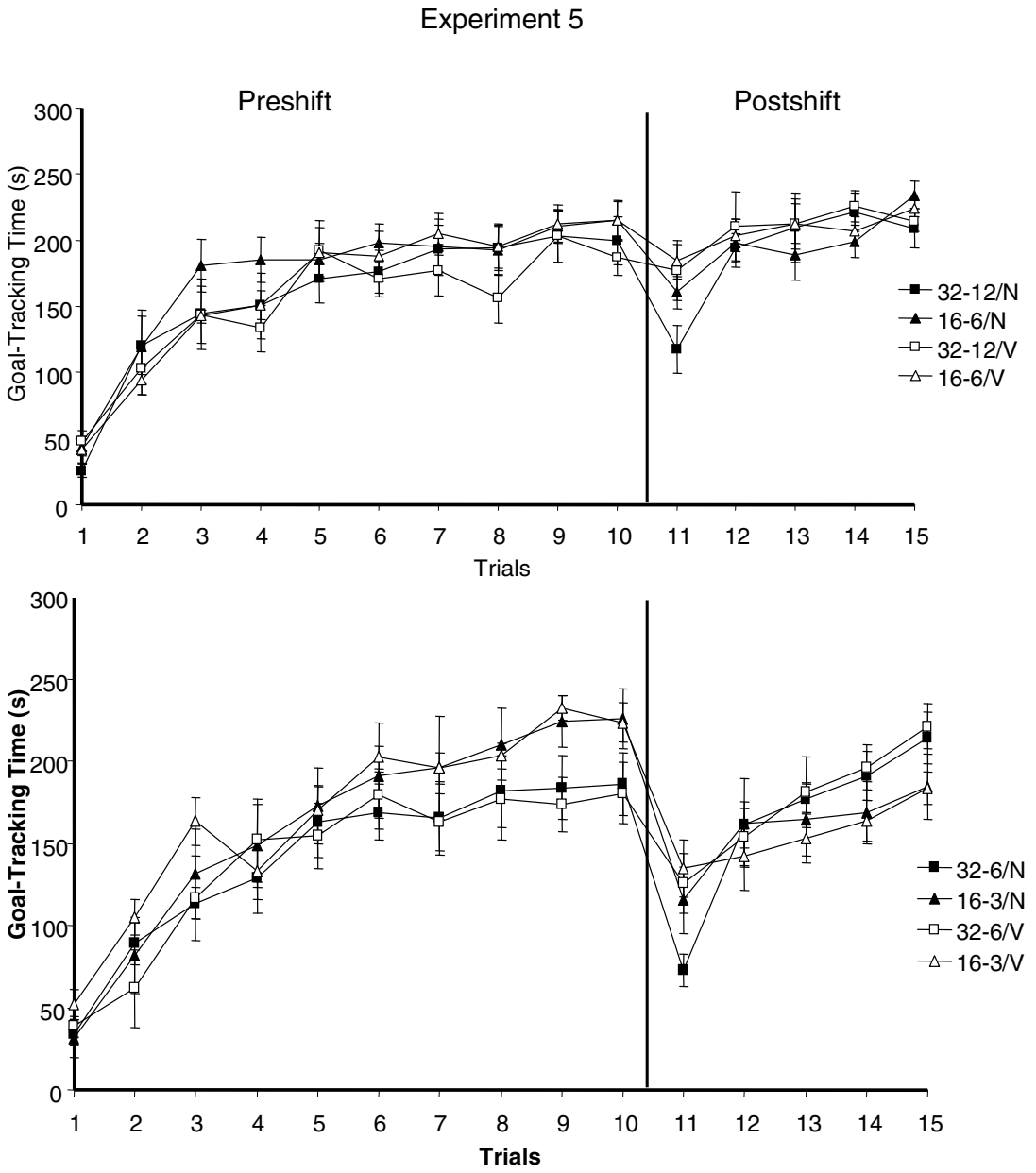

Figure 7. Mean (± SEM) goal-tracking time for Experiment 5, organized by ratios of 0.375 (top panel) and 0.188 (bottom panel). Groups 32-12/NAL, 32-6/NAL, 16-6/NAL, and 16-3/NAL received naloxone (2.0 mg/kg, i.p.) 15 min before trial 11, and Groups 32-12/V, 32-6/V, 16-6/V, and 16-3/V received vehicle.

the scaling property of cSNC reported previously. First, the higher ratio is associated with less suppression of consummatory behavior. Second, equal ratios yield similar levels of suppression on trial 11. An overall analysis of postshift performance with a 2 X 2 X 2 X 5 (Preshift Solution X Ratio X Drug X Trial) ANOVA supported this description, with main effects of trial ($F(4, 65) = 50.89$, $p < 0.01$) and ratio ($F(1, 65) = 16.24$, $p < 0.01$), and significant trial by preshift ($F(4, 65) = 5.65$, $p < 0.01$) and trial by drug ($F(4, 65) = 4.52$, $p < 0.01$) interactions. All other effects and interactions were nonsignificant (Fs < 2.08, ps > 0.08). The effects of naloxone on consummatory behavior were restricted to trial 11.

Further analyses of trial 11 revealed that Group 32-6/V tended to drink less than Group 16-6/V, and Group 32-6/N drank less than Group 16-6/N, providing evidence for the special case of contrast. Furthermore, while Groups 16-6/N and 16-6/V did not differ significantly from each other, Group 32-6/V drank more than 32-6/N, indicating that naloxone more strongly affected the shift with the highest absolute discrepancy between solutions. A one-way ANOVA comparing Groups 32-6/N, 32-6/V, 16-6/N, and 16-6/V with LSD pairwise posthoc comparisons verified these observations, with an overall difference between groups ($F(3, 29) = 10.77$, $p < 0.01$). Group 32-6/V drank less than Group 16-6/V ($p < 0.02$), and Group 32-6/N drank less than Group 16-6/N ($p < 0.01$). 16-6/N and 16-6/V did not differ ($p > 0.28$), whereas Group 32-6/V drank more than 32-6/N ($p < 0.02$).

Finally, a 2 X 2 (Preshift Solution X Ratio) ANOVA for only the vehicle groups on trial 11 exactly replicated the scaling property reported by Papini and Pellegrini (2006), with a significant main effect of ratio ($F(1, 31) = 8.15$, $p < 0.01$). The preshift

solution and the preshift by ratio interaction were nonsignificant (Fs < 1, ps > 0.65). However, a 2 X 2 (Preshift Solution X Ratio) ANOVA on only the naloxone groups indicates that, like the vehicle groups, there is a significant effect of ratio ($F(1, 33) = 8.16$, $p < 0.01$), but preshift also becomes significant ($F(1, 33) = 7.41$, $p < 0.02$). The interaction remains nonsignificant ($F < 1$, $p > 0.99$). This result illustrates that normally the ratio determines the level of behavioral decrement during cSNC, but when naloxone is administered, the absolute disparity between the preshift and postshift solutions becomes important in determining the level of responding. Figure 8 shows goal-tracking time on trial 11 by the absolute discrepancy between pre- and postshift solutions. Vehicle groups exhibit similarity based upon the postshift/preshift ratio, but suppression in the naloxone groups become more linear with regard to the absolute discrepancy.

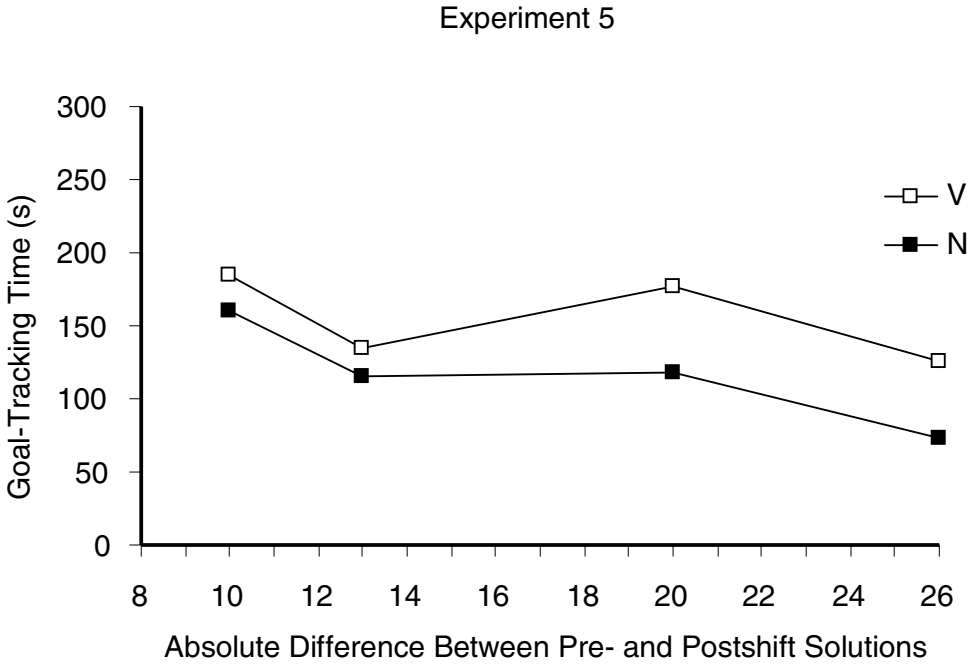

Figure 8. Mean goal-tracking time for trial 11 of Experiment 5 with regard to the absolute disparity between pre- and postshift solutions. Absolute disparity refers to the difference between preshift and postshift solutions (e.g., a shift from 32-12 yields an absolute disparity of 20), as opposed to a ratio of the solutions. Naloxone (2.0 mg/kg, i.p.) was administered 15 min before trial 11 for the naloxone groups (N), and equal volume saline injections were administered to the vehicle groups (V).

The present experiment replicated both the special case of contrast and the scaling property of cSNC reported by Papini and Pellegrini (2006). Shifts with greater absolute disparities were more sensitive to the effects of naloxone, providing the first evidence supporting the hypothesis that the influence of opioids on cSNC is related to the value comparison between expected and received rewards.

General Discussion

The present experiments explored the role of several potential mechanisms that could explain the effects of opioids in situations involving surprising reward loss. Experiments 1 and 2 showed that nonselective and δ-selective opioid drugs, which are known to influence cSNC when administered at the same doses before downshift trials, have no measurable effect when administered immediately after the downshift trials. Experiments 3 and 4 generalized this finding to the autoshaping paradigm, using different reinforcers, different trial distributions, anticipatory (rather than consummatory) behavior, and a downshift to no reward (rather than to a smaller reward). Experiment 4 found no effects of posttrial naloxone on extinction, whether administered early or late in the extinction phase, even when extensive extinction has taken place. Finally, Experiment 5 demonstrated that naloxone disrupts the scaling property of cSNC such that the absolute discrepancy becomes important in addition to the ratio between pre- and postshift solutions.

Three possible opioid mechanisms were proposed: comparison between expected and received rewards, the intensity of primary frustration, and the acquisition of secondary frustration. Modulation of the intensity of primary frustration cannot be directly dissociated from the other possibilities, thus the others must be eliminated to

provide support for that possibility, and it was not explored directly. The present experiments broadly demonstrate that the opioid system does not appear to modulate the acquisition of secondary frustration, through consolidation of the frustration memory, in surprising reward loss situations. Thus, the working hypothesis that emerges from this line of research is that the opioid system affects incentive relativity by modifying the intensity of primary frustration and/or affecting the incentive comparison process.

Nonselective opioid drugs have been implicated in modulating the intensity of primary frustration and the strength of secondary frustration, but not in the conditioning of secondary frustration. Pre- and posttrial manipulations have narrowed the influence of the δ-opioid receptor subsystem to modulation of the intensity of the UR, given that DPDPE and naltrindole affect only the first downshift trial in pretrial administration (Wood et al., 2005) and have no effect on consolidation after posttrial 11 administration (present Experiment 2). The possibility that the κ-opioid subsystem modulates primary frustration can be safely discarded, given the lack of effect of pretrial 11 administration of U-50 on cSNC (Wood, 2006). However, the bidirectional effects of U-50,488H administered before trial 12 show that it may be involved in the acquisition of secondary frustration. The μ-opioid receptor subsystem has yet to be explored with selective opioid agonists and antagonists, but the nonselective nature of naloxone used in the present experiments suggests that it is unlikely that μ-selective drugs would influence memory consolidation.

In previous studies in which posttrial 11 injections have influenced trial 12, the interpretation has been that the treatment enhanced the memory for the downshift (Bentosela, Ruetti, et al., 2006). It is possible that the treatment actually reduced the

memory of the downshift event, resulting in greater primary frustration on trial 12 (i.e., as though trial 11 never happened), thus increasing cSNC. To discriminate the two from each other, the selectivity of DPDPE to reduce contrast on trial 11 can be exploited. If a compound interferes with consolidation when administered after trial 11, then the experience of trial 11 is minimized. An injection of DPDPE before trial 12 would, in this case, attenuate contrast on trial 12, as if trial 11 never occurred. This prediction could be tested using corticosterone as the posttrial 11 manipulation that increases cSNC (Bentosela, Ruetti, et al., 2006).

Finally, the results of Experiment 5 support the idea that naloxone influences the comparison of expected and received rewards, possibly by inflating the absolute discrepancy between solutions and distorting the scaling property. The comparison concept is not as obvious with regard to frustration theory, which assumes that a comparison is made but does not specifically implement a theoretical component to accomplish this. Amsel's (1992) frustration theory falls short in capturing this kind of comparison, but allows formulation of many specific empirical predictions. Future revisions of frustration theory should attempt to incorporate the comparison mechanism, as in the detection phase described by Flaherty's (1996) multistage hypothesis.

One problem for future studies to explore is the generality of the present findings in Pavlovian procedures described here to instrumental procedures, such as iSNC or runway extinction. Opioids do not seem to be involved in learning about reward loss situations, per se, but may modulate learning via the intensity of primary frustration or incentive comparison. While Experiment 5 provided evidence for the latter, the intensity of primary frustration cannot yet be discarded until the empirical tools are developed to

dissociate it from the reward comparison itself. Future research on this dissociation problem will be particularly valuable.

Because of the extensive parallels between physical pain and psychological pain from loss, connections have been drawn between the opioid system and psychological disorders arguably related to loss, such as anxiety disorders and depression (Nyhuis & Gastpar, 2005; Papini et al., 2006; Sher, 1998). Among the evidence supporting this connection is that patients with anxiety disorders are more likely to use opioids for self medication (Sher, 1998). In addition, some treatments for anxiety rely upon activation of endogenous opioids, such as acupuncture, psychotherapy, and placebos (Sher, 1998). Although cSNC in rats is significantly less complex than depression or anxiety in humans, using cSNC as an animal model of loss-induced anxiety and depression allows for an understanding of opioid function. The present research adds to a growing body of evidence suggesting that the opioid system modulates resilience or vulnerability to reward loss. Because there are substantial individual differences in opioid sensitivity, even in the cSNC situation (Pellegrini et al. 2005), and known genetic variations in opioid receptors in rodents and humans (Mayer & Höllt, 2001), this opens the possibility for development of diagnostic tools for vulnerability to loss.

References

Agranoff, B. W., Davis, R. E., & Brink, J. J. (1966) Chemical studies on memory fixation in goldfish. *Brain Research,* **1,** 303–309

Amsel, A. (1992). *Frustration theory*. Cambridge, UK: Cambridge University Press.

Becker, H. C. (1986). Comparison of the effects of the benzodiazepine midazolam and three serotonin antagonists on a consummatory conflict paradigm. *Pharmacology, Biochemistry, and Behavior*, **24**, 1057-1064.

Becker, H. C., Jarvis, M. F., Wagner, G. C., & Flaherty, C. F. (1984). Medial and lateral amygdalectomy differentially influence consummatory negative contrast. *Physiology and Behavior,* **33,** 707-712.

Bentosela, M., D´Ambros, M. A., Altamirano, A., Muzio, R. N., Baratti, C., & Mustaca, A. E. (2005). Effects of Cholinergic Drugs on Consummatory Successive Negative Contrast (cSNC) in Rats. *International Journal of Comparative Psychology,* **18,** 91-99.

Bentosela, M., D´Ambros, M. A., Mustaca, A. E., & Papini, M. R. (2006). Consummatory successive negative contrast in young and middle-aged rats. *International Journal of Psychology and Psychological Therapy,* **6, 2**91-300.

Bentosela, M., Ruetti, E., Muzio, R. N., Mustaca, A. E., & Papini, M. R. (2006). Administration of corticosterone after the first downshift trial enhances consummatory successive negative contrast. *Behavioral Neuroscience,* **120,** 371-376.

Boughner, R. L. & Papini, M. R. (2006). Survival of the partial reinforcement extinction effect after contextual shifts. *Learning and Motivation,* **37,** 304-323.

Bower, G. H. (1961). A contrast effect in differential conditioning. *Journal of Experimental Psychology,* **62**, 196-199.

Breen, R. A. and McGaugh, J. L. (1961). Facilitation of maze learning with posttrial injections of picrotoxin. *Journal Francais de Medecine et Chirurgie Thoraciques,* **54,** 498-501.

Brown, R. T. & Wagner, A. R. (1964). Resistance to punishment and extinction following training with shock or nonreinforcement. *Journal of Experimental Psychology,* **68,** 503-507.

Cahill, L. & McGaugh, J. L. (1991). NMDA-induced lesions of the amygdaloid complex block the retention enhancing effect of posttraining epinephrine. *Psychobiology,* **19,** 206-210.

Capaldi, E. J. (1972). Successive negative contrast effect: Intertrial interval, type of shift, and four sources of generalization decrement. *Journal of Experimental Psychology,* **96**, 433-438.

Cleland, E. A., Williams, M. Y., & DiLollo, V. (1969). Magnitude of negative contrast effect in relation to drive level. *Psychonomic Science,* **15**, 121-122.

Collerain, I., & Ludvigson, H. W. (1972). Aversion of conspecific odor of frustrative nonreward in rats. *Psychonomic Science,* **1,** 54-56.

Collerain, I., & Ludvigson, H. W. (1977). Hurdle-jump responding in the rat as a function of conspecific odor of reward and nonreward. *Animal Learning and Behavior,* **5**, 177-183.

Crespi, L. (1942). Quantitative variation of incentive and performance in the white rat. *American Journal of Psychology,* **55**, 467-517.

Daly, H. B. (1974). Reinforcing properties of escape from frustration aroused in various learning situations. *Psychology of Learning and Motivation*, **8**, 187-231.

Daniel, A. M., Wood, M. D., Pellegrini, S. M., Norris, J. N., & Papini, M. R. (2007). *Contextual control of consummatory successive negative contrast.* Manuscript in preparation.

Davis, M. (1998). Are different parts of the extended amygdala involved in fear versus anxiety? *Biological Psychiatry,* **44,** 1239–1247.

Delamater, A. R. (2004). Experimental extinction in Pavlovian conditioning: Behavioural and neuroscience perspectives. *Quarterly Journal of Experimental Psychology,* **57B,** 97-132.

Demarest, J., & MacKinnon, J. R. (1978). Effects of chlordiazepoxide and reward magnitude on the acquisition and extinction of a partially reinforced response. *Physiological Psychology,* **6**, 78-82.

Di Lollo, V., & Beez, V. (1966). Negative contrast effect as a function of magnitude of reward decrement. *Psychonomic Science,* **5**, 99-100.

Duncan, C. P. (1949). The retroactive effect of electroshock on learning. *Journal of Comparative and Phsyiological Psychology,* **42,** 32-44.

Elkins, R. L., & Hobbs, S. H. (1979). Forgetting, preconditioning, CS familiarization, and taste-aversion learning: An animal experiment with implications for alcoholism treatment. *Behavioral Research and Theory,* **17,** 567-573.

Elliott, M. H. (1928). The effect of changes of reward on the maze performance of rats. *University of California Publications in Psychology,* **4**, 19-30

Flaherty, C. F. (1996). *Incentive Relativity*. New York: Cambridge University Press.

Flaherty, C. F., Becker, H. C., & Pohorecky, L. (1985) Correlation of corticosterone elevation and negative contrast varies as a function of postshift day. *Animal Learning and Behavior*, **13**, 309-314.

Flaherty, C.F., Clarke, S., & Coppotelli, C. (1996) Lack of tolerance to the contrast-reducing actions of chlordiazepoxide with repeated reward reductions. *Physiology and Behavior*, **60**, 645-652.

Flaherty, C. F., Grigson, P. S., & Rowan, G. A. (1986). Chlordiazepoxide and the determinants of contrast. *Animal Learning and Behavior*, **14**, 315-321.

Flaherty, C. F., Grigson, P. S., Demetrikopoulos, M. K., Weaver, M. S., Krauss, K. L., & Rowan, G. A. (1990). Effect of serotoninergic drugs on negative contrast in consummatory behavior. *Pharmacology, Biochemistry, and Behavior*, **36**, 799-806.

Flaherty, C. F., Hrabinsky, K., & Grigson, P. S. (1990). Effect of taste context and ambient context changes on successive negative contrast. *Animal Learning and Behavior,* **18,** 271-276.

Flaherty, C. F., & Kelly, J. (1973). Effect of deprivation state on successive negative contrast. *Bulletin of the Psychonomic Society*, **1**, 365-367.

Flaherty, C. F., & Lombardi, B. R. (1977). Effect of prior differential taste experience on retention of taste quality. *Bulletin of the Psychonomic Society,* **9**, 391-394.

Flaherty, C. F., Lombardi, B. R., Wrightson, J., Deptula, D. (1980). Conditions under which chlordiazepoxide influences successive gustatory contrast. *Psychopharmacology*, **67**, 269-277.

Flaherty, C. F., & Rowan, G. A. (1988). Effect of intersolution interval, chlordiazepoxide, and amphetamine on anticipatory contrast. *Animal Learning and Behavior,* **16**, 47-52.

Flaherty, C. F., & Rowan, G. A. (1989). Rats selectively bred to differ in avoidance behavior also differ in response to novelty stress, in glycemic conditioning, and in reward contrast. *Behavioral and Neural Biology*, **51**, 145-164.

Flaherty, C. F., Troncoso, B., & Deschu, N. (1979). Open field behaviors correlated with reward availability and reward shift in three rat strains. *American Journal of Psychology*, **92**, 385-400.

Fowler, S. C. (1974). Some effects of chlordiazepoxide and chlorpromazine on response force in extinction. *Pharmacology, Biochemistry and Behavior,* **2**, 155-160.

Frenk, H. & Rogers, G. H. (1979). The suppressant effects of naloxone on food and water intake in the rat. *Behavioral and Neural Biology,* **26,** 23-40.

Gallagher, M., Bostock, E., & King, R. A. (1985). Effects of opiate antagonists on spatial memory in young and aged rats. *Behavioral and Neural Biology,* **44,** 374-385.

Gallagher, M., & Kapp, B. S. (1978). Manipulation of opiate activity in the amygdala alters memory processes. *Life Sciences,* **23,** 1973-1978.

Gallup, G. G. (1965). Aggression in rats as a function of frustrative nonreward in a straight alley. *Psychonomic Science,* **3**, 99-100.

Garcia, J., & Koelling, R. A. (1966). The relation of cue to consequence in avoidance learning. *Psychonomic Science,* **4,** 123-124.

Gleitman, H. & Steinman, F. (1964). Depression effect as a function of retention interval before and after shift in reward magnitude. Journal of Comparative and *Physiological Psychology,* **57,** 158-160.

Goddard, G. V. (1964). Amygdaloid stimulation and learning in the rat. *Journal of comparative and physiological psychology,* **58,** 23-30.

Gonzalez, R. C., Fernhoff, D., & David, F. G. (1973). Contrast, resistance to extinction, and forgetting in rats. *Journal of Comparative and Physiological Psychology,* **84,** 562-571.

Gonzalez, R. C., Gleitman, H., & Bitterman, M. E. (1962). Some observations on the depression effect. *Journal of Comparative and Physiological Psychology,* **55**, 578-581.

Goodrich, K. P. (1959). Performance in different segments of an instrumental response chain as a function of reinforcement schedule. *Journal of Experimental Psychology,* **57**, 57-63.

Gordon, W. C., Flaherty, C. F., & Riley, E. P. (1973). Negative contrast as a function of the interval between pre-shift and post-shift training. *Bulletin of the Psychonomic Society,* **1,** 25-27.

Gray, J. A. (1987). *The psychology of fear and stress*. Cambridge, UK: Cambridge University Press.

Grigson, P. S., Spector, A. C., & Norgren, R. (1992). Microstructural analysis of successive negative contrast in free-feeding and deprived rats. *Physiology and Behavior*, **54**, 909-916.

Hunskaar, S., Odd-Geir, B., and Hole, K. (1986). A modified hot-plate test sensitive to mild analgesics. *Behavioural Brain Research,* **21,** 101-108.

Hutchinson, A. C., Simpson, G. R., Randall, J. F., Zhang, X., Calderon, S. N., Rice, K. C., & Riley, A. L. (2000). Assessment of SNC 80 and naltrindole within a conditioned taste aversion design. *Pharmacology Biochemistry and Behavior,* **66,** 779–787.

Jones, E. C. (1970). Correlational study of pre- and postgoal competing responses. *Psychonomic Science,* **21**, 25-27

Kirk, R. E. (1968). *Experimental design: procedures for the behavioral sciences.* Belmont: Brooks/Cole, 577 pp.

Lester, P. A. & Traynor, J. R. (2006). Comparison of the in vitro efficacy of μ, d, κ and ORL1 receptor agonists and non-selective opioid agonists in dog brain membranes. *Brain Research,* **1074,** 290-296.

Lett, B. T. (1988). The painlike effect of gallamine and naloxone differs from sickness induced by lithium chloride. *Behavioral Neuroscience,* **99,** 145-150.

Liang, K. C., Juler, R. G., & McGaugh, J. L. (1986). Modulating effects of posttraining epinephrine on memory: involvement of the amygdala noradrenergic system. *Brain Research,* **368,** 125–133.

Liang, K. C., & McGaugh, J. L. (1983). Lesions of the stria terminalis attenuate the enhancing effect of posttrial epinephrine on retention of an inhibitory avoidance response. *Behavioural Brain Research,* **9,** 49-58.

Mansour, A., Fox, C. A., Akil, H., & Watson, S. J. (1995). Opioid-receptors mRNA expression in the rat CNS: Anatomical and functional implications. *Trends in Neurosciences,* **18,** 22-29.

Mayer, P. & Höllt, V. (2001). Allelic and somatic variations in the endogeneous opioid system of humans. *Pharmacological Therapy,* **91,** 167–77.

McGaugh, J. L. (1973). Drug facilitation of learning and memory. *Annual Review of Pharmacology,* **13,** 229-241.

McGaugh, J. L. (2000). Memory-A century of consolidation. *Science,* **247,** 248-251.

McHose, J. H., & Ludvigson, H. W. (1966). Differential conditioning with nondifferential reinforcement. *Psychonomic Science,* **6**, 485-486.

McNally, G. P., & Akil, H. (2002). Role of corticotropin-releasing hormone in the amygdala and bed nucleus of the stria terminalis in the behavioral, pain modulatory, and endocrine consequences of opiate withdrawal. *Neuroscience,* **112**, 605-617.

Mellgren, R. L., Fouts, R. S., & Martin, J. W. (1973). Approach and escape to conspecific odors of reward and nonreward in rats. *Animal Learning and Behavior,* **1**, 129-132.

Meng, F., Taylor, L. P., Hoversten, M. T., Ueda, Y., Ardati, A., Reinscheid, R. K., Monsma, F. J., Watson, S. J., Civelli, O. and Akil, H. (1996). Moving from the orphanin FQ receptor to an opioid receptor using four point mutations. *Journal of Biological Chemistry,* **271 (50)**, 32016–32020.

Mikulka, P. L., Lehr, R., & Pavlik, W. B. (1967). Effect of reinforcement schedule on reward shifts. *Journal of Experimental Psychology*, **74**, 57-61.

Miller, N. E. (1944). Experimental studies of conflict. In J. M. Hunt (Ed), *Personality and the behaviour disorders*, (pp. 431-465). New York: Ronald.

Miller, R. R., Kasprow, W. J., & Schachtman, T. R. (1986). Retrieval variability: Sources and consequences. *American Journal of Psychology,* **99,** 145-218.

Miller, R. R. & Springer, A. D. (1972). Induced recovery of memory in rats following electroconvulsive shock. *Physiology and Behavior,* **8,** 645-651.

Mitchell, C., & Flaherty, C. (1998). Temporal dynamics of corticosterone elevation in successive negative contrast. *Physiology and Behavior,* **64,** 287-292.

Müller, G. E. and Pilzecker, A. (1900). Experimentelle Beiträge zur Lehre vom Gedächtnis. *Zeitschrift fuer Psychologie. Erganzungband.* **1,** 1-300.

Mustaca, A. E., Bentosela, M., & Papini, M. R. (2000). Consummatory successive negative contrast in mice. *Learning and Motivation,* **31,** 272-282.

Mustaca, A. E., & Papini, M. R. (2005). Consummatory successive negative contrast induces hypoalgesia. *International Journal of Comparative Psychology,* **18,** 255-262.

Nyhuis, P. W. & Gastpar, M. (2005). Opiate treatment in ECT-resistant depression. *European Neuropsychopharmacology,* **15,** S420.

Papini, M. R. (2003). Comparative psychology of surprising nonreward. *Brain, Behavior and Evolution,* **62,** 83-95.

Papini, M. R., & Dudley, R. T. (1995). Pavlovian performance of rats following unexpected reward omissions. *Learning and Motivation,* **26**, 63-82.

Papini, M. R., & Dudley, R. T. (1997). Consequences of surprising reward omissions. *Review of General Psychology*, **1**, 175-197.

Papini, M. R., Ludvigson, H. W., Huneycutt, D., & Boughner, R. L. (2001). Apparent incentive contrast effects in autoshaping with rats. *Learning and Motivation,* **32,** 434-456.

Papini, M. R. & Pellegrini, S. P. (2006). Scaling relative incentive value in consummatory behavior. *Learning and Motivation*, **37,** 357-378.

Papini, M. R. & White, N. I. (1994). Performance during signals for reward omission. *Learning and Motivation,* **25,** 45–64.

Papini, M. R., Wood, M., Daniel, A. M., & Norris, J. N. (2006). Reward loss as psychological pain. *International Journal of Psychology and Psychological Therapy,* **6,** 189-213.

Pellegrini, S., Muzio, R. N., Mustaca, A. E., & Papini, M. R. (2004). Successive negative contrast after partial reinforcement in the consummatory behavior of rats. *Learning and Motivation*, **35**, 303-321.

Pellegrini, S. & Papini, M. R. (2007). Scaling relative incentive value in anticipatory behavior. *Learning and Motivation,* **38,** 128-154.

Pellegrini, S., Wood, M., Daniel, A., & Papini, M. R. (2005). Opioid receptors modulate recovery from consummatory successive negative contrast. *Behavioural Brain Research,* **164,** 239-249.

Reynolds, G. S. (1961). Behavioral contrast. *Journal of the Experimental Analysis of Behavior,* **4**, 57-71.

Riley, E. A., & Dunlap, W. P., (1979). Successive negative contrast as a function of deprivation condition following shifts in sucrose concentration. *American Journal of Psychology*, **92**, 59-70.

Rowan, G. A., & Flaherty, C. F. (1987). The effects of morphine in the consummatory contrast paradigm. *Psychopharmacology*, **93**, 51-58.

Salinas, J. A., Introini-Collision, I. B., Damlmaz, C., & McGaugh, J. L. (1997). Posttraining intra-amygdala infusion of oxotremorine and propranolol modulate storage of memory for reductions in reward magnitude. *Neurobiology of Learning and Memory,* **68,** 51-59.

Sher, L. (1998). The role of the endogenous opioid system in the pathogenesis of anxiety disorders. Medical Hypotheses, 50, 473-474.

Spear, N. E., & Spitzner, J. H. (1966). Simultaneous and successive contrast effects of reward magnitude in selective learning. *Psychological Monographs: General and Applied,* **80**, 31.

Stolerman, I . P ., Pilcher, C. W. T ., & D'Mello, G. D. (1978). Stereospecific aversive property of narcotic antagonists in morphine-free rats. *Life Sciences,* **22,** 1755-1762.

Terrace, H. S. (1971). Escape from S-. *Learning and Motivation,* **2**, 148-163.

Thorndike, E. L. (1911). *Animal intelligence*. New York: Macmillan.

Tinklepaugh, O. L. (1928). An experimental study of representative factors in monkeys. *Journal of Comparative Psychology,* **8**, 197-236.

Uhl, G. R., Childers, S., Pasternak, G. (1994). An opiate-receptor gene family reunion. *Trends in Neurosciences,* **17 (3)**, 89-93.

Vogel, J. R., Mikulka, P. J., & Spear, N. E. (1968). Effects of shifts in sucrose and saccharin concentrations on licking behavior in the rat. *Journal of Comparative and Physiological Psychology*, **66**, 661-666.

Wasserman, E. A., & Jensen, D. D. (1969). Olfactory stimuli and the "pseudo-extinction" effect. *Science,* **166**, 1307-1309.

Watson, J. B. (1917). An Attempted formulation of the scope of behavior psychology. *Psychological Review,* **24**, 329-352.

Wood, M. D. (2006). *Role of κ-opioid receptor agonist U-50,488H in consummatory successive negative contrast.* Unpublished doctoral dissertation.

Wood, M. D., Daniel, A. M., & Papini, M. R. (2005). Selective effects of the δ opioid receptor agonist DPDPE on consummatory successive negative contrast. *Behavioral Neuroscience,* **119,** 446-454.

Zeaman, D. (1949). Response latency as a function of the amount of reinforcement. *Journal of Experimental Psychology,* **39**, 466-483.

Zimprich, A., Simon, T., & Höllt, V. (1995). Cloning and expression of an isoform of the rat μ opioid receptor (rMOR1B) which differs in agonist induced desensitization from rMOR1. *FEBS Letters,* **359,** 142–6.

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Wood, M. D., Daniel, A. M., Daniels, E., & Papini, M. R. (2006). Effects of housing on consummatory successive negative contrast in rats: wire-bottom cages versus polycarbonate tubs. *Lab Animal,* **35,** 34-38.

Pellegrini, S., Wood, M., Daniel, A. M., & Papini, M. R. (2005). Opioid receptors modulate recovery from consummatory successive negative contrast. *Behavioral Brain Research,* **164,** 239-249.

Wood, M., Daniel, A. M., & Papini, M. R. (2005). Selective effects of the δ-opioid receptor agonist DPDPE on consummatory successive negative contrast. *Behavioral Neuroscience,* **119,** 446-454.

Burns-Cusato, M., Cusato, B. M., & Daniel, A. (2005) A new model for sexual conditioning: the ring dove (Streptopelia risoria). *Journal of Comparative Psychology,* **119,** 111-6.

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ABSTRACT

ROLE OF OPIOIDS IN MEMORY CONSOLIDATION DURING CONSUMMATORY SUCCESSIVE NEGATIVE CONTRAST

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Previous research has shown that the opioid system is engaged during surprising reward loss events. Frustration theory predicts that the opioid system's modulatory influence on such situations is attributable to three potential mechanisms: comparison of received and expected rewards, intensity of the frustrative emotional response induced by this comparison, or consolidation of the frustration memory. Four experiments provide no supportive evidence for the hypothesis that the opioid system participates in the consolidation of the frustration memory. These experiments involved situations varying in terms of the type of reward reduction (complete or partial), the type of reward (solid food pellets or sucrose solutions), and the type of behavior system (anticipatory or consummatory behavior). A fifth experiment suggests that opioids distort the comparison between expected and received rewards, narrowing the possible opioid mechanisms to modulation of incentive comparison and/or the intensity of primary frustration.