ROLE OF K-OPIOID RECEPTOR AGONIST U-50,488H IN CONSUMMATORY SUCCESSIVE NEGATIVE CONTRAST

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Role of κ-Opioid Receptors in Consummatory Successive Negative Contrast

The behavioral and emotional consequences following reward loss may seem trivial when discussed in terms of laboratory manipulations such as downshifted sucrose solutions. However, when reward loss is translated into every-day-life events, it becomes a topic of broad application and much concern. For example, the Social Readjustment Rating Scale has been used as a reliable tool for ranking stressful life events. Of the top ten most stressful life events, many arguably involve loss, including the death of a spouse, jail term, divorce, death of a family member, being fired from work, and retirement (Scully, Tosi, & Banning, 2000). Clinical research shows that separation from or loss of a loved one are often followed by affective disorders, disruption of autonomic function, changes in appetite, disruption of sleep patterns, general health deterioration, suppression of the immune system, and increased mortality (Bartrop, Luckhurst, Lazarus, Kiloh, & Peny, 1977; Hall & Irwin 2001; Rando, 1993; Stein & Trestman, 1990).

Consummatory successive negative contrast (cSNC) addresses reward loss in the laboratory and has become a model to ask behavioral, pharmacological, and neurobiological questions that arise as a consequence of reward downshift. Studies focused on addressing these questions indicate that surprising reward loss has an emotional component, which is reduced or eliminated by partial reinforcement prior to reward loss (Pellegrini, Muzio, Mustaca, & Papini, 2004), by opioid treatment (Rowan & Flaherty, 1987; Wood, Daniel, & Papini, 2005; Pellegrini, Wood, Daniel, & Papini, 2005), by anxiolytic treatments (Flaherty, 1996; Becker & Flaherty, 1982, 1983; Flaherty, Grigson, & Rowan, 1986), and by certain limbic ablations (Flaherty, Capobianco, &

Hamilton 1973; Flaherty, Powell, & Hamilton, 1979; Gaffan, 1992; Kesner, & Andrus, 1982; Murphy & Brown, 1970).

Evidence indicates that the opioid system may be a component of a larger mechanism that mediates the response to reward loss. For example, the nonselective opioid receptor agonist morphine attenuates both the initial impact and the recovery from reward downshift (Rowan & Flaherty, 1987), while the anxiolytic chlordiazepoxide (CDP) assists in recovery from reward downshift without affecting the initial impact (Flaherty, Lombardi, Wrightson, & Deptula, 1980; Flaherty et al., 1990). Additional evidence suggests that activity among the different opioid receptors may mediate the response to reward loss differently than the recovery that follows. For example, the selective δ-opioid receptor agonist DPDPE attenuates the initial impact, but fails to affect recovery from reward downshift (Wood et al., 2005). A more detailed view of the opioid system reveals that centrally active endogenous enkephalins have affinity for the μ and δ opioid receptors, while dynorphins have affinity for the κ-opioid receptor. In addition, κ-opioid receptors activate glutamatergic neurons in the amygdala, while μ and δ activate GABAergic neurons (Zhu & Pan, 2004). This dichotomy also exists in behavior. For example, μ- and δ-opioid receptor antagonists enhance fear-like responses, while κ-opioid receptors antagonists decrease fear-like responses (Osaki et al., 2003).

An exploration of the effects κ-opioid receptors is needed to understand how the opioid system mediates the initial impact of reward downshift and the recovery that follows. The experiments reported here were designed to test the effects of the κ -opioid receptor agonist U-50,488H (U-50) on cSNC. Experiment 1 will address the effects of U-50 when administered before postshift trials 11 and 12. Experiment 2 will address the

effects of U-50 administered only before postshift trial 12. Experiment 3 will address the effects of U-50 on a general activity task. Finally, Experiment 4 will evaluate the effects of U-50 when administered immediately after postshift trial 11.

Early Developments in the Study of Incentive Contrast

Learning theorists such as Thorndike (1911), Watson (1913), Hull (1943), and Tolman (1951) sought answers to the question, "What is learned?" Thorndike (1911) and Hull (1943) theorized that learning was due to the development of an association between a stimulus (S) and a response (R). The strength of the S-R association was suggested to be dependent upon experience (amount of training) and reinforcement (magnitude frequency). Importantly, the reward was treated as a catalyst for the S-R connection that was not encoded in the associative structure. Rather, Simmons (1924, p. 1) emphasized a goal-oriented or incentive-related motivation by stating that "the end serves to arouse, to direct, and to bring to a conclusion some persistent activity." Simmons (1924) compared qualitatively different incentives, including milk, bread, sunflower seeds, opportunity for escape, and sex in terms of their effects on complex maze learning. Hull (1943) interpreted Simmons' findings as evidence for S-R associations, regardless of the individual incentives strengthening such associations.

Grindley (1929; cited in Hull, 1943) demonstrated the effects of reward magnitude on acquisition in chicks. When given larger amounts of boiled rice, chicks ran faster than when given small amounts of boiled rice. Hull (1943) interpreted the effect of reward amount as influencing the asymptotic level of performance, rather than acquisition rate. Animals were thought to be motivated by food deprivation and by the capacity of the reinforcing substance to reduce drive. Hull's theory yielded predictions

for gradual changes in behavior, but failed to account for the type of sharp changes demonstrated by Tinklepaugh (1928) and Elliott (1928). Interestingly, neither paper was cited in Hull's (1943) book.

Tinklepaugh (1928) studied delayed-reaction learning in monkeys. Monkeys observed the experimenter placing banana slices under one of two cans, which were then hidden behind a raised wooden platform. Following a delay, monkeys were allowed to choose one of the two cans to obtain the reward. However, in some test trials, the experimenter replaced the banana with lettuce (an acceptable, but less preferred reward) when the monkey was unable to see it. Observations yielded these comments (Tinklepaugh, 1928, pp. 224-225):

> She extends her hand to seize the food. But her hand drops to the floor without touching it. She looks at the lettuce, but (unless very hungry) does not touch it. She looks around the cup and behind the board. She stands up and looks under and around her. She picks up the cup and examines it thoroughly inside and out. She has on occasions turned toward the observers present in the room and shrieked at them in apparent anger.

In the interpretation of another experiment, Tinklepaugh (1928, p. 228) stated that "the banana had predisposed him to a certain expectancy which was frustrated on uncovering the lettuce." Tinklepaugh performed a similar experiment with 4-year old boys, who responded with "surprise" and then "disappointment." The main conclusion was that monkeys and children had developed a representation of the goal object.

Elliott (1928) compared response speed and errors of two groups of rats trained to find food in a complex maze. The experimental group received bran mash on trials 1-10,

followed by sunflower seeds on trials 11-20. The control group received sunflower seeds over the 20 trials of training. Results showed a rapid decrease in response speed and an increase in errors when the rats in the first group were downshifted to sunflower seeds on trial 11. Elliott (1928, p. 28) suggested that the reaction to the downshift reflected "an emotional upset of some sort." Hull's (1943) theory failed to explain the immediate reaction exhibited after a reward downshift, characterized by greater suppression of responding than in an unshifted control group.

Tolman (1951) also theorized about the importance of reward on maze performance, but his views differed from those of Thorndike (1911), Watson (1913), and Hull (1943). Tolman (1951) interpreted reward as a goal object and suggested that animals learned to expect and anticipate a specific reward. This theory contrasted with other interpretations of reinforcement that suggested that rewards promote the S-R connection, but are not themselves encoded. Tolman's theory explained the immediate reaction to reward downshift as an expectancy violation after a change in the goal object or incentive. It was Tolman's explanation that influenced Amsel's (1958) development of frustration theory based on the notion of incentive, rather than reinforcement. Thus, the term "incentive" will be used (instead of "reinforcement") to connote the presence of cognitive processes during associative learning (i.e., learning about the goal event).

Crespi (1942) demonstrated that Hull's theory, which excluded an explanation for the rapid change of behavior following reward shift, was incomplete. Crespi (1942) measured the running speed of rats reinforced with different amounts of Purina dog biscuit. Rats that were downshifted from a large to a small amount showed a "depression" effect, resulting in suppressed responding below unshifted controls given

reinforced with the small amount. In addition, animals upshifted from a small to a large amount of reward showed an "elation" effect, compared to their own performance before the incentive upshift. Hull's (1943) theory predicted a gradual change until responding was appropriate to the reward amount, but failed to account for the overshooting and undershooting described by Crespi (1942). Zeaman (1949) also demonstrated rapid changes in behavior following reward shifts that mimicked Crespi's (1942) finding. However, rather than using "depression" and "elation" to describe the effects of incentive shifts, Zeaman (1949) coined the terms "negative contrast effect" and "positive contrast effect," respectively, which have been widely accepted to describe these phenomena.

Amsel's frustration theory (1992) has traditionally offered a coherent framework to explain successive negative contrast, as well as a variety of other incentive effects. Bower (1961) first suggested that the anticipatory mechanisms used by frustration theory to explain the partial reinforcement extinction effect also applied to contrast. Amsel (1958) postulated that an emotional reaction (frustration) is induced by the violation of an appetitive expectancy. At that time, others postulated similar theories. For example, Brown and Farber (1951) hypothesized frustration as an intervening variable resulting from conflicting response tendencies in the immediate presence of reward reduction, either by barrier separation of reward or by nonreward. However, no reference was made to the conditioned form of frustration produced by experience with reward reduction or nonreward (Amsel, 1992). Amsel (1958) termed the immediate reaction to surprising nonreward primary frustration, referring to it as an emotional state. The initial impact is created by a negative discrepancy between the expected and actual incentives. A Pavlovian connection created between prevailing stimuli and primary frustration gave

rise to an anticipatory emotional state named secondary frustration. Amsel (1992) originally developed frustration theory (as applied to the incentive contrast effect) as a third component of Hull's two-process theory of inhibition, which included reactive inhibition and conditioned inhibition. Later, Hull (1952) and Spence (1956) accepted frustration (an emotional factor) to account for the incentive contrast effect as demonstrated by Crespi (1942) and Elliot (1928). However, additional contrast procedures were developed in the years following these theoretical proposals, as described in the next section.

Classification of Contrast Procedures

There are four variants of the contrast procedure: anticipatory, simultaneous, behavioral, and successive. In anticipatory contrast the incentive is alternated within a trial from large to small (positive anticipatory contrast), or from small to large (negative anticipatory contrast). For example, Flaherty and Checke (1982) administered a 0.15% saccharin solution (3 min) followed 15 s later by a more rewarding 32% sucrose solution (5 min), and found that deprived rats decreased 0.15% saccharin consumption on subsequent trials, compared to a group that received 0.15% saccharin in both trials.

Simultaneous contrast is similar to anticipatory contrast in that each subject is exposed to different incentives. However, simultaneous is distinguished from anticipatory contrast by the use of a different context or spatial location for each incentive. For example, Bower (1961) used white and black runways as the discriminative stimuli signaling different incentive magnitudes. The goal box at the end of the white runway contained 1 pellet, whereas the goal box at the end of the black runway contained 8 pellets. The results showed that such rats run slower for 1 pellet than a control group

receiving 1 pellet in both runways, and run faster for 8 pellets than a control group receiving 8 pellets in both runways. These findings are known as simultaneous negative and positive contrasts, respectively (Bower, 1961).

Behavioral contrast is an operant procedure differentiated from other contrast procedures because it manipulates the frequency of reinforcement, rather than incentive value. In behavioral contrast, when reinforcement frequency is downshifted to extinction in one component, the rate of responding in the other component increases. This is known as positive behavioral contrast. Reynolds (1961) was the first to implement behavioral contrast in studies using multiple schedules.

Successive contrast is distinguished from simultaneous and anticipatory contrast because it involves a single shift in reward quality or quantity across trials (as in Crespi's, 1942 experiment described previously). Successive contrast procedures were further subdivided by the difference in the dependent variable: instrumental and consummatory. A common instrumental measure is running speed in runway or maze (Flaherty, 1996), or lever pressing in a Skinner box (Papini, Ludvigson, Huneycut, & Boughner, 2001). A typical cSNC procedure consists of 10 trials prior to the incentive downshift and 5 trials after the downshift. In the procedure used in the present experiments, each trial started by placing the animal in the conditioning box. After approximately 30 s, a sipper tube was inserted into the box. Novelty of the sipper tube instigates an approach response followed by a lick response. Sucrose solution (either 32% or 4%, depending on the group) served to reinforce the approach and lick response. In the following nine preshift trials the sipper tube and surrounding areas became a cue that instigates an expectation for sucrose. On trial 11 the subjects in the experimental group experience incentive downshift (instead of

the 32% sucrose, they receive access to the 4% sucrose). This downshift induces search behavior and decreases consumption. Their performance is compared to that of a control group that receives access to 4% sucrose across all trials. During trials 12 to 15, suppression of drinking decreases gradually until behavior reaches the level of the unshifted control group.

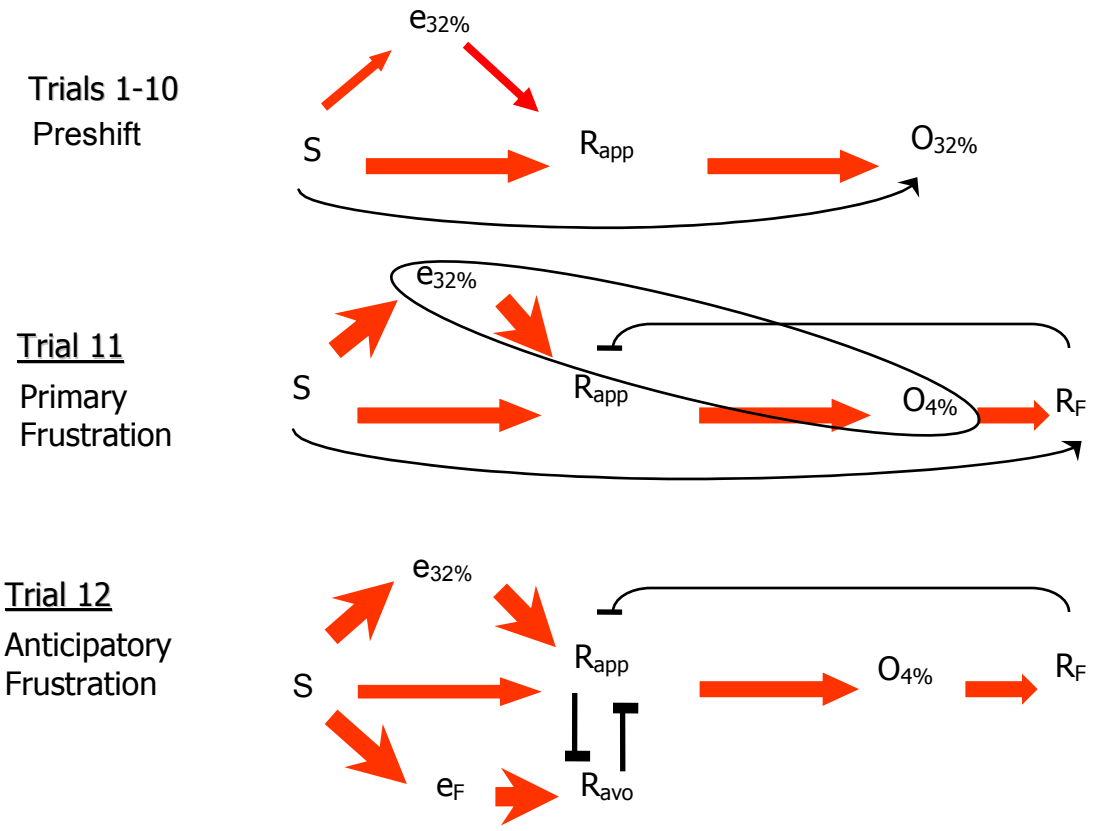

Figure 1. A graphic depiction of Amsel's (1992) theory of frustration and how it relates to the cSNC procedure. The symbol ***S*** represents the stimulus or sipper tube delivering sucrose. ***e*** represents the animal's expectancy for the given 32% or 4% sucrose solution. ***R*** represents the animal's response and indicates the direction of that response approach (***app***) or avoidance (***avo***). ***O*** represents the reward outcome of a given 32% or 4% sucrose.

The phenomenon of cSNC can be explained in terms of Amsel's (1992) frustration theory, as depicted in Figure 1 (Wood et al., 2005). The initial stimuli (S)

during preshift trials 1-10 represents the conditioning box and sipper tube, which serves as a signal for the 32% sucrose ($O_{32\%}$). Successive preshift trials strengthen an expectation for 32% sucrose ($e^{32\%}$) when S is present. However, when the 32% sucrose is downshifted to 4% sucrose during trial 11 the subject exhibits a frustrative response (R_F), that suppresses drinking behavior. In addition, R_F is also paired with S in a Pavlovian fashion. During trial 12 the S comes to elicit competing expectations for the 32% sucrose solution ($e^{32\%}$) and for the frustrative experience (e_F), resulting in the development of an approach-avoidance conflict.

Amsel's (1992) theory was used to explain the behavioral reaction denoted as emotional by Crespi (1942). Other procedures, such as limbic ablations, have provided evidence for this claim.

Neurobiology of cSNC

Septal lesions have been shown to increase consumption of saccharine and sucrose as well as exacerbate rejection of quinine-adulterated solutions (Beatty & Schwartzbaum, 1967, 1968), and produced deficiencies in active (Donovick, 1968) and passive avoidance (McCleary, 1961) and free operant discrimination (Dickinson, 1972). This evidence suggests a potential contribution of the septal area to incentive contrast effects. However, septal lesions failed to affect lick rate when rats were downshifted from 32% to 4% sucrose, as compared to unshifted controls (Flaherty et al., 1973, 1979).

The hippocampus has been implicated as a primary element of a larger mechanism enabling response inhibition (Gray, 1982, 1987), which suggests an influence on cSNC. However, the data on this issue are unclear. Murphey and Brown (1970) reported the elimination of the iSNC effect after extensive electrolytic lesions of the

hippocampus that spared only the ventral tips. However, Kramarcy, Mikulka, and Freeman (1973) ablated the dorsal hippocampus via electrolytic procedure and found no effect on lick frequency or consumption following a shift from 32% to 4% sucrose. A chemolytic procedure damaging granule cells of the hippocampus by applying the neurotoxin colchicine had no effect on cSNC (Flaherty, Rowan, Emerich, & Walsh, 1989). Additional chemolytic procedures that resulted in a more complete ablation of hippocampal cells via the neurotoxin ibotenic acid also had no effect on cSNC (Flaherty, Otto, Hsu, & Coppotelli, 1994). A more detailed analysis of these lesion studies identified major differences. For example, in the study of Murphy and Brown, (1970), the hippocampal lesion included tissue adjacent to the hippocampal formation. Additionally, Murphy and Brown (1970) used subjects that had previous exposure to reward reduction via the sucrose consumption test, and repetitive deprivation cycles, which may have influenced the results. Based on these data, it was assumed that previous exposure to incentive downshift and extent of the lesion in areas adjacent to the hippocampus could be the driving influence responsible for the difference in contrast, rather than the mechanistic properties of the hippocampus. However, there are additional factors. For example, fibers passing through the hippocampus that are not affected by chemolytic procedures may contribute to cSNC. Lastly, instrumental contrast may be more sensitive to hippocampal lesions than consummatory contrast. However, further analyses are needed before a more definitive interpretation is possible.

Considerable evidence indicates that amygdala is a major structure mediating cSNC. Lesions in the basolateral amygdala reduced contrast, whereas corticomedial lesions prevented it (Becker, Jarvis, Wagner, & Flaherty, 1984). Findings cited earlier

about the reducing effects of benzodiazepines and opioid drugs on cSNC may relate to the fact that the central nucleus of the amygdala contains a high density of these two kinds of receptors (Mohler & Okada, 1977). The central nucleus also receives taste fibers form the pontine and cortical taste areas (Norgren, 1984). Hodges, Green, and Glenn (1987) investigated the role of the amygdala in anticonflict by direct injection of benzodiazepine antagonist flumazenil, the inverse agonist FG-7142, and CDP. Flumazenil and FG-7142 blocked increased consumption rates during punished responding created by CDP. Moreover, Liao, and Chuang (2003) found attenuation of cSNC following direct injection of diazepam into the amygdala.

Flaherty (1996) suggested five potential psychological deficits created by amygdala damage that could apply to cSNC: diminished neophobic response, response perseveration, diminished emotional reactivity, diminished memory of reward, and inability to compare rewards. What is the evidence for each of these potential deficits? Concerning neophobic responses, Bagshaw and Benzies (1968) found that monkeys given amygdalectomies reported less autonomic orienting (heart rate, respiratory rate, EEG activity) to novel stimuli. Furthermore, rats given amydalectomies show diminished neophobic responses to taste stimuli than sham animals (Kemble & Schwartzbaum, 1969). It may be assumed that animals failed to distinguish the difference between 32% and 4% sucrose. However, lick frequencies remained similar during the first preshift phase when solutions were novel, while shift from 32% to 4% led to a decrease in responding in nonlesioned groups (Becker et al., 1984).

Another possible explanation for the effect of amygdalectomy lies in the possibility that it may render animals incapable of remembering characteristics of the

preshift reward. The influence of the amygdala on memory during reward reduction has long been speculated (Gaffan, 1992; Kesner & Andrus, 1982; McGaugh et al., 1992). These speculations are not entirely resolved. Becker (1984) performed corticomedial lesions in rats prior to incentive downshift but found that their performance was similar to that of the 4% unshifted controls on the first postshift trial (see Flaherty, 1996). However, an absolute deficit is unreasonable considering that rats with corticomedial lesions rats showed normal acquisition during the preshift phase. Thus, the role of amygdalectomy and memory during reward downshift is unclear.

In addition, amygdalectomized animals may respond to the absolute value of the current reward, failing to compare it with previous rewards. Support for this hypothesis may be provided by haloperidol, which failed to affect contrast but decreased the overall responding to reward (Flaherty, 1996). Dopamine activity was more accurately assessed by Genn, Soyon, and Phillips (2004) who measured dopamine efflux from the nucleus accumbens in rats undergoing cSNC. They found that unshifted rats showed significantly higher efflux than shifted animals. This indicates that the response to reward incentive and the reaction to reward reduction may be mediated by different mechanisms.

Lastly, GABAergic and glutamatergic connections in the amygdala may play a role in the mediation of cSNC. Zhu and Pan (2004) found that the basolateral amygdala contained a high density of glutamatergic neurons where the central nucleus contained both glutamatergic and GABAergic neurons. The effects of κ-opioid agonists appear to be mediated by glutamatergic neurons, whereas μ agonists predominantly activate GABAergic neurons. In support, Jackson and Nutt (1991) found that μ and δ opioid receptor inhibition by naloxone and naltrindole respectively, increased seizure threshold,

which was unaltered by the κ-opioid antagonist nor-binaltorphimine. This evidence suggests that opioid and GABAergic activity may play a valuable role in the mediation of cSNC.

The parabrachial nucleus (PBN) is another structure, which plays a role in cSNC. The PBN is the secondary relay circuit for ascending taste fibers. Taste fibers are first sent to the solitary tract, then to the PBN, thalamus, gustatory cortex, and amygdala. Grigson and Norgren (1994) showed that animals with lesions in the PBN failed to show the abrupt decrease in response to reward downshift, gradually decreasing their drinking behavior after the downshift from 32% to 4% sucrose. Animals in this study showed response differences between 32% and 4% during the preshift phase, but responded equally during the postshift phase.

Anxiolytics and Contrast

Several pharmacological agents have been tested in procedures involving incentive contrast. Flaherty (1996) classified many of these agents in one of two categories: effective or ineffective in attenuating cSNC. This classification was based upon an effectiveness ratio quantified by calculating the response average of the unshifted vehicle minus the downshifted vehicle, divided by the response average of the unshifted group receiving drug minus the downshifted drug. Among the most effective drugs classified were a series of benzodiazepine anxiolytics and morphine. Thus, this review will address the effects of anxiolytics (this section) and opioids (next section) on cSNC, since these two drug classes are known to mediate the effects of reward downshift.

The barbiturate sodium amobarbital reduced cSNC on both the first and second postshift trials, but the reduction of contrast was considerably less than that produced by

chlorodiazepoxide (CDP; Flaherty, Becker, & Driscoll, 1982). However, sodium amobarbital activates other mechanisms in addition to the GABA-receptor complex. The effectiveness of more selective GABA receptor agonists indicates that the attenuation of contrast produced by sodium amobarbitol on the first postshift trial may be the product of side effects.

The anxiolytic ethanol marginally reduced cSNC (Becker & Flaherty, 1982, 1983), but was ineffective when given on the first postshift trial. A GABA chloride channel blocker, picrotoxin (Becker & Anton, 1990), and Ro 15-4513, a partial inverse agonist of the benzodiazepine receptor (Becker & Hale, 1991), significantly reduced the effect of ethanol. Interestingly, a combination of ineffective doses of CDP plus ethanol significantly reduced cSNC on the second postshift day, suggesting that the GABA/benzodiazepine/chloride channel receptor is one of the mechanisms mediating cSNC.

The effects produced by benzodiazepine anxiolytics (e.g., CDP, midazolam, diazepam, and flurazepam), support Elliot's (1928) hypothesis that a negative emotional reaction occurs following surprising incentive downshift. However, arguments challenging this hypothesis arise from the side effects of these anxiolytics, such as, muscle relaxation, anticonvulsant action, sedative action, and, of most concern, appetite stimulation action (Cooper & Estall, 1985). Flaherty and Rowan (1986) and Flaherty et al. (1990) have found evidence supporting a negative emotional reaction as opposed to the influence of these side effects on contrast. An initial concern for the use of CDP was its appetite stimulation effects. Flaherty, Grigson, and Rowan (1986) administered CDP to downshifted and unshifted groups and found that it failed to reduce contrast during the

first postshift trial, indicating no relationship between the effects of CDP on appetite and contrast. Similar effects were obtained with diazepam in mice (Mustaca, Bentosela, & Papini, 2000).

Flaherty (1996) debated several hypotheses concerning the time of effectiveness of CDP administration. Among the alternative hypotheses generated were memory retention, prior experience with reward shift, and length of trial. Consider the hypothesis that the effects of CDP on contrast are restricted by the drug's action on memory retention. Flaherty et al. (1986) injected CDP in two groups downshifted either 24 or 48 h after the last trial of preshift, and found that CDP had the same effect on cSNC at both retention intervals. This result suggests that the effects of CDP are not due to the impairment of the memory for the preshift incentive.

A second hypothesis is that prior experience with incentive downshift may be necessary for CDP to be effective. However, is the effectiveness of CDP due to prior experience with the 4% sucrose or the loss experienced during the first postshift trial? To test these ideas, Flaherty et al. (1990) exposed animals to 4% sucrose prior to cSNC testing. During cSNC, half of the animals were given CDP and half saline on the first postshift trial. Animals previously exposed showed a slight attenuation as opposed to unexposed controls. A second test was performed on animals which previously experienced reward downshift with CDP and saline. These animals showed attenuation of cSNC when compared to novice controls. Thus, it appears that the effectiveness of CDP was not due to previous incentive downshift experience.

Lastly, the amount of experience with the downshifted solution may determine CDP's effectiveness. Flaherty et al. (1986) extended the length of a standard 5 min trial

to 20 min (equal to the length of 4 trials) and recorded lick frequency on a minute-to-minute basis. Results showed that CDP did not affect licking in the first 5 min of the trial, but it significantly increased licking during the last 5 min period, indicating that the effectiveness of CDP was dependent upon the amount of previous exposure to the downshifted solution.

Opioids and Contrast

Opioids are the second group of pharmacological agents that can influence cSNC. Morphine has a small but reliable reducing effect on cSNC during the first and second postshift trials (Rowan & Flaherty, 1987). The nonselective opioid antagonist naloxone eliminated the effects of morphine on cSNC (Rowan & Flaherty, 1987), and also enhanced cSNC during subsequent postshift trials when administered alone (Pellegrini et al., 2005). Both morphine and naloxone are considered nonselective opioids as far as their receptor-binding properties. However, some have reported a greater affinity for the μ receptor in both drugs (Bodnar & Hadjimarkou, 2003). If this were correct, then it would indicate a potentially nonselective role of the μ receptor in cSNC, as far as its action on the first and second postshift trials.

Unlike for morphine and naloxone, there appears to be a selective effect of the δ-opioid receptor in the mediation of contrast during the first postshift trial. Wood, Daniel, and Papini (2005) found that the δ-opioid receptor agonist DPDPE attenuated cSNC when administered before the first postshift trial, but had no effect when administered before the second postshift trial. In addition, administration of the δ-opioid receptor antagonist naltrindole before trials 11 and 12 attenuated cSNC only on trial 11 (Pellegrini et al., 2005). All together, these findings suggest that δ and μ opioid receptors may have

different functions during the early and later portions of the postshift phase of cSNC. Understading the role of the opioid system in cSNC requires additional information. Thus, it becomes necessary to discuss the opioid system itself.

Opium and other related opioid analogues have been used as a medicinal treatment for pain, insomnia, headaches, gallbladder maladies, colic, and kidney stones, among others, dating as far back as 2000 BCE (Snyder, 1986). A well known opioid agonist, morphine, was first isolated from the poppy plant by Friedrich Sertuerner, in 1805, and later named morphine (after Morpheus, the Greek God of dreams, for some of its side effects). More recently, Goldstein (1967, cited in Snyder, 1986), commented on the effects of opium, and hypothesized the existence of endogenous opioids. Subsequently, Hughes et al. (1975) isolated a putative endogenous opioid and then reinjected it into novice animals. The results showed similar effects to those of morphine. This substance was thereafter named enkephalin (meaning "in the head").

Opioids are a family of neuroactive peptides known as the enkephalins, β-endorphins, and dynorphins. Each of these three classes of opioid peptides is generated from three distinct genes: proenkephalin gene, proopiomelanocortin gene, and prodynorphin gene, respectively. These genes are members of the G-protein class (Kendall, 2000). Leucine and methionine enkephalin (leu- and met-enkephalin) are smaller peptides (5-amino acids), that activate both μ and δ opioid receptors, and are widely distributed throughout the brain. β-endorphins are expressed primarily in the pituitary, mediating the release of adrenocorticotropic hormone as part of the stress response. Dynorphins activate κ-opioid receptors and are widely distributed throughout the central nervous system (Colombo, 1993). An additional set involves the opioid

receptor-like orphan receptors, OFQ/N and ORL-1 that also respond to endogenous opioids (Bodnar & Hadjimarkou, 2003).

In a review of the role of opioids on motivation, Stolerman (1985) asserted that endorphins and enkephalins mediate motivational mechanisms of reward and aversion on procedures of drug self-administration, drug-induced conditioned place preference, and conditioned taste aversion. Stolerman (1985) indicated that the opioid agonist nalorphine and the antagonist cyclozocine had no effect on operant escape/avoidance conditioning in the rhesus monkey. In contrast, some studies have countered Stolerman's claim. For example, Schulteis (1992) injected mice with DAGO (μ-opioid receptor agonist) and CTOP (μ-opioid receptor antagonist) during one-way active avoidance conditioning and found that DAGO enhanced acquisition, while CTOP impaired acquisition. In addition, Schulteis, Martinez, and Hurby (1988) found that the δ-opioid receptor agonist DPDPE enhanced passive avoidance learning.

The κ-opioid receptor system also appears to be involved in Pavlovian fear conditioning. For example, Fanselow et al. (1991) reported an increase in freezing responses in Pavlovian fear conditioning following injection of u antagonist CTOP, but a decrease in freezing response following injection of κ antagonist nor-binaltorphimine. However, injection of cyprenorphine and naltrindole (δ-opioid receptor antagonists), revealed no effect when compared to saline controls. Thus, there are important differences between the μ, δ, and κ-opioid receptor systems in the mediation of Pavlovian fear conditioning.

Endogenous opioids have been indicated as a potential mechanism modulating fear. The midbrain structures known as periaqueductal gray and inferior colliculus are

involved in the generation of defensive behavior. Osaki et al. (2003) tested the effects of naloxonazine (μ antagonist) and nor-binaltorphimine (κ antagonist) administered into these midbrain areas and then measured fear-like behaviors (running and jumping) after electrical stimulation of the central nucleus of the inferior colliculus. Results showed that naloxonazine increased the defensive threshold whereas nor-binaltorphimine decreased the defensive threshold, suggesting that the κ-opioid receptor may be a part of a regulatory mechanism for μ-mediated behaviors. This evidence also suggests that activation of the κ-opioid receptor may increase defensive threshold.

Similar experiments have revealed that κ agonists produced opposite effects on avoidance learning compared to μ and δ agonists. Ilyutchenok and Dubrovina (1995) demonstrated that naloxone (μ antagonist), and ICI 174,864 (δ antagonist) enhanced reacquisition of one-trial passive avoidance, while dynorphin (κ agonist) also enhanced reacquisition, implying a regulatory function for the κ-opioid receptor system.

Anatomical evidence shows differences in the distribution of μ, δ, and κ opioid receptors, a fact consistent with the opposing function of κ-opioid receptors in the opioid system. The hippocampus is one site of activation for the κ agonist U-50, which produces anxiogenic effects that can be blocked by intrahippocampal injections of nor-binaltorphimine (κ antagonist; Privette, 1995). U-50 inhibits the release of hippocampal glutamate (Privette, 1995), while dynorphin (κ agonist) activates the release of hypothalamic corticotropic releasing hormone (Taylor et al., 1996). Consequently, chronic levels of corticosterone reduced hippocampal dynorphin release in the dentate granular cells (Privette, 1995), suggesting a biofeedback mechanism in which increased levels of corticosteroids would inhibit the release of dynorphins. This evidence implicates

anxiogenic opposition to the euphoric and/or analgesic activity produced by μ and δ opioid activity.

Additional support for opposing systems comes from addiction and substance abuse literature. Studies with κ-opioid receptor knock-out mice revealed that the κ-opioid receptor did not contribute to morphine analgesia in the formalin, tail pressure, abdominal constriction, tail immersion, and hot plate tests. However, animals lacking the κ-opioid receptor did suppress withdrawal symptoms following naloxone-induced withdrawal (Simonin et al., 1998). These findings indicate increased κ-opioid receptor activity following μ- and δ-opioid receptor activated withdrawal. Narita, Suzuki, Misawa, and Nagase (1993) found that the straub tail reaction induced by intracerebroventricular injections of morphine was significantly antagonized by beta-funaltrexamine (μ antagonist), and by U-50 (κ agonist). Glick et al. (1996) trained rats in an operant chamber to self-administer cocaine or morphine. Results showed that self-administration was dose-dependently (2.5, 5, 10 mg/kg i.p.) inhibited by U-50.

The following four experiments were organized to address the role of the κ-opioid receptor agonist U-50 on cSNC. The following findings suggest that U-50 has a bidirectional effect on cSNC that is selective for the recovery period (trials 12-15). The low dose of U-50 reduced cSNC on trial 12, while the medium and high doses enhanced cSNC on trials 12 and 13. The κ opioid system appears to mediate mechanisms that are involved in the posttrial processing of the initial downshift event following trial 11, enhancing consolidation of the incentive downshift memory. The evidence presented below provide a valuable understanding of the role of the opioid system in mediating incentive loss.

Experiment 1

The κ-opioid system may regulate cSNC in an opposite manner to that of the μ- and δ-opioid receptor systems. For example, Walker, Thompson, Frascella, and Friederich (1987) found that activation of the κ receptor by U-50 suppressed the firing of neurons activated by the μ agonist morphine in the substantia nigra. At the behavioral level, Pearl and Glick (1996) found that U-50 attenuated locomotor behavior induced by morphine challenge in the rat. In addition, preadolescent rats showed impaired morphine-induced place preference following U-50 injection (Bolanos, Garmsen, Clair, & McDougall, 1996). Other behaviors, including ultrasonic vocalizations, were also sensitive to U-50 treatment. Nazarian, Krall, Osburn, and McDougall (2001) showed increased vocalizations in preadolescent rats following administration of U-50 and suppressed vocalizations following administration of the κ-opioid receptor antagonist nor-binaltorphimine.

In addition to U-50 showing opposite effects to those of μ and δ opioid receptor agonists, the level of dose appears to affect behavior differentially. For example, Schnur and Walker (1990) administered a low dose of U-50 (1 mg/kg) and found locomotor hypoactivity, while a high dose (10 mg/kg) induced hyperactivity in hamsters. Apart from the potential species differences, it seems that the first step in this research should be to identify an effective dose of U-50 in the cSNC situation.

Experiment 1 addresses the effectiveness of U-50 following the administration of three dosage levels: 1, 3, and 10 mg/kg U-50, administered before postshift trials 11 and 12. Based upon previous findings suggesting that U-50 opposes the effects of morphine, and considering that morphine reduces cSNC when injected before trials 11 and 12

(Rowan & Flaherty, 1987), it was hypothesized that U-50 would enhance cSNC in a dose-dependent manner. However, based on the seemingly differential effects of U-50 on activity at various drug doses (see previous paragraph), it was considered plausible that U-50 would have opposite effects on cSNC depending on the dose.

Method

Subjects. The subjects were 64 adult Long-Evans rats, 90-110 days old at the start of the experiment. Thirty two males and 32 females were used. Rats were bred and housed in the TCU vivarium under a 12:12 h light:dark cycle (lights on at 07:00 h) and deprived of food to an 81-84% of their ad libitum body weight. Animals were tested during the light phase of the cycle and had free access to water in their home cage. Animals were transferred from polycarbonate tubs into wire-bottom cages at about 70 days of age, since such housing was found to enhance the cSNC effect (Wood, Daniel, Daniels, & Papini, 2006).

Apparatus. Animals were tested in 4 conditioning boxes (MED Associates, Vermont) constructed of aluminum and Plexiglas, 29.3 cm long, 21.3 cm high, and 26.8 cm wide. The floor, made of steel rods 0.4 cm in diameter and 1.6 cm apart, ran parallel to the feeder wall. A tray filled with corncob bedding was placed below the floor to collect fecal pellets and urine. An elliptical hole in the feeder wall was, 1 cm wide, by 2 cm high, and located 4 cm from the floor. A sipper tube (1 cm in diameter and flush against the feeder wall when fully inserted) was automatically inserted and retracted to deliver the sucrose solution. Contact with the sipper tube was recorded automatically by the closing of an electric circuit between the sipper tube and the steel floor.

The conditioning box was enclosed in a sound-attenuating chamber 57.5 cm long, 36.9 cm high, and 39.4 cm wide. This chamber also had a speaker and a fan, which register 80.1 db (SPL, scale C). The control of the sipper tube and recording of the response were performed by a computer located in an adjacent room.

Procedure. Animals were exposed to the conditioning box for a total of 15 trials, 10 preshift trials and 5 postshift trials. Testing consisted of a 5-min exposure to sucrose via sipper tube and counted starting after the first contact. Each trial started and ended with a variable interval averaging 30 s during which the sipper tube was retracted. Downshifted animals received access to 32% sucrose on preshift trials 1-10 and then 4% on postshift trials 11-15. Unshifted animals received 4% in both pre- and postshift trials. One trial per day was administered. Sucrose solutions were prepared (w/w) by mixing 4 g (or 32 g) of sucrose per 96 g (or 68 g) of distilled water. Thirty two animals received a 32–4 shift and were randomly assigned to four drug conditions (n = 8). All drugs were administered i.p. 20 min before the start of trials 11 and 12. Group 32/S, 32/U1, 32/U3, and 32/U10 received, respectively, injections of saline, 1, 3, or 10 mg/kg. These doses were based on Schnur and Walker (1990). Thirty two animals were assigned to the unshifted 4-4 control conditions and randomly distributed in four groups (n = 8). Drug treatments were identical to those described previously for the 32-4 groups. Table 1 summarizes the design used in Experiment 1.

Table 1
Design of Experiment 1.

		Postshift trial injections		
Groups	**Contrast Condition**	**11**	**12**	***n* =**
32/U1	32 – 4	1 mg U-50	1 mg U-50	8
32/U3	32 – 4	3 mg U-50	3 mg U-50	8
32/U10	32 – 4	10 mg U-50	10 mg U-50	8
32/S	32 – 4	Saline	Saline	8
4/U1	4 – 4	1 mg U-50	1 mg U-50	8
4/U3	4 – 4	3 mg U-50	3 mg U-50	8
4/U10	4 – 4	10 mg U-50	10 mg U-50	8
4/s	4 – 4	Saline	Saline	8

Trans-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclo-hexyl]-benzeneacetamide (U-50,488H) was prepared by mixing the appropriate amount of powder with 1 ml of saline. The stock solution was then diluted to the appropriate doses. Doses were prepared 48 h prior to the first postshift trial (trial 11). Saline was 85% sodium chloride solution. Drugs were purchased from Sigma-Aldrich Chemicals (Saint Louis, MO).

Results

Due to experimental error, 2 females in the 4% sucrose condition received fewer than 10 trials of testing; the data from these two animals were excluded. A computer malfunction during the last trial of the experiment, trial 15, resulted in the loss of 6 data points, 2 from Group 32/U1, 1 from 32/U3, 1 from 32/U10, and 2 from 32/S. For plotting purposes, these data were replaced with the group average on that trial; however, trial 15 was not included in any of the statistical analyses reported below. Figure 2 illustrates the group average goal tracking time for each preshift (1-10) and postshift (11-15) trial. A Sucrose (32%, 4%) x Trial (1-10) mixed-model analysis of variance (ANOVA), with

repeated measures for the factor trial, was computed to test for preshift effects. The preshift sucrose conditions were comprised of pooled groups receiving 4% (n = 30) and 32% (n = 32) sucrose. All animals increased goal tracking time across the preshift trials, $F(9, 540) = 101.89$, $p < 0.001$. However, groups receiving 32% showed a higher average goal tracking time when compared to 4% controls, $F(1, 60) = 6.86$, $p < 0.01$, and increased consumption faster, as shown by a significant contrast by trial interaction, $F(9, 540) = 3.56$, $p < 0.001$.

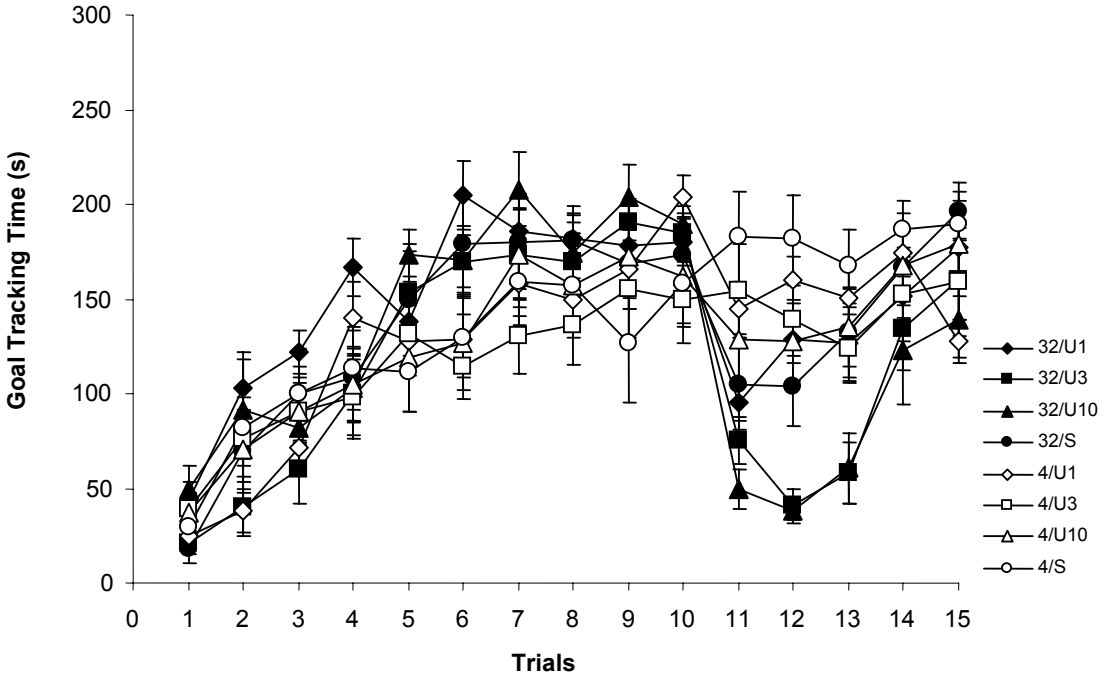

Figure 2. The graphic illustrates consummatory performance over 15 trials. During trials 1-10 animals were given either 32% sucrose (32/U1, 32/U3, 32/U10, 32/S), or 4% sucrose (4/U1, 4/U3, 4/U10, 4/S). During trials 11-15 all animals were given 4% sucrose. The κ-opioid receptor agonist U-50 and saline were administered before trials 11 and 12 of the postshift phase, in the following amounts: 1, 3, and 10 mg/kg (labeled ***U1***, ***U3***, and ***U10***). Saline was labeled as ***S***.

The results of greatest interest are those involving postshift trials. However, the complexity of these results suggested a different way to visualize the effects of the

various doses on cSNC. This alternative depiction is presented in Figure 3, in which the trial performance of each group is presented in separate panels each corresponding to the five critical trials of this experiment: trials 10 to 14. There were four major results. First, the administration of U-50 dose-dependently suppressed consummatory behavior. This is most clearly seen in the performance of the unshifted control groups on trials 11 and 12, the two trials scheduled after drug administration. Second, a comparison of downshifted vs. unshifted groups administered saline solution indicates considerable consummatory suppression on trials 11 and 12, followed by recovery of normal levels of behavior. Third, administration of 1 mg/kg of U-50 before trial 11 had little or no effect on cSNC, other than the generally suppressive influence on consummatory behavior noted above. Forth, the effects of U-50 were noticed on trials 12-14 and appeared to be opposite depending on the dose. At the 1 mg/kg dose, U-50 reduced the size of the cSNC effect, whereas at 3 and 10 mg/kg it actually enhanced the cSNC effect. The following statistical analyses confirmed these four general conclusions.

The following is a global analysis of postshift performance using a Contrast (32%, 4%) x Drug (0, 1, 3, 10 mg/kg) x Trial (11-14) ANOVA. A significant trial effect was found, $F(4, 216) = 37.39$, $p < 0.001$, indicating differences in group performance over the 4 postshift trials. A significant contrast by trial interaction, $F(4, 216) = 14.61$, $p < 0.001$, indicated that the performance of downshifted groups changed at a different rate across postshift trials than that of the unshifted controls. The drug by trial interaction was significant, $F(12, 216) = 2.09$, $p < 0.02$, indicating a significant change of groups receiving U-50 over the five postshift trials. A condition by drug by trial interaction, $F < 1$, failed to reach significance. Between subjects analyses revealed a main effect of

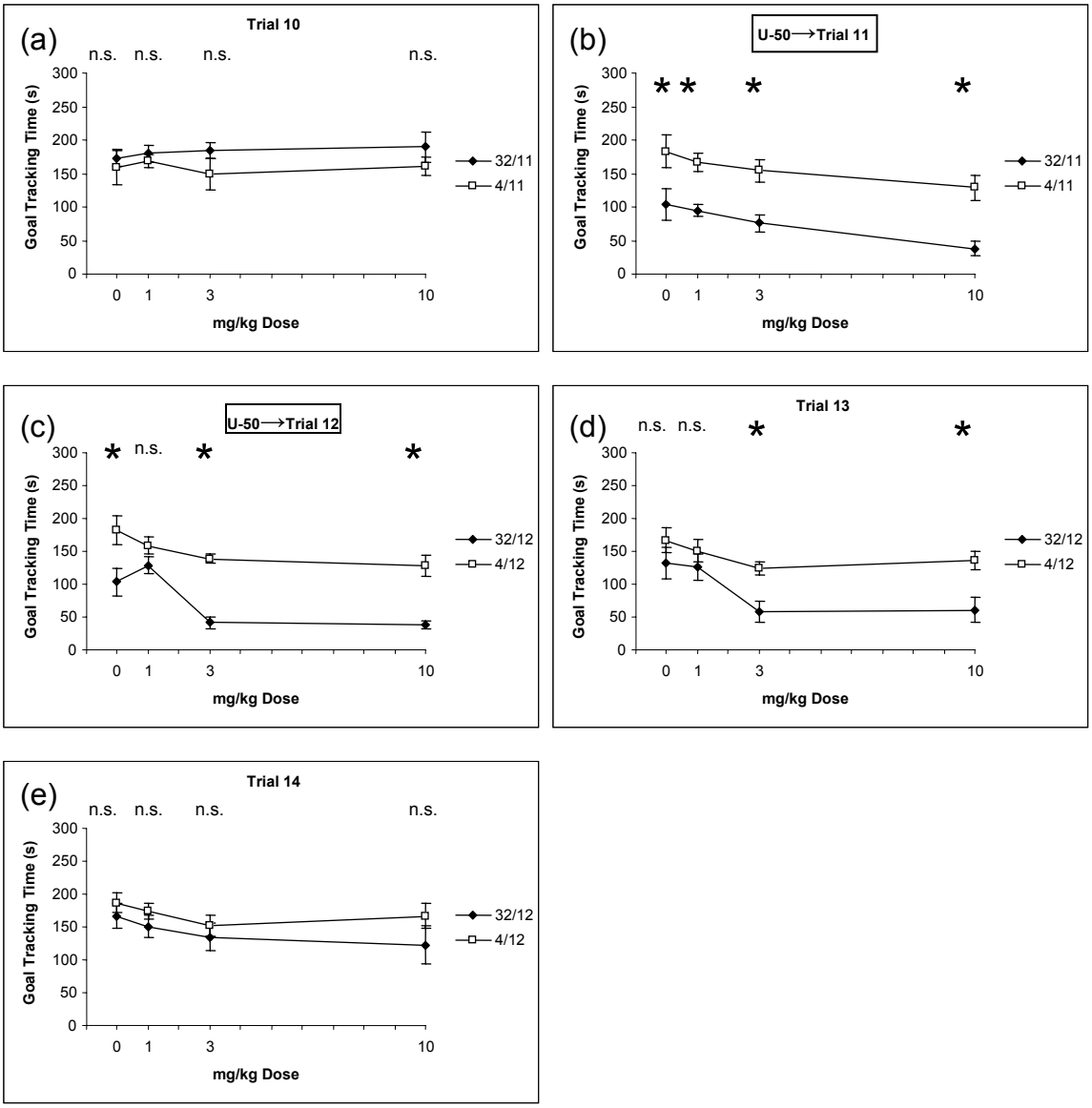

Figure 3. The following graph illustrates a dose by goal tracking time comparison of downshifted and unshifted groups on trial 10 of preshift and trials 11-14 of postshift. The κ-opioid receptor agonist U-50 and saline (***S***) were administered before trials 11 and 12 of the postshift phase, in the following amounts: 1, 3, and 10 mg/kg (labeled ***U1***, ***U3***, and ***U10***). ***n.s***: nonsignificant difference between downshifted and unshifted conditions of the given dose. *: significant difference between conditions of a given dose, $p < 0.05$.

Contrast, $F(1, 54) = 23.09$, $p < 0.001$, indicated greater suppression of responding in the downshifted (32-4) groups relative to the unshifted (4-4) groups. A main effect of drug, $F(3, 54) = 5.57$, $p < 0.01$, was also found. However, the contrast by drug interaction

failed to reach significance, $F < 1$. The significant drug by trial interaction warranted further analyses at each postshift trial.

As already mentioned, Figure 3 illustrates the effect of U-50 on consummatory performance on the last preshift trial (trial 10) and on postshift trials 11 to 14. A one-way ANOVA and Fisher's post hoc LSD pairwise tests were calculated for each of these trials. The group effect was not significant for trial 10, $F < 1$ (Figure 3a). Although goal tracking times are higher for groups exposed to the 32% than the 4% sucrose pairwise comparisons revealed nonsignificant differences for all the drug conditions (0, 1, 3, and 10 mg/kg), ps > 0.05.

On trial 11 (Figure 3b) there was a significant group effect, $F(7, 61) = 7.50$, $p < 0.001$. Pairwise comparisons revealed significant differences between the two contrast groups (32% vs. 4%) for all the drug conditions (0, 1, 3, 10 mg/kg), ps < 0.01. LSD post hoc analysis of the downshifted groups (32-4) revealed a significantly greater suppression of response in Group 32/U10 than in Group 32/S, $p < 0.05$. Similarly in the unshifted control groups (4-4), Group 4/U10 showed response suppression when compared to Group 4/S, $p < 0.05$, indicating a suppressive effect of U-50 on consummatory behavior. Groups 32/U3, 32/U1, 4/U3, and 4/U1 did not differ significantly, $p > 0.05$, from their respective saline controls, Groups 32/S and 4/S. Thus, the downshifted vs. unshifted comparison indicated a significant cSNC effect for each drug dose level, but the largest dose (10 mg/kg) also caused significant consummatory suppression in both downshifted and unshifted groups.

Trial 12 (Figure 3c) revealed a significant group effect, $F(7, 61) = 12.57$, $p < 0.001$, indicating differences between downshifted and unshifted conditions. Pairwise

comparisons revealed significant differences between the two contrast groups (32% vs. 4%) for drug conditions 0, 3, and 10 mg/kg, *p*s < 0.05. The performance of Groups 32/U1 and 4/U1 was not significantly different, $p > 0.05$, indicating the attenuation of the cSNC effect. LSD post hoc analyses of downshifted groups showed a significant suppression of responding in Groups 32/U3 and 32/U10 relative to Group 32/S, *p*s < 0.05. Similarly, there was a significant suppression of responding in Groups 4/U3 and 4/U10 when compared to Group 4/S, *p*s < 0.05. These comparisons indicate a suppressive effect of U-50 on consummatory behavior for the 3 and 10 mg/kg doses. However, Groups 32/U1 vs. 32/S, and 4/U1 vs. 4/S were not different from each other, *p*s > 0.05.

Trial 13 (Figure 3d) revealed a significant group effect, $F(7, 61) = 6.74$, $p < 0.001$, indicating differences between downshifted and unshifted condition. Pairwise comparisons revealed significant differences between the two contrast groups (32% vs. 4%) for the 3 and 10 mg/kg doses, *p*s < 0.05. LSD post hoc analyses of unshifted groups showed nonsignificant differences in suppression of responding in Groups 4/U1, 4/U3, and 4/U10, when compared to 4/S, *p*s > 0.05. LSD post hoc analyses of the downshifted groups showed a significant suppression of responding in Groups 32/U3 and 32/U10 when compared to Group 32/S, *p*s < 0.05. These results indicate an enhancement of cSNC. Groups 32/S and 32/U1, showed a nonsignificant difference from their respective unshifted controls, Groups 4/S and 4/U1, *p*s > 0.05. Group 32/U1 showed no difference from Group 32/S, $p > 0.05$.

Trial 14 (Figure 3e) revealed a nonsignificant group effect, $F(7, 61) = 1.04$, $p > 0.05$, indicating recovery from cSNC in all the drug conditions. Post hoc pairwise

comparisons revealed a nonsignificant difference between the two contrast groups (32% vs. 4%) for all drug conditions (0, 1, 3, 10 mg/kg), *p*s > 0.05.

Discussion

The preceding results introduce the first known opioid drug to selectively affect trial 12 performance in the cSNC situation (see Wood et al., 2005). More specifically, a low dose of U-50 (1 mg/kg) attenuated cSNC on trial 12, but did not affect cSNC on trial 11. The absence of an effect of the low U-50 dose on unshifted controls implies that the drug effect cannot be attributed to factors related to the contextual environment, including sensory-perceptual, motivational, or motor influences on consummatory behavior. However, the design tested does not allow for further speculation of selectivity, because trial 12 effects may be influenced by the administration of U-50 on trial 11. Lynch and Burns (1990) suggested that consecutive administration of U-50 may enhance consummatory responsiveness to sucrose solutions. Thus, the degree of trial selectivity in the cSNC situation can only be clearly determined by testing U-50 on trial 12 alone (see Experiment 2).

The medium dose of U-50 (3 mg/kg) also failed to influence cSNC on trial 11, but led to significant cSNC effects on trials 12 and 13. This dose also had a general suppressive effect on consummatory behavior. Similar results were also found in animals given high dose of U-50 (10 mg/kg). Both medium and high doses of U-50 administered before trials 11 and 12 prolonged cSNC for one additional trial, relative to the saline groups. Thus, the effects of these doses on cSNC cannot be accounted for in terms of U-50's effects on consummatory behavior.

The opioid system has shown involvement in feeding behavior, taste palatability, and gustatory responses, by influencing activity in specific nuclei receiving taste afferents. For example, feeding behavior, taste palatability, and gustatory responses have all been reduced by morphine administration in the parabrachial nucleus (Saper & Loewy, 1980). Li, Davis, and Smith (2003) found that neurons in the NST involved in taste perception were suppressed following microinjections of met-enkephalin. Blancquaert, Lefebvre, and Willems (1986) found that κ-opioid agonist ethylketocyclazocine showed consistent antiaversive effects on conditioned taste aversion, induced by apomorphine, lithium chloride, and copper sulphate. These data may account for the suppression of consummatory behavior in unshifted controls, but cannot explain the extension of cSNC to trial 13. If U-50 affected only consummatory behavior, the cSNC effect should have been evident only on trials when the saline controls (Groups 32/S and 4/S) showed the effect.

Motivational factors in consummatory behavior are also sensitive to opioid drugs. Drewnowski, Krahn, Demitrack, Nairn, and Gosnell (1991) found that naloxone reduced overall consumption of bulimic patients and nonbulimic individuals, indicating opioid involvement in motivational factors related to palatability. Lynch and Burns (1990) suggested that the level of U-50 dose may differentially affect oroexogenic properties. Their findings revealed that 10 days of successive 1 mg/kg U-50 treatment produced no difference in the volume of 20% sucrose consumed. However, animals given 0.3 mg/kg of U-50 under the same conditions showed increased consumption of 20% sucrose. Badiani, Rajabi, Nencini, and Stewart (2001) found that 4 mg/kg U-50 increased the consumption of high sucrose (30%, 40%) and decreased the consumption of low sucrose

(1%, 4%) over a 30 min period. Data collected on trial 12 seem to show a similar pattern of responding revealing a dose-dependent suppression (Figure 3c) implicating an effect on oroexogenic properties. However, if oroexogenicity were the only mechanism affected by U-50 one would expect to see this same pattern also on trial 11. Moreover, an oroexogenic effect would require an enhancement of consummatory behavior also in Group 4/U1, which was not observed.

Schnur and Walker (1990) found a similar dose-dependent bidirectional response to U-50 on locomotor activity. A high dose of U-50 (10 mg/kg) significantly suppressed running wheel activity, whereas a low dose (1 mg/kg) significantly increased it. Changes in locomotor activity could affect consummatory behavior. However, the results reported by Schnur and Walker (1990) are in the opposite direction to what would be predicted for the cSNC situation. For example, if the 10 mg/kg dose suppressed activity, than this should allow for a greater amount of consummatory behavior, when, in fact, consummatory suppression was observed in this experiment. Similarly, if the 1 mg/kg dose increased activity, then one would expect suppression of consummatory behavior, when, again, the opposite was observed (see Figure 3c).

The present results suggest a dose-dependent bidirectional influence of U-50 on cSNC. For example, the medium and high doses of U-50 enhanced cSNC, whereas the low dose attenuated it. With saline injections, cSNC was observed on trials 11 and 12; however, with 1 mg/kg U-50, cSNC was observed only on trial 11, whereas with 3 and 10 mg/kg, cSNC was observed on trials 11, 12, and 13. All the drug groups showed recovery from cSNC on trial 14, thus demonstrating that these effects were transient.

The possibility cannot be discarded that the results of trial 12 and 13 may be altered by exposure to U-50 on trial 11. This scenario seems unlikely given the pharmacokinetics of U-50. Bhargava and Thorat (1994) found, for example, that the analgesic effects of 25 mg/kg of U-50 was no different from saline after 240 min, implicating a short half life, which would not allow for accumulation of the drug in the cSNC procedure. The 24-h interval between the administration of U-50 before trials 11 and 12 was probably sufficiently long to allow for the complete elimination of the drug. Thus, drug accumulation does not appear to explain the results of Experiment 1. Nonetheless, the issue of the trial and dose selectivity effects of U-50 was studied in Experiment 2 by delivering the drug only before trial 12.

Experiment 2

The selective properties of opioid receptors in cSNC has been suggested by Wood et al. (2005), who found that the δ-opioid receptor agonist DPDPE attenuated cSNC on postshift trial 11 without affecting cSNC on postshift trial 12. Moreover, Pellegrini et al. (2005) reported that the δ-opioid receptor antagonist naltrindole enhanced cSNC on trial 11, but not on trial 12. In addition, the nonselective antagonist naloxone had a greater suppressive effect on consummatory behavior than naltrindole and saline, enhancing cSNC on postshift trials 11, 12, and 13. This suggests that μ, δ, and κ opioid receptors may differentially mediate the cSNC effect during the initial impact and subsequent recovery trials.

Experiment 2 was designed to determine the effects of U-50 on trial 12 performance by administering the low and medium doses (1 and 3 mg/kg) only before trial 12. If the opposite effects of these doses on cSNC during trial 12 were not caused by

drug accumulation or some other effect of drug administration on trial 11, then these opposing effects of U-50 should be replicated in Experiment 2.

Methods

Subjects. The subjects used were 50 male rats, tested between 90-120 days of age. Experiment 2 was run in two replications, the first included 29 animals bred at the TCU vivarium (as in Experiment 1), while the second replication used 21 animals purchased from Harlan. Upon arrival food deprivation started when all rats were 90 days old. Harlan rats were housed in wire cages for 10 days prior to food deprivation.

Apparatus. The apparatus as used in Experiment 2 were the same described in Experiment 1.

Procedure. The present experiment used a similar procedure to that of Experiment 1. Groups 32/S, 32/U1, and 32/3 (n = 8) received, respectively, an injection of saline, 1, or 3 mg/kg of U-50. Twenty four animals were assigned to the unshifted 4-4 control conditions and randomly distributed in 3 groups (n = 8). Drug treatments were identical to those described previously for the 32-4 groups except that only one injection was administered before trial 12. Table 2 summarizes the design used in Experiment 2. U-50 and saline were prepared and administered as described in Experiment 1.

Table 2
Design of Experiment 2.

Group	Contrast Condition	Injection Trial 12	*n* =
32/U1	32 – 4	1 mg/kg U-50	8
32/U3	32 – 4	3 mg/kg U-50	8
32/S	32 – 4	Saline	8
4/U1	4 – 4	1 mg/kg U-50	8
4/U3	4 – 4	3 mg/kg U-50	8
4/S	4 – 4	Saline	8

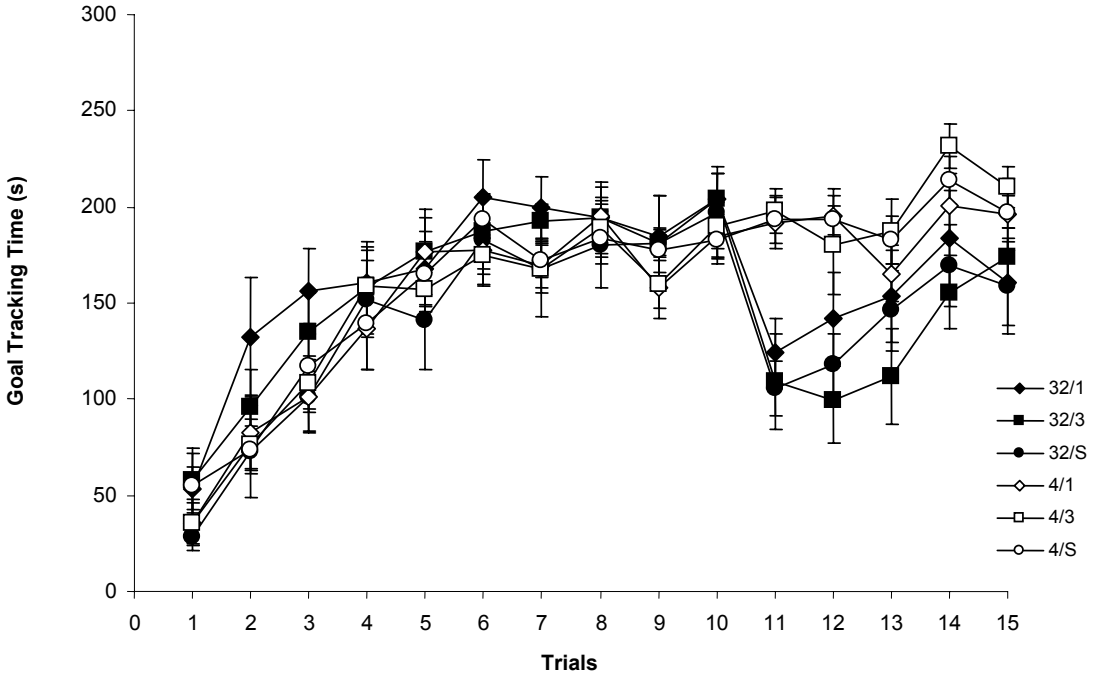

Figure 4. The graphic illustrates consummatory performance over 15 trials. During trials 1-10 animals were given either 32 % sucrose (32/U1, 32/U3, 32/S), or 4 % sucrose (4/U1, 4/U3, 4/S). During trials 11-15 all animals were given 4% sucrose. The κ-opioid receptor agonist U-50 and saline (***S***) were administered before trial 12 of the postshift phase, in the following amounts: 1 and 3 mg/kg (labeled ***U1*** and ***U3***).

Results

Due to computer malfunction, data were lost for 6 trials from Groups 32/U3 (3 trials in different animals), 4/U1, 4/U3, and 4/S (all in preshift trials, except for one on trial 15). These data were replaced with the group average on that trial, as specified by Kirk (1968). One animal in Group 32/U1 (n = 7) was eliminated because of a recording malfunction that occurred on trial 12. Figure 4 illustrates the group average goal tracking time for each preshift (1-10) and postshift (11-15) trial. A Contrast (32%, 4%) x Trial (1 - 10) mixed-model ANOVA with repeated measures for trials, was computed to test for preshift effects. The preshift sucrose conditions were comprised of pooled groups

receiving 4% (n = 24) and 32% (n = 23) sucrose. All animals increased goal tracking time across preshift trials, $F(9, 405) = 100.54$, $p < 0.001$. However, a nonsignificant contrast effect and contrast by trial interaction were found for preshift data, $F < 1$.

The trials of interest in Figure 4 are trials 11 to 15. Trial 11 confirms the cSNC effect in downshifted groups validated by a significant suppression in responding following the 32–4% sucrose downshift. Most importantly, the average tendencies in the results of trials 12-14 were consistent with the findings of Experiment 1. When each downshifted group is compared to its respective nonshifted control, 1 mg/kg U-50 tended to attenuate cSNC, whereas 3 mg/kg U-50 tended to enhance cSNC. Trial 15 revealed complete recovery in all the downshifted groups relative to the responding of their respective unshifted controls.

Postshift trials (11-15) were analyzed using a Contrast (32%, 4%) x Drug (0, 1, 3, 10mg/kg) x Trial (11-15) ANOVA. A significant effect of trial, $F(4, 164) = 16.10$, $p < 0.001$, indicated significant suppression of responding of downshifted (32-4%) groups when compared to unshifted (4-4%) groups. A significant contrast by trial interaction, $F(4, 164) = 4.60$, $p < 0.01$, indicated the rapid change in the performance of downshifted groups over the five postshift trials. However, the postshift trial by drug interaction was not significant, $F(8, 164) = 1.27$, $p > 0.05$. The three way interaction between contrast, drug, and trial also failed to reach significance, $F < 1$. There was also a significant main effect of contrast, $F(1, 41) = 23.20$, $p < 0.001$, but no effect of drug, $F < 1$, or of the interaction of drug by contrast, $F < 1$.

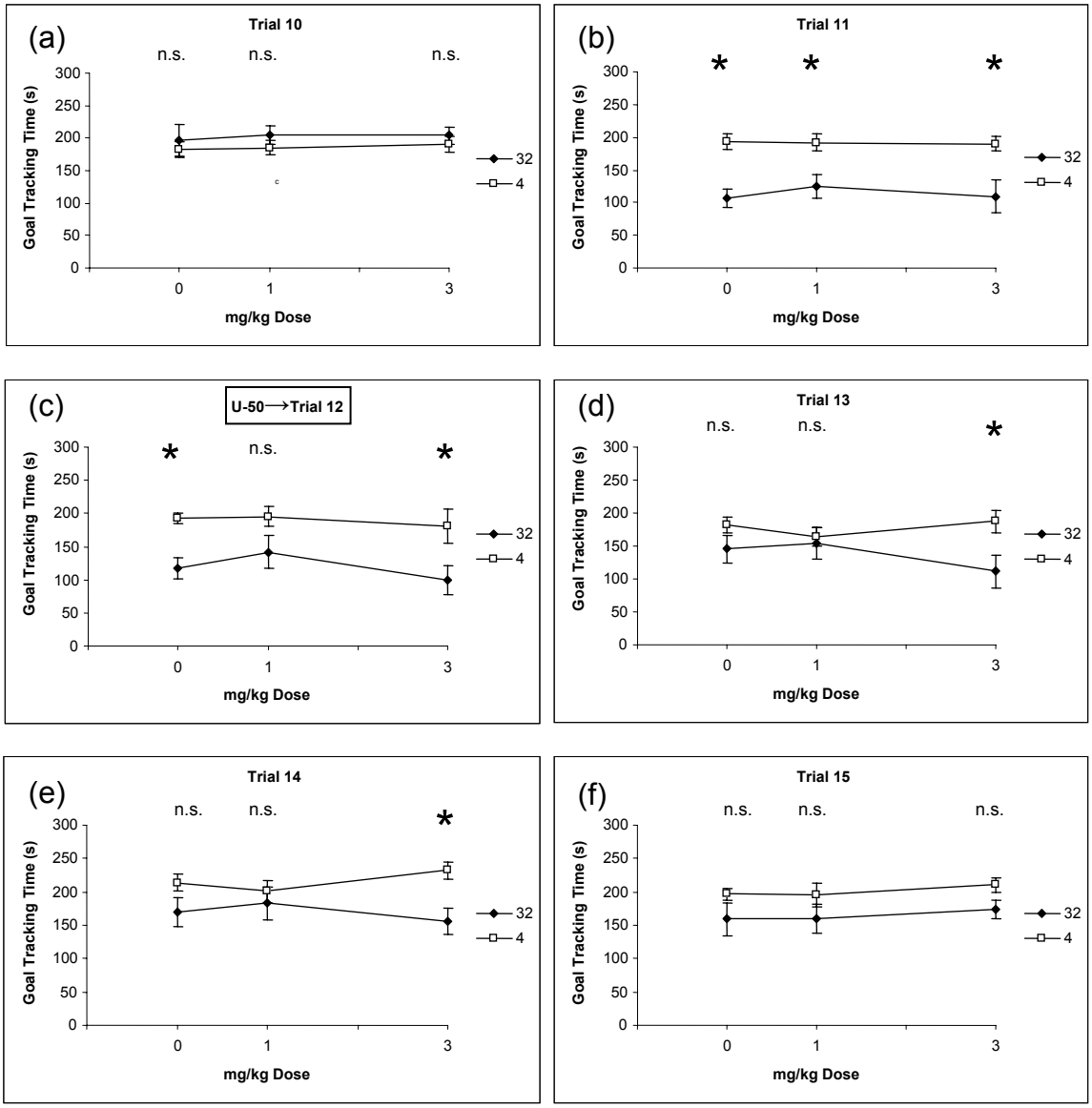

Figure 5. The following graph illustrates a dose by goal tracking time comparison of downshifted and unshifted groups on trial 10 of preshift and trials 11-15 of postshift. The κ-opioid receptor agonist U-50 and saline (***S***) were administered after trial 11 of the postshift phase, in the following amounts: 1 and 3 mg/kg (labeled ***U1*** and ***U3***). ***n.s***: nonsignificant difference between downshifted and unshifted conditions of the given dose. *: significant difference between conditions of a given dose, $p < 0.05$.

Figure 5 illustrates the effect of U-50 dose on consummatory performance on the last preshift trial 10 and postshift trials 11–15. As done in the case of Experiment 1, these data were subjected to one-way ANOVAs for trials 10-15, followed by post hoc LSD

pairwise comparisons. On trial 10 (Figure 5a), there was a nonsignificant group effect, $F < 1$. Pairwise comparisons revealed nonsignificant differences between the two contrast groups (32% vs. 4%) for all the drug conditions (0, 1, 3, 10 mg/kg), ps > 0.05. As expected, trial 11 (Figure 5b) yielded a significant difference between groups, $F(5, 46) = 8.17$, $p < 0.05$. Pairwise comparisons revealed significant differences between the two contrast groups (32% vs. 4%) for all drug conditions (0, 1, and 3 mg/kg), ps < 0.01. LSD post hoc analysis of the downshifted (32-4) and unshifted (4-4) conditions revealed nonsignificant differences between animals receiving 1 or 3 mg/kg of U-50 when compared to downshifted saline, ps > 0.05.

The key results correspond to trials 12-15. On trial 12 (Figure 5c), the analysis revealed a significant group effect, $F(5, 46) = 4.89$, $p < 0.001$. Pairwise comparisons showed significant differences between the two contrast groups (32% vs. 4%) of the 0 and 3 mg/kg drug conditions, ps < 0.05. However, Group 32/U1 was not significantly different from Group 4/U1, $p > 0.05$, indicating an attenuation of cSNC in the 1 mg/kg U-50 condition. LSD post hoc analyses of the downshifted conditions (32-4) revealed nonsignificant differences between Groups 32/S, 32/U1, and 32/U3, ps > 0.05, and also between Groups 4/S, 4/U1, and 4/U3, ps > 0.05.

An analysis of trial 13 (Figure 5d) showed a marginally significant group effect, $F(5, 46) = 2.31$, $p = 0.06$. However, post hoc LSD analyses revealed a significant in Group 32/U3 when compared to 4/U3, $p < 0.05$. This enhancement of cSNC after a single administration of 3 mg/kg U-50 before trial 12 continued into trial 14. The comparison between Groups 32/S vs. 4/S and between Groups 32/U1 vs. 4/U1 on trial 13 was nonsignificant, ps > 0.05

Trial 14 (Figure 5e) revealed a significant group effect, $F(5, 46) = 3.01$, $p < 0.05$, combined with a significant difference between Groups 32/U3 and 4/U3, $p < 0.05$. The comparison between Groups 32/S vs. 4/S and between Groups 32/U1 vs. 4/U1 on trial 14 was nonsignificant, ps > 0.05.

Finally, trial 15 (Figure 5f) revealed a nonsignificant group effect, $F(5, 46) = 1.81$, $p > 0.05$, followed by nonsignificant pairwise differences, ps > 0.05.

Discussion

The evidence from Experiment 2 supports previous findings from Experiment 1. Specifically, the low dose of U-50 selectively attenuated contrast on trial 12, whereas the intermediate dose of U-50 selectively enhanced cSNC on trials 13 and 14. The theoretical implications and future directions underpinning the actions of the low dose of U-50 on cSNC will be discussed in greater detail in the General Discussion.

As for the effect of the medium dose of U-50 on cSNC, Experiment 1 had shown that 3 mg/kg U-50 administered before trials 11 and 12 enhanced cSNC for one additional trial relative to the saline controls (see Figure 3d). Similarly, Experiment 2 revealed that 3 mg/kg U-50 administered before trial 12 enhanced cSNC for an additional two trials, relative to the saline controls (see Figures 5d and 5e). By trial 14 in Experiment 1 and trial 15 in Experiment 2, the effects of 3 mg/kg U-50 on cSNC had dissipated (see Figures 3e and 5f). Two explanations of this effect will be explored in Experiments 3 and 4. The first explanation implies that 3 mg/kg U-50 may decrease goal tracking time through an indirect activation of locomotor activity. Other experiments have indicated that U-50 can influence locomotion (Schnur & Walker, 1998). However, the activity tests used on those experiments did not measure the effects of U-50 within a

period of time similar to consummatory trials implemented in these experiments. This implication will be addressed in Experiment 3. The second explanation suggests a potential effect of U-50 on mechanisms affecting posttrial processing of the downshift experience. Bentosela, Ruetti, Muzio, Mustaca, and Papini (2006) found that trial 12 performance in the cSNC situation is sensitive to the posttrial 11 injection of corticosterone, implicating an effect on associative properties that mediate the processing of the downshift experience. These speculations will be addressed in more detail in Experiment 4.

Experiment 3

U-50 has an affect on locomotor activity. Schnur and Walker (1990) reported that U-50 exhibits a dose-dependent bidirectional effect on running wheel activity. Animals were tested 10 min following a U-50 injection and for a total of 120 min. Activity was then averaged into 10-min bins. Overall activity showed that low dose of U-50 (1 mg/kg) induced hyperactivity while high dose (10 mg/kg) induced hypoactivity in the golden syrian hamster. An additional medium dose (3 mg/kg) showed no difference from saline controls. However, Schnur and Walker (1990) failed to show a significant effect of the low dose of U-50 until 50 min after the injection. This indicates that the effects of U-50 on locomotor activity may influence consummatory behavior by either a slow onset or an independent effect on the exposure to sucrose.

Experiment 1 identified a suppression of consummatory behavior induced by the medium dose of U-50 in unshifted groups. Thus, it could be argued that U-50 may promote a slight locomotor effect within the 5-min trial duration in the cSNC experiment. An increase in activity by U-50 may lead to a reduction in consumption of sucrose during

cSNC by a simple mechanism of response competition. This idea is not novel. In a conventional weight loss treatment, the prescription of locomotor stimulants (e.g., amphetamine) is followed by suppressed food consumption. κ-opioid mechanisms underlying the effects of amphetamine have been discussed by Nencini and Valeri (1992), who found that amphetamine decreased the consummatory effects generated by 4 mg/kg of U-50 within the first 2 h following amphetamine injection, while prolonging the anorexic effects to approximately 5 h after administration in free feeding rats. Henry, Walker, and Marjules (1985) reported that metabolic weight loss was generated by U-50, an effect accompanied by locomotor activity. All behaviors induced by U-50 were reversed by the κ-opioid antagonist MR2663. Despite these implications, U-50 is classically known and used for its small locomotor effect in the analgesic preparation of horses (Kamerling, Weckman, Donahoe, & Tobin, 1988). Thus, the possibility that U-50 may interfere with consummatory behavior via increased activity levels is an option that needs to be considered in detail. Experiment 3 explored the possibility that the medium dose of U-50 may have some locomotor influence on the rat within a similar time period as cSNC (5 min).

Method

Subjects. The subjects were 15 adult Long-Evans hooded rats (9 male and 6 female), 90-130 days old, at the start of the experiment. Rats were bred and housed in the TCU vivarium as described in previous experiments. Animals were tested during the light phase of the cycle. Animals were transferred from polycarbonate breeding tubs to wire-bottom cages 7-10 days prior to testing. Previous to testing, all animals were used for breeding. All males fathered pups, approximately three weeks prior to wire mesh caging.

Females were housed in wire mesh cages approximately 10 days after weaning and tested approximately 17-20 days after weaning.

Apparatus. Activity was assessed using a rectangular runway 160.4 cm in length, 15.5 cm in width, and 21.5 cm in height. Thus, this apparatus lacked any open spaces. The runway was constructed of wood, including hinged panels on top for placement and removal of the subject. Activity was recorded by three photocells located 61.2 cm apart, one in the middle, the other two were located 15 cm from the end wall on either end of the runway. No lights were used, allowing animals to be tested in a dark environment. A computer located in an adjacent room accumulated photocell interruptions.

Procedure. Groups were matched for sex and randomly assigned into two groups, one receiving 3 mg/kg U-50 (n = 8) the other saline (n = 7). An extra male was assigned to the U-50 condition. Saline and U-50 were injected i.p. 20 min prior to the activity test. An activity score was obtained by adding the number of times each photocell was activated during a 30-min trial and also by accumulating activity scores every 30 s. Animals were placed in the activity chamber facing an end wall and were allowed to explore for 30 s prior to the trial. All lights in the testing room were turned off and the door to the room was closed to produce a dark testing environment. U-50 and saline were prepared as in Experiment 1.

Results

Figure 6 illustrates the group average of photocell crossings collected in 30-s bins during the initial 5 min of the test. A Drug (3 mg/kg U-50, Saline) x Bin (1–10) ANOVA revealed nonsignificant differences across groups and bins, Fs< 1, indicating no detectable adaptation within the first 5 min. The drug by bin interaction also yielded a

nonsignificant effect, $F(9, 117) = 1.46$, $p > 0.05$. Figure 6 reveals considerable divergence between groups in the first 30 s. However, an ANOVA computed on these scores yielded only a marginal difference between groups, $F(9, 117) = 3.78$, $p = 0.07$.

Figure 7 illustrates group averages of photocell crossing collected in 5-min bins. A Drug x Bin (1–60) ANOVA revealed a nonsignificant group effect, $F < 1$. However, a signficant effect of bin, $F(5, 65) = 20.64$, $p < 0.001$, indicated that animals adapted to the environment over the 30-min testing period. The drug by bin interaction was nonsignificant, $F(5, 65) = 1.49$, $p > 0.05$, indicating similar adaptation rates across drug groups over the 30-min period.

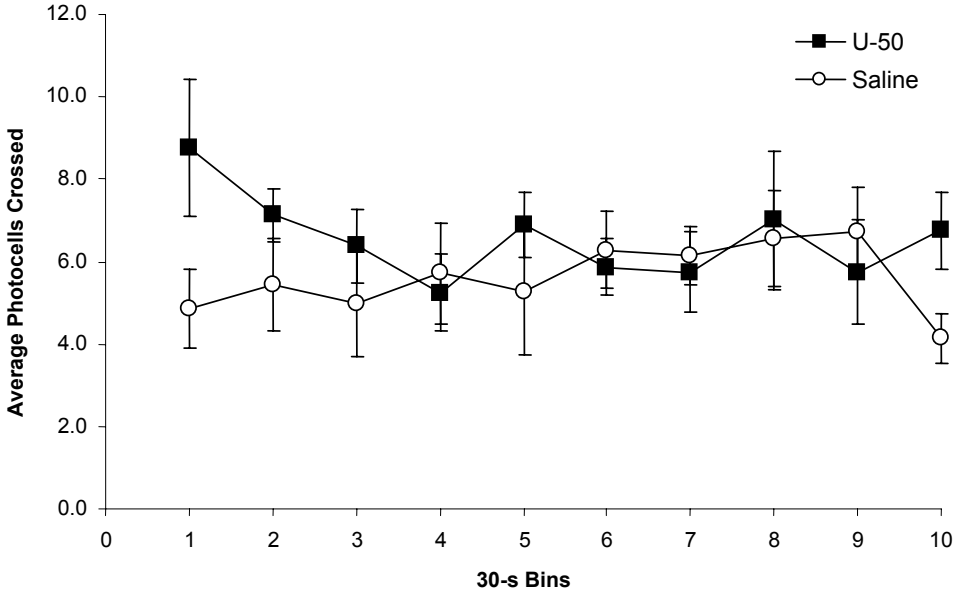

Figure 6. The graphic illustrates average number of photocells crossed in the activity runway 30-s bins. Represented are groups receiving U-50 (3 mg/kg) or Saline before the activity test.

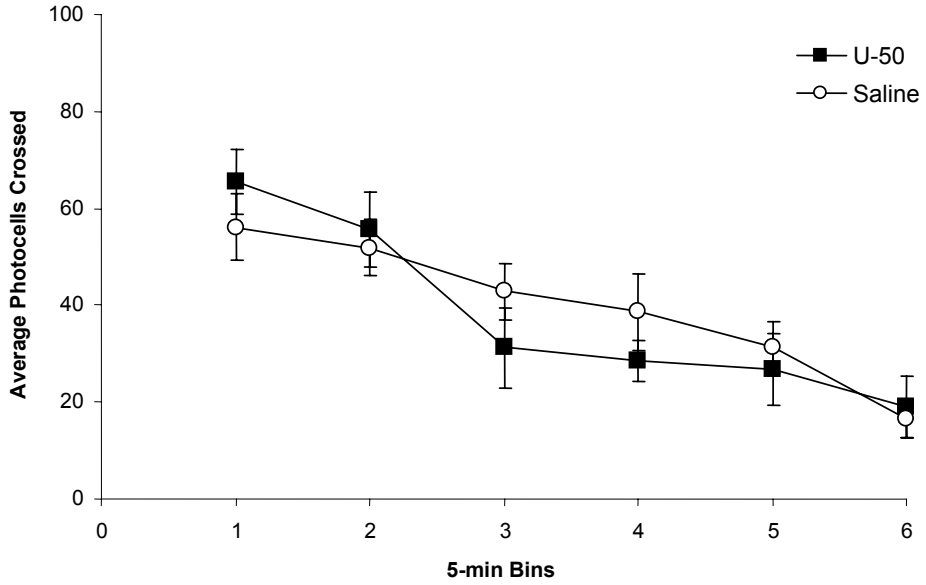

Figure 7. The graphic illustrates average number of photocells crossed in the activity runway in 5-min bins. Represented are groups receiving U-50 (3 mg/kg) and Saline.

Discussion

The medium dose of U-50 failed to affect locomotor activity. These data suggest that the effects of medium dose of U-50 in Experiments 1 and 2 are probably not the result of locomotor activation. However, it may have been better to measure activity in a similar testing apparatus as that used to test for cSNC and collect data from other behaviors (in addition to activity) potentially elicited by U-50. These data are consistent with the findings of Schnur and Walker (1990) who showed that running wheel activity was unaffected by 3 mg/kg U-50.

Experiment 4

Experiment 4 addresses the possibility that U-50 may influence the memory of the downshift experience that occurs on trial 11. In Experiment 2, the low dose of U-50 reduced the contrast effect by one trial, while the medium dose of U-50 prolonged it by

two trials, relative to the saline control groups. However, it is unclear how these effects were accomplished. One possibility is that U-50 can influence the memory mechanisms involved in the establishment of the relevant associations after reward downshift.

The opioid system is involved in the learning of aversive events. Gallagher and Kapp (1978) reported that amygdala injections of the morphine analogue levorphanol and of the opioid antagonist naloxone following passive avoidance training affected retention in a time- and dose-dependent manner. Thus, levorphanol decreased retention when injected immediately following passive avoidance training, while naloxone injected immediately following passive avoidance training increased retention. Injections given 6 h posttrial were just as ineffective as saline controls. Hiramatsu, Hyodo, and Kameyama (1996) found antiamnesia effects in the step-down passive avoidance task in mice subjected to amnesia induced by carbon monoxide. Findings revealed that U-50 administration before passive avoidance testing ameliorated carbon monoxide-induced deficits. Based on this evidence, one may expect that U-50 injection following postshift trial 11 would enhance memory of the aversive reward-downshift experience and induce increased suppression of consummatory responding on postshift trial 12.

The actual role of the κ-opioid system in memory is not well understood. Addressed above were examples of enhanced memory of aversive events. However, alternative evidence indicates κ-opioid involvement in memory deficits. For example, McDaniel, Mundy, and Tilson (1990) identified spatial memory impairments on radial arm maze learning following κ-opioid agonist dynorphin injection into the dorsal but not ventral hippocampus (an effect reversible by naloxone). An applied example emerges from the Alzheimer's literature that identifies an increased number of κ-opioid receptors

in limbic areas (Hiller, Itzhak, & Simon, 1987), putamen, and cerebellar cortex (Mathiew-Kia et al., 2001), in postmortem studies of Alzheimer's patients.

The effects of a medium dose of U-50 on the recovery phase of cSNC may be the result of the activation of alternative systems, rather than direct mediation. For example, Taylor et al. (1996) found that U-50 stimulates pituitary-adrenal function via hypothalamic arginine-vasopressin and corticosterone releasing factor, which increased the levels of corticotropic releasing hormone and corticosterone in blood plasma as compared to saline controls. Bentosela et al. (2006) identified a retardation of recovery of cSNC on postshift trials 12-15 when animals were injected with corticosterone immediately after trial 11. The effect was not present when corticosterone was administered 3 h after trial 11.

Experiments 1 and 2 suggest that U-50 has a bidirectional influence on cSNC depending on the dose. Thus, two effects were predicted following posttrial 11 injections: (1) A low dose of U-50 was predicted to enhance recovery on trial 12 when injected posttrial 11, and (2) A medium dose of U-50 was expected to impair recovery after trial 11.

Method

Subjects. The subjects were 48 rats (25 females and 23 males). Housing and maintenance conditions were as described in Experiment 1.

Apparatus. The same conditioning boxes described in Experiment 1 were used.

Procedure. The downshift procedure used in Experiment 3 was similar to that of Experiment 1. The design included three downshifted drug Groups (32/S, 32/U1, and 32/U3; $n = 8$) receiving, respectively, an i.p. injection of saline, 1, or 3 mg/kg U-50

immediately following trial 11. Twenty four animals were assigned to the unshifted 4-4 control conditions and randomly distributed in 3 groups with the same drug regimens (n = 8). Males and females were equally represented in all the groups, except for Group 4/U1, which was composed of 3 males and 5 females. Table 3 summarizes the design used in Experiment 3. U-50,488H and saline were prepared as in Experiment 1.

Table 3
Design of Experiment 4.

Group	Contrast Condition	Injection Posttrial 11	n =
32/U1	32 - 4	1 mg/kg U-50	8
32/U3	32 - 4	3 mg/kg U-50	8
32/S	32 - 4	Saline	8
4/U1	4 - 4	1 mg/kg U-50	8
4/U3	4 - 4	3 mg/kg U-50	8
4/S	4 - 4	Saline	8

Results

A computer malfunction occurred during trial 8 affecting one animal in Group 32/U3 and on trial 15 affecting one animal in Group 4/S. Lost data were replaced with the group average as specified by Kirk (1968). Figure 8 illustrates the group average goal tracking time for preshift (1-10) and postshift (11-15) trials. A Contrast (32%, 4%) x Trial (1-10) mixed-model ANOVA, with repeated-measures for the factor trial, was computed to test for preshift effects. The preshift sucrose conditions were comprised of pooled groups receiving 4% (n = 24) and 32% (n = 24) sucrose. All animals increased goal tracking time across the preshift trials, $F(9, 414) = 69.93$, $p < 0.001$. Animals receiving 32% were not signficantly different from 4% controls, $F < 1$, over preshift trials. In addition, the interaction between contrast and trial failed to reach significance, $F < 1$.

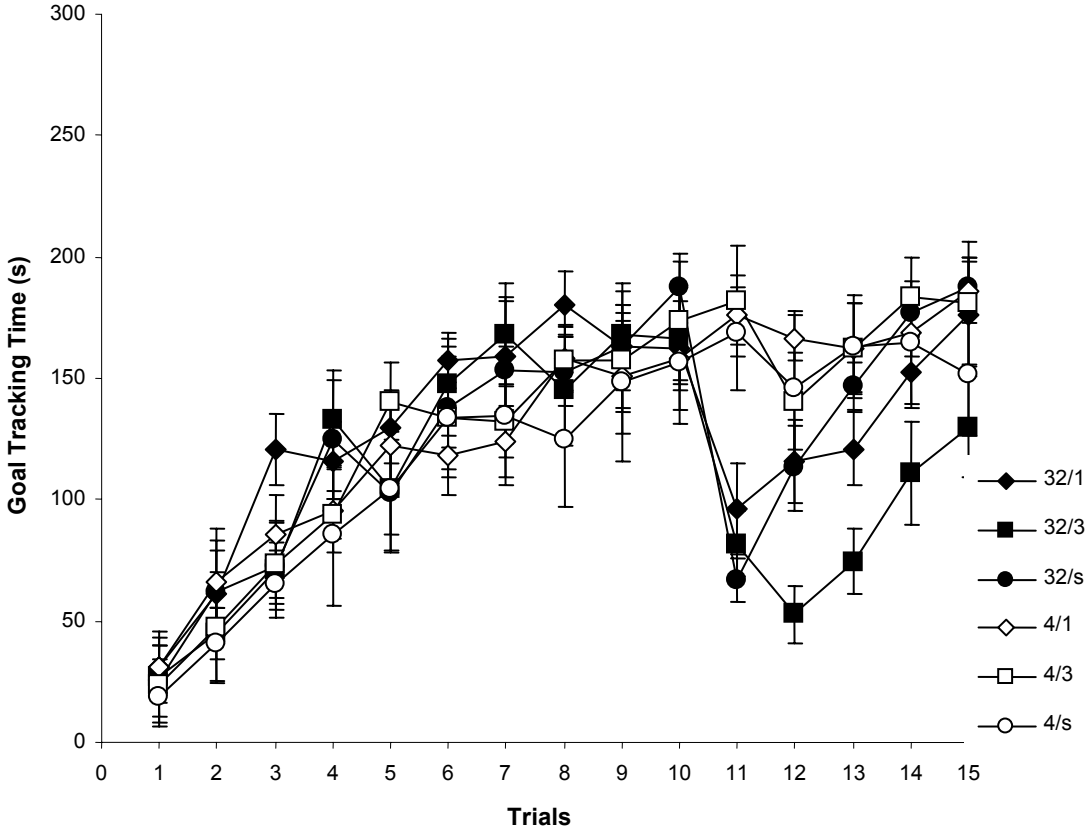

Figure 8. The following graph illustrates average consummatory performance over 15 trials. On trials 1-10, animals were either given 32 % or 4 % sucrose. On trials 11-15, all animals were given 4% sucrose. On posttrial 11, U-50 was administered in low (1 mg/kg) and medium (3 mg/kg) levels, and denoted in the legend as 32(1, 3, S), or 4(1, 3, S).

The results of most interest in Figure 8 occurred during the postshift trials. Significant effects generated on the postshift trials revealed that U-50 had an effect on recovery from cSNC. All downshifted groups showed a significant suppression in comparison to their respective unshifted controls, confirming an effect of downshifted sucrose. During trial 12, Group 32/U3 showed an enhancement of contrast relative to Group 32/S, while Group 4/U3 showed no difference from Group 4/S, indicating an effect on cSNC as opposed to consummatory behavior. Group 32/U1 showed no

difference from Group 32/S, indicating no detectable effect of 1 mg/kg of U-50 on posttrial 11 adminsitration.

The following is a global analysis of postshift trials using a Contrast (32%, 4%) x Drug (0, 1, 3 mg/kg) x Trial (11-15) ANOVA. A significant effect of trial, $F(4, 168) = 19.35$, $p < 0.001$, indicated a recovery across postshift trials. A significant contrast x trial interaction, $F(4, 168) = 12.96$, $p < 0.001$, indicated that the performance of downshifted groups changed at a different rate across postshift trials than that of the unshifted controls. A significant drug x trial interaction, $F(8, 168) = 2.26$, $p < 0.05$, indicated a change of groups receiving U-50 (1 or 3 mg/kg) over the five postshift trials. However, a three way contrast x drug x trial interaction, $F(8, 168) = 1.47$, $p > 0.05$, failed to reach significance. The between-subject factors revealed a main effect of contrast, $F(1, 42) = 14.01$, $p < 0.001$, but not of drug, $F(2, 42) = 1.23$, $p > 0.05$. Also, the condition x drug interaction failed to reach significance, $F(2, 42) = 1.96$, $p > 0.05$.

The trial of emphasis in this experiment is trial 12. However, all contrast research is dependent upon a significant suppression of responding on trial 11 and recovery to the responding level of the 4% controls on the remaining 4 postshift trials. Thus, a more detailed analysis of postshift trials (11-15) was carried out using a one-way ANOVAs for each postshift trial, combined with LSD post hoc pairwise comparisons.

Figure 9 illustrates the effect of U-50 dose on consummatory performance on the last preshift trial 10 and postshift trials 11–15. On trial 10 (Figure 9a) a nonsignificant group effect, $F < 1$ was found. Although goal tracking times are higher for groups exposed to the 32% than the 4% sucrose, pairwise comparisons revealed nonsignificant differences for all the drug conditions (0, 1, 3, and 10 mg/kg), ps > 0.05.

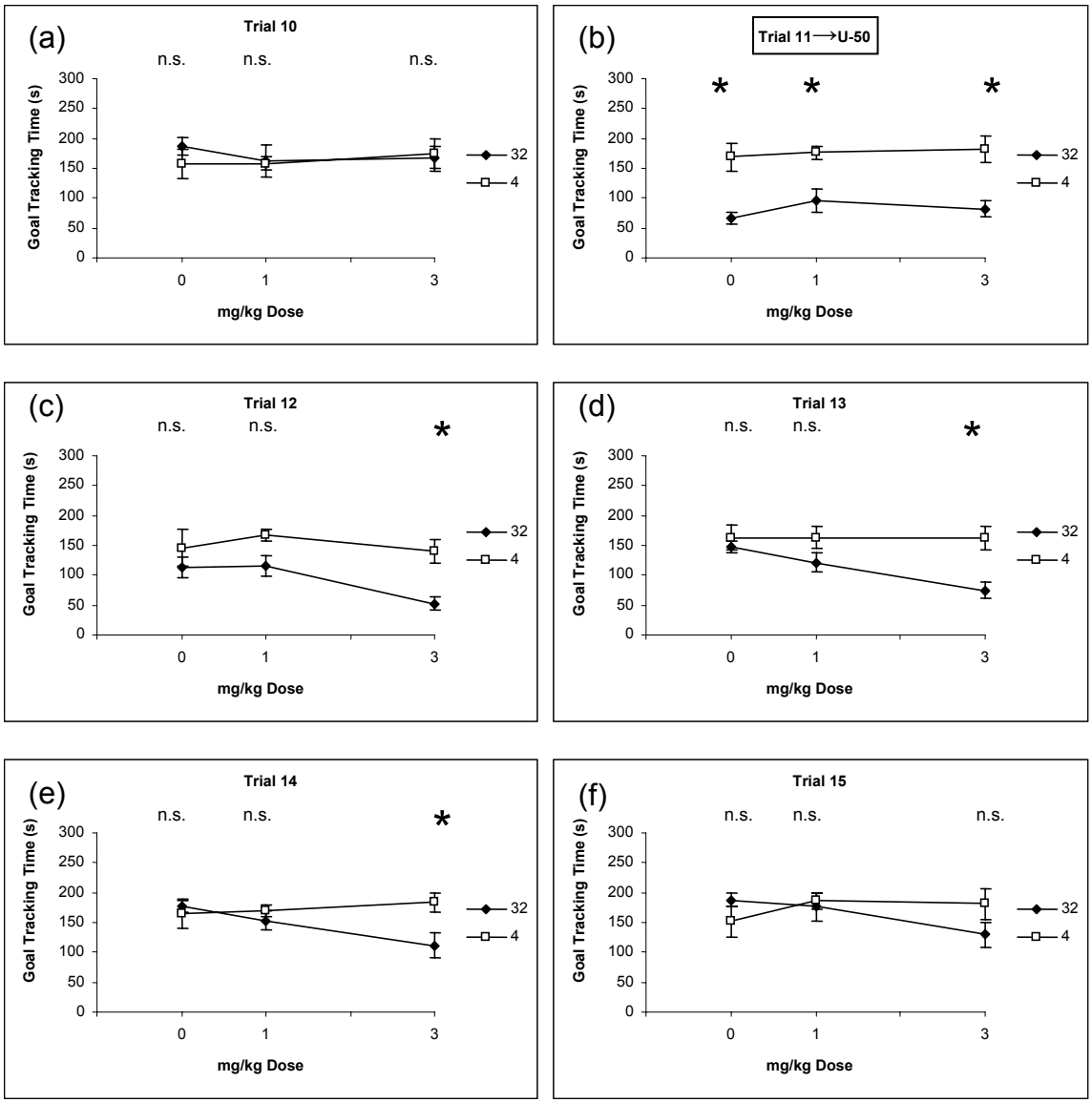

Figure 9. The following graph illustrates a dose by goal tracking time comparison of downshifted and unshifted groups on trial 10 of preshift and trials 11-15 of postshift. The κ-opioid receptor agonist U-50 and saline were administered immediately after trial 11 of the postshift phase, in the following amounts: 1 and 3 mg/kg (labeled ***U1*** and ***U3***). ***n.s***: nonsignificant difference between downshifted and unshifted conditions of the given dose. *: significant difference between conditions of a given dose, $p < 0.05$.

The analysis of trial 11 (Figure 9b) performance revealed a significant group effect, $F(5, 47) = 8.91$, $p < 0.001$. Pairwise comparisons revealed significant differences between the two contrast groups (32% vs. 4%) for all drug conditions (0, 1, and 3 mg/kg),

*p*s < 0.01. LSD post hoc analyses of the downshifted condition (32-4) revealed no significant difference between groups, $p > 0.05$. Likewise LSD post hoc analysis if the unshifted condition (4-4) revealed no significant differences between groups, $p > 0.05$.

Trial 12 (Figure 9c) revealed a significant group effect, $F(5, 48) = 4.31$, $p < 0.01$, indicating differences between downshifted and unshifted condition. Pairwise comparisons revealed nonsignificant differences between the two contrast conditions (32% vs. 4%) for drug groups 0, and 1 mg/kg, *p*s > 0.05. LSD post hoc comparisons of groups within condition revealed that Group 32/U3 showed a significant suppression of responding in comparison to Group 32/S, $p > 0.05$, indicating enhancement of contrast following posttrial 11 administration of 3 mg/kg U-50. LSD post hoc comparison of unshifted groups revealed no significant differences between U-50 drug groups 0, 1, and 3 mg/kg, $p > 0.05$.

Trial 13 (Figure 9d) revealed a significant group effect, $F(5, 47) = 4.55$, $p < 0.01$, indicating differences between downshifted and unshifted condition. Pairwise comparisons revealed significant differences between contrast groups (32% vs. 4%) for the 3 mg/kg drug condition, $p < 0.05$. Group 32/U1 was not different from Group 32/S, $p > 0.05$. LSD post hoc analysis of unshifted groups showed nonsignificant suppression of responding across groups, *p*s > 0.05.

Trial 14 (Figure 9e) revealed a nonsignificant group effect, $F(5, 47) = 2.29$, $p > 0.05$, indicating recovery from contrast in all drug conditions. However, pairwise comparisons revealed significant differences between contrast groups (32% vs. 4%) for the 3 mg/kg drug condition, $p < 0.05$. Groups 32/S and 32/U1 showed a nonsignificant difference from Groups 4/S and 4/U1, respectively, *p*s > 0.05, implicating full recovery

for these groups. LSD post hoc analysis of Group 32/U3 showed a significant suppression of responding, $p < 0.05$, when compared Group 4/U3.

Trial 15 (Figure 9f) revealed a nonsignificant group effect, $F(5, 47) = 1.28$, $p > 0.05$, indicating full recovery of all drug conditions. Pairwise comparisons revealed nonsignificant differences between all contrast groups (32% vs. 4%) of drug conditions (0, 1, and 3 mg/kg), ps > 0.05.

Discussion

Administration of the medium dose of U-50 on posttrial 11 enhanced the contrast effect on trial 12, and retarded recovery on subsequent trials 13 and 14, while the low dose of U-50 was not different from saline. The nonsignificant effect found in nonshifted and drug control groups indicates that the effect of U-50 cannot be attributed to factors relating to contextual environment, sensory mechanisms which influence consummatory behavior, motivational influence, or motor impairments. Furthermore, the attenuation effect produced by low dose of U-50 in Experiments 1 and 2 cannot be attributed to an effect on memory consolidation of the downshift experience, since the posttrial administration had no effect. However, the enhancement of contrast by medium dose of U-50 in Experiments 1 and 2 can be attributed to an effect of U-50 on memory consolidation of the downshift experience, due to the increased suppression of consummatory behavior found following posttrial injection. These data are consistent with the conclusions addressed in Experiment 3, suggesting that the effects of 3 mg/kg of U-50 in Experiments 1 and 2 are not due to alteration in motor behavior. Therefore these data indicate that the medium dose of U-50 has a direct effect upon mechanisms that mediate the associative connections procured following the downshift experience.

These results add to the findings of Bentosela et al. (2006) who reported that posttrial 11 administration of corticosterone impaired recovery on trial 12 when injected immediately following trial 11, as opposed to 3 h after trial 11. Their conclusions indicated that corticosterone enhanced the processing of an internal state of arousal influenced by the initial downshift experience. The effects generated in Experiment 4 support Bentosela's et al. (2006) conclusions and introduce the κ-opioid system as a mechanism which may mediate associative properties generated by the downshift experience.

General Discussion

The κ-opioid receptor agonist U-50 had a variety of effects on the cSNC situation. All together, Experiments 1 and 2 showed that while a high dose of U-50 (10 mg/kg U-50) impaired consummatory behavior independently of the effects of incentive downshift, all three doses also affected the cSNC effect per se. Both the attenuation (by the low dose of U-50) and the enhancement of the cSNC effect (by the medium dose of U-50) were observed. Moreover these effects of U-50 were selectively apparent on trial 12: the agonist had no effect on cSNC when administered before trial 11. Experiment 3 confirmed that the effects of the medium dose of U-50 are probably not due to increased locomotor activity. Experiment 4 elaborated on the effects generated by U-50 to implicate mechanisms involved in cSNC following the experience of downshift, possibly related to the consolidation of the aversive recovery of the downshift event.

The theory chosen to explain these effects has been mentioned in the Introduction, Amsel's (1992) frustration theory. Figure 1 illustrates frustration theory as a linear model in which an expectancy for 32% sucrose is developed during the first 10 preshift trials.

Trial 11 introduces a downshift in sucrose value $S_{4\%}$, resulting in a Pavlovian connection between antecedent stimuli and primary frustration (R_F). The trial 12 experience is represented by two competing expectations, one for 32% (e_{32}) sucrose and the other for frustrative 4% sucrose (e_F). The result is an internal state of conflict between the hedonically appealing e_{32} and the aversive e_F. Amsel (1992) suggested that this internal state resulted in an approach-avoidance conflict, which has been shown to be susceptible to many benzodiazepine anxiolytics (Flaherty, 1996). The evidence presented in this paper suggests that a low dose of U-50 possesses anxiolytic like properties that enhance recovery from cSNC. Similar doses of U-50 have shown to attenuate anxiety-related behaviors. Privette (1995) found that 1 mg/kg of U-50 increased exploratory behavior in the elevated plus maze. The κ-opioid receptor agonist U-69,593 showed similar effects to U-50 in the same test, indicating that the κ-opioid system appears to have anxiolytic properties at low levels of activation.

The similarity between the low dose of U-50 and the effects of anxiolytics suggests that these drugs may be mediating the response to anticipatory contrast through similar mechanisms. Several studies have identified a relationship between the effects of benzodiazepine and opioid drugs. For example, naloxone blocks the anxiolytic effects of benzodiazepines in several models of anxiety (Ågmo, Galvan, Heredia, & Morales, 1995). In addition, naloxone has shown to potentiate anxiogenic-like behavior (Cancela, Bregonzio, & Monzella, 1994; Koks et al., 1998; Schulties, et al., 1998). Ågmo and Belzung (1998) suggested that this effect may be in part mediated by the κ-opioid receptor system. They found that the κ-opioid receptor antagonist nor-BNI dose dependently blocked the effects of the benzodiazepine chlordiazepoxide (CDP). These

data implicate that the κ-opioid system must be active for the benzodiazepine CDP to be effective and that the effects generated by the benzodiazepine CDP are mediated through the κ-opioid system. In contrast, Nemmani, and Ramarao (2002) found that diazepam dose-dependently attenuated U-50 (40 mg/kg) induced analgesia. The high dose of U-50 (40 mg/kg) in Nemmani, and Ramarao's (2002) experiment implicates a new dimension not addressed in the preceding paragraph, that these drugs (κ-opioid agonists and benzodiazepine anxiolytics) may perform functions that balance each other depending upon their level of activation.

The κ-opioid system has shown differential and opposing functions based upon its level of activation. For example, Schnur and Walker (1990) found that 1 mg/kg of U-50 induces hyperactivity, while 10 mg/kg induces hypoactivity on the running wheel. Consummatory behavior also exhibits a similar parallel, Lynch and Burns (1990) found that 0.3 mg/kg of U-50 increased consummation of 20% sucrose while 1 mg/kg decreased consumption over 10 days of successive pretrial treatment. However, κ-opioid opposition to all μ and δ opioid receptor mediated behaviors is not exact. For example, analgesia produced by morphine, and more selective μ and δ opioid receptor agonists, is also produced by U-50 in high levels, while a low dose of U-50 fails to influence analgesia (Nemmani, and Ramarao, 2002).

The κ-opioid system may function as a mechanism that balances the activity of the μ and δ opioid systems. For example, U-50 appears to influence cSNC in a bidirectional fashion within a limited dosage range (1 and 3 mg/kg). Morphine attenuates cSNC on trial 12, similar to low dose of U-50, while naloxone prolongs recovery similar

to medium dose of U-50. Thus, future studies may require the combined administration of a medium dose of U-50 with either morphine or naloxone during trial 12 of cSNC.

The retardation of recovery generated by a medium dose of U-50 may be the result of an altered perception of the loss component, or a general enhancement of consolidation of the association between the perceived loss and apparatus was, thereby prolonging memory. These topics are left undissected in Experiment 4. These data are the first to clarify that opioid drugs affect mechanisms which mediate the posttrial experience of cSNC.

Conclusions

As an animal model, cSNC has opened the way to study the neurochemical systems that regulate the adjustment of situations involving incentive loss. Traditionally, the GABAergic system had been implicated in a selective engagement during the conflict phase of cSNC, represented by trial 12 performance in the typical experiment. More recently, work with opioid agents has uncovered a complex regulation of cSNC. The δ-opioid receptor system appears to be involved in the initial reaction (trial 11). The μ-opioid receptor system appears to be involved in both the initial reaction and the conflict phase of cSNC (trials 11 and 12). The present series of experiments uncover yet another selective role, in this case for the κ-opioid receptor system. The agonist U-50,488H has shown two types of selectivity, both unique so far as we know for any drug studied in the context of the cSNC effect. First, U-50,488H showed trial selectivity, acting on trial 12, but not on trial 11. In this sense, it complements the selectivity shown by the δ-opioid receptor agonist DPDPE, which shows selectivity for trial 11. Second, U-50,488H showed dose selectivity, having opposite effects on cSNC depending on the dose. At the

1 mg/kg, U-50,488H reduced cSNC, whereas at the 3 and 10 mg/kg, U-50,488H enhanced cSNC. These effects cannot be attributed to an action of U-50,488H on consummatory behavior per se (Experiments 1 and 2) or to activity (Experiment 3). Finally, the cSNC-enhancing action of the 3 mg/kg dose of U-50,488H may be the result of an action on memory consolidation of the downshifted episode (Experiment 4). These experiments contribute another piece of the puzzle to an understanding of the role of the opioid system on cSNC in particular, and on the adjustment to incentive loss in general.

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ABSTRACT

ROLE OF K-OPIOID RECEPTOR AGONIST U-50,488H IN CONSUMMATORY SUCCESSIVE NEGATIVE CONTRAST

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The role of κ agonist U-50,488H in cSNC was explored in four experiments. Experiment one revealed that U-50 failed to influence cSNC on trial 11 but showed both an attenuation with low dose of U-50 and exaggeration of cSNC in medium and high dose of U-50 by impairing recovery. Experiment two confirmed a selective attenuation for trial 12 of cSNC in groups given low dose, while the medium dose impaired recovery. Experiment three explored the possibility that the effects of U-50 on cSNC may be the result of an influence on alternative mechanisms such as locomotor activity. Results revealed that U-50 showed no influence from saline control. Experiment four explored the effects of U-50 on posttrial 11 and revealed that U-50 signficantly enhanced contrast, on trial 12, indicating an effect on associative mechanisms involved in the memory of the downshift experience.