BEHAVIORAL CONSEQUENCES OF REWARD DOWNSHIFT:

ROLE OF THE PREFRONTAL CORTEX

by
LEONARDO A. ORTEGA MURILLO

Psychologist, 2005
National University of Colombia
Bogotá D. C., Colombia

Master of Science, 2008
Texas Christian University
Fort Worth, Texas

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ABBREVIATIONS

ACC: Anterior cingulate cortex

ACTH: Adrenocorticotropic hormone

BNST: Bed nucleus of the stria terminalis

CeA: Central nucleus of the amygdala

CN: Cranial nerves.

cSNC: Consummatory successive negative contrast

DPDPE: D-Pen2,D-Pen5-Enkephalin

GABA: γ-aminobutyric acid

HPA axis: Hypothalamic-pituitary-adrenal axis

iSNC: Instrumental successive negative contrast

LH: Lateral hypothalamus

mPFC: Medial prefrontal cortex

NMDA receptor: N-methyl-D-aspartate receptors

NST: Nucleus of the solitary tract

PbN: Parabrachial nucleus

PFC: Prefrontal cortex.

PRAE: Partial reinforcement acquisition effect

VLO: Ventrolateral orbital cortex.

VPM: Ventral posteriomedial nucleus of the thalamus

An unexpected change in a reward triggers behavioral adjustments to a new environmental situation. A complex set of psychobiological mechanisms underlies these behavioral adjustments (Gray & McNaughton, 2000). Reward downshift is a special case of an alteration of the environment in which the animal is presented with a reward that has unexpectedly become less valuable than previously experienced (Elliot, 1928). The purpose of the present experiments is to assess the role of three areas in the PFC of the rat, ACC, mPFC, and VLO, in the modulation of the behavioral processes underlying a type of reward downshift, cSNC.

The present manuscript approaches the experimental problem by discussing:

(1) cSNC as a paradigm to study incentive relativity.

(2) Experimental findings on the neural basis of cSNC.

(3) Some interpretations of the role of the proposed cortical areas in behavior and cSNC.

Incentive relativity

Some of the behavioral and biological effects following unexpected reward downshifts depend upon the characteristics of the reward itself, while other effects depend upon the environmental and organismic context in which the reward is presented. Flaherty (1996) referred to such distinction in terms of the absolute vs. relative effects of incentives. The absolute effects of incentives are related to the physical properties of the reward, such as its caloric value, size, or nutrients. The relative effects of incentives are related to previous or concurrent experience with incentives of different absolute value (Flaherty, Krauss, Rowan, & Grigson, 1994). The notion of incentive relativity was proposed largely in the context of reward downshift (i.e., the shift from a more preferred reward to a less preferred reward), although it also applies to the less well-studied case of reward upshift

(Flaherty, 1996). cSNC is an animal paradigm of incentive relativity, including preshift trials followed by postshift trials. Typically, the preshift phase consists of one daily trial during 10 days. During the preshift, food-deprived rats are assigned to groups in which they receive two different levels of a reward, for example, 32% (downshifted group) and 4% (unshifted group) sucrose solution. Rats lick sucrose from sipper tubes while consummatory behavior is automatically measured in terms of contact with the sipper tube. The postshift phase lasts 5 days, one daily trial, in which all rats receive 4% sucrose. Thus, during the postshift phase rats in the downshifted group are presented with a less valuable reward (4% sucrose) than previously received (32% sucrose), while rats in the unshifted condition receive the same level of reward as during the preshift (4% sucrose). As shown in Figure 1, rats in the downshifted group (32-4) show a sharp suppression of consummatory behavior during Trial 11 (first postshift trial), when compared to unshifted rats (4-4). This suppression of behavior, relative to the unshifted rats, is defined as the cSNC effect. During Trial 12 (second postshift trial) and subsequent trials, downshifted rats usually show a gradual recovery of consummatory behavior to the level of the unshifted rats.

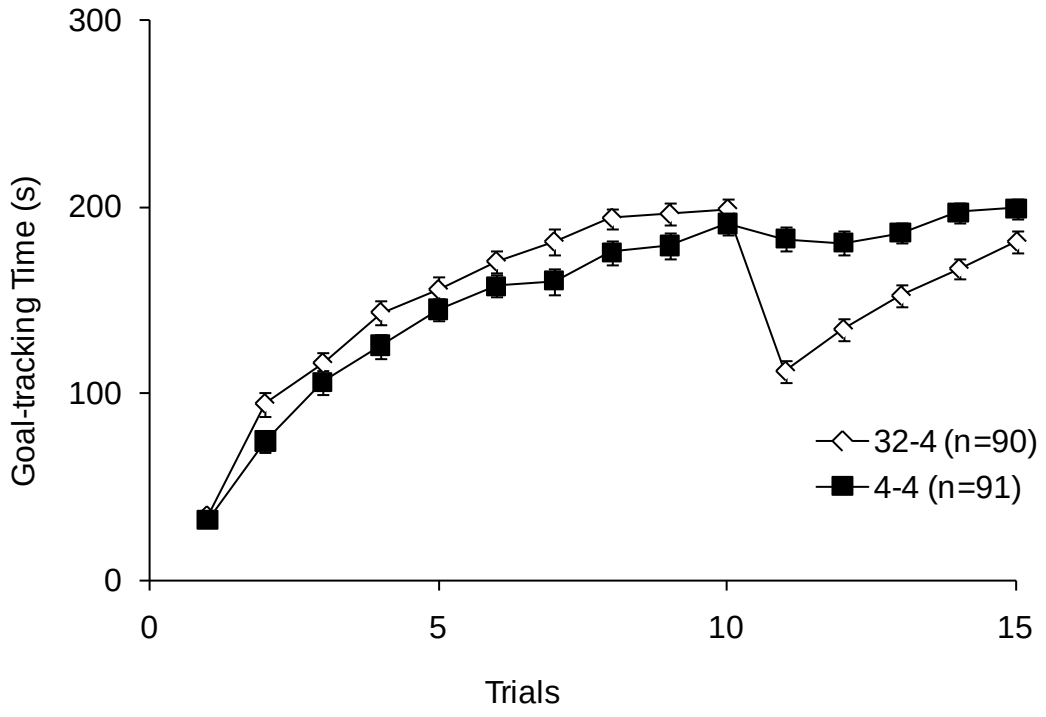

Figure 1. Example of cSNC obtained by averaging the performance of animals from different experiments treated in identical manner. The dependent variable is the cumulative time in contact with the sipper tube, called goal-tracking time and expressed in seconds (± SEM).

A sequence of behavioral processes is triggered by reward downshift in the cSNC situation, captured by Flaherty's (1996) multistage model shown in Figure 2. According to this model, the processes underlying the postshift phase of cSNC can be organized along two main consecutive stages. During Stage 1, peaking on Trial 11, rats detect the discrepancy between the expected reward (32%) and the obtained reward (4%). This in turn triggers a rejection of the novel unexpected reward and the search for the reward received during the preshift. This stage would be mainly reflected in the consummatory performance

during Trial 11. Papini and Pellegrini (2006) found that the detection of the reward downshift was controlled by the ratio between the sucrose concentration received during the postshift and the sucrose concentration received in the preshift (except when concentrations were extreme). Equal postshift/preshift ratios resulted in similar levels of consummatory suppression. For example, groups exposed to 32-4 or 16-2 downshifts in sucrose concentration exhibited similar levels of suppression, even though the absolute value and the absolute difference of the concentration were different. Thus, cSNC is a function of the downshift incentive ratio rather than the absolute value of the preshift solution or the difference between preshift and downshift solutions. In agreement with Flaherty's Stage 1, Papini and Pellegrini (2006) suggested that the reported scaling effect modulates the emotional reaction following the detection of the reward downshift. Such emotional reaction accompanies the rejection of the new, relatively less valuable, 4% sucrose and it is suggested by increased corticosterone levels following reward downshift (Pecoraro, de Jong, & Dallman, 2009), as well as by a set of behavioral aftereffects following reward omission that could be described as emotional (see Papini & Dudley, 1997). A behavioral process involving search for the missing reward is suggested by differential activity behavior for downshifted rats, when compared to unshifted rats, in ambulation and rearing behaviors in the usual contrast box (Pellegrini & Mustaca, 2000), and in the open field situation (Flaherty, Blitzer, & Collier, 1978), and increased exploration of the arms of a radial arm maze (Flaherty et al., 1994) and elevated plus maze (Pecoraro, Timberlake, & Tinsley, 1999) after rats underwent reward downshift in one of the arms.

During Stage 2, peaking on Trial 12, there is a behavioral conflict between the simultaneous tendencies to approach and avoid the downshifted reward, which is less valuable in comparison, but still valuable in itself. The conflicting tendencies may involve stress, as suggested by increased corticosterone levels in anticipation to and after Trial 12 (Flaherty, Becker, & Pohorecky, 1985; Mitchell & Flaherty, 1998). Finally, a recovery of consummatory behavior is driven by the absolute value of the new reward. There are individual differences in the rate of recovery from cSNC (Pellegrini, Wood, Daniel, & Papini, 2005). Faster rates of recovery can also be artificially selected and are related to an array of phenotypic characteristics (Ortega, Norris, Lopez-Seal, Ramos, & Papini, in preparation).

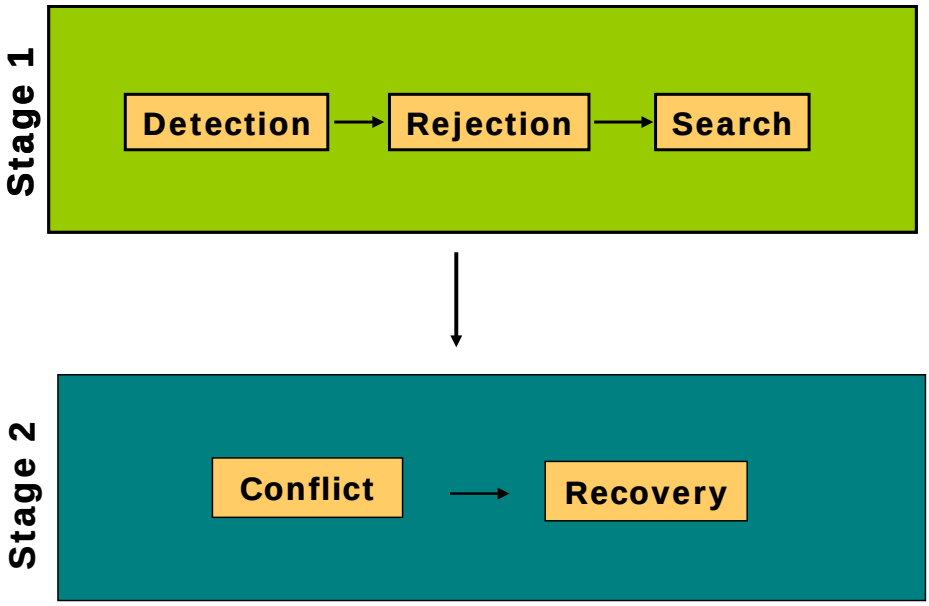

Figure 2. Flaherty's (1996) multistage hypothesis of cSNC.

Neural basis of cSNC

Psychopharmacology

The experimental strategy used most extensively to shed light on the neural basis underlying cSNC involves pharmacological manipulations. Studies using systemic drug administration have provided a general picture of the neurochemical systems relevant to cSNC. In addition, research has suggested the operation of different neurochemical systems for Flaherty's proposed stages. A review of the literature points to four types of drugs that modulate, in a specific fashion, different aspects of cSNC: opioids, anxiolytics (including ethanol and benzodiazepine anxiolytics), memory enhancers (D-cycloserine), and stress hormones (including corticosterone and ACTH).

Opioid receptors. Opioids are a family of neuroactive peptides that bind to four types of receptors: μ, δ, κ, and ORL-1 receptors (review on Papini & Ortega, in press). These receptors are widely, but differentially distributed in the mammalian brain (Mansour, Fox, Akil, & Watson, 1995). Nonspecific opioid receptor agonists and antagonists modulate both stages of Flaherty's model. Rowan and Flaherty (1987) found that administration of the nonspecific opioid receptor agonist morphine before Trials 11 or 12 resulted in the attenuation of cSNC. Except when large doses were used, the effect of morphine was specific to the reward downshift condition, as consummatory behavior in the unshifted group was not affected. In addition, morphine modulation of cSNC was eliminated when the nonspecific opioid receptor antagonist naloxone was coadministered. However, Rowan and Flaherty (1987) found no detectable effects when naloxone was administered by itself. Later, Pellegrini et al. (2005) reported that naloxone indeed enhanced consummatory suppression following cSNC when administered at a higher dose. Interestingly, they also reported that rats showing a slower rate of recovery from reward

downshift showed higher sensitivity to naloxone administration before an activity test, when compared to rats showing a faster rate of recovery. The results of blocking opioid receptors with naloxone can be attributed to at least four processes (Papini, 2009): (a) modulation of the downshift experience via either amplification of the rejection process or by reducing the incentive value of the sucrose; (b) disruption of the downshift detection process; (c) consolidation of the memory of the downshift event; and (d) induction of a conditioned taste aversion to the downshifted solution.

Daniel, Ortega, and Papini (2009) tested hypothesis (b), (c), and (d) with the following results. Naloxone modulated the comparison process that triggers cSNC, shifting the comparison from ratio-based to a difference-based comparison. Thus, opioid-receptor blockage enhanced consummatory suppression as a direct function of the absolute difference between preshift and postshift sucrose concentrations. In addition, they showed that posttrial 11 naloxone administration had no detectable effects on subsequent trials, suggesting that opioid receptors are not involved in the consolidation of the memory for the downshift event. Daniel et al. (2009) also showed that naloxone administration failed to support conditioned taste aversion to a novel 4% sucrose solution. Thus, processes (c) and (d) can be tentatively discarded as explanations for the effects of naloxone on cSNC. However, more research is needed to distinguish between opioid modulation on processes (a) and (b).

Specific opioid receptors differentially modulate Flaherty's Stage 1 or 2. For instance, δ opioid receptors seem to affect the behavioral processes involved in Stage 1, Trial 11, of cSNC. Wood, Daniel and Papini (2005) administered DPDPE, a selective δ receptor agonist, before Trials 11 or 12 in the cSNC situation. DPDPE attenuated the

consummatory suppression following reward downshift during Trial 11, but had no effect during Trial 12. Conversely, administration of naltrindole, a selective δ receptor antagonist, during Trial 11 and 12 resulted in enhanced suppression of consummatory behavior on Trial 11, but not on Trial 12 (Pellegrini et al., 2005). These results support the hypothesis that δ opioid receptors are selectively involved in the rejection process during Stage 1.

Processes involved in Stage 2 can be pharmacologically manipulated by the administration of drugs immediately after Trial 11 and before Trial 12. κ opioid receptors are involved differentially in the modulation of Stage 2 as suggested by Wood, Norris, Daniel, and Papini (2008). They administered U-50,488H, a κ opioid receptor agonist before Trial 11 or 12. No drug effects were detected on Trial 11, but administration before Trial 12 resulted in complex effects depending upon the dose. A low dose (1 mg/kg) attenuated cSNC while higher doses (3 and 10 mg/kg) enhanced it. Administration of U-50,488H immediately after Trial 11 resulted in an enhanced cSNC effect during Trial 12 with the 3 mg/kg dose, but no effect was observed with the 1 mg/kg dose. The enhancing effect of the 3 mg/kg dose could eventually be explained in terms of a conditioned taste aversion to the 4% solution induced by the drug. Thus, it appears that κ opioid receptors are involved in conflict and/or recovery from cSNC, but only at low doses.

Anxiolytics and ethanol. A second neurochemical system related to the Stage 2 is the GABAergic, as suggested by the administration of benzodiazepines. There are two types of GABA receptors, GABA-A and GABA-B. Of special interest are GABA-A receptors, which are allosterically modulated by a number of pharmacological manipulations. Benzodiazepines modulate GABA-A activity by binding to the benzodiazepine site in the receptor (Goetz, Wulff, & Wisden, 2009). Like benzodiazepines,

ethanol also acts as a positive allosteric modulator for GABA-A receptors. From a behavioral point of view, this kind of receptors is known to be connected to the modulation of anxiety-related behaviors. For instance, systemic administration of the benzodiazepine chlordiazepoxide to rats before placing them in an elevated plus maze resulted in a higher percentage of time spent in the open arms, when compared to vehicle rats (Pellow, Chopin, File, & Briley, 1985). Also, pre-training intra-amygdala administration of the benzodiazepine midazolam had no detectable effects on passive avoidance training, but impaired its retention 48 hours after training (Dickinson-Anson & McGaugh, 1993).

Administration of benzodiazepines before Trial 12 attenuates cSNC, increasing consummatory behavior for the downshifted group (e.g., Flaherty, Clark, & Coppotelli, 1996; Flaherty, Grigson, & Rowan, 1986). Interestingly, the benzodiazepine chlordiazepoxide had an effect during the first downshift trial, but only when using a procedure in which rats experienced several reward downshifts, giving them previous experience with the downshift situation (Flaherty, Clark, & Coppotelli, 1996). Ethanol also resulted in attenuation of the development of cSNC only when applied before the third downshift in a procedure involving several reward downshifts (Kamenetzky, Mustaca, & Papini, 2008). Early studies suggested that administration of a number of benzodiazepines before Trial 11 has no detectable effect on cSNC (e.g., Flaherty, Clark, & Coppotelli, 1996). An interpretation of the effects of benzodiazepines on Trial 12 in anxiolytic terms would suggest that benzodiazepines are involved in the conflict process of cSNC (Flaherty, 1996). However, at least one other study reported a chlordiazepoxide effect on Trial 11 (Genn, et al., 2004). Infusion of the benzodiazepine diazepam into the amygdala, but not into the hippocampus, reduced cSNC on Trial 12 (Liao & Chang, 2003). Thus, GABAergic transmission in the amygdala is an important part of the neural circuit underlying cSNC.

Memory modulation. Posttrial pharmacological manipulations are used to study memory consolidation (e.g., McGaugh, 2002). The facilitating effects on fear extinction of D-cycloserine (Bouton, Vurbic, & Woods, 2008), a partial agonist at the glycine site of NMDA receptors, made this drug of special interest to test the hypothesis of the formation of an aversive memory following cSNC. Norris, Ortega, and Papini (submitted) administered D-cycloserine after Trial 11 and found that it enhanced the aversive memory formed during cSNC, as it retarded the recovery following the reward downshift. Similar results were found with pretrial 11 administration. Also, the effect was specific to the aversive memory, as D-cycloserine administration did not affect consummatory behavior unrelated to cSNC.

McGaugh, Ferry, Vazdarjanova, and Roozendaal (2000) proposed that norepinephrine was a key player for memory modulation of aversive memories by the amygdala. Despite this role for aversive memory modulation, Flaherty (1996) reviewed some published and unpublished experiments suggesting that cSNC seems to be impervious to manipulations involving norepinephrine. Blockage of neither α- nor β-adrenergic receptors had no detectable effect on the course of cSNC. This suggests a major difference in the neurochemical mechanisms of cSNC and aversive procedures such as passive avoidance. The consolidation of the aversive memory following passive avoidance learning is both modulated by the manipulation of norepinephrine levels, via systemic epinephrine administration, and posttrial administration of opioids (Izquierdo & Dias, 1983, 1985), neither of which have been shown to modulate cSNC (Daniel et al., 2009; Flaherty, 1996).

Finally, although Daniel et al. (2009) reported that posttrial naloxone did not seem to modulate memory consolidation in cSNC, GABA-A and opioid receptors are involved in the modulation of memory consolidation in passive avoidance (McGaugh, Ferry, Vazdarjanova, & Roozendaal, 2000; Izquierdo & Dias, 1983, 1985).

Stress hormones. The stress hormones of interest are corticosterone and ACTH. Corticosterone, a hormone widely associated with stress responses (e.g., Herman, 2009), is produced in the adrenal cortex, a component of the HPA axis. The release of corticosterone is controlled by ACTH, a hormone produced in the anterior pituritary gland, which is in turn modulated by the hypothalamus. The HPA axis is a key mechanism underlying behavioral and physiological adjustments to environmental demands (Pecoraro & Dallman, 2009).

Two types of evidence link stress hormones to cSNC. The first type of evidence, mentioned earlier, is that levels of plasma corticosterone and ACTH were higher in rats undergoing cSNC than in unshifted controls after Trial 11 (Pecoraro et al., 2009) and before and after Trial 12 (Flaherty et al., 1985; Mitchell & Flaherty, 1998). Early work by Flaherty et al. (1985) reported no differences in corticosterone levels after Trial 11 due to the reward downshift, which suggested that corticosterone was selectively involved in the modulation of Stage 2. However, recent evidence (Pecoraro et al., 2009) of changes in corticosterone levels after Trial 11 expands the role of corticosterone to processes involved in Stage 1, possibly to the emotional activation underlying the rejection process.

The second type of evidence connecting corticosterone to cSNC involves its administration immediately after Trial 11, which results in prolonged consummatory suppression in later trials (Bentosela, Ruetti, Muzio, Mustaca, & Papini, 2006) This effect was observed in consummatory, but not in anticipatory contrast; it was found to depend on a large, but not small reward downshift; and could not be explained in terms of conditioned taste aversion (Ruetti, Justel, Mustaca, & Papini, 2009).

Dopaminergic modulation of cSNC? Dopaminergic release in the nucleus accumbens has been related to different aspects of incentive motivation (e.g., Phillips, Vacca, & Ahn, 2008), including the rewarding effects of sucrose solutions (Hajnal & Norgren, 2008). Also, administration of quinpirole, a dopamine D2 receptor agonist, resulted in retardation of behavioral extinction after nose-poke training using water as reinforcer (Kurylo & Tanguay, 2003). Evidence of dopaminergic modulation specific to cSNC was provided by Genn, Barr, and Phillips (2002), who applied amilsupride before Trial 11 and found that a higher dose (60 mg/kg), but not a lower dose (10 mg/kg), reduced cSNC. Amilsupride binds preferentially to D2 and D3 dopamine receptors and has a biphasic effect depending upon the dose; low doses facilitate dopamine transmission by blocking presynaptic receptors and high doses block postsynaptic D2 receptors (Genn et al., 2002). However, Flaherty, Becker, Checke, Rowan, and Grigson (1992) found that administration of the dopamine antagonists haloperidol and chlorpromazine (antagonizing D2 and D1-D4 receptors, respectively) before the Trial 11 in cSNC resulted in nonspecific modulation of consummatory behavior (i.e., the effect was not unique to the downshifted group). These contradictory results render unclear the role of dopamine in cSNC. In spite of Flaherty's (1996) proposal that dopaminergic function is related to absolute reward value rather than to relative reward value in the cSNC situation, there are not enough data to

discard a dopaminergic involvement specific to cSNC, as suggested by measurement of DA in the nucleus accumbens (see relevant brain studies below).

The use of pharmacological manipulations has helped identify some of the neurochemical systems responsible for cSNC. However, virtually all the research has focused on systemic manipulations, which leaves open the question of which brain areas are responsible for the reported effects of the various pharmacological manipulations.

Brain areas involved in cSNC

There is evidence for the involvement of a number of particular brain areas in different aspects of cSNC. The measured behavioral output in the cSNC situation usually involves some variable associated with licking for the sucrose solution. The motor mechanisms of licking for sucrose in rats, as part of the consummatory phase of fluid ingestion, have been described using the concept of a central pattern generator (Travers, Dinardo, & Karimnamazi, 1997). Cycles of licking in rats typically occur in bursts organized in species-typical modal frequencies. The organization of the cycles of motor movement depends upon the kind of sapid stimuli provided to the rat (e.g., sucrose solutions).

Discriminatory motor responses to taste stimuli in general, and to sucrose in particular, seem to be organized at the caudal brainstem level, as suggested by a series of studies using a taste reactivity test that measured facial responses to the oral infusion of gustatory stimuli. Grill and Norgren (1978a) reported that administration of sucrose in the oral cavity was accompanied by a series of rhythmic mouth movements, followed by tongue protrusions and lateral tongue movements, which can be interpreted as appetitive behaviors. The administration of quinine (a bitter substance) provoked a sequence of movements, such as gaping and different body responses, that appeared to facilitate the

removal of the stimulus from the mouth, and were interpreted as aversive responses. The control by the caudal brainstem of these appetitive and aversive responses to liquid stimuli was suggested after Grill and Norgren (1978b) showed that drinking-related behavior, as measured by a taste reactivity test, of rats that underwent a chronic decerebrate preparation was similar to the performance of intact rats; decerebrated rats showed a similar sequence of appetitive and aversive behaviors to sucrose and quinine, respectively, compared to sham controls. In addition, the pattern of responses to sucrose solutions of decerebrated rats was modulated by food deprivation and was tightly related to the stimuli (Grill & Norgren, 1978c). Finally, neurons in the reticular formation within the brainstem send descending connections to tongue motor neurons (Norgren, 2005).

On the input side of the consumption of fluids, sucrose is detected by taste receptors in the tongue and this information is conveyed to the brain by cranial nerves VII (facial), IX (glosopharyngeal), and X (vagus). Several nuclei within the brainstem are related to gustatory information. The first brainstem area to receive ascending projections from the periphery is the NST, which has been considered the first central relay for taste information (Norgren, 2005). The PbN receives ascending information from peripheral gustatory receptors via the NST. In turn, the PbN sends ascending fibers that make synaptic connection with the BNST.

Brain correlates for cSNC have been reported at the level of the brainstem. Grigson, Specter, and Norgren (1994) reported that rats that underwent bilateral electrolytic lesions of the PbN failed to show cSNC. The pattern of the variables measuring several aspects of licking behavior during the preshift and postshift phases suggested that the elimination of cSNC was not due to disruption of motivational, memory, or reward comparison factors. However, motor effects of the lesions are possible as rats with PbN lesions showed a

generally lower number of licks when compared to sham rats. In addition, blunted responsiveness to sapid solutions following PbN lesions (e.g., Reilly & Trifunovic, 2000) may affect the interpretation of PbN's role in the cSNC situation, as rats with PbN lesions showed lower number of licks for the 32% sucrose during the preshift, potentially affecting the comparison between the preshift and postshift sucrose solutions. Gustatory processing of sucrose solutions on the PbN is modulated by activity in the CeA, the lateral hypothalamus, and the gustatory cortex (Lundy & Norgren, 2004). In particular, the PbN is heavily connected to the CeA, an area that seems to be critical for cSNC as well (Becker, Jarvis, Wagner, & Flaherty, 1984). Thus, Grigson et al. (1994) suggested that lesions of the PbN result in a devaluation of the hedonic value of the sucrose reward following cSNC that depends on the interaction between the PbN and forebrain areas, particularly the CeA.

Taste information from the PbN also ascends to the VPM (also referred as the gustatory thalamus), in the diencephalon, which is considered as the central relay area for taste information before the cortex (Norgren, 2005). Bilateral electrolytic lesions of the VPM also eliminated the cSNC effect (Reilly & Trifunovic, 1999). The elimination of cSNC did not seem to be related to the response to the absolute values of the sucrose rewards or the processing of the downshift memory (Reilly & Trifunovic, 2000). Thus, it seems plausible that VPM lesions disrupt the reward comparison mechanism activated by the downshift event. Such comparator role for the VPM seems to be specific to sucrose solutions as chemical lesions of the same area did not have any measurable effect on iSNC, which uses food pellets as reward (Sastre & Reilly, 2006).

The role of several telencephalic areas on cSNC has also been studied. Bilateral electrolytic lesions of the septal nuclei did not have noticeable effects on the initial response to the reward downshift in the cSNC situation (Flaherty & Hamilton, 1971).

Further studies using similar lesions resulted in the disruption of the cSNC effect when a longer retention interval (4 days vs. 1 day) between the last preshift trial (Trial 10) and the first postshift trial (Trial 11) was used, relative to sham controls (Flaherty, Capobianco, & Hamilton, 1973). In addition, search behavior following cSNC was enhanced by bilateral electrolytic lesions of the septal area (Flaherty, Powell, & Hamilton, 1973). The pattern of these results suggest a role for the septal area on the discrimination between preshift and postshift sucrose values, the memory of the preshift reward, or the reward comparison mechanism. However, the lack of an effect of septal lesions on cSNC using the usual procedural parameters suggests that further studies are needed to characterize the function of the septal area on cSNC.

The nucleus accumbens seems to modulate the current incentive value of the sucrose, but not the generation of expectancies during the downshift in the cSNC situation. Genn, Ahn, and Phillips (2004) measured dopamine efflux in the nucleus accumbens using brain dialysis and high performance liquid chromatography during the first downshift trial of cSNC. Dopamine efflux was similar for downshifted and unshifted groups immediately before the downshift trial. An increase on dopamine efflux was observed for the unshifted but not the downshifted group during the trial (10 min). In addition, activation of the nucleus accumbens after Trial 11 was observed using c-fos immunoreactivity (Pecoraro & Dallman, 2005).

However, bilateral excitotoxic (Eagle et al., 1999) or electrolytic (Leszczuk & Flaherty, 2000) lesions of the nucleus accumbens did not affect cSNC. Some procedural differences between the Genn et al. (2004) study and the usual cSNC situation may have contributed to the divergent results between dopamine efflux measurement and lesions in the nucleus accumbens. Genn et al. (2004) reported a milder (85-90%) food deprivation

level than that typical of cSNC studies (80-85%). Also, the sipper tube may have included a more salient instrumental component, as rats had to push a valve with each lick response to obtain sucrose.

A role of the amygdala in reward value for sucrose consumption is suggested by an experiment showing that sucrose solution, but not distilled water administration, resulted in differential CeA activation, as measured by functional magnetic resonance imaging in rats (Tabuchi et al., 2002). Direct evidence of amygdala involvement in cSNC was reported by Becker et al. (1984). They found that bilateral electrolytic lesions of the CeA completely eliminated the cSNC effect. In addition, basolateral amygdala lesions attenuated the cSNC effect. A plausible role of the amygdala on cSNC is of a reward comparison deficit similar to observed with VPM lesions (Reilly & Trifunovic, 1999). The potential role of CeA in the detection process was also discussed by Becker et al. (1984) in their work on CeA lesions. Taste reactivity can be an issue in the interpretation of their data as it is not clear why there were differences between the 32% and 4% sucrose licking in sham controls but not in the two lesioned groups during preshift trials. In addition, the elimination of cSNC by CeA lesions could be related to a diminished neophobic response to the novel 4% solution, as suggested by the lesions in the insular cortex (Lin et al., 2009). In short, although lesions of the CeA eliminated cSNC, the precise functional role of this area is still not clear. Interestingly, a double dissociation between the roles of the amygdala and the hippocampus for cSNC and iSNC is suggested by a series of experiments in which ibotenic acid lesions in the central or basolateral amygdala produced no detectable effects on iSNC (but disrupted cSNC; see above), although instrumental performance was affected during subsequent trials (Salinas, Parent, & McGaugh, 1996), while similar lesions of the hippocampus disrupted iSNC but not cSNC (Flaherty, Coppotelli, Hsu, & Otto, 1998).

Recent results suggest that two cortical areas, the insular cortex and the mPFC, play a role in cSNC. Lin, Roman, and Reilly (2009) tested the effects of bilateral chemical lesions (induced by NMDA infusion) in the insular cortex on cSNC. Lesioned rats showed no evidence of cSNC while sham lesioned rats showed the typical consummatory suppression following reward downshift. Interestingly, insular lesions resulted in a gradual reduction of performance following reward downshift to the level of unshifted controls. Lin et al. suggested that this pattern of responding could be explained in terms of a neophobic reaction to the solution presented during the downshift and a differential role for this area compared to the VPM. However, the gradual reduction of consummatory behavior can be also explained in terms of an adjustment to the new reward conditions, in which rats simply started to respond to the absolute value of 4% sucrose and gradually decreased their behavior to levels supported by the new absolute value of the reward (i. e., rats failed to show an emotional response to the downshift; Papini, 2003). In turn, the mPFC may play a role on the recovery from cSNC. Using a reward downshift paradigm similar to cSNC, Pecoraro, de Jong, Ginsberg, and Dallman (2008) reported that rats undergoing bilateral excitotoxic lesions of the mPFC showed no differences in the degree of consummatory suppression following reward downshift, when compared to sham rats, but did show higher levels of sucrose consumption during the second half of an extended postshift phase (12 trials). Following the postshift, rats were presented with 32% sucrose again during 6 trials and later were downshifted for a second time. No behavioral differences were reported during the second downshift. Although it may be tempting to conclude from this pattern of results that mPFC lesions resulted in faster recovery from reward downshift, the reported study did not include unshifted controls, thus limiting the interpretation of the results to the effects of the lesion on the rate of change of consummatory behavior following reward

downshift. Thus, it is not possible to determine whether the effect of mPFC lesions were specific to the downshift event or more generally related to sucrose consumption. Further studies addressing explicitly the comparison between downshifted and unshifted rats during the postshift phase are needed to clarify the role of the mPFC on recovery from cSNC.

Integration of neurobiological results

A simplified model of the neural pathways underlying cSNC is proposed in Figure 3. This model is derived from the general brain model for sucrose consumption proposed by Jones, Fontanini, and Katz (2006), the studies evaluating several brain areas underlying cSNC, and a study in which c-fos-like immunoreactivity was measured following cSNC (Pecoraro & Dallman, 2005). Four main descriptive divisions are proposed, starting with the input and output mechanisms in the periphery, and then ascending to brainstem, diencephalic, and telencephalic areas.

During the preshift, when downshifted rats have access to 32% sucrose, the brain activation pattern seems to be related to the control of functions such as palatable feeding, viscerosensory processing, and parasympathetic control (Pecoraro & Dallman, 2005). Brain activation was observed mainly in brainstem nuclei, such as the NST, and in areas of the hypothalamus. Although differential dopaminergic release in the nucleus accumbens increases as a function of sucrose concentration (Hajnal, Smith, & Norgren, 2004), there was no activation during terminal preshift behavior in the nucleus accumbens. Finally, the CeA showed a weak pattern of activation during the terminal preshift.

Pecoraro and Dallman (2005) also reported widespread brain activation during the downshift (Trial 11). The pattern of activation is consistent with areas that were evaluated with lesions, such as the mPFC, insular cortex, lateral amygdala, lateral and medial septum, PbN, and VPM. In addition, c-fos measurement also confirmed the role of areas consistent

with the proposed sucrose consumption model, such as the lateral hypothalamus. The nucleus accumbens was activated, an effect consistent with decreased dopamine release for the downshifted group during Trial 11 (Genn et al., 2004), but inconsistent with the lesion studies targeting this area, which found no detectable effects (Leszczuk & Flaherty, 2000). Finally, the activation of some unexplored brain areas suggests new avenues for research on the neural basis of cSNC, such as the ACC, orbital cortex, lateral and anterodorsal preoptic area, superior colliculus, dorsal raphe, and the pontine central gray. Unexpectedly, almost no c-fos activity was observed during Trial 12. The only area activated was the pontine central gray (Pecoraro & Dallman, 2005).

Pecoraro and Dallman (2005) suggested that the event of unexpected reward downshift is modulated by a top-down activation of the cortex and selected subcortical nuclei that results in an extensive reorganization of sucrose solution-related consummatory behavior. In other words, cortical mechanisms modulate the reorganization of brainstem-based mechanisms for sucrose solution consumption. It is unclear what brain areas would be critical for the modulation of the processes described in Flaherty's (1996) multistage hypothesis (Figure 2). The effects of brain lesions of the VPM, CeA, and insular cortex are consistent with a role in the detection of the downshifted solution. Decreased dopaminergic expression in the nucleus accumbens during downshift and elimination of cSNC by CeA lesions may be both responsible for the rejection of the novel, relatively less valuable, 4% sucrose solution. Finally, search behavior may be regulated by the septal area, as lesions there resulted in increased search behavior following cSNC (Flaherty et al., 1973).

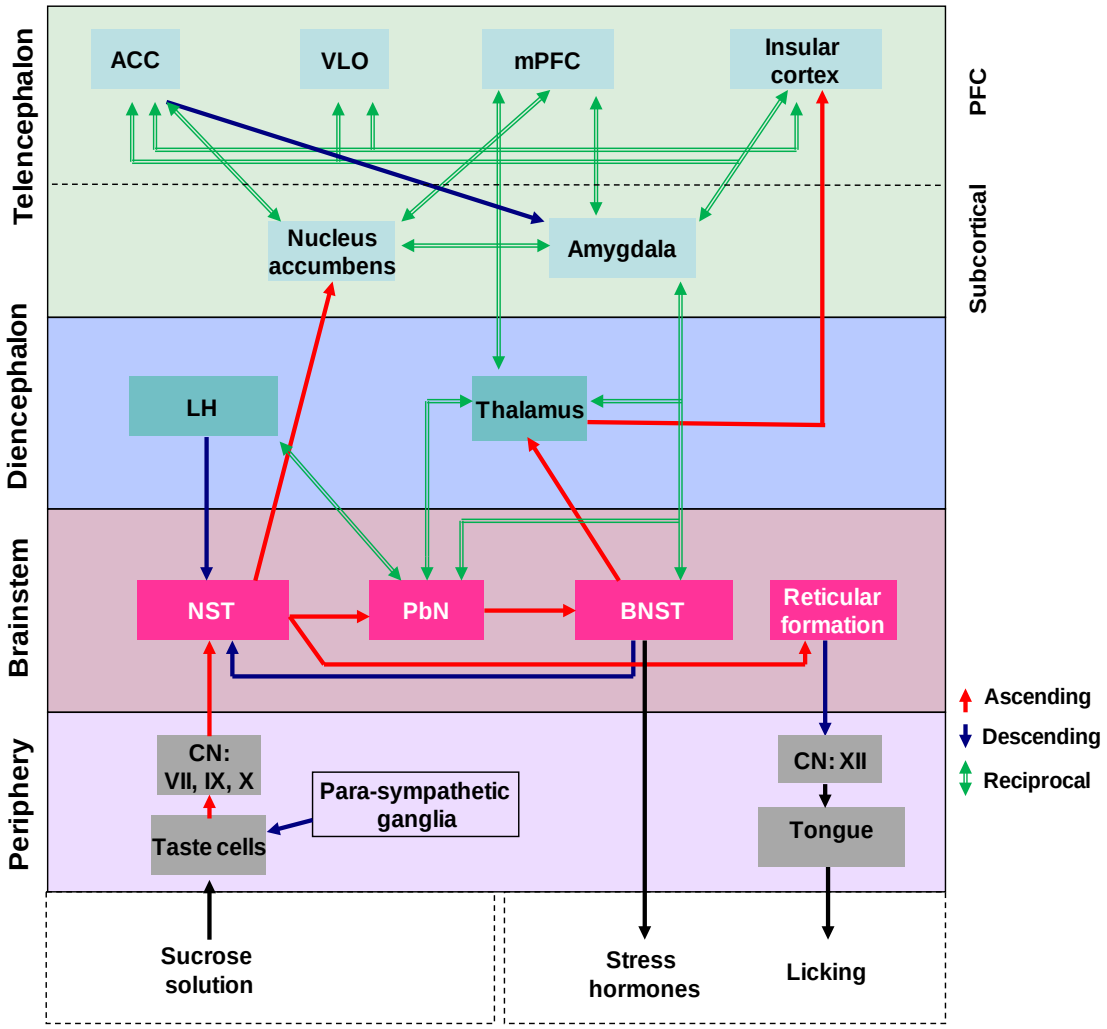

Figure 3. Simplified model of the neural circuitry involved in cSNC, as modified from the gustatory system model proposed by Jones, Fontanini, and Katz (2006). ACC: Anterior cingulate cortex. BNST: Bed nucleus of the stria terminalis. CN: Cranial nerves. LH: Lateral hypothalamus. mPFC: Medial prefrontal cortex. NST: Nucleus of the solitary tract. PbN: Parabrachial nucleus. VLO: Ventrolateral orbital cortex. VPM: Ventral posteriomedial nucleus of the thalamus.

Prefrontal modulation of cSNC

The PFC is a structurally and functionally complex cortical region. The limits of the PFC can be defined by bidirectional connections to thalamic areas, which are relevant for PFC development (Uylings, Groenewegenb, & Kolb, 2003). Several cortical fields within the rat PFC can be roughly divided in three regions: dorsolateral, medial, and orbital. Not surprisingly, different areas within the PFC are associated to different behaviors and a particular area can be associated to more than one behavior. For instance, lesions of the ventromedial PFC (including infralimbic areas) retarded fear conditioning extinction (Quirk, Russo, Barron, & Lebron, 2000), while lesions of the orbitofrontal cortex impaired the formation of a negative incentive value for a conditioned stimulus paired with food (unconditioned stimulus), when the food was later devaluated using lithium chloride (Gallagher, McMahan, & Schoenbaum, 1999). In addition, lesions of the orbitofrontal cortex impaired autoshaping acquisition (Chudasama & Robbins, 2003).

The PFC is involved in neural networks including, but not limited to, thalamic nuclei, basal ganglia, and sensory and motor cortices (Uylings et al., 2003). As suggested by Miller and Cohen (2001), brain networks associated to the PFC are in an exceptional location to coordinate a wide array of neural processes, with the potential for dynamic control of various types of behaviors. Furthermore, the PFC may be especially relevant for the control of behavior in a top-down manner, as in situations requiring dynamic responses that in turn may involve the organization of sensory/internal inputs, cognitive information, and motivated responses. Thus, the PFC may underlie the coordination of some of the behavioral and motivational adjustments in sucrose-related consummatory behavior following cSNC.

The role of the PFC on behavioral processes underlying cSNC has not been thoroughly studied. Although the effects of insular cortex and mPFC lesions on cSNC have been reported, the functional, and perhaps dissociable, role of the different areas of the PFC is still not clear. From the complex neural pattern of activation following cSNC, a particularly promising approach is suggested by the hypothesis of cortical top-down control of consummatory behavior. In particular, a set of functionally distinct areas in the PFC may underlie some of the behavioral processes associated with cSNC. In the present work, it is hypothezised that three prefrontal areas may be associated to cSNC on the basis of evidence from several sources, such as c-fos activity in the PFC after reward downshift, opioid receptor density, and parallels suggested by Gray's fear=frustration theory (Gray, 1982).

PFC candidate areas for further research are suggested by Pecoraro and Dallman (2005), who reported c-fos activation following cSNC in the ACC, orbital cortex, and mPFC. In addition, the concentration of mu and delta opioid receptors in the neocortex is very high, as shown by Mansour, Fox, Akil, and Watson (1995). As reviewed above, there is evidence pointing to a role of mu and delta opioid receptors in the modulation of behavioral processes underlying cSNC. In particular, modulating the rejection process to the novel 4% sucrose, and/or disrupting the downshift detection process (Rowan & Flaherty, 1987; Daniel, Ortega, & Papini, 2009; Pellegrini et al., 2005; Wood et al., 2005). Moreover, Gray (1982) proposed that similar neural circuits are involved in the emotional anticipation to physical pain and psychological pain, known as the fear=frustration hypothesis. Extending this hypothesis, Papini, Wood, Daniel, and Norris (2006; Papini, 2003) suggested mechanistic parallels between brain pathways underlying the unconditioned response to reward downshift (primary frustration) and the unconditioned response to electric shock (physical pain). One implication of this hypothesis is that

physical pain and frustration should interact, as indeed seems to be the case (Mustaca & Papini, 2005; Ortega, Daniel, Davis, Fuchs, & Papini, submitted). For example, Ortega et al. reported that peripheral pain caused by the formalin test (intradermal formalin injection in a hindpaw) added to the psychological pain induced by cSNC, enhancing response suppression selectively in animals experiencing reward downshift. Moreover, Mustaca and Papini (2005) reported increased hypoalgesia, as measured in the hot plate test, following the second downshift trial during cSNC. A second implication of this hypothesis is that some brain areas known to modulate physical pain may be involved in the frustration response following cSNC.

The ACC is associated to some forms of physical pain. Bilateral ACC lesions resulted in a decrease of pain responses following formalin administration, but had no effect on physical pain caused by ligation of a spinal nerve (Donahue, LaGraize, & Fuchs, 2001). As described, formalin administration enhanced cSNC, which suggests that the ACC may be related to this kind of psychological pain. Further relations between the ACC and cSNC are suggested by reports of ACC lesions modulating instrumental responses guided by differential reward expectancies. Schweimer and Hauber (2005) reported that ACC lesions biased rat responding in a maze to an option involving a smaller reward and less work, while the lesion had no detectable effect on the ability to discriminate between different magnitudes of reward. Bilateral lesions of the ACC may impair endogenous opioid function, thus causing retardation of recovery from cSNC.

The VLO is a set of areas within the orbital cortex also related to physical pain. A role of the VLO on modulation of pain-related responses following the formalin test is suggested by Xie, Wang, Huo, Jia, and Tang (2004), who reported that morphine, a nonselective opioid receptor agonist, administered unilaterally into the VLO attenuated

pain-related behaviors induced by the formalin test. Interestingly, bilateral lesions of the orbitofrontal cortex (including VLO and infralimbic areas of the cortex) impaired sign-tracking autoshaping acquisition and lead to perseveration of the original trained response in a discrimination reversal procedure, while mPFC lesions (including infralimbic and prelimbic areas of the cortex) did not affect autoshaping performance (Chudasama & Robbins, 2003). These data are of special interest given that a functional dissociation is suggested between the VLO and mPFC and thus a possible dissociation in their function in cSNC. Although lesions of the mFC (Pastoriza, Morrow, & Casey, 1996) and microinjections of amphetamine within the mPFC had not detectable effects on pain-related behavior after formalin administration (Altier & Stewart, 1993), Pecoraro et al. (2008) reported that mPFC lesions affected behavioral performance following reward downshift. Thus, although the mPFC seems to be involved in cSNC, it is possible that its role is unrelated to physical pain.

Bilateral electrolytic lesions before cSNC training are proposed as the strategy to clarify the functional significance of different PFC areas on cSNC. The present experiments aim at addressing two specific questions:

(a) What are the effects of bilateral lesions of three functionally distinct prefrontal areas, ACC, VLO, and mPFC, in the cSNC situation?

(b) Would the effects of the bilateral lesions of VLO and mPFC extend to open field behavior, sucrose sensitivity, and appetitive acquisition (autoshaping)?

Experiment 1

A role of the PFC on cSNC is suggested by the disruption of the initial response to cSNC following lesions of the insular cortex (Lin, Roman, & Reilly, 2009) and widespread prefrontal activation, as measured by c-fos expression after Trial 11 (Pecoraro & Dallman,

2005). Interestingly, insular cortex activation was found during the formalin test, as measured by positron emission tomography (Shih et al., 2008). Insular cortex involvement in both cSNC and peripheral pain is in agreement with the pain=frustration hypothesis (Papini, 2003). Following this hypothesis, a potential relationship between ACC and cSNC is suggested by diminished pain-related behavior following the formalin test of lesions on the ACC (Donahue et al., 2001) and formalin-induced enhancement of cSNC (Ortega et al., submitted). In addition, the ACC was activated following Trial 11 in cSNC (Pecoraro & Dallman, 2005) and ACC lesions diminished iSNC (Gurowitz, Rosen, & Tessel, 1970). Thus, bilateral electrolytic lesions of the ACC before cSNC allow for an evaluation of the role of the ACC on the initial response to and the recovery from cSNC.

Method

Subjects. Forty Long-Evans male rats were used for this experiment. The animals were purchased from Harlan Laboratories (Indianapolis, IN). The subjects were 90 days old, maintained at 81-84% of their free-food weight, and housed in individual wire-bottom cages with free access to water. Each cage contained a rodent retreat for enrichment. During the experiment, animals were under a 12 h light/12 h dark schedule (lights on at 07:00 h), in noise-controlled rooms with constant room temperature (22-23 °C) and humidity between 50 and 65%. They were fed with standard laboratory rat chow administered at least 15 min after the end of training trials.

Apparatus. cSNC training was conducted in 4 conditioning boxes (MED Associates, St. Albans, VT) made of aluminum and Plexiglas (29.3 × 21.3 × 26.8 cm, L×H×W). The floor consisted of steel rods running parallel to the feeder wall. A tray with corncob bedding was placed below the floor to collect feces and urine. In the feeder wall was a hole 1 cm wide, 2 long, and 4 cm from the floor through which a sipper tube, 1 cm in

diameter, was inserted. When fully inserted, the sipper tube was flush against the wall. Diffuse light was provided by a house light located in the center of the box's ceiling. A computer located in an adjacent room controlled the presentation and retraction of the sipper tube. When the rats made contact with the sipper tube, a circuit involving the steel rods in the floor was closed and the signal was recorded by the computer. This provided a measure of cumulative contact with the sipper-tube, called goal-tracking time (measured in 0.05-s bins). Each conditioning box was placed in a sound-attenuating chamber containing a speaker to deliver white noise and a fan for ventilation. Together, the speaker and fan produced noise with an intensity of 80.1 dB (SPL, scale C).

Surgery. Rats received bilateral electrolytic lesions of the ACC. Animals were deeply anesthetized by IP ketamine (50 mg/kg) and xylazine (2.61 mg/kg). Animals were then positioned in a stereotaxic frame with blunt-tipped ear bars. A midline incision was made in the scalp and two burr holes were drilled to insert a single electrode twice, one for the ACC in each hemisphere. The coordinates were 0.0 mm (i. e., on bregma), +/-0.9 mm lateral to bregma, and -3.2 mm D/V at a 15 degree angle (coordinates from Paxinos & Watson, 2007). Bilateral electrolytic lesions were performed by passing a 0.5 mA current, during 15 s, using a 0.3 mm electrode, which was insulated except for the tip. Rats were allowed 5-8 days to recover from surgery. Notice that performing electrolytic lesions will also disrupt any fibers of passage within the targeted area. Antibiotics were applied as needed. Food and water was continuously available in the cage. After recovery from surgery, animals were deprived of food to 81-84% of their free-feeding weight. Behavioral training started when the weight of all rats reached the target range.

Behavioral procedure. Four groups of rats (*n*=10) were established by random assignment. In groups 32/ACC and 32/Sham rats had acess to 32% sucrose solution during Trials 1-10 (preshift), but this solution was replaced by a less sweet 4% solution during Trials 11-15 (postshift). In Groups 4/ACC and 4/Sham rats had access to the 4% sucrose solution throughout the 15 trials. Solutions were prepared weight/weight, by mixing 32 g (or 4 g) of sucrose for every 68 g (or 96 g) of distilled water. Rats were randomly assigned to the lesion conditions (ACC vs. Sham), and then rats within each lesion condition were randomly assigned to the contrast conditions (downshifted vs. unshifted).

Two types of control conditions were included in this experiment. First, behavioral controls involved groups not exposed to the reward downshift (i.e., always given 4% sucrose; unshifted controls). Second, sham controls were exposed to the surgery procedures and electrode insertion, but no current was delivered.

Histology. When all behavioral testing was finished (after Trial 15), animals were sacrificed using CO_2. The brains were removed and stored in 10% formaldehyde for at least 24 h. Using a microtome, 80 µm coronal sections were sliced, mounted on gelatin coated glass slides and stained with thionin. An experimenter blinded to behavioral outcome performed histological analysis under 40X magnification to determine the location and extent to tissue damage relative to plates form the atlas of Paxinos and Watson (2007). Animals whose lesions were not located in the target ACC were discarded from the analyses.

Results and discussion

Histology. One animal (ACC) presented a lesion that extended to motor cortices, and was not considered for statistical analysis. Nineteen animals had at least 75% bilateral damage to the ACC. Histological analysis (Figure 4) indicated that the average anterior-

posterior extent of the damage for the largest percentage of animals was localized between -0.3 to -0.4 mm relative to bregma. More than 75% of the animals had damage between 0.2 and -0.92 mm relative to bregma. The distribution of damage for animals in the downshifted vs. unshifted conditions was similar.

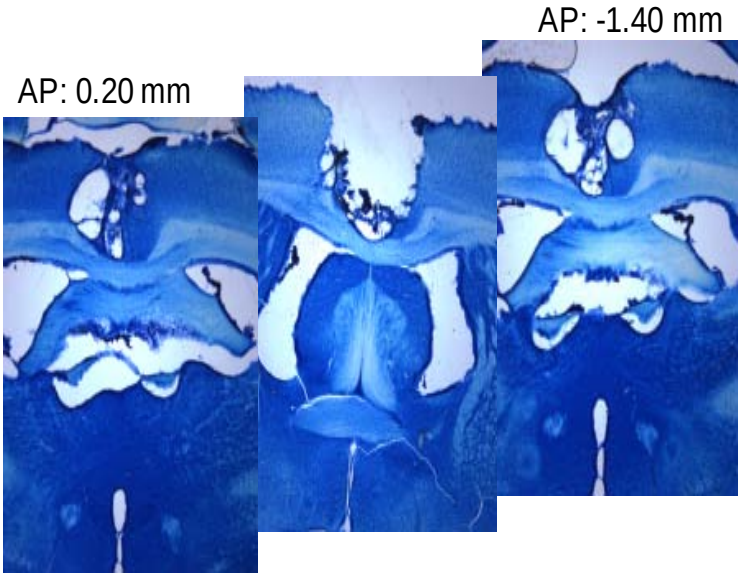

Figure 4. Example of ACC lesions from Experiment 1.

cSNC. Results can be seen in Figure 5. During the preshift phase, rats learned to drink the sucrose solutions, with no differences between the experimental groups. This was supported by a Lesion (ACC, Sham) x Contrast (32%, 4%) x Preshift Trial (1-10) analysis of variance (ANOVA), which revealed a significant effect of trial, $F(9, 315) = 116.84$, $p < 0.01$, but nonsignificant main effects of lesion, contrast, or their interaction, Fs < 1.01, ps > 0.37. During the postshift phase, there was an effect of the ACC lesions on cSNC. There was a triple interaction between lesion (ACC, Sham), contrast (32%, 4%), and postshift trial (11-15), $F(4, 140) = 2.86$, $p < 0.03$. Also significant were the trial by contrast interaction, $F(4, 140) = 6.56$, $p < 0.01$, the trial main effect, $F(4, 140) = 19.16$, $p < 0.01$,

and the contrast main effect, $F(1, 35) = 14.33$, $p < 0.01$. All other effects were nonsignificant, Fs < 1.54, ps > 0.19.

For further analyses of the recovery of lesioned and sham groups, one-way ANOVAs were conducted between the downshifted and unshifted groups for each postshift trial and independently for each lesion condition. Consistent with the preshift cSNC data, sham rats showed a nonsignificant effect for Trial 10, $F(1, 19) < 0.01$, $p > 0.96$. Sham rats showed significant cSNC effects on Trials 11-13, Fs(1, 19) > 4.12, ps < 0.02, but not for Trials 14-15, Fs(1, 19) < 0.43, $p > 0.52$. ACC rats also showed a nonsignificant effect for Trial 10, $F(1, 18) < 0.91$, $p > 0.35$, whereas all cSNC effects were significant for Trials 11-15, Fs(1, 18) > 8.10, ps < 0.01.

Further information about the effect of the ACC lesions on cSNC can be gathered from the behavior within each postshift trial. If rats formed an emotional memory of the downshift event during Trial 11, such memory should promote suppression of consummatory behavior during the initial section of subsequent trials. Indeed, posttrial 11 administration of CDP (Ortega et al., in preparation) and D-cycloserine (Norris, Ortega, & Papini, submitted) resulted in consummatory suppression during the initial 100 s of later postshift trials; these effects were interpreted as pharmacological enhancement of the emotional memory (i. e., an associative effect) triggered by the downshift event. In addition, suppression of consummatory behavior during the later section of the postshift trials, but not the initial part, may indicate a motivational effect (i. e., nonassociative) in that contact with the downshifted sucrose solution during the initial section of the trial triggers a negative reaction that in turn results in suppression of consummatory behavior later in the trial.

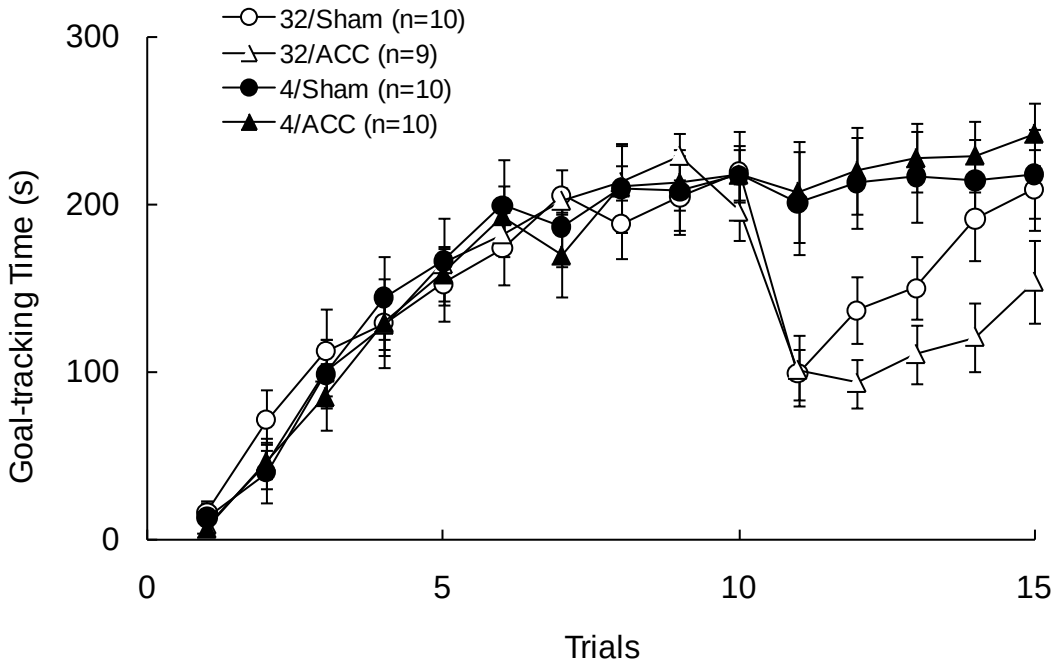

Figure 5. Mean (± SEM) goal-tracking time for Experiment 1. Groups 32 received 32% sucrose during the preshift and downshifted to 4% sucrose during the postshift. Groups 4 received 4% sucrose during preshift and postshift. ACC groups received bilateral electrolytic lesions targeted to the ACC, while Sham groups received a similar surgical treatment but no current was passed through the electrode or into the brain tissue.

Figure 6 depicts consummatory behavior during the initial and last 100 s of trials 10-15. For the initial 100 s, trial-by-trial, one-way ANOVAs for the 32/Sham vs. 4/Sham groups revealed nonsignificant effects for Trials 10-15, Fs(1, 19) < 2.09, ps > 0.16, a result that replicates prior findings with intact animals (Norris, Daniel, & Papini, 2008). Similar analyses for 32/ACC vs. 4/ACC groups revealed nonsignificant effects for Trials 10, 11, and 15, Fs(1, 18) < 3.10, ps > 0.10, but significant effects for Trials 12-14, Fs(1, 18) > 6.76, ps < 0.02. For the last 100 s, trial-by-trial, one-way ANOVAs for 32/Sham vs.

4/Sham groups revealed significant effects for Trials 11 and 12, Fs(1, 19) > 9.07, ps < 0.02, but nonsignificant effects for Trials 10 and 13-15, Fs(1, 19) < 0.14, ps > 0.71, also consistent with previous results (Norris et al., 2008). Finally, one-way ANOVAs for the last 100 s of the 32/ACC vs. 4/ACC groups revealed a nonsignificant effect for Trial 10, F(1, 18) < 0.62, p > 0.44, and significant effects for Trials 11-15, Fs(1, 18) > 12.78, ps < 0.01.

The pattern of results from Experiment 1 suggests that bilateral ACC lesions retarded the recovery from cSNC (i.e., extended cSNC two more trials). The ACC lesion had no measurable effects on the consummatory performance of animals before the downshift (i.e., the lesion did not affect consumption of 32% vs. 4% sucrose); on the performance of unshifted, 4% sucrose animals; and on the first downshift trial. In addition, ACC lesions resulted in an enhancement of the emotional memory of the downshift event, as shown by 32/ACC vs. 32/Sham decreased performance during the initial 100 s of Trials 12-14, and an extended motivational effect, in that 32/ACC consummatory suppression during the last 100 s lasted three additional trials beyond the performance of the sham controls. However, these two hypothesized effects of ACC lesions on cSNC (i. e., memory and drive effects) cannot be unambiguously distinguished on the basis of the present data.

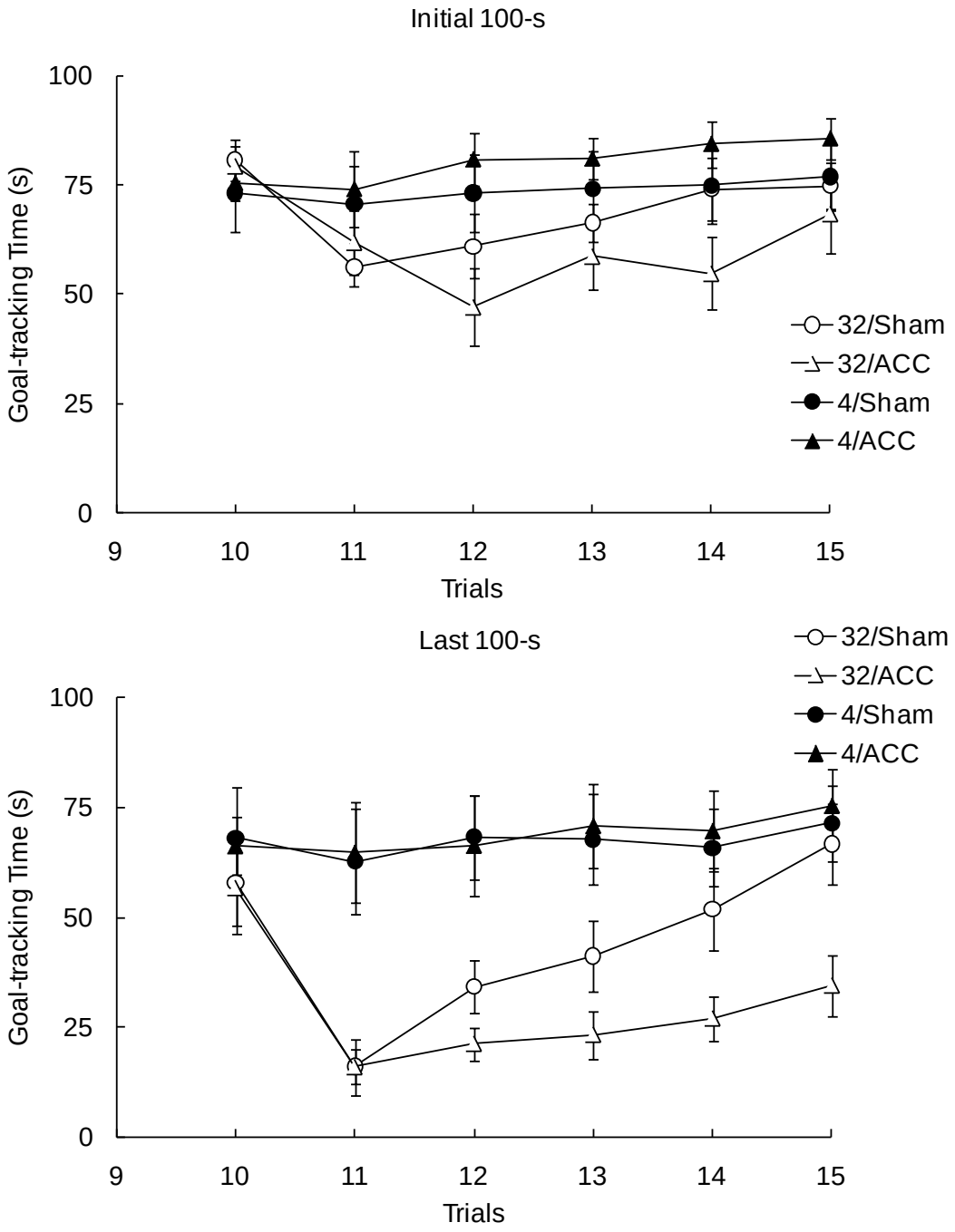

Figure 6. Within-trial mean (± SEM) goal-tracking time for Experiment 1. Figures show the initial 100 s (top) and the last 100 s (bottom) from Trials 10-15.

Experiment 2

The pain=frustration hypothesis can be extended to another PFC area, the VLO. Microinjections of morphine into the VLO diminished pain-related behavior induced during the formalin test (Xie et al., 2004). Systemic administration of opioid receptor antagonists or agonists also modulates cSNC in a trial selective and opiod receptor specific way, although the brain areas involved are still unknown (Papini, 2009). Bilateral lesions of the VLO before cSNC were used to evaluate the involvement of this prefrontal area in reward downshift situations. Specifically, it is expected that VLO lesions impair recovery from cSNC, having an effect similar to that of ACC lesions, as manipulations on both ACC and VLO resulted attenuation of formalin-induced pain behavior (Donahue et al., 2001; Xie et al., 2004).

The role of the mPFC in pain modulation is unclear. However, lesions of this prefrontal area affected consummatory performance following a reward downshift (Pecoraro et al., 2008). An evaluation of bilateral lesions of the mPFC may modulate cSNC without being clearly involved in pain behaviors. Moreover, the possibility of mPFC modulation of recovery from cSNC cannot be properly evaluated on the basis of Pecoraro et al.'s (2008) data, because they did not include unshifted controls (i. e., 4% lesion and 4% sham groups). Thus, it is unclear whether the reported effects of the mPFC lesion were specific to reward downshift or simply affected consummatory behavior.

Lesions of the mPFC, but not the lateral PFC, increase exploratory behavior, as measured by open field center activity (Lacroix, Spinelli, Heidbreder, & Feldon, 2000). To assess the possibility of modulation of activity, relevant to the search stage of cSNC, rats with both VLO and mPFC lesions were also tested in the open field. Finally, deep brain stimulation in the ventromedial PFC (including the prelimbic and infralimbic cortices)

attenuated the suppression of sucrose consumption following footshock stress in a model of learned helplessness (Hamani et al., 2010). These results suggest that the mPFC may play a role in the detection of sucrose solutions. Thus, a sucrose sensitivity test will also be administered to evaluate if VLO and/or mPFC lesions affect the detection of sucrose.

Autoshaping acquisition following continuous or partial reinforcement was also evaluated after VLO and mPFC lesions in the present experiment. Chudasama and Robbins (2003) reported impaired autoshaping acquisition following orbitofrontal lesions. However, there was no evaluation of the effects of orbitofrontal lesions on autoshaping acquisition under partial reinforcement. Behavior under partial reinforcement acquisition may also be related to recovery from cSNC. In a selective breeding study that targeted recovery rates from cSNC, three strains of rats were bred for 5 filial generations according to their rate of recovery: high, low, or random. Whereas the low and random lines continued to show significant cSNC effects across generations, the high line exhibited a progressive reduction in the size of the cSNC effect across generations. When animals from the 5th filial generation were tested under partial vs. continuous reinforcement conditions, the emerging pattern of results was consistent with their cSNC performance. Thus, rats in the randomly breeding condition showed higher acquisition performance after partial rather than continuous reinforcement training (the so-called PRAE; Amsel, 1992). However, rats selectively bred for higher recovery rates from cSNC showed no acquisition differences under partial and continuous reinforcement (Ortega, Norris, Lopez-Seal, Ramos, & Papini, in preparation). The absence of cSNC and the two effects of partial reinforcement in high recovery rats from the 5th filial generation are consistent with an interpretation based on a reduced role of emotional (frustration) activation in these animals. Therefore, a similar

design was used in the present experiment to determine whether VLO and mPFC lesions had similar effects, if any, on both cSNC and partial reinforcement training.

Method

Subjects. Fifty-eight Long-Evans rats were used. They were reared and housed as described in the previous experiment.

General procedure. cSNC training was similar to those described for the Experiment 1. Additionally, rats were tested on sucrose sensitivity, activity behavior, and autoshaping. Bilateral lesions were performed in two brain areas, VLO and mPFC. Six groups of rats were used. In groups 32/VLO, 32/mPFC, and 32/Sham rats learned to consume 32% sucrose solution during Trials 1-10 (preshift), but this solution was replaced by a less sweet 4% solution during Trials 11-15 (postshift). In groups 4/VLO, 4/mPFC, and 4/Sham rats learned to consume the 4% sucrose solution throughout the 15 trials. Rats were randomly assigned to the lesion conditions (VLO vs. mPFC vs. Sham), and then rats within each lesion condition were randomly assigned to the contrast conditions (downshifted vs. unshifted).

Sucrose sensitivity. This test assessed possible changes in sucrose sensitivity due to the lesions. Following cSNC testing and during 3 consecutive days, two-bottle tests, 24 h per sucrose concentration, were administered in the home cage, according to a procedure described by Dess (2000). One bottle contained either 0.5%, 1%, or 2% sucrose solutions (weight/volume; e. g., 0.5% was prepared by mixing 5 g of sucrose for every liter of distilled water). The other bottle contained distilled water. The order of the sucrose concentrations administered across the three days was counterbalanced across subjects, as well as the position of the sucrose vs. water bottles. Each concentration was left in the cage

for 24 h. Sucrose sensitivity was measured in terms of the following ratio: Sucrose consumption over sucrose consumption plus water consumption (mL).

Open field testing. Following the sucrose sensitivity test, activity behavior in the open field was assessed to test for the possibility of motor effects following lesions in the VLO and mPFC. Four open field chambers were used (Med Associates, St. Albans, VT). The dimensions of each chamber were 43 × 30 × 43 cm (L×H×W). Testing took place between at 10:00 and 16:00 h. Rats were tested in squads of four. At the start of the trial, each rat was placed in the center of the open field. Overall and center locomotor activity was automatically recorded in 5-min bins during a single 20-min trial. The open field was swept immediately after each trial with a wet paper towel.

Autoshaping testing. Autoshaping training was administered to test if lesions in the VLO and mPFC affect another kind of reward downshift, appetitive extinction. Four standard conditioned boxes were used (MED Associates, St. Albans, VT). The dimensions of each chamber were 28 × 20.5 × 20.1 cm (L × H × W). The floor was made of steel rods running parallel to the feeder wall. A tray with corncob bedding was placed below the floor to collect feces and urine. A recessed magazine, 2 cm from the floor, was located in the center of the front wall, into which the pellets (45-mg Noyes, rat formula A/I) were delivered automatically. A retractable lever made of aluminum (4.8 cm wide, 1.9 cm deep, and 7 cm above the floor) was located 2 cm to the left of the magazine. Insertion and retraction of the lever took 0.2 s. A light bulb (GE 1820) attached to the ceiling of the chamber and positioned opposite to the magazine, provided diffuse illumination. Each conditioning box was placed in a sound-attenuating chamber containing a speaker to deliver white noise and a fan for ventilation (SPL 80.1 dB, scale C).

Autoshaping testing consisted of 5 daily sessions. Each session started with the onset of the house light and ended when the house light was turned off. Within each session, 10 training trials were presented, separated by a variable intertrial interval averaging 90 s (range: 60-120 s). However, an error in the program was discovered in that rats in the continuous reinforcement condition actually received 9 trials per session. Thus, rats in the continuous reinforcement condition received a total of 45 trials, whereas rats in partial reinforcement condition received 50 trials. Each trial began with the insertion of the retractable lever for 10 s. A computer recorded lever-pressing responses and goal tracking while the lever was inserted in the chamber. Rats within each lesion condition were randomly assigned to two groups, matched by weight and cSNC experience. For the continuous reinforcement groups, each trial ended with the delivery of 5 pellets on the magazine cup. For the partial reinforcement groups, a random half of the trials ended with the delivery of 5 food pellets. Pellets were delivered at a rate of one pellet per 0.2 s. In nonreinforced trials, the lever was retracted, but no pellets were delivered.

Surgery, lesions, and histology. General surgical, recovery from surgery, and histological procedures were identical to those described for Experiment 1. However, the placement of the lesions varied. Bilateral electrolytic lesions of the VLO were performed by passing a 0.5 mA current, during 15 s, using a 0.3 mm electrode (AP 3.0–3.7; ML 1.5–2.5; DV 4.0–5.0). Bilateral electrolytic lesions of the mPFC were performed by passing a 0.5 mA current, during 15 s, using a 0.3 mm electrode (AP 3.0; ML 0.4; DV 4.9; at a 15 degree angle; all coordinates from Paxinos & Watson, 2007). Each sham group (32/Sham, 4/Sham) was exposed to the surgery procedures and electrode insertion, but no current was delivered. Five rats within each sham group had the electrode inserted in the VLO, while the other five rats had the electrode inserted in the mPFC.

Results and discussion

Histology. Fourteen animals (8 VLO and 5 mPFC) presented lesions that extended to adjacent areas, were unilateral, or were too small, and were not considered for statistical analyses. Twelve animals had at least 75% bilateral damage to the VLO. Average anterior-posterior extent of the damage for the largest percentage of animals was localized between 4.2 to 2.7 mm relative to bregma, while thirteen animals had at least 75% bilateral damage to the mPFC, average anterior-posterior extent of the damage for the largest percentage of animals was localized between 3.7 to 2.2 mm relative to bregma (Figure 7). The distribution of damage for animals in the downshifted vs. unshifted conditions was similar.

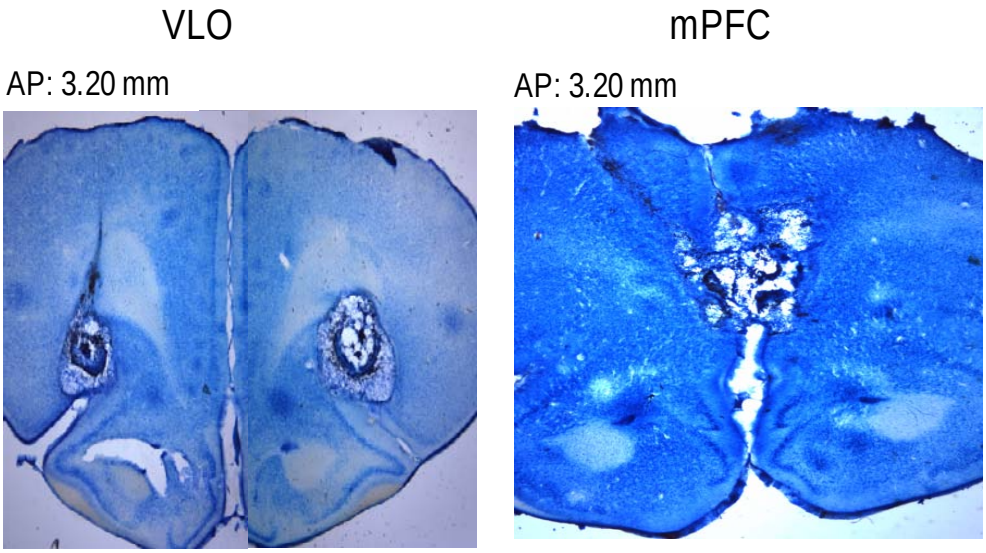

Figure 7. Examples of VLO and mPFC lesions from Experiment 2.

cSNC. Results can be seen in Figure 8. One rat in Group 32/Sham failed to acquire consummatory behavior during the preshift and was discarded from the analysis. Preliminary analyses showed similar consummatory performance during the preshift and posthift phases for the two sham operations (VLO vs. mPFC sham). During the preshift

phase, a Sham (VLO, mPFC) x Contrast (32%, 4%) x Preshift Trial (1-10) analysis of variance (ANOVA) revealed a significant effect of trial, $F(9, 135) = 35.62$, $p < 0.01$, contrast, $F(1, 15) = 15.08$, $p < 0.01$, and trial by contrast, $F(18, 135) = 3.30$, $p < 0.01$. All other effects were nonsignificant, Fs < 1.67, ps > 0.22. During the postshift phase, a cSNC effect is suggested by a trial by contrast interaction, $F(4, 60) = 4.12$, $p < 0.01$, and a trial effect, $F(4, 60) = 8.60$, $p < 0.01$. There was no evidence for effects of the sham operations on cSNC, as revealed by nonsignificant effects for all factors, Fs < 0.44, ps > 0.78. Thus, VLO and MPFC sham groups were pooled together for further cSNC analyses.

During the preshift phase, rats learned to drink the sucrose solutions, presenting differential acquisition for the 32% vs. 4% solutions. This effect was similar for all lesion conditions, as yield by a Lesion (VLO, mPFC, Sham) x Contrast (32%, 4%) x Preshift Trial (1-10) ANOVA, which revealed significant effects for trial, $F(9, 342) = 65.79$, $p < 0.01$, contrast, $F(1, 38) = 46.32$, $p < 0.01$, and trial by contrast, $F(18, 342) = 8.83$, $p < 0.01$. All other effects and interactions were nonsignificant, Fs < 3.00, ps > 0.06. During the postshift phase, a cSNC effect is suggested by a trial by contrast interaction, $F(4, 152) = 7.31$, $p < 0.01$, and a trial effect, $F(4, 152) = 13.84$, $p < 0.01$. However, no evidence for effects of the VLO or mPFC lesions on cSNC were found, as revealed by nonsignificant effects for all factors, Fs < 2.38, ps > 0.11.

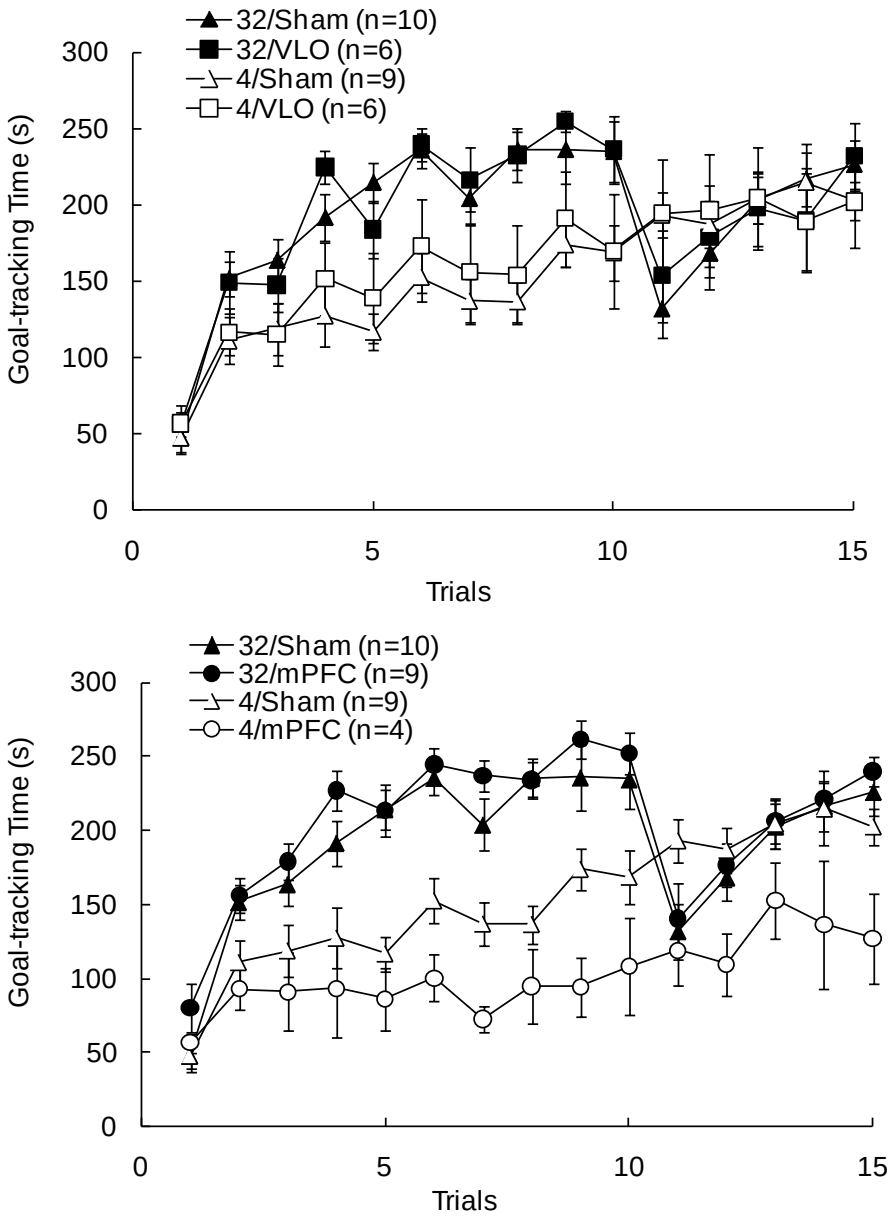

Figure 8. Mean (± SEM) goal-tracking time for Experiment 2. Groups 32 received 32% sucrose during the preshift and downshifted to 4% sucrose during the postshift. Groups 4 received 4% sucrose during preshift and postshift. VLO and mPFC groups received bilateral electrolytic lesions targeted to the VLO and mPFC, respectively, while Sham groups received a similar surgical treatment but no current was passed through the electrode or into the brain tissue.

Further analyses of the postshift using one-way ANOVAs for each lesion condition provided the following results. Comparisons between the 32/Sham vs. 4/Sham groups showed significant effects for Trials 10 and 11, *F*s(1, 18) > 5.97, *p*s < 0.03, but not for Trials 12-15, *F*s(1, 18) < 1.29, *p*s > 0.27. Postshift 32/VLO vs. 4/VLO ANOVAs revealed nonsignificant differences for Trials 10-15, *F*s(1, 11) < 2.37, *p*s > 0.16. Finally, 32/mPFC vs. 4/mPFC comparisons revealed significant effects on Trials 10, 12, 14, and 15, *F*s(1, 12) > 6.56, *p*s < 0.03, but not for Trials 11 and 13, *F*s(1, 12) < 3.63, *p*s > 0.08.

Consummatory behavior during the initial 100 s of trials 10-15 is shown in Figure 9. For the initial 100 s, trial by trial one-way ANOVAs for the 32/Sham vs. 4/Sham groups revealed a significant Trial 10 effect, *F*(1, 18) > 12.18, *p* < 0.01, but nonsignificant effects for Trials 11-15, *F*s (1, 18) < 1.24, *p*s > 0.28. Similar analyses for 32/VLO vs. 4/VLO groups showed nonsignificant effects for Trials 10-15, *F*s (1, 11) < 3.76, *p*s > 0.08. In addition, 32/mPFC vs. 4/mPFC comparisons revealed significant Trial 10-15 effects, *F*s(1, 12) > 5.32, *p*s < 0.04. However, notice that these effects were produced by a very low consummatory performance in group 4/mPFC. For the last 100 s (Figure 10), one-way ANOVAs for the 32/Sham vs. 4/Sham groups showed a significant Trial 11 effect, *F*(1, 18) > 24.97, *p* < 0.01, but nonsignificant effects for Trials 10, and 12-15, *F*s (1, 18) < 2.82, *p*s > 0.11. Comparisons between groups 32/VLO vs. 4/VLO revealed nonsignificant Trial 10-15 effects, *F*s(1, 11) < 1.19, *p*s > 0.30. Finally, 32/mPFC vs. 4/mPFC comparisons revealed significant effects for Trials 10 and 15, *F*s(1, 12) > 10.93, *p*s < 0.01., but nonsignificant effects for Trials 11-14, *F*s (1, 12) < 3.25, *p*s > 0.10.

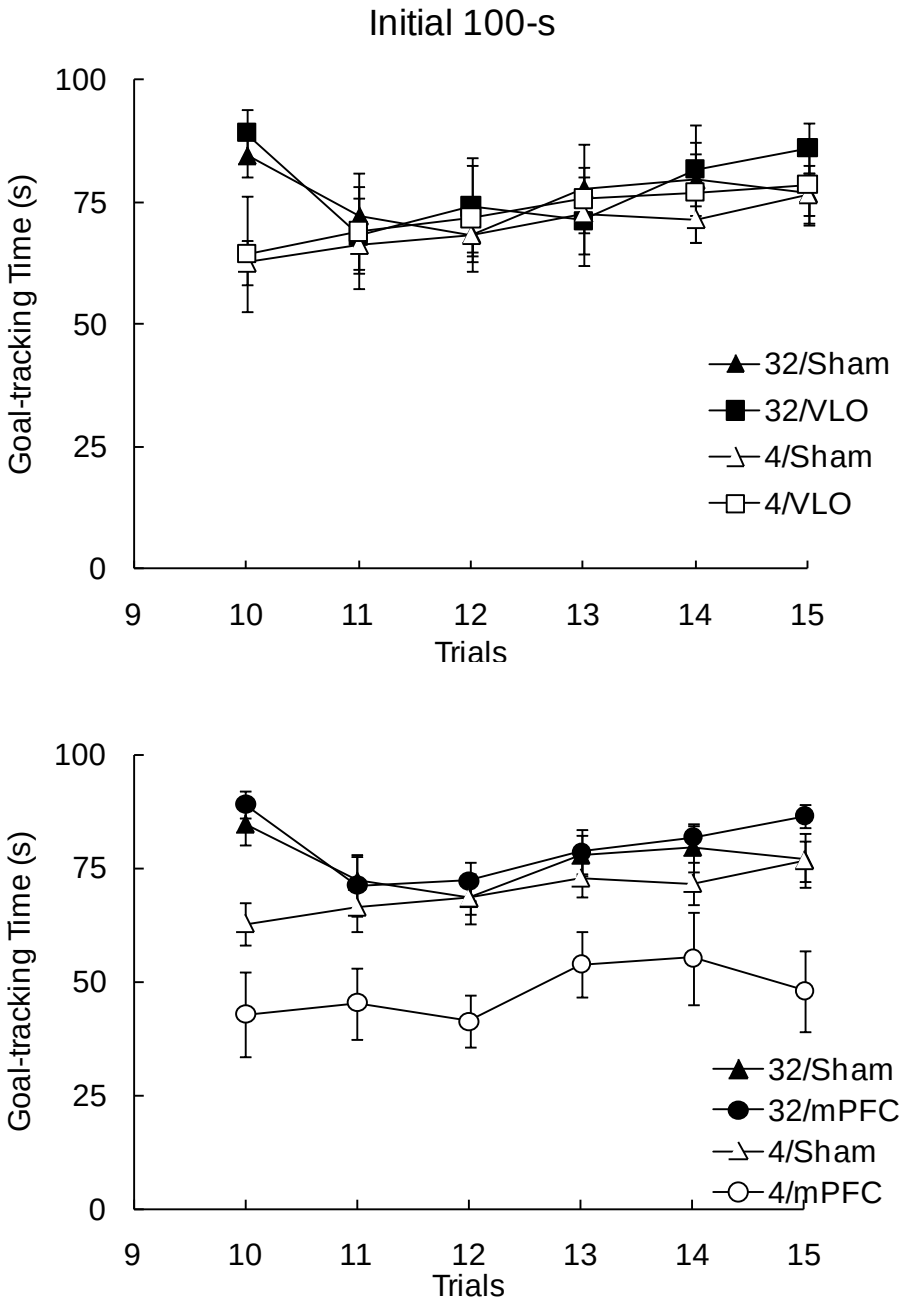

Figure 9. Within-trial mean (± SEM) goal-tracking time for Experiment 2. Figures show the initial 100 s (top) and the last 100 s (bottom) from each postshift trial.

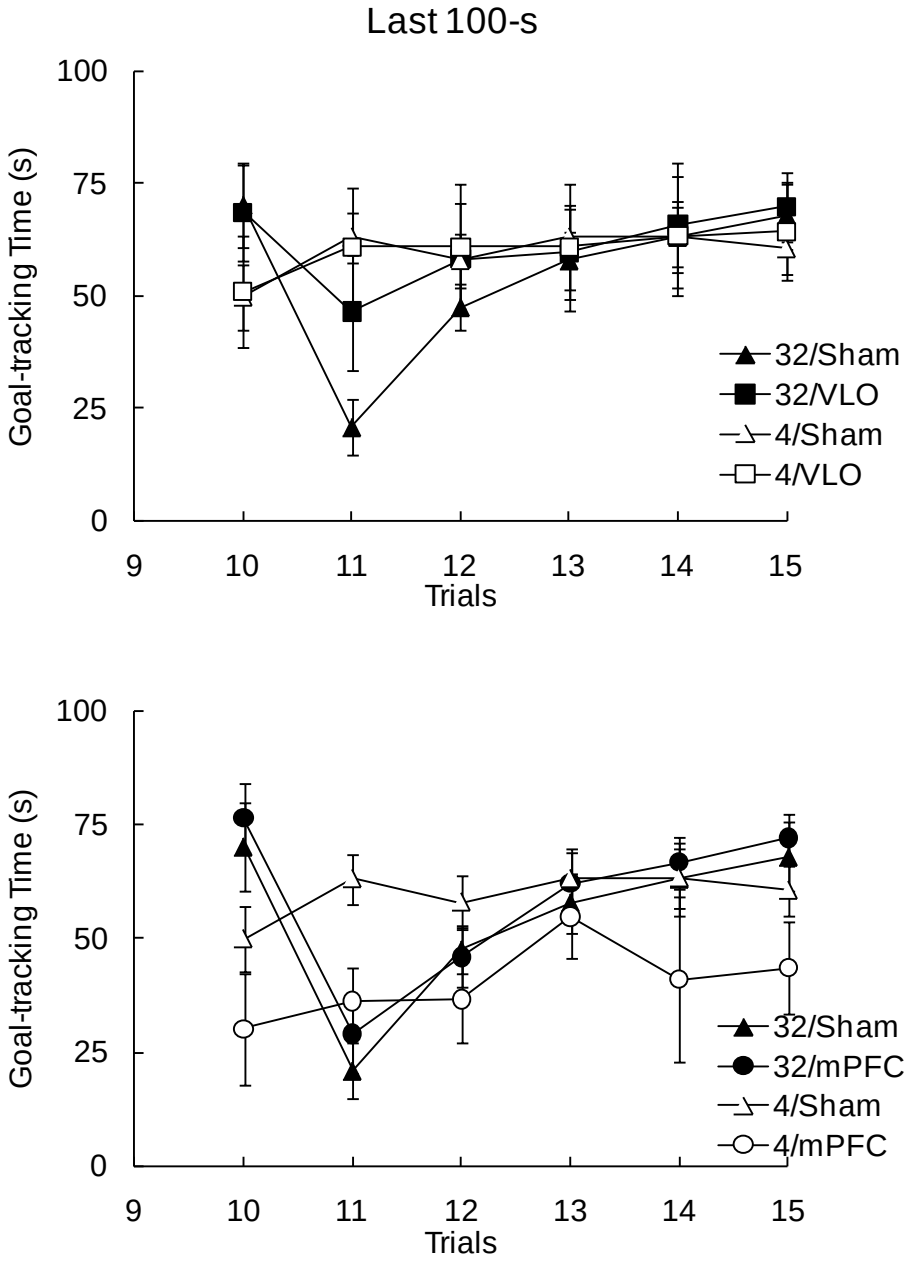

Figure 10. Within-trial mean (± SEM) goal-tracking time for Experiment 2. Figures show the last 100 s from each postshift trial.

The effects of VLO and mPFC lesions on cSNC were not very clear. First, VLO lesions had no measurable effects on consummatory behavior during the preshift, while mPFC lesions seem to have an effect on preshift and postshift consummatory performance

for the 4% animals, but not for 32% animals. However, a note of caution should be raised on the effects related to the 4/mPFC condition, as there was a low number of animals in this group (n=4) after histological analysis. Second, within-trial analyses pointed to effects of both lesions on the last 100 s of Trial 11. VLO rats showed an attenuated response to cSNC during the last 100 s of Trial 11, relative to sham controls, an effect that could be motivational. The effects of mPFC lesions do not seem to be related to the downshift, but rather to a low performance in Group 4/mPFC.

Sucrose sensitivity. The statistical analysis was performed using VLO vs. Sham and mPFC vs. Sham one-way ANOVAs for each sucrose concentration (0.5, 1.0, 2.0). Figure 11 shows the preference for the tested sucrose concentrations by lesion. VLO rats showed nonsignificant effects for all concentrations, $Fs(1, 30) < 3.81$, $ps > 0.06$. Rats in the mPFC condition showed a significantly lower sucrose preference than sham rats for the 0.5 sucrose concentration, $Fs(1, 32) > 4.46$, $p < 0.04$, but there were nonsignificant effects for the other concentrations, $Fs(1, 32) < 0.24$, $ps > 0.63$. These results showed no evidence for an effect of VLO lesions on sucrose sensitivity, whereas mPFC lesions disrupted the preference for low concentration sucrose (0.5%) solutions. This effect may be related to the unusually low level of consummatory behavior in Group 4/mPFC during cSNC testing (see Figure 7, lower panel)

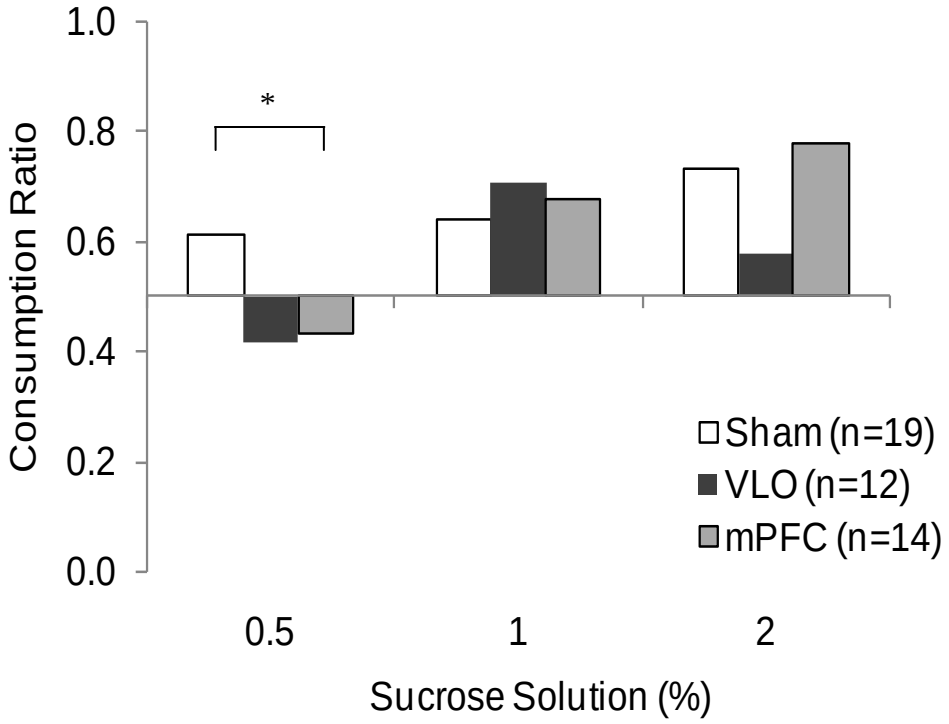

Figure 11. Mean (± SEM) sucrose consumption ratio. Sucrose sensitivity was measured in terms of the following ratio: Sucrose consumption over sucrose consumption plus water consumption (mL).

Open field. For the reported data in this section, overall and center ambulatory distance was evaluated using Lesion (VLO, mPFC, Sham) x Bin (1-4) ANOVAs. Due to equipment malfunction, data for three rats were lost. As seen in Figure 12, all rats showed a decreasing level of overall and center ambulation along the test, suggesting habituation to the activity chamber. For both overall and center performance, lesions did not seem to affect ambulatory behavior. This description is supported, for the overall data, by an activity bin effect, $F(3, 117) = 261.21$, $p < 0.01$), and nonsignificant activity bin by lesion and lesion effects, Fs < 1.17, ps > 0.33.Similarly, center data showed an activity bin effect,

$F(3, 117) = 136.00$, $p < 0.01$), and nonsignificant activity bin by lesion and lesion effects, Fs < 1.45, ps > 0.20. In short, animals in all conditions and for overall and center activity showed similar levels of ambulation and habituation to the testing chamber.

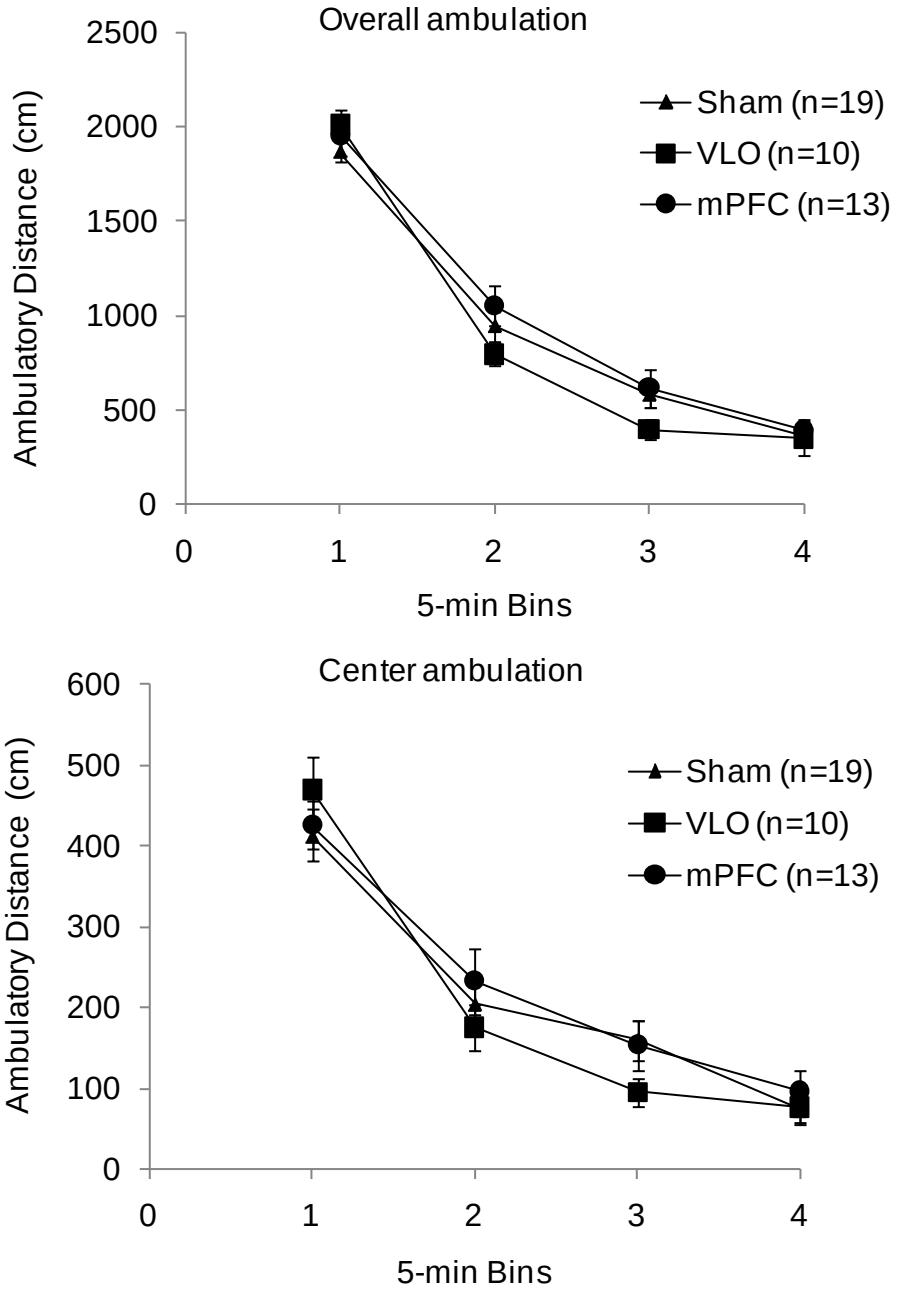

Figure 12. Mean (± SEM) overall (top) and center (bottom) ambulatory distance during a 20-min open-field session.

Autoshaping. A key analysis for autoshaping is that of the performance under the two tested schedules of reinforcement. Thus, analyses were performed independently for each lesion condition. Thus, autoshaping performance was evaluated for each lesion condition using Session (1-5) x Schedule (Continuous vs. Partial) ANOVAs.

Lever press autoshaping acquisition performance for all lesion conditions is shown in Figure 13. Rats in the VLO condition failed to acquire the lever pressing response for the two reinforcement conditions, as supported by nonsignificant effects for all comparisons, Fs < 1.73 ps > 0.16. Rats in the mPFC condition acquired the lever pressing response, but their performance was higher in the partial reinforcement condition than in the continuous reinforcement condition, as indicated by a schedule effect, $F(1, 11) = 10.47$, $p < 0.04$. There was also a significant session effect, $F(4, 44) = 8.92$, $p < 0.01$, but the session by schedule interaction was not significant, $F(4, 44) = 3.10$, $p < 0.03$. Similarly, rats in the sham condition acquired the lever-pressing response at a higher rate in the partial reinforcement condition than in the continuous reinforcement condition, as shown by a schedule effect, $F(1, 17) = 5.13$, $p < 0.04$. There was also a session effect, $F(4, 68) = 7.85$, $p < 0.01$. However, the session by schedule interaction was not significant, $F(4,68) = 1.01$, $p > 0.37$.

Analyses for the initial response during each autoshaping session were performed using the response rate during Trial 1 on Sessions 1-5 (Figure 14). Trial 1 performance is not influenced by food presentations and omissions, thus providing a relatively clean view of effects dependent upon the signal (i. e., the lever as a conditioned stimulus).VLO rats showed an increased of the number of initial responses across sessions that was similar for the two reinforcement conditions, as indicated by a session effect, $F(4, 40) = 7.85$, $p < 0.01$, but nonsignificant schedule effect, $F(1, 10) = 0.03$, $p > 0.88$, and session by schedule

interaction, $F(4, 40) = 0.30$, $p > 0.88$. mPFC rats showed higher levels of initial responding for partially than continuously reinforced conditions, as shown by a session effect, $F(4, 44) = 10.89$, $p < 0.01$, and a schedule effect, $F(1, 11) = 4.86$, $p < 0.05$; however, the session by schedule interaction was not significant, $F(4,44) = 1.98$, $p > 0.11$. Sham rats also responded at higher levels in the partially reinforced condition, as indicated by a session effect, $F(4, 68) = 14.15$, $p < 0.01$, a schedule effect, $F(1, 17) = 6.33$, $p < 0.02$, and a session by schedule interaction, $F(4,68) = 3.11$, $p < 0.02$.

Goal tracking performance for all lesion conditions is shown in Figure 15. Rats in all conditions showed higher goal tracking performance under partial reinforcement than under continuous reinforcement. For VLO rats, this is supported by a session effect, $F(4, 40) = 2.90$, $p < 0.01$, and schedule effect, $F(1, 10) = 94.58$, $p < 0.01$, but nonsignificant session by schedule interaction, $F(4, 40) = 2.57$, $p > 0.51$. For mPFC rats, there were significant session effect, $F(4, 44) = 2.82$, $p < 0.04$, schedule effect, $F(1, 11) = 121.89$, $p < 0.01$, and session by schedule interaction, $F(4,44) = 2.98$, $p < 0.03$. Lastly, sham rats showed a schedule effect, $F(1, 17) = 92.34$, $p < 0.01$, but nonsignificant session effect, $F(4, 68) = 0.86$, $p > 0.47$, and session by schedule interaction, $F(4,68) = 0.77$, $p > 0.54$.

In short, sham rats showed a PRAE, with rats in the partial reinforcement schedule responding at higher levels than rats in the continuous reinforcement schedule. Similarly, mPFC lesions resulted in a PRAE, which was absent in animals with VLO lesions. The disruption of autoshaping by VLO lesions was observed from the first trial of the session.

The results from autoshaping could be explained in terms of three hypotheses. (a) By Amsel's (1992) frustration theory, in terms of the disruption of generalized drive induced by partial reinforcement in VLO rats. (b) A competing goal tracking response that would interfere with lever press responding in continuously reinforced VLO rats. However,

goal tracking was actually lower in continuously reinforced than in partially reinforced groups (Figure 15). It is still possible, though, that other responses compete with lever pressing behavior. (c) Within-session satiation, as rats in the continuously reinforced schedule received more food pellets in each session than the partially reinforced rats (45 vs. 25 food pellets, respectively). However, the decrease of lever pressing behavior was observed on the first trial of each session, in which performance is not influenced by reward presentations and omissions and, therefore, the animals have a similar food deprivation level. Thus, an interpretation in terms of drive induction by partial reinforcement is more consistent with the pattern of results from autoshaping acquisition. It is suggested that the VLO plays a key role in drive induction during reward downshift.

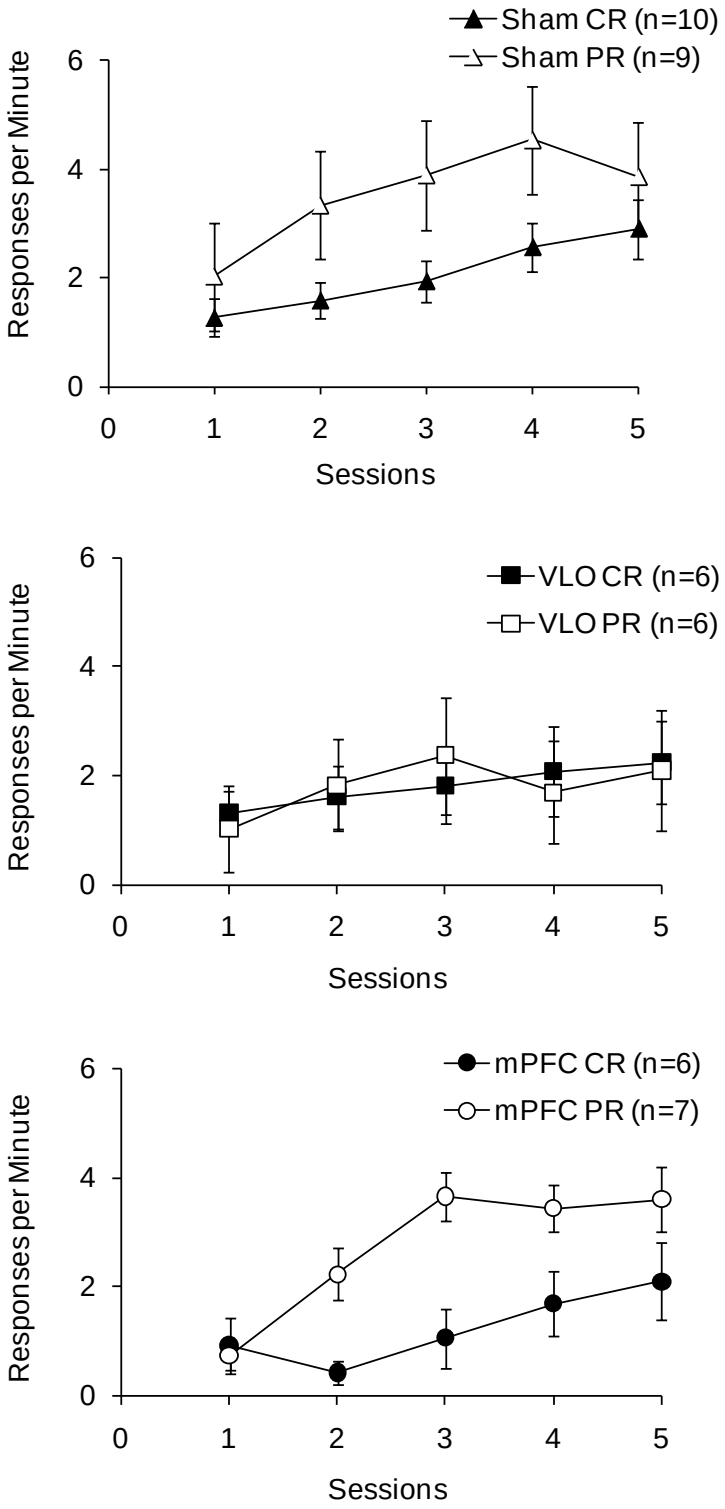

Figure 13. Mean (± SEM) responses per minute for each session during autoshaping.

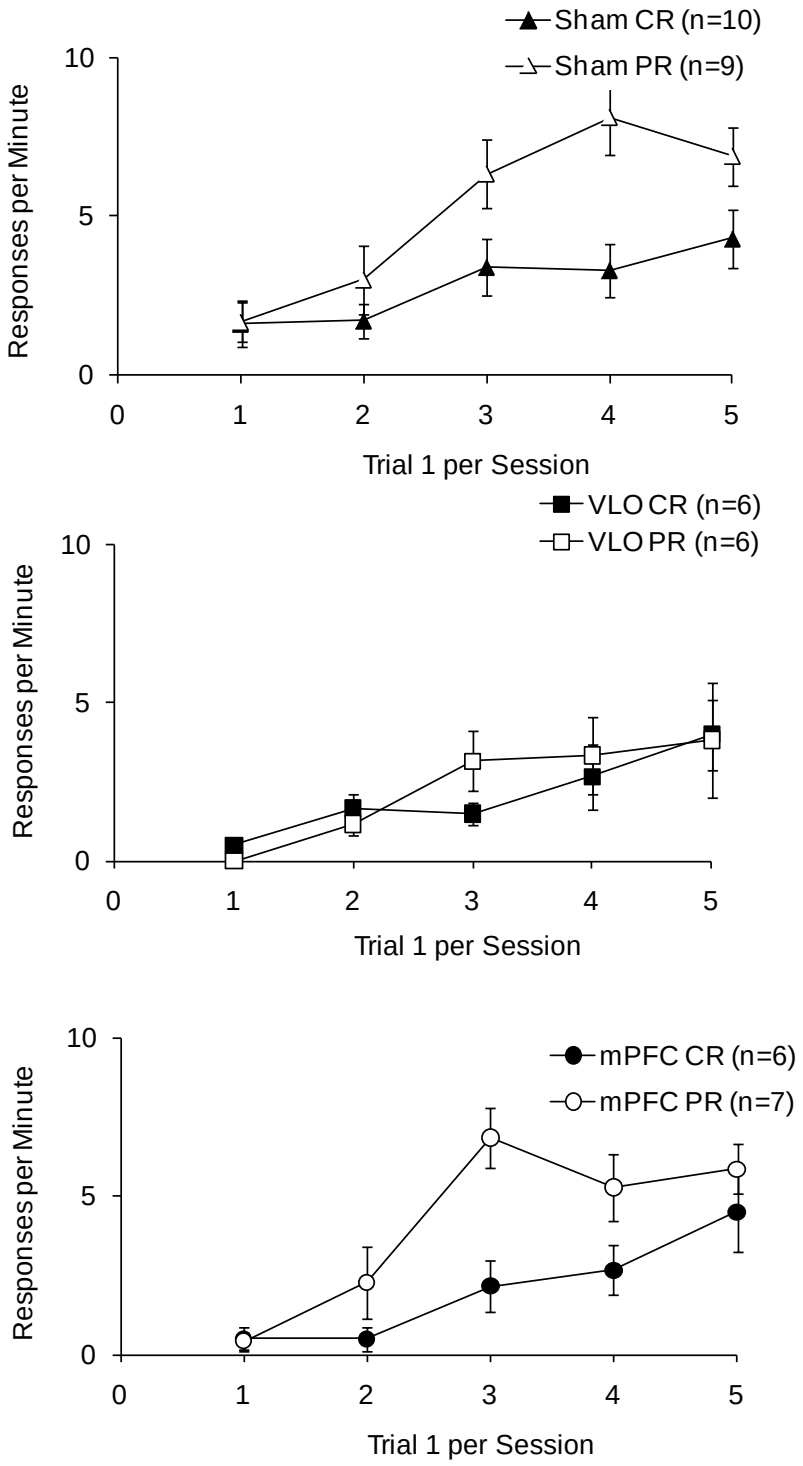

Figure 14. Mean (± SEM) autoshaping performance during the first Trial for Sessions 1-5.

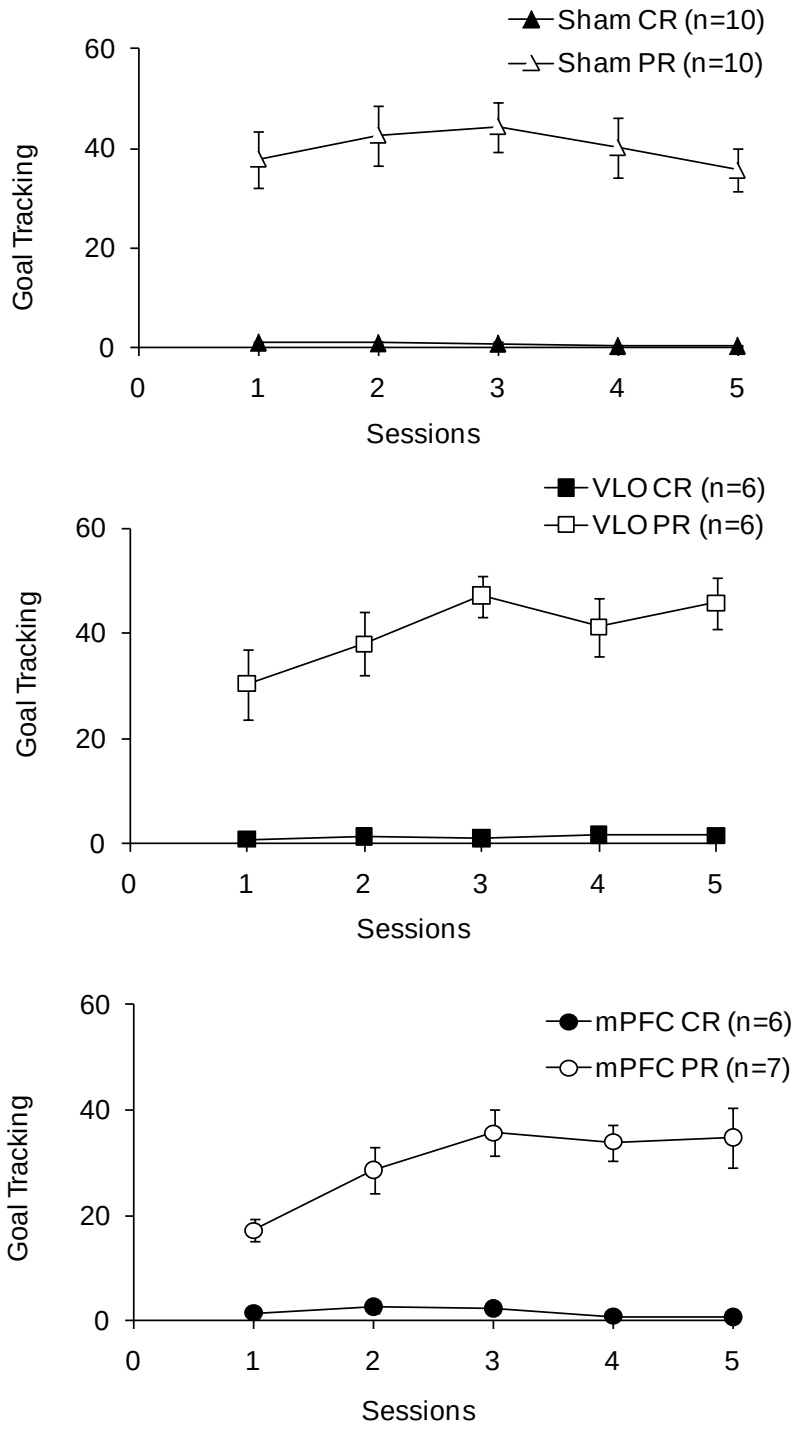

Figure 15. Mean (± SEM) autoshaping Goal Tracking performance.

General discussion

The present experiments evaluated the role of several PFC regions on cSNC and some additional behavioral measures. The results can be summarized as follows: (1) ACC lesions resulted in the retardation of recovery from cSNC. (2) VLO lesions did not affect cSNC, although they attenuated cSNC on the later part of the first downshift trial. (3) There was no evidence that mPFC lesions affected cSNC performance, whereas they decreased consummatory behavior in the unshifted condition. (4) Neither VLO nor mPFC lesions seemed to affect activity behavior on an open-field situation. (5) VLO lesions had no detectable effect on sucrose sensitivity, while mPFC lesions reduced preference for the lowest sucrose solution tested. (6) Autoshaping responding was higher under partial reinforcement than continuous reinforcement, except in animals with VLO lesions.

Consider first the effects of ACC lesions on cSNC. This is the first report of a brain area whose lesion selectively impairs recovery from cSNC, while leaving the response to sucrose solutions (Trials 1-10) and to the reward downshift (Trial 11) unimpaired. Previous studies reported that the VPM and amygdala lesions attenuated or eliminated the initial response to the reward downshift (Grigson et al., 1994; Reilly & Trifunovic, 1999; Becker et al., 1984), while lesions of the insular cortex completely disrupted cSNC (Lin et al., 2009). These effects are related to the initial reaction to the downshift event (Flaherty's Stage 1; Figure 2). The evaluation of the effect of VPM and amygdala lesions on recovery (Flaherty's Stage 2) using irreversible lesions is complicated by the effects of these lesions in the initial response to the downshift. Thus, it is unclear whether these lesions affect recovery directly, or via their effects on the early response to the downshift event. Pecoraro et al. (2008) found no effects of mPFC lesions on consummatory suppression following reward downshift, but they reported higher levels of consummatory behavior during the

later part of an extended postshift phase. Although a role of the mPFC on recovery from cSNC is possible, this study did not include unshifted controls, which raises the possibility that the lesions had a nonspecific effect on consummatory behavior, rather than specifically affecting recovery from cSNC. Indeed, a mPFC lesion effect on 4% consummatory performance (i. e., Group 4/mPFC) was found on Experiment 2. Thus, the present study on ACC lesions and cSNC is unique in that the lesion effect can be unambiguously interpreted as one affecting recovery from cSNC.

The effect of ACC lesions on recovery can be interpreted as evidence for a selective involvement of this brain area on Flaherty's Stage 2 of cSNC. Previous research suggests differing neurochemical profiles for Flaherty's Stages 1 and 2. Specifically, recovery from cSNC was selectively modulated by systemic manipulations with benzodiazepines (Flaherty et al., 1986) and a κ opioid receptor agonist drug (Wood et al., 2008). The present within-trial data suggest that the effect of ACC lesions on recovery from cSNC is related to both an enhancement of the emotional memory of the downshift event and an extended motivational effect, as shown by the early and late suppression of consummatory behavior, when compared to the performance of sham rats (see Figures 6 and 7). Together, the present and previous studies suggest new avenues of research involving recovery from cSNC. For instance, intra-ACC administration of benzodiazepines and κ opioid receptor modulators before Trial 12, but not before Trial 11, should affect recovery from cSNC. This hypothesis assumes that these systemic drug effects are mediated by activation of GABA and opioid receptors within the ACC. There is evidence for such presence (Mansour et al., 2005; Chu et al., 1990).

Further research is needed to characterize the molecular mechanisms by which electrolytic lesions of the ACC affect recovery from cSNC. The associative and motivational effects related to the retardation of recovery from cSNC following ACC lesions are consistent with enhanced fear reactivity, as shown by enhancement of fear acquisition to the CS and the context, and an increase of the number of days necessary for fear extinction, following lesions of the dorsal mPFC (including the ventral ACC; Morgan & LeDoux, 1995). It is possible that the extension of the lesion to the ventral ACC is mainly responsible of the enhancement of fear, because more restricted mPFC lesions do not affect fear reactivity (Gewirtz, Falls, & Davis, 1997). However, an interpretation based on enhancement of negative emotional components of cSNC conflicts with data showing that: (a) reversible (Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004) and irreversible (Liu, heng, & Li, 2009) lesions on the ACC disrupt remote (test after 1day vs. at least 7 days) fear memory; (b) bilateral ACC administration of a NMDA receptor antagonist specific for the NR2B subunit reduces contextual fear memory, but not auditory fear memory (Zhao et al., 2005), although the lack of data for shock-only responses obscures an associative interpretation of the results; and (c) ACC lesions resulted in diminished pain-related behavior following the formalin test, an effect that was interpreted in terms of disruption of affective and motivational processes (Donahue et al., 2001). One hypothesis is that ACC lesions modulate cSNC by damaging cells with opioid receptors located in this area, thus causing an enhancement of the associative and motivational components of the negative emotional reaction to the downshift event that would be normally modulated by the conflict-solving and recovery-enhancing effects of endogenous opioids. As suggested, opioid receptor pharmacological manipulations within the ACC or

reversible inactivation of the ACC during recovery from cSNC may help to clarify of mechanisms underlying the behavioral effects observed on Experiment 1.

Bilateral lesions in the VLO had no detectable effects on cSNC or consummatory behavior, although there was a within-trial effect in that VLO rats showed higher consummatory performance during the later part of the first downshift trial (Trial 11). It is possible that such an effect of the VLO lesion is related to a decreased negative emotional response to the downshift that takes a few minutes to peak on Trial 11. The lack of evidence for a general effect of the VLO lesions on cSNC suggests that the within-trial effects may be related to the enhanced c-fos expression after the downshift trial for the orbitofrontal cortex (Pecoraro & Dallman, 2005). Alternatively, divergent results between VLO lesions and orbitofrontal cortex activation on cSNC may be related to procedural differences between Pecoraro and Dallman (2005) and the present Experiment 2, similar to the studies on the nucleus accumbens and cSNC. Sucrose downshift enhanced c-fos expression in the nucleus accumbens (Pecoraro & Dallman, 2005), and blunted dopamine efflux during Trial 11 (Genn, et al., 2004). However, lesions in this area failed to show any effect on cSNC (Eagle et al., 1999; Leszczuk & Flaherty, 2000). Differences in the cSNC procedure across labs were described above as possibly contributing to the conflicting results. For the VLO case, Pecoraro and Dallman (2005) used 14 preshift trials and presented the sucrose in the home cage of rats, while in Experiment 2 rats received 10 preshift trials and training took place on automated conditioning boxes.

There was no evidence for an effect of mPFC lesions on reward downshift, although an interpretation on terms of cSNC is complicated by the effect of the lesion on unshifted performance. mPFC lesions decreased consummatory behavior to 4% sucrose, but did not seem to affect consummatory behavior to 32% sucrose. These data are partially consistent

with Pecoraro et al. (2008), who reported no effects of mPFC lesions on early postshift (postshift trials 1-3), but an increase of performance in an extended postshift phase. However, as noted above, they did not include unshifted controls, as done in the present Experiment 2. mPFC lesions in Experiment 2 resulted in decreased consummatory behavior for 4% sucrose, a fact suggesting that the late recovery effect reported by Pecoraro et al. (2008) may not be specific to reward downshift. Similar to the VLO lesion, mPFC lesions did not affect the initial response to cSNC. Notice that mPFC lesions on Experiment 2 were located mainly in the prelimbic cortex, while Pecoraro et al. (2008) mPFC lesions were located mainly in the infralimbic cortex. Taken together, these data provide no evidence that the mPFC (prelimbic and infralimbic cortices) plays a role in the cSNC effect.

Activity testing showed no detectable performance differences for VLO and mPFC lesions. Lacroix et al. (2000) reported that mPFC lesions increase exploratory behavior in the center of the open field. However, their lesions were more focused on the infralimbic cortex, although with important extensions to the prelimbic cortex, while mPFC lesions in Experiment 2 were mainly restricted to the prelimbic cortex. The present experiment also provides no evidence of an involvement of the VLO on general activity behavior, including activity in the central area of the open field.

There was an effect of mPFC on sensitivity for sucrose solutions of low concentrations. Hamani et al. (2010) reported that deep brain stimulation in the ventromedial PFC attenuated sucrose consumption after footshock stress. Data from the present Experiment 2 indicated a similar attenuation of preference for sucrose solutions of low concentration. Disruption of sensitivity for low sucrose solutions is also consistent with the mPFC effect on performance under unshifted conditions. Such a gustatory effect further complicates the evaluation of a role for the mPFC on reward downshift. VLO lesions did

not seem to affect sucrose sensitivity, which, together with the lack of effect of VLO lesions in consummatory behavior of the unshifted animals in Experiment 2, suggests that the VLO effect on the later part of the reward downshift (Trial 11) is not mediated by gustatory processes.

VLO rats showed a disruption of the partial PRAE. The decreased responding during continuously reinforced acquisition adds to the reported impaired acquisition of autoshaping following orbitofrontal lesions (Chudasama & Robbins, 2003). In addition, disruption of partially reinforced acquisition extends the role of the VLO on autoshaping performance. The impaired autoshaping acquisition under both continuous and partial reinforcement schedules and the decrease of performance during the initial part of all sessions are consistent with an explanation in terms of frustration theory (Amsel, 1992). Under normal circumstances, partial reinforcement training (i. e., unpredictable reward conditions) invigorates behavior by eliciting higher levels of generalized drive. VLO lesions seem to disrupt generalized drive induced by partial reinforcement, a nonassociative process. An interpretation of VLO lesion effects in terms of the impairment of drive induction by reward downshift is consistent with the reduced response in autoshaping (Figures 13 and 14, middle panel), as well as reduced suppression of consummatory behavior in the cSNC situation (Figure 10, top panel). A role of the VLO on both cSNC and autoshaping is also consistent with results connecting selective breeding for high rates of recovery and a disruption of the PRAE (Ortega et al., in preparation) and increased persistence on extinction from food rewarded responses following VLO lesions (Butter, Mishkin, & Rosvold, 1963).

By contrast, mPFC lesions resulted in a PRAE similar to sham rats. Rhodes and Killcross (2004) reported that rats with mPFC lesions (infralimbic cortex) had similar

acquisition profiles than sham rats. The results from Experiment 2 (prelimbic cortex) are consistent with Rodhes and Killcross (2004), and extend the lack of evidence for a role of the mPFC on partially reinforced autoshaping. Interestingly, the same area does not seem to play a role on reward downshift.

Conclusions

The pain=frustration hypothesis applied to reward downshift **(**Papini, 2003; Papini et al., 2006) results in the hypothesis that some brain areas known to modulate physical pain may be involved in the frustration response following reward downshift. Results from the present experiments are consistent with this general hypothesis, as areas that were reported to play a role on physical pain (ACC and VLO; Donahue et al., 2001; Xie et al., 2004) affected some aspect of reward downshift performance, whereas the mPFC, which has an unclear role in physical pain, does not seem to play a role on reward downshift. An interesting issue that arises from this hypothesis is the extension of the overlap between shared neural mechanisms and behavioral processes. For instance, ACC lesions decreased pain scores via disruption of affective and motivational processes (Donahue et al., 2001). However, ACC effects on cSNC (Experiment 1) seem to be related to an enhancement of the emotional memory of the downshift event and an extended motivational effect.

Brain mechanisms underlying reward downshift can be described in terms of a top-down activation of the cortex and critical subcortical nuclei that reorganizes brainstem-based mechanisms for sucrose-related consummatory behavior (Pecoraro & Dallman, 2005). PFC areas are in an exceptional location to coordinate several neural processes (Miller & Cohen, 2001). In the case of cSNC, it seems that the ACC and the insular cortex are the more promising areas for the control of the behavioral mechanisms that underlie the sudden reorganization of consummatory behavior following cSNC (Figure 3). Flaherty's

multistage hypothesis may help clarify the neurochemical basis of cSNC. As noted, functional effects of brain areas consistent with the detection and rejection of the downshifted solution during cSNC are the VPM, CeA, and insular cortex, while the septal area seems to be related to cSNC-induced search behavior (Stage 1). Results from the present experiments are consistent with a role of the ACC on conflict/recovery from cSNC (Stage 2). In addition, the present results suggest that the VLO may play a motivational role in cSNC. The mPFC is a less promising area for further studies on the neural basis of cSNC. Future research may focus on the model of PFC top-down modulation of subcortical and brainstem mechanisms to shed light on the brain circuitry underlying the behavioral mechanisms for cSNC. For instance, are there functional connections between the VPM, amygdala, and insular cortex necessary for the modulation of Stage 1? Is the insular cortex modulating in a top-down manner subcortical circuits of detection and rejection of reward downshift? The ACC has reciprocal connections with the nucleus accumbens and the insular cortex, as well as descending connections to the amygdala. This suggests some potential avenues of modulation of recovery from cSNC: Via nucleus accumbens, by controlling the incentive value of the novel downshifted sucrose solution, and/or via amygdala, by controlling the negative emotional value emerging from the cSNC situation.

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VITA

Personal

Leonardo A. Ortega Murillo

Education

Psychologist, National University of Colombia, Bogotá, Colombia, 2005.
Master of Science, Texas Christian University, Fort Worth, TX, 2008.

Publications

Papini, M. R. & Ortega, L. A. (In press). Endogenous opioids, opioid receptors, and incentive processes. International handbook of behavior, diet and nutrition. Springer.

Ortega, L. A. (In press). [Evolution, development, and behavior]. Gutiérrez G. & Papini M. R. (Eds.), *Darwin and the behavioral sciences*.(In Spanish).

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Awards

Dean's Graduate Student Teaching Award in Psychology. College of Science and Engineering. Texas Christian University, 2010.

H. W. Ludvigson Award for outstanding paper presentation. In Recognition of the paper presentation titled: Paradoxical reward effects in the feeding behavior system of japanese quail. Southwestern Comparative Psychology Association, 2009.

Meritorious mention for undergraduate honor thesis by the National University of Colombia.

Experience

Teaching Assistant, Texas Christian University, 2006-present.
Auxiliary Professor of Psychology, Unisangil, San Gil, Colombia, 2005-2006.

ABSTRACT

BEHAVIORAL CONSEQUENCES OF REWARD DOWNSHIFT: ROLE OF THE PREFRONTAL CORTEX

by Leonardo A. Ortega, 2011
Department of Psychology
Texas Christian University

Dissertation Advisor: Mauricio R. Papini, Professor of Psychology

The present experiments were designed to determine whether bilateral electrolytic lesions on three prefrontal areas, anterior cingulate cortex (ACC), ventrolateral orbital cortex (VLO), and the medial prefrontal cortex (mPFC), modulate performance during reward downshift. ACC lesions retarded the recovery from a type of reward downshift, consummatory successive negative contrast (cSNC). VLO lesions had a small effect, in that they decreased cSNC during the late part of the downshift trial. There was no evidence that mPFC lesions played a role on cSNC, although they disrupted consummatory behavior and sucrose preference. Further testing showed that VLO lesions did not have effects on sucrose preference, but affected autoshaping acquisition under partial reinforcement. The present experiments, together with previous research, suggest that the ACC and the insular cortex are critical areas within the prefrontal cortex for the control of the response to and the recovery from cSNC.