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Lesions of the Ventral Tegmental Area Disrupt Drug-induced Appetite Stimulating Effects but Spare Reward Comparison

Robert C. Twining, Andras Hajnal, Li Han,
Penn State University College of Medicine, U.S.A.

Kristy Bruno, Ellen J. Hess, and
Johns Hopkins University School of Medicine, U.S.A.

Patricia S. Grigson
Penn State University College of Medicine, U.S.A.

Dopamine neurons in the ventral tegmental area (VTA) - nucleus accumbens (NAC) pathway track both absolute and relative properties of reward. The present study used 6-hydroxydopamine lesions of the VTA to test the obligate role of this nucleus in morphine- and cocaine-induced suppression of conditioned stimulus (CS) intake and in chlordiazepoxide- and morphine-induced appetite stimulating effects. The results showed that an 80% reduction in accumbens DA fully prevented drug-induced appetite stimulating effects, augmented a latent inhibition-like effect, but failed to disrupt drug-induced suppression of CS intake. These data demonstrate that, while the VTA is essential for responding to the reward-enhancing effects of chlordiazepoxide and morphine, it does not contribute to cocaine- or morphine-induced devaluation of the lesser saccharin reward cue.

Reward comparison is commonplace (Flaherty, 1996). Rats, monkeys, and man readily compare rewards over time. The earliest observations occurred between different levels of the same reward type, such as a piece of lettuce and a more palatable piece of banana, a large and small number of food pellets, or a low and a high concentration of sucrose (for a review see Flaherty, 1996). More recently, however, it is becoming clear that animals also compare rewards across different modalities. As a consequence, it is now evident that a rewarding drug of abuse can come to devalue a natural reward and a natural reward, in turn, can come to devalue a drug of abuse. Thus, in rats, the opportunity to self-administer cocaine reduces running in a running wheel, and the opportunity to run in a running wheel, in turn, reduces cocaine self-administration behavior (Cosgrove, Hunter, & Carroll, 2002). Likewise, access to a nondrug reinforcer, like a glucose and saccharin mixture, reduces cocaine self-administration and drug-induced relapse, while the removal of the sweet results in an immediate 4-fold increase in cocaine self-administration (Carroll, Lac, & Nygaard, 1989; Liu & Grigson, 2005). When food and cocaine are concurrently available, monkeys also make fewer choices for cocaine as the value of the food alternative is increased (Nader & Woolverton, 1991).

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Finally, drug addicted humans weigh less, are more often absent from work, and more often have their children removed from the home due to neglect (Jones, Casswell, & Zhang, 1995; Nair et al., 1997; Santolaria-Fernandez et al., 1995). Even so, the availability of natural rewards such as money (Donny, Bigelow, & Walsh, 2003, 2004) or vouchers for community based activities (e.g., ski lift passes, course credits) can serve to greatly reduce cocaine self-administration in addicted humans (Higgins et al., 1994).

Drugs of abuse, then, can devalue natural rewards and the availability of a natural reward can reduce drug self-administration behavior. Despite the pervasive and fundamental nature of this bi-directional phenomenon, relatively little is known about the underlying neural substrates. One potential player is accumbens dopamine. Dopamine is increased in the nucleus accumbens (NAC) following consumption of a natural reward such as saccharin or sucrose (Colantuoni et al., 2002; Hajnal & Norgren, 2001, 2002; Hajnal, Smith, & Norgren, 2004), food (Mirenowicz & Schultz, 1996; Richardson & Gratton, 1996; Smith & Schneider, 1988), sex (Everitt, 1990; Meisel, Camp, & Robinson, 1993; Pfaus et al., 1990), and following ingestion of salt when salt hungry (Roitman, Patterson, Sakai, Bernstein, & Figlewicz, 1999). It also is increased following the administration of drugs of abuse including morphine, cocaine, and ethanol (Di Chiara, 2002; Di Chiara et al., 1999; Ito, Dalley, Howes, Robbins, & Everitt, 2000). Finally, accumbens dopamine is increased in response to cues that predict cocaine administration (Ito et al., 2000; Ito, Dalley, Robbins, & Everitt, 2002; Phillips, Stuber, Heien, Wightman, & Carelli, 2003; Schultz, 1998) and its release can elicit approach in cocaine experienced rats (Phillips et al., 2003).

Of course, the involvement of dopamine in response to the absolute properties of a given reward does not necessitate the involvement of dopamine in response to relative reward properties (e.g., in response to the comparison of different levels of a given reward over time). Indeed, evidence suggests that accumbens dopamine tracks the comparison of different levels of the same reward over time, but that it is not essential for the phenomenon. For example, rats with a history of access to a highly preferred 32% sucrose solution consume far less of a 4% sucrose solution than do rats that have only experienced the lesser 4% sucrose reward (for a review see Flaherty, 1996; Flaherty & Checke, 1982; Flaherty & Rowan, 1986). This reduction in responding for the lesser reward is referred to as a successive negative contrast effect and is thought to occur because the perceived value of the 4% sucrose solution is reduced in comparison to the memory of the preferred 32% sucrose reward. Genn et al. have used this phenomenon to provide evidence that accumbens dopamine tracks relative reward value (Genn, Ahn, & Phillips, 2004). Specifically, they have shown that downshifting rats from 32% to 4% sucrose served to blunt the accumbens dopamine peak to the 4% sucrose solution, relative to unshifted rats that only experienced the 4% sucrose reward. Even so, other data suggest that dopamine is not necessary for comparing two levels of the same reward over time. For example, successive negative contrast effects are not altered by pretreatment with dopamine antagonists such as chlorpromazine or haloperidol (Flaherty et al., 1992). Likewise, these contrast effects in consummatory behavior also are not affected by extensive bilateral 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens (Leszczuk & Flaherty, 2000). Finally, this same lesion also fails to prevent the development of another form of contrast, anticipatory con-

trast, where rats avoid intake of a lesser saccharin reward cue when it comes to predict the availability of the preferred 32% sucrose reward following once daily saccharin-sucrose pairings (Flaherty & Checke, 1982; Leszczuk & Flaherty, 2000).

Although not essential for the comparison of two different levels of a natural reward, dopamine may be required for the comparison of rewards from different modalities (e.g., for comparing a natural reward with a drug of abuse). In this paradigm, rats avoid intake of an otherwise palatable saccharin cue when it predicts the availability of a drug of abuse following once daily taste-drug pairings (Cappell & LeBlanc, 1971; Cappell, LeBlanc, & Endrenyi, 1973; Carey & Goodall, 1974; Goudie & Dickins, 1978; Goudie, Dickins, & Thornton, 1978; Grigson, Twining, & Carelli, 2000; Le Magnen, 1969). This phenomenon was first interpreted as a conditioned taste aversion (Nachman, Lester, & Le Magnen, 1970; Riley & Tuck, 1985). More recent data, however, point to a reward comparison mechanism suggesting that rats reduce intake of the saccharin cue because it pales in comparison to the highly rewarding properties of the drug that is anticipated in the near future (Grigson, 1997; Grigson & Freet, 2000; Grigson, Lyuboslavsky, & Tanase, 2000; Grigson & Twining, 2002; Grigson, Wheeler, Wheeler, & Ballard, 2001; Schroy et al., 2005). As with the successive negative contrast paradigm, the results from a microdialysis study show that dopamine tracks this cross-modal reward comparison process over time. In particular, the dopamine peak that typically is associated with the consumption of saccharin was fully blunted when the saccharin cue came to predict morphine following a single saccharin-morphine pairing (Grigson, Acharya, & Hajnal, 2004). The data gleaned from neurotoxic lesions, on the other hand, are more mixed. Neurotoxic lesions induced by the administration of 6-OHDA into the lateral ventricles eliminated drug-induced suppression of CS intake (Wagner, Foltin, Seiden, & Schuster, 1981), while more selective 6-OHDA lesions of the nucleus accumbens did not (van der Kooy, Swerdlow, & Koob, 1983). The following experiments will revisit this important issue in an effort to determine whether accumbens dopamine simply tracks this cross-modal reward comparison process (as described) or whether it is, in fact, essential for it. The resulting data will be considered in light of the relative effectiveness of the same lesion on appetite stimulating effects induced by chlordiazepoxide and by morphine.

Experiment 1a (Saccharin-Morphine)

As alluded to above, methylamphetamine-induced suppression of CS intake was prevented by the administration of 6-OHDA into the lateral ventricles (Wagner et al., 1981) and morphine and ethanol-induced suppression was prevented by the ip administration of alpha-methyl-para-tyrosine (Sklar & Amit, 1977). Apomorphine-induced suppression of CS intake, however, was not disrupted by the more selective administration of 6-OHDA into the nucleus accumbens (van der Kooy et al., 1983). Experiment 1, then, was designed to revisit this issue by testing whether morphine-induced suppression of saccharin intake is disrupted by bilateral 6-OHDA lesions of the ventral tegmental area (VTA). The VTA is the source of dopaminergic innervation for the nucleus accumbens and for the prefrontal cortex (Dallvechia-Adams, Kuhar, & Smith, 2002; Dallvechia-Adams, Smith, & Kuhar, 2001).

Method

Subjects. The subjects were 34 naïve, male Sprague-Dawley rats (Charles River Laboratories, Massachussetts, U.S.A.) weighing between 275 and 300 g at the start of testing. All rats were individually housed in stainless steel cages in a colony room where temperature (21°C), humidity, and lighting (12L:12D) were controlled automatically. All experimental manipulations began 3 h into the light phase of the cycle. Food and water were available ad libitum, except where noted otherwise. In Experiment 1, 20 rats received bilateral, stereotaxically-guided 6-OHDA lesions of the VTA (Group VTAx) and 14 rats served as control subjects (Group SHAM): Six of these SHAM rats received vehicle infusions of 1% ascorbic acid into the VTA (SC), while 8 served as non-surgical controls (NSC).

Surgery. Twenty minutes prior to anesthesia, the rats were injected intraperitoneally (i.p.) with atropine sulfate (0.25 mg/rat) and Gentamicin (6 mg/rat). They were then anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and supplemented as necessary throughout surgery. To protect norepinephrine containing neurons, the norepinephrine reuptake inhibitor, protriptyline (15 mg/kg), was administered (i.p.) 30 ± 10 min before the 6-OHDA infusion. The rat's head was then mounted in a stereotaxic instrument, using nontraumatic earbars, with the skull level between bregma and lambda. The skin over the skull was opened with a midline incision. Using a 4 mm diameter trephine, a hole was drilled in the skull on either side of the midline, about 4.0 mm posterior to bregma. The dura mater was left intact and kept moist throughout the surgery with physiological saline. The coordinates for placement of the Hamilton (10 µl) syringe into the VTA were -4.8 to -5.0 mm posterior to bregma; ± 0.8 to ± 1.0 mm lateral to the midsagittal suture; -8.7 mm below the skull surface. Three minutes after the syringe was lowered in place, 3 µl of 6-OHDA was infused over 10 min followed by a 10 min diffusion period before removing the syringe. The same procedure was followed for the opposite side. The surgical control rats were treated identically, except that 3 µl of 1% ascorbic acid was infused instead of 6-OHDA. After removal of the Hamilton syringe, the hole in the skull was filled with Gelfoam and the wound closed with wound clips.

Recovery. In general, the animals recovered from the initial surgery over about 2 days and body weight returned to presurgical levels within a week. After 7 days, however, 6 of 72 rats treated with 6-OHDA did not eat enough regular chow to maintain body weight. These rats were given high calorie sweetened condensed milk mixed with a powdered chow to offset their weight loss. Four of these rats continued to lose weight and were then tube fed sweetened condensed milk (5–8 ml) twice daily by an oral gavage until they recovered. Tube feeding lasted no more than 7 days and, although some rats were lost (2/36 in Experiment 1 and 3/36 in Experiment 2), the surviving rats were healthy and eating normally by the third week post surgery.

Apparatus. Experiments 1a and 1b were conducted using inverted Nalgene-graduated cylinders with silicone stoppers and stainless steel spouts affixed to the front of each home cage with springs. Fluid intake was recorded to the nearest 0.5 ml.

Solutions. Sodium saccharin and L-alanine were obtained from Sigma Chemical Co., St. Louis, MO, and sucrose (saccharose) was obtained from Fisher Chemical (Pennsylvania, U.S.A.) All solutions were prepared at least 24 h in advance and presented at room temperature. Morphine sulfate and cocaine hydrochloride were generously provided by the National Institute on Drug Abuse (NIDA). Both were mixed in saline immediately before testing. Cocaine was injected in a stock solution (1.5 mg/ml), adjusted for body weight to avoid necrosis (Durazzo et al., 1994). The 6-hydroxydopamine (6-OHDA; 2 µg/µl in 1% ascorbic acid vehicle, Sigma, Missouri, U. S. A.) was prepared fresh each day and maintained on dry ice between injections. Chlordiazepoxide (CDP) was obtained from the Hershey Medical Center Pharmacy and was mixed with 0.9% saline 1-2 hrs before injection.

Deprivation State. Once recovered, all rats were weighed and handled daily and placed on a water deprivation schedule in which they received access to distilled water (dH_2O) for 5 min in the morning and for 1 h in the afternoon. Once morning intake stabilized (5-10 days), rats were matched into groups on the basis of morning intake over the final 2 days of baseline and assigned to one of two US conditions: saline or 10 mg/kg morphine, i.p. (SHAM: n = 7/cell; VTAx: n = 10/cell). When

testing began, all rats were weighed and given 5 min access to a 0.15% saccharin solution. After a 5-min interstimulus interval they were injected i.p. with saline or 10 mg/kg morphine. One such CS-US pairing occurred every other day for a total of eight trials. In addition to their daily 1 h afternoon rehydration period, all rats were given 5 min access to water on mornings between conditioning trials.

Analysis. All statistical analyses were conducted using 3-way, mixed factor analyses of variance (ANOVAs) varying the between factors (drug and lesion) by a single within factor (trials). When appropriate, all posthoc tests were conducted using Newman-Keuls with alpha set at 0.05. Preliminary statistical analyses were conducted between the surgical control and the non-surgical control groups. No significant differences were obtained. As a consequence, these groups were collapsed and will, hereafter, be referred to as group SHAM.

Brain Dissection. The rats were sacrificed by decapitation and their brains were rapidly removed and placed on the dorsal surface (about 3 min). The brains were dissected on a cold microtome stage with a glass surface (about 3 min). The initial coronal slice was taken approximately 2.0 mm anterior to the hypothalamus. The next slice was taken directly anterior to the hypothalamus. The striatum was then removed from the caudal surface of this slice of brain, based on its distinct morphological appearance. The caudate putamen included tissue dorsal to the anterior commissure, ventral to the corpus callosum, and medial to the external capsule. The medial prefrontal cortex also was dissected. The samples were immediately put in separate cold microcentrifuge tubes and weighed.

Homogenization. 500 µl of 0.1 M ice-cold PCA containing 0.01% cysteine, and DHBA was added to the samples and then homogenized for 2 min, and centrifuged at 12,000 x g for 20 min at 4°C. Supernatants (300 µl) were transferred onto a 0.2 µm-pore filtering tube and frozen at -72°C. For experiment 2, tissue was sonified in 0.1M NaOAc (pH 4.0) at 10 µL/mg. The homogenate was centrifuged at 14,000 x *g*, 4°C, for 10 min. Supernatant was filtered through 0.45 µm microspin filter tubes (Alltech Associates, Illinois, U.S.A.) by centrifuging at 14,000 x *g*, 4°C, for 2 min, and a 20 µL aliquot was analyzed.

Biochemical Determinations. Concentrations of dopamine (DA), norepinephrine (NE), serotonin (5-HT), and the metabolites 3,4-dihydroxyphenyl-acetic acid (DOPAC), Homovanillic acid (HVA), and 5-Hydroxyindoleacetic acid (5-HIAA) were analyzed by reverse-phase HPLC with coulometric detection. Samples (15 µl) were injected with an autosampler (ESA 540, Massachusetts, U.S.A.) to a 15-cm column with 3-mm bore and 3-µm C-18 packing (ESA MD-150). The mobile phase contained 60 mM sodium phosphate, 100 µM EDTA, 1.24 mM heptanesulfonic acid (Sigma, Missouri, U.S.A.), and 6% (v/v) methanol at pH 3.6. Once separated, the compounds were measured with a Coulochem II system (ESA; analytic cell: model 5014B, electrode 1 -175 mV, electrode 2 +175 mV; guard cell: model 5020, + 300 mV). The system detection limit for DA is about 2.0 fmol/15µl standard sample. In brain microdialysates, DOPAC levels typically are >100-fold higher than DA, so detection limits are not an issue. For Experiment 2, samples were analyzed by HPLC equipped with a C18, MD-150 column (150 mm length x 3 mm interior diameter, ESA, Massachusetts, U.S.A.) and 4 coulometric electrochemical detectors. Electrochemical sensor potentials were set at 150, 250, 350 and 500 mV. Mobile phase consisted of 75 mM sodium dihydrogen phosphate, 1.7 mM 1-octanesulfonic acid sodium salt, 25 µM EDTA and 8% acetonitrile (pH 2.9) at a flow rate of 0.6 ml/min. Compounds were identified and quantified by comparing retention time, sensor ratio measures and peak height to known standards.

Results and Discussion

HPLC Analysis. Relative to SHAM controls, the infusions of 6-OHDA into the VTA led to an 80% depletion of DA in the nucleus accumbens and an approximate 35% depletion of NE and 5-HT. This depletion profile was similar in the dorsal striatum. In the medial prefrontal cortex, however, the VTA lesion led to an approximate 15% depletion of DA and NE and a 25% depletion of 5-HT.

Table 1
Effect of VTA 6-OHDA Infusions on Brain Tissue Concentration of Monoamines Relative to SHAM Controls for the Rats in Experiment 1.

	NAC		dSTR		mPFC	
	AVG %	**±SEM**	**AVG %**	**±SEM**	**AVG %**	**±SEM**
DA	20.0	7.0	25.3	5.2	84.5	13.3
NE	71.7	7.6	64.7	5.8	84.6	14.0
5HT	77.5	12.4	83.3	16.8	75.1	8.8
DOPAC	109.8	10.4	171.6	12.5	113.4	1.9
HVA	86.5	12.6	48.3	9.8	116.0	33.1
5HIAA	95.4	10.2	68.1	7.2	93.5	10.4

Note. Data are expressed as a percent of the SHAM group's average tissue concentration (± 4.9-10% S.E.M.) for the respective monoamine and metabolite. The concentrations were collapsed for both right and left hemispheres. NAC, nucleus accumbens; dSTR, dorsal striatum; mPFC, medial prefrontal cortex; ND, not detectable.

Saccharin-CS Intake. All rats (SHAM and VTAx) suppressed intake of the saccharin cue following pairings with the 10 mg/kg dose of morphine, see Figure 1, left and right panels.

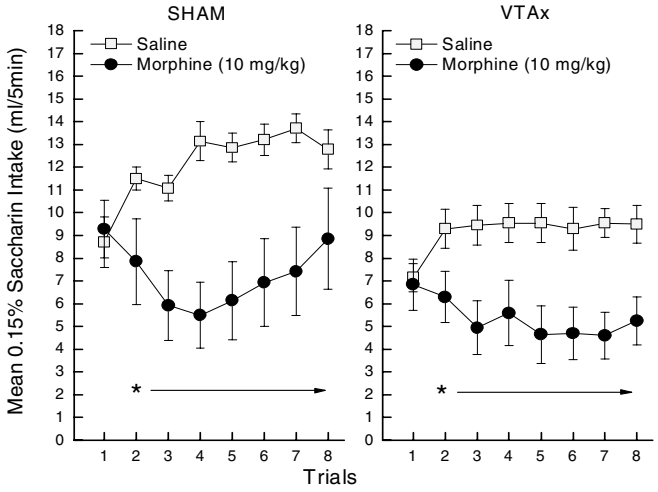

Figure 1. Mean (± SEM) intake (ml/5 min) of 0.15% saccharin in SHAM and VTA-lesioned (VTAx) rats injected intraperitoneally with either saline or morphine (10 mg/kg) across 8 taste-drug pairings.

This conclusion was supported by the results of a 2 x 2 x 8 mixed factorial ANOVA varying lesion (VTAx or SHAM), drug (morphine or saline) and trials (1-8). The results showed that the Drug x Trials interaction was significant, $F(7, 210) = 13.41$, $p < 0.0001$. Post hoc tests of this two-way interaction showed that all morphine treated rats, SHAM and VTAx, consumed less saccharin than their saline injected controls on trials 2-8, ps < .05. Neither the Lesion x Drug, $F < 1$, nor the Lesion x Drug x Trials interaction, $F(7, 210) = 1.64$, $p < 0.12$, however, attained

statistical significance, indicating that the lesion of the VTA had no impact on morphine-induced suppression of CS intake. The main effect of lesion, on the other hand, was significant, $F(1, 30) = 5.27$, $p < 0.03$, showing that the VTAx rats consumed less saccharin than the SHAM rats overall.

Experiment 1b (Alanine-Cocaine)

It is clear that the dopamine lesion (about 80% depletion) did not affect morphine induced suppression of CS intake in the present report. Although morphine administration causes a substantial increase in dopamine in the nucleus accumbens (Di Chiara et al., 1999), μ opioid receptors are widely distributed outside of the VTA, most notably in the NAC and lateral hypothalamus (MacDonald, Billington, & Levine, 2004). Thus, it is plausible that many of the effects of morphine, including the rewarding effects, are conserved in rats with a selective depletion of accumbens dopamine. Therefore the following experiment used the SHAM and the VTAx rats from Experiment 1a in a crossover design to test whether the lesion would disrupt the suppressive effects of cocaine. Cocaine is an indirect DA agonist and its rewarding effects are generally thought to be mediated by inhibition of the DA transporter on presynaptic terminals of VTA neurons in the NAC (Giros, Jaber, Jones, Wightman, & Caron, 1996). Direct administration of the neurotoxin into the VTA, then, would be expected to damage these terminals and, in so doing, may more selectively disrupt cocaine-induced suppression of CS intake.

Method

Subjects. The subjects were the same as those described in Experiment 1a.

Procedure. Experiment 1b began one week after completion of Experiment 1a. Water deprivation was maintained and a complete crossover design was employed whereby the rats that had received the saccharin CS paired with morphine in Experiment 1a were now presented with a novel sweet tasting amino acid, 0.3 M alanine. This CS was paired with saline (VTAx: $n = 10$; SHAM: $n = 7$). Rats that previously served in the saccharin-saline condition in Experiment 1a, on the other hand, also received access to the alanine CS, but this CS was now paired with cocaine (VTAx: $n = 10$; SHAM: $n = 7$). During testing, all rats were weighed and given 5 min access to the 0.3 M alanine solution. After a 5 min interstimulus interval they were injected subcutaneously with saline or a 10 mg/kg dose of cocaine. One such CS-US pairing occurred every other day for a total of eight trials. To maintain proper hydration, all rats received 1 h access to water each afternoon and 5 min access each morning between conditioning trials.

Results and Discussion

Alanine-CS Intake. Unlike the SHAM rats, the VTAx rats did not suppress intake of the alanine CS following 8 pairings with the 10 mg/kg dose of cocaine. This conclusion was supported by the results of a 2 x 2 x 8 mixed factorial ANOVA varying lesion (VTAx or SHAM), drug (cocaine or saline), and trials (1-8). The results showed that the Lesion x Drug x Trials interaction was significant, $F(7, 210) = 3.27$, $p < 0.003$. Post hoc tests of this 3-way interaction revealed that the cocaine treated SHAM rats exhibited a significant reduction in intake of the alanine CS on trials 5, 7, and 8 relative to their saline treated controls, ps < 0.05. The cocaine treated VTAx rats, in comparison, actually consumed significantly

more of the alanine CS than their saline treated controls on trials 1 and 4, *p*s < 0.05. The Drug x Trials interaction also was significant, $F(7, 210) = 8.33$, $p < 0.0001$. Post hoc tests of this 2-way interaction indicated that all of the rats receiving saline (VTAx and SHAM) consumed significantly less of the alanine CS than their cocaine injected controls on trials 1 and 2. This finding probably was due to carryover effects (see General Discussion) for the rats previously serving in the saccharin-saline condition in Experiment 1a. While there was a significant main effect of Lesion, $F(1, 30) = 6.01$, $p < 0.02$, indicating that the VTAx rats consumed significantly less alanine overall, neither the main effect of Drug, $F < 1$, nor the Lesion x Drug interaction, $F(1, 30) = 2.14$, $p = 0.15$, attained statistical significance. Thus far, these results suggest that bilateral lesions of the VTA may disrupt cocaine- but not morphine-induced suppression of CS intake. Experiment 2a will directly test the validity of this conclusion.

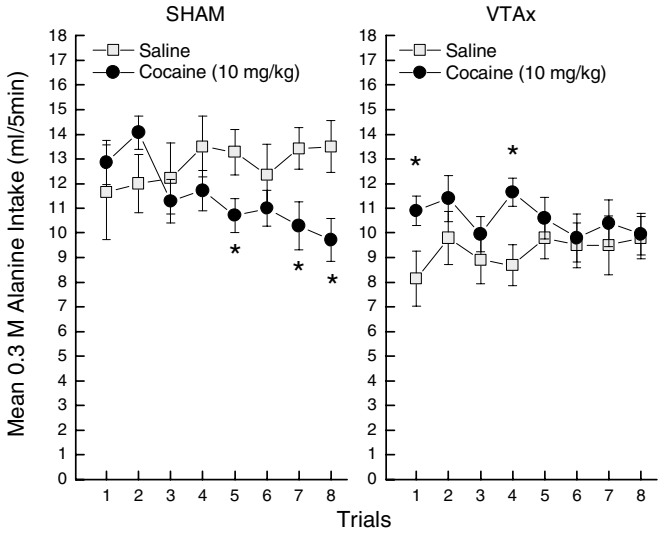

Figure 2. Mean (± S.E.M) intake (ml/5 min) of 0.3 M alanine in SHAM and VTA-lesioned (VTAx) rats injected subcutaneously with saline or cocaine (10 mg/kg) across 8 taste-drug pairings.

Experiment 1c (CDP-Induced Appetite)

Although it is possible that the VTA lesion selectively disrupts the suppressive effects of cocaine, but not morphine, it also is possible that the lesion was simply too small to adequately disrupt both phenomena. The overall reduction in saccharin and alanine intake by the VTAx rats in both Experiment 1a and 1b may be indicative of a good VTA lesion (e.g., a motivational deficit) or it may, on the other hand, reflect damage to the neighboring substantia nigra (i.e., a motor deficit). Thus, it is crucial to the current investigation to implement a behavioral test that is sensitive to the motivational deficit induced by DA depletion of the VTA-accumbens pathway, while at the same time ruling out a potential motor deficit. The present experiment, then, will employ a task known to be disrupted by a similar VTA lesion. Specifically, pretreatment with a benzodiazepine induces appetite stimulating effects when measured in an intake test and increases appetitive taste

reactivity (Berridge & Treit, 1986; Shimura, Kamada, & Yamamoto, 2002). Dopaminergic lesions of the VTA have been shown to block midazolam-induced increases in intake of a 0.1 M sucrose solution using a 24 h two-bottle intake test (Shimura et al., 2002). This earlier report, however, did not control for a potential motor deficit, did not verify DA levels in either the dorsal or ventral striatum, and did not employ stimulus parameters that are relevant to the current investigation. For the current experiment, we modified the parameters to match those employed in Experiments 1a and 1b, controlled for a potentially confounding motor deficit, and measured extracellular DA concentrations in both the dorsal and ventral striatum. Specifically, we used a 0.1 M sucrose solution and a 5 min one-bottle test in non-deprived rats to determine whether the present VTA lesion, which appears to have disrupted cocaine-induced suppression of CS intake, is sufficient to prevent chlordiazepoxide-induced appetite stimulating effects.

Methods

Subjects. The subjects were the same as those described in Experiment 1a and 1b. In this experiment, however, all rats were given access to food and water ad libitum.

Apparatus. Testing was conducted in one of four modular operant chambers (MED Associates, Vermont, U.S.A.) measuring 30.5 x 24.0 x 29.0 cm, housed in a light- and sound-attenuating cubicle equipped with a ventilation fan. All chambers had a clear Plexiglas top, front, back, and one side wall (the side with the sipper tubes). The remaining side wall was made of aluminum. The grid floors consisted of nineteen 4.8-mm stainless steel rods spaced 1.6 cm apart (center to center). Each chamber was equipped with a retractable sipper tube that could enter the chamber through 1.3-cm diameter holes. A stimulus light was located 6 cm above the tube. In the extended position, the tip of the sipper tube was aligned in the center of the hole, flush with the wall. A lickometer circuit (0.3 μA) was used to monitor licking. A shaded houselight reflected light off the ceiling. Each chamber was also equipped with a tone generator (Sonalert Time Generator, 2900 Hz, Mallory, Indiana, U.S.A.) and a speaker for white noise (75 dB) on the wall opposite the sipper tubes. Events in the chamber and collection of the data were controlled on-line with a Pentium computer. Programs were written in Medstate notation language from Med-PC for windows.

Procedure. For trials 1-10, each SHAM or VTAx rat was taken from his home cage, weighed and placed in the operant chamber. When the trial was initiated, the house light was illuminated and a bottle containing a palatable 0.1 M sucrose solution was advanced for 5 minutes. After the 5 min access period, the bottle retracted, the house light was turned off and the subject was removed from the operant chamber and placed back in his home cage. Baseline responding for 0.1 M sucrose was determined across trials 1-5. On trials 6-7, the rats were habituated to an i.p. injection of saline. Approximately, 35 min (± 5 min) later, they were given 5 min access to 0.1 M sucrose. Test trials were conducted over trials 8-10. Using a standard ABA design, 5 min intake of the 0.1 M sucrose solution was assessed following an i.p. injection of saline (trial 8), a 10 mg/kg dose of chlordiazepoxide (trial 9), and saline (trial 10).

Results and Discussion

Bilateral 6-OHDA lesions of the VTA were uniformly successful in disrupting the appetite stimulating effects of chlordiazepoxide. Thus, the SHAM rats, but not the VTA lesioned rats, significantly increased intake of the 0.1 M sucrose solution following the injection of CDP.

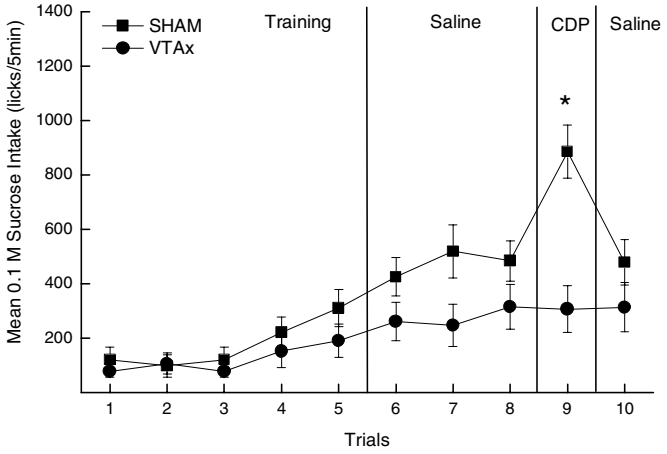

Figure 3. Mean (± S.E.M) intake (licks/5 min) of 0.1 M sucrose in SHAM and VTA-lesioned (VTAx) rats. Baseline intake was assessed on trials 1-5. Intake on the remaining trials was assessed 35 min after an intraperitoneal injection of either saline (trials 6-8 &10) or a 10 mg/kg dose of chlordiazepoxide (trial 9).

This conclusion was supported by the results of a 2 x 10 mixed factorial ANOVA varying lesion (SHAM or VTAx) and trials (1-10). The Lesion x Trials interaction was highly significant, $F(9, 288) = 6.82$, $p < 0.0001$. Posthoc tests of this 2-way interaction revealed that the SHAM rats consumed significantly more 0.1 M sucrose after the CDP injection on trial 9 than they did after saline injections either before (trial 8) or after (trial 10) the test trial, ps < 0.05. This effect was completely abolished in the VTAx rats. In addition, there was a tendency for the SHAM rats to drink more sucrose than the VTAx rats overall, as indicated by a significant main effect of lesion, $F(1, 32) = 4.14$, $p < 0.05$. However, posthoc tests of the two-way Lesion x Trials interaction described above revealed that the SHAM rats drank significantly more 0.1 M sucrose only on trial 9, after CDP was injected. We conclude, therefore, that the significant main effect is carried by the increase in intake exhibited by the SHAM rats following the injection of CDP on trial 9. Further, it is important to note that the failure of the VTAx rats to increase sucrose consumption after the CDP injection cannot be attributed to a motor deficit or to a ceiling effect. In Experiments 1a and 1b, the same VTAx rats demonstrated much higher lick rates during equivalent 5 min sessions when tested under water-deprived conditions. Specifically, given that rats take approximately 5 µl/lick (Corbit & Luschei, 1969), the VTA lesioned rats made between 1000–2000 licks/5 min when tested in the water-deprived state in Experiments 1a and 1b. These rats, then, could have made more licks/5 min in the present experiment, but they did not. Thus, while the lesion exerted an apparent mixed disruptive effect on drug-induced suppression of CS intake, it was sufficient to fully eliminate the appetite stimulating effect induced by the i.p. administration of CDP.

Experiment 2a (Alanine-Cocaine)

The results of Experiment 1 suggest that bilateral lesions of the VTA disrupt avoidance of a palatable taste cue when paired with cocaine, but not when

paired with morphine. If true, this would indicate that the suppressive effect of cocaine, but not morphine, relies on intact dopaminergic transmission within the striatum. While a seemingly parsimonious conclusion, confidence is diminished by the relatively small size of the suppressive effect of cocaine in the SHAM subjects. In our hands, this dose of cocaine generally supports a substantial reduction in CS intake in naïve rats (Grigson, 1997; Grigson, Cornelius, & Wheeler, 2001; Grigson et al., 2001). Thus, it appears that prior experience with the saccharin-saline condition in Experiment 1a may have disrupted cocaine-induced suppression of alanine intake in both the intact and the lesioned subjects in Experiment 1b. The purpose of the present experiment was to eliminate this interpretive confound by replicating cocaine-induced suppression of alanine intake in a set of naïve VTA lesioned rats. If an intact VTA is essential for the development of cocaine-induced suppression of alanine intake, then the effect should be disrupted even when both the CS and the US are novel. If, on the other hand, the disruptive effect of the lesion found in Experiment 1b was due to carry-over effects from Experiment 1a, then cocaine-induced suppression of CS intake should be robust in both the SHAM and the VTA lesioned rats.

Method

Subjects. The subjects were 33 naïve, male Sprague-Dawley rats obtained and maintained as described. They weighed between 275-300 g at the start of testing. Fourteen rats received bilateral, stereotaxically-guided 6-OHDA lesions of the VTA and 19 rats served in group SHAM: Twelve received vehicle infusions of 1% ascorbic acid into the VTA (SC) and 7 served as nonsurgical controls (NSC).

Apparatus. Testing was conducted in the home cage as described in Experiment 1a and 1b.

Deprivation State. The rats (SHAM: n = 9/cell; VTAx: n = 7/cell) were tested as described in Experiment 1b.

Table 2
Effect of VTA 6-OHDA Infusions on Brain Tissue Concentration of Monoamines Relative to SHAM Controls for the Rats in Experiment 2.

	NAC		dSTR		mPFC	
	AVG %	**±SEM**	**AVG %**	**±SEM**	**AVG %**	**±SEM**
DA	22.9	5.8	20.7	7.8	62.6	13.3
NE	75.0	10.3	37.1	6.6	68.8	6.5
5HT	72.6	4.6	55.9	5.2	73.4	6.2
DOPAC	21.3	8.3	21.8	11.8	127.6	43.3
HVA	24.8	5.7	23.2	7.2	ND	-
5HIAA	97.6	4.9	89.6	7.6	91.1	9.9

Note. Data are expressed as a percent of the SHAM group's average tissue concentration (± 4.9-10% S.E.M.) for the respective monoamine and metabolite. The concentrations were collapsed for both right and left hemispheres. NAC, nucleus accumbens; dSTR, dorsal striatum; mPFC, medial prefrontal cortex; ND, not detectable.

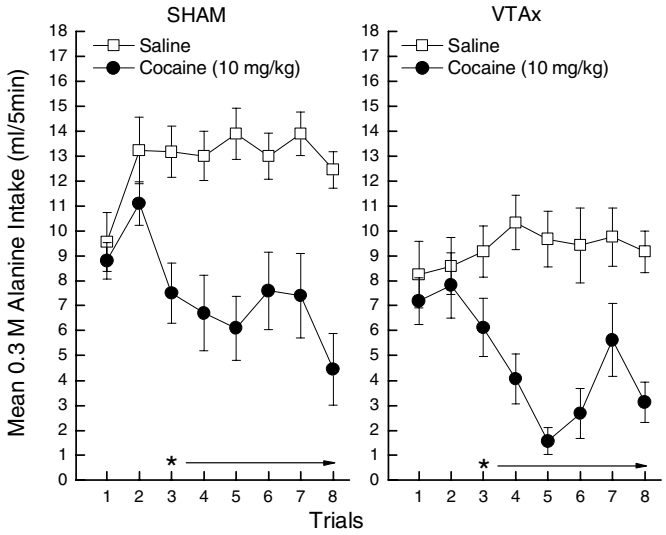

Figure 4. Mean (± S.E.M) intake (ml/5 min) of 0.3 M alanine in SHAM and VTA-lesioned (VTAx) rats injected subcutaneously with saline or cocaine (10 mg/kg) across 8 taste-drug pairings.

Results and Discussion

HPLC Analysis. The HPLC results for the NAC are similar to those reported in Experiment 1. Bilateral 6-OHDA lesions of the VTA led to an approximate 80% depletion of DA in the accumbens and a 25%-30% depletion of NE and 5-HT. The VTA lesion also led to an 80% depletion of DA in the dorsal striatum and a 73% and a 44% depletion of NE and 5-HT, respectively. Finally, the VTA lesion led to a 37% depletion of DA and a 31% depletion of NE in the prefrontal cortex.

Alanine-CS Intake (Experiment 2a). Similar to the results of Experiment 1a with morphine, all rats (SHAM and VTAx) suppressed intake of the alanine cue following pairings with the 10 mg/kg dose of cocaine, see Figure 4. This conclusion was supported by the results of a 2 x 2 x 8 mixed factorial ANOVA varying lesion (VTAx or SHAM), drug (cocaine or saline), and trials (1-8). The results showed that the Drug x Trials interaction was significant, $F(7, 203) = 11.90$, $p < 0.0001$. Post hoc tests of this two-way interaction showed that all cocaine treated rats, SHAM and VTAx, consumed less alanine than their saline injected controls on trials 3-8, $p < 0.05$. Neither the Lesion x Drug, $F < 1$, nor the Lesion x Drug x Trials interactions, $F < 1$, however, attained statistical significance indicating that the lesion of the VTA had no impact on cocaine-induced suppression of CS intake. The main effect of Lesion, $F(1, 29) = 9.64$, $p < 0.004$, on the other hand, was significant, showing that the VTAx rats consumed less alanine than the SHAM rats overall. When taken with the results of Experiment 1, these data demonstrate that depletion of accumbens dopamine via 6-OHDA lesions of the VTA is not sufficient to disrupt either morphine- or cocaine-induced suppression of CS intake in naïve rats.

Experiment 2b (Saccharin-Morphine)

In Experiment 1, the suppressive effects of morphine were intact for all of the rats while those of cocaine were eliminated in the rats with bilateral 6OHDA lesions of the VTA. The results of Experiment 2a suggest that this dissociation had more to do with the order of testing than with the nature of either the conditioned or unconditioned stimuli employed. Experiment 2b was designed to test the validity of this conclusion.

Method

Subjects. The subjects were the same as those described in Experiment 2a.

Procedure. Experiment 2b began one week after completion of Experiment 2a. Water deprivation was maintained and a complete crossover design was employed whereby the rats that had received the alanine CS paired with cocaine in Experiment 2a were now presented with a novel palatable CS, 0.15% saccharin. This CS was paired with saline (VTAx: n = 10; SHAM: n = 7). Rats that previously served in the alanine-saline condition in Experiment 2a, on the other hand, also received access to the saccharin CS, but this CS was now paired with morphine (VTAx: n=10; SHAM: n=7). During testing, all rats were weighed and given 5 min access to the 0.15% saccharin solution. After a 5 min interstimulus interval they were injected i.p. with saline or a 10 mg/kg dose of morphine. One such CS-US pairing occurred every other day for a total of eight trials. To maintain proper hydration, all rats received 1 h access to water each afternoon and 5 min access each morning between conditioning trials.

Results and Discussion

As predicted, the SHAM, but not the VTAx rats, suppressed intake of the saccharin CS following pairings with a 10 mg/kg dose of morphine.

This conclusion was supported by the results of a 2 x 2 x 8 mixed factorial ANOVA varying lesion (VTAx or SHAM), drug (morphine or saline), and trials (1-8). The results showed that there was a highly significant Lesion x Drug x Trials interaction, $F(7, 189) = 3.84$, $p < 0.0006$. Post hoc tests of this 3-way interaction revealed that the VTAx rats in the morphine condition actually consumed more saccharin than their saline controls on trial 1. This effect was also exhibited by the SHAM rats and persisted until trial 2. Thereafter, only the SHAM rats injected with morphine demonstrated significant avoidance of the saccharin CS relative to their saline injected controls (e.g., on trials 5-8). Neither the main effect of drug, $F < 1$, nor the main effect of trials, $F < 1$, was significant. The main effect of lesion approached statistical significance, $F(1, 27) = 4.09$, $p < 0.053$, consistent with a tendency for the VTAx rats to drink less saccharin than the SHAM rats overall. Taken together, the results demonstrate that naïve rats, with or without VTA lesions, will learn to avoid a palatable taste cue paired with either morphine or cocaine. However, conditioned avoidance of a palatable taste paired with either morphine or cocaine is retarded by prior nonreinforced experience with a different sweet-tasting CS.

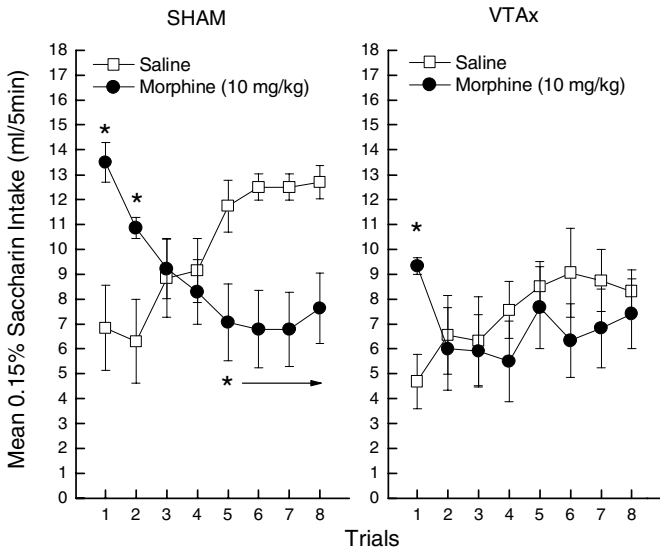

Figure 5. Mean (± S.E.M) intake (ml/5 min) of 0.15% saccharin in SHAM and VTA-lesioned (VTAx) rats injected intraperitoneally with either saline or morphine (10 mg/kg) across 8 taste-drug pairings.

Experiment 2c (Morphine Induced Appetite)

The results thus far show that, while an intact VTA is not essential for morphine- or cocaine-induced suppression of saccharin intake, it is required for the expression of a CDP-induced appetite stimulating effect. This pattern of data suggests that the dopaminergic pathway is not essential for responding to the devaluation that occurs following saccharin-drug or saccharin-sucrose (Leszczuk & Flaherty, 2000) pairings, but is essential for responding to the increase in palatability that occurs following the administration of a benzodiazepine. The final experiment examined this dichotomy more directly using morphine to induce the appetite. Like CDP, morphine increases food intake, and the associated appetitive taste reactivity behaviors, and these effects have been shown to depend upon opiate receptors in the nucleus accumbens (Doyle, Berridge, & Gosnell, 1993; Soderpalm & Berridge, 2000). Shimura et al. (2002) showed that the appetite stimulating effect of morphine also was disrupted by VTA lesions, but again, controls for motor deficits were not included and accumbens and striatal dopamine were not measured. If an intact VTA is required for the expression of appetite stimulating effects in general, then our rats with VTA lesions also should fail to exhibit a morphine-induced appetite stimulating effect. Such a finding would be striking, given that the same drug (morphine) readily suppressed CS intake in naïve rats with similar VTA lesions in Experiment 1a. In the present experiment, powdered chow intake was measured in nondeprived rats over a 1-h test period. This approach allowed ample time for the VTAx rats to demonstrate an increase in food consumption on the day of the morphine injection. Finally, a manipulation was conducted at the end of testing to verify that any disruption in intake was not simply due to a motor deficit. This control is particularly important because our VTA lesion also led to an 80%

depletion of DA in the dorsal striatum and a general reduction in 5-min intake was obtained in all of the conditioning experiments.

Method

Subjects. Two surgical control rats that served in the first two phases of Experiment 2 were lost due to infection. The remaining 31 rats were healthy and served in this final experiment. The subjects had access to both food and water ad libitum, except where noted otherwise.

Apparatus. All testing was conducted in the home cage as described.

Procedure. For trials 1-5, each rat was taken from his home cage, weighed, and returned. While the rats were being weighed, their food pellets were removed and a food dish containing powdered chow was weighed and placed in the center of the home cage for 1 h. After this 1h-access period to powdered chow, both the food dish and the rat were weighed again. Spillage was minimal, but when it occurred, was brushed into the dish before the final weight measurement was taken. Again, a standard ABA design was used to evaluate the hyperphagic effects of a 4 mg/kg dose of morphine administered ip. Thirty minutes prior to the start of the 1 h test session, all rats were injected with saline on trial 3, morphine on trial 4, and saline on trial 5. In order to test for potential motor deficits, all rats were food-deprived for 24 h on trial 6 and then were given 1 h access to powdered food.

Results and Discussion

Once again, the bilateral 6-OHDA lesions of the VTA were successful. Only the SHAM rats significantly increased intake of powered food following the injection of morphine.

This conclusion was supported by the results of a 2 x 5 mixed factorial ANOVA varying Lesion (SHAM or VTAx) and Trials (1-5). The Lesion x Trials interaction was highly significant, $F(4, 116) = 8.76$, $p < 0.0001$. Post-hoc tests of this 2-way interaction revealed that the SHAM rats consumed significantly more food after the morphine injection on trial 4 than they did after saline injections on trial 3 or trial 5, ps < 0.05. This effect was completely absent in the VTAx rats. Powdered chow intake did not differ between the VTAx rats and the SHAM lesioned rats on any trial, except after the morphine injection on trial 4. The main effect of lesion was not significant, $F < 1$, indicating that the SHAM and the VTAx rats consumed roughly equivalent amounts of the powdered chow when collapsed across trials. The failure of the VTAx rats to increase food consumption after the injection of morphine cannot be attributed to a motor deficit or to a ceiling effect as they, and their SHAM counterparts, exhibited much higher consumption of powdered chow on trial 7 following 24 h of food deprivation than on any other day. A t-test confirmed that intake by the SHAM and the VTA lesioned rats did not differ following 24-h food deprivation, $p > 0.05$. Taken together, the results show that dopaminergic transmission in the ventral striatum is essential for the expression of appetite stimulating effects, whether elicited by CDP or by morphine and whether measuring consumption of a sucrose solution or powdered chow.

General Discussion

Bilateral 6-OHDA lesions of the VTA led to an 80% reduction in dopamine in the NAC and to a 15–37% depletion of dopamine in the prefrontal cortex

in rats. This lesion was sufficient to fully eliminate the appetite stimulating effects induced by both a 10 mg/kg dose of CDP and a 4 mg/kg dose of morphine. The lesion also served to augment the disruptive effects of prior nonreinforced exposure to a sweet-tasting CS (i.e., prior experience with a sweet paired with saline). Even so, use of a counterbalanced design confirmed that this effect was not dependent upon either the nature of the CS or the nature of the US. Finally, while the lesion was sufficient to eliminate the appetite stimulating effects of both CDP and morphine and to augment the disruptive influence of prior experience, the same lesion had no impact whatsoever on either morphine- or cocaine-induced suppression of CS intake in naïve rats. We conclude that the dopamine projection from the VTA to the NAC need not be intact to compare rewards over time. It is, however, required for the increase in sustained intake that occurs following the administration of a benzodiazepine or morphine and for new learning following a period of nonreinforced exposure to a similar CS.

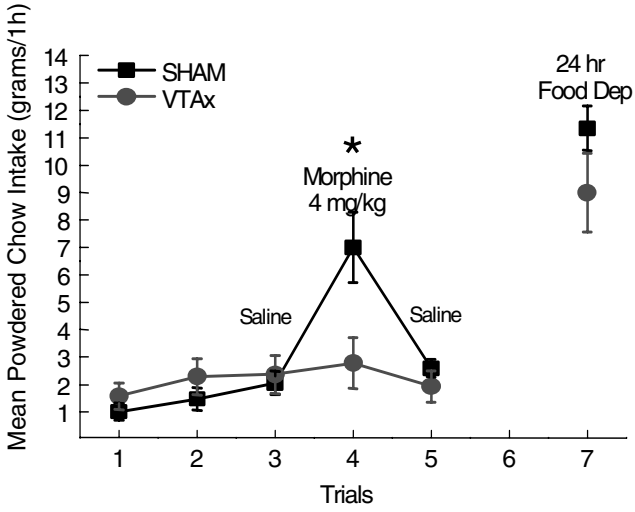

Figure 6. Mean (± S.E.M) intake (g/h) of powdered chow in SHAM and VTA-lesioned (VTAx) rats. Baseline feeding was assessed on trials 1 & 2. Food intake on the remaining trials was assessed 30 minutes after an intraperitoneal injection of either saline (trials 3 & 5), a 4 mg/kg dose of morphine (trial 4), or after 24 h food deprivation (trial 7).

The VTA-NAC pathway clearly tracks both absolute and relative reward properties. It is not, however, an obligate relay. As discussed, neurochemical and electrophysiological data show that NAC dopamine tracks stimuli of import (rewarding, aversive, or when neutral stimuli become associated) and the neural response to these stimuli is altered by experience (Carelli & Deadwyler, 1997; Datla, Ahier, Young, Gray, & Joseph, 2002; Di Chiara et al., 1999; Hajnal et al., 2004; Kiyatkin & Stein, 1996; Kiyatkin, Wise, & Gratton, 1993; Mark, Smith, Rada, & Hoebel, 1994; Roitman, Wheeler, & Carelli, 2005; Tobler, Fiorillo, & Schultz, 2005; Young, 2004; Young et al., 1998). The accumbens also tracks relative reward properties. The activity of some single cells in the NAC is reduced for a low concentration of sucrose when alternated with the availability of a preferred, higher concentration (simultaneous negative contrast, Taha & Fields, 2005; Wheeler, Roitman, Grigson, & Carelli, 2005) and it is increased for a high concentration of

sucrose when alternated with a less preferred, low concentration (simultaneous positive contrast, Wheeler et al., 2005). Similarly, Tobler et al. (2005) recently showed in the Macaque monkey that the activity of VTA dopamine neurons for a given reward magnitude depends upon the relative magnitude of the alternative reward that is predicted. Finally, as described, the peak in accumbens dopamine following the ingestion of saccharin or sucrose is blunted for the same solution if the rats are expecting, on the basis of prior experience, to have access to a more palatable, more concentrated sucrose solution or a drug of abuse (Genn et al., 2004; Grigson et al., 2004).

In contrast to a critical role identified for the gustatory thalamus and cortex (Geddes, Han, Baldwin, & Grigson, 2004; Grigson, et al., 2000; Mackey, Keller, & van der Kooy, 1986; Reilly & Pritchard, 1996; Schroy et al., 2005), however, the VTA-NAC pathway need not be intact for the expression of such reward processing in behavior. Indeed, in many instances extensive lesions of the dopaminergic VTA-NAC pathway do not disrupt responding to either absolute or relative reward properties. Regarding absolute reward properties, rats with accumbens DA depletions (Berridge & Robinson, 1998), or with no forebrain at all for that matter (Grill & Norgren, 1978a), exhibit normal positive hedonic orofacial movements that increase lawfully with the concentration of sucrose infused into the oral cavity (Grill & Norgren, 1978b). Further, drug-induced increases in appetitive taste reactivity measures can be augmented by the administration of either morphine or benzodiazepines (Berridge & Treit, 1986; Doyle et al., 1993), even when rats are depleted of almost 99% of DA in the NAC (Berridge & Robinson, 1998). Similar lesions of the VTA only minimally reduce intake of increasing concentrations of rewarding and aversive taste stimuli in a 24 h 2-bottle intake test (Shimura et al., 2002). Finally, regarding relative reward properties, bilateral 6-OHDA lesions of the NAC fail to disrupt successive negative contrast effects in consummatory behavior (Leszczuk & Flaherty, 2000) and similar lesions of this pathway fail to disrupt avoidance of a saccharin cue when paired with a rewarding sucrose solution (Leszczuk & Flaherty, 2000). The results from the present report are in keeping. Large bilateral lesions of the VTA failed to prevent avoidance of a palatable taste cue following pairings with either cocaine or morphine. The dopaminergic projection from the VTA to the NAC, then, need not be intact for drug-induced suppression of CS intake.

Although large lesions do not disrupt responding to either absolute or relative properties of reward, several other behaviors are disrupted, and as such, provide insight into the obligate function of the VTA-NAC pathway in behavior. Of most relevance to the current report, Leszczuk and Flaherty (2000) showed that the same NAC lesion that failed to disrupt consummatory successive negative contrast effects following the unexpected downshift from 32% to 4% sucrose, completely prevented the successive negative contrast effect in instrumental behavior in a runway. An intact NAC, then, is not required to detect the gustatory stimuli, accurately identify their relative value, remember the value of the previously received reward, compare the available 4% sucrose reward with the memory of the preferred 32% sucrose reward, or to suppress consummatory behavior accordingly (steps also required for the comparison of a saccharin cue with a drug of abuse). The lesion-induced deficit occurs when the loss of reward should impact upon instrumental performance. In this case, the lesioned rats decreased running speed in

the runway, but only to the level of the unshifted controls. Interestingly, this finding parallels that obtained with bilateral excitotoxic lesions of the hippocampus which also selectively disrupt successive negative contrast effects in instrumental (runway), but not consummatory, behavior (Flaherty, Coppotelli, Hsu, & Otto, 1998). The hippocampus is known to be critical for context learning (Honey & Good, 1993) and regulates "up" and "down" states of neurons in the NAC (Goto & O'Donnell, 2002). Taken together, these observations may suggest that the failure to demonstrate contrast in instrumental performance following a lesion that disrupts the VTA-NAC pathway relates to the context dependent nature of the instrumental behavior.

The most obvious lesion-induced deficit in our hands was the complete elimination of the appetite stimulating effects of chlordiazepoxide and morphine. These effects could not be attributed to a general motor deficit because the same lesioned rats exhibited a higher level of food and water intake when food- or water-deprived. In the same light, the data cannot be attributed to a general motivation deficit, as the VTA lesioned rats clearly were motivated to consume the stimuli when hungry or thirsty. According to Kelley (2004) and Berridge and Robinson (1998), opiates and benzodiazepines serve to increase the perceived palatability of sweets, leading to an increase in the associated appetitive orofacial responses for the stimuli when infused directly into the oral cavity. These drugs also are thought to lead to an increase in motivation as reflected by an increase in the animal's willingness to work for access to the sweet on a progressive ratio schedule of reinforcement (Zhang, Balmadrid, & Kelley, 2003). As briefly mentioned above, rats with extensive lesions of the NAC continue to demonstrate a benzodiazepine-induced appetite stimulating effect in taste reactivity behavior following intraoral delivery (Berridge & Robinson, 1998). The lesioned rats, then, are appropriately sensitive to the drug-induced palatability shift, if the stimuli are infused directly into the oral cavity. They fail, apparently, when having to seek access to the fluid. Thus, even though changes in perceived palatability are likely intact, the present data show that the VTA lesioned rats fail to increase consumption of the rewarding stimulus when having to approach the location of the reward.

As stated, the VTA lesioned rats had no difficulty approaching the same rewarding stimuli when motivated by internal cues such as food or water deprivation. Hanlon, Baldo, Sadeghian, and Kelley (2004), however, have shown that the motivation underlying these drug-induced appetite stimulating effects differs from that induced by food deprivation. Of course, the VTAx rats did approach and sample the stimuli in the present report. Indeed, with the drug on board, they consumed a volume that did not differ from that consumed on nondrug trials. This exposure to this presumably more palatable stimulus, however, was not sufficient to facilitate a drug-induced increase in intake. One possible explanation relates to "work". Salamone has published a number of papers clearly showing that accumbens dopamine is required to appropriately respond to increasing work requirements (Salamone, Wisniecki, Carlson, & Correa, 2001). It would seem unlikely, however, that licking a spout on a continuous reinforcement schedule such as this would constitute "work". Indeed, Salamone, et al. (2001) showed that a similar lesion of the NAC disrupted lever pressing for food pellets when using a Fixed Ratio (FR) 20 or greater, but not when using a FR5 schedule of reinforcement. A second possibility relates to Pavlovian-to-instrumental transfer (PIT). Specifically,

selective lesions of the nucleus accumbens have been found to disrupt PIT, where responding for a reward is increased in the presence of a reward-associated cue (Hall, Parkinson, Connor, Dickinson, & Everitt, 2001). Were this interpretation to provide an explanation, we would have to conclude that the context plays a critical role in drug-induced appetite stimulating effects and that the lesioned rats are either unable or unmotivated to use this information about the context to direct their behavior following the administration of the drug. Some support for this conclusion is provided by the finding that contextual cues support conditioned feeding following repeated intraaccumbens injections of morphine (Kelley, Bakshi, Fleming, & Holahan, 2000). Whether the phenomenon is, or is not, dependent upon contextual cues, it would appear that the drug-treated VTA lesioned rats perceive the food as more palatable when it comes into contact with the oral cavity, but that this experience is not sufficient to either maintain the ingestive behavior or to facilitate approach to the stimulus during an "interburst" or "intermeal" interval. Interestingly, Higgs and Cooper (2000) have shown that benzodiazepines increase intake by increasing burst length and that this effect is dopamine mediated. Thus, accumbens dopamine may be required to sustain burst length during voluntary consumption, even though the palatability of the stimulus is appropriately augmented by the drug pretreatment. Perhaps accumbens dopamine is required to sustain consumption, even on a continuous reinforcement schedule, when the licking behavior is motivated in a given context (perhaps via incentive salience) by exteroceptive, rather than interoceptive, stimuli (i.e., by the incentive associated with the perceived value of the reward rather than by the drive induced by hunger or thirst).

Finally, the VTA lesion also exerted disruptive effects on drug-induced suppression of CS intake by augmenting carry-over effects from Experiments 1a and 2a. The retarded development of a CS-US association as a function of prior nonreinforced experience is referred to as latent inhibition (Lubow, 1973, 1989; Lubow & Moore, 1959). Although the present experiments did not include a specific CS preexposure group, the present report is consistent with literature showing augmented latent inhibition in rats with targeted disruption of dopaminergic signaling in the NAC via accumbens 6-OHDA lesions or intra-NAC infusions of the DA antagonist haloperidol or chronic interferon alpha (Bethus, Stinus, & Goodall, 2003; Gray et al., 1997; Joseph et al., 2000; Weiner & Feldon, 1997). Of course, this would suggest that stimulus generalization occurred between the two CSs (saccharin and alanine). Even so, it is unlikely that disrupted performance by the lesioned rats is due solely to an increase in stimulus generalization. Were the VTA lesioned rats to exhibit greater CS generalization, then they should have exhibited greater carry-over effects (i.e., greater suppression of CS intake) on the first trial following the switch from the "drug-associated" to the "safe" CS than the SHAM controls. This, however, was not the case in either Experiment 1b or 2b. Rather, the lesion-induced deficit became evident over the latter trials during reversal learning. This finding is consistent with the view posed by Joseph et al. (2000) that dopamine signaling is required during conditioning to "learn that the to-be-conditioned stimulus is familiar" (page 929). From a Schultz perspective (Tobler et al., 2005), dopamine signaling may be required during conditioning to recognize that presentation of the CS led to a consequence that differed from that which was expected. Dopamine's role in the tracking of rewards and in the development of expectancies, then, may be critical when behavior needs to change in some exterocep-

tive/context-dependent manner. Interestingly, latent inhibition is context dependent (Honey & Good, 1993) and is similarly augmented by lesions of the hippocampus (Reilly, Harley, & Revusky, 1993). Since neural activity in the NAC is modulated by input from both the hippocampus (Goto & O'Donnell, 2002) and VTA, and producing lesions of either structure augments latent inhibition, the NAC may be the final common pathway for the proper expression of reversal learning. Indeed, the accumbens is a more likely focal point of the two major dopaminergic projections from the VTA because evidence suggests that the PFC is not involved in cases where prior non-reinforced experience disrupts subsequent learning (Broersen, Heinsbroek, de Bruin, & Olivier, 1996; Ellenbroek, Budde, & Cools, 1996; Lacroix, Broersen, Weiner, & Feldon, 1998; Weiner & Feldon, 1997).

In sum, we have found that an 80% depletion of accumbens dopamine was sufficient to fully eliminate the appetite stimulating effects induced by a benzodiazepine and morphine. Internal data showed that this finding could not be explained by either a general motor impairment or a general motivational deficit. The lesion also augmented the disruptive influence of a latent inhibition-like effect, where prior non-reinforced exposure to one sweet-tasting CS retarded the development of drug-induced suppression of intake of another sweet-tasting CS. Use of the cross-over design demonstrated that the disruptive effect of the lesion in this paradigm was not due to a general inability to detect or respond to either the gustatory CS or the drug US. Finally, while the lesion was sufficient to eliminate the appetite stimulating effects of both CDP and morphine and to augment the disruptive influence of a latent inhibition-like effect, it had no impact on either morphine- or cocaine-induced suppression of CS intake in naïve rats. This expands upon earlier findings (van der Kooy et al., 1983) by demonstrating that the suppressive effects of morphine and cocaine are intact following extensive lesions of the VTA-NAC pathway. Similar lesions of the NAC also failed to alter both successive and anticipatory contrast effects in consummatory behavior (Leszczuk & Flaherty, 2000). Thus, while dopamine neurons in the VTA-NAC pathway track this drug-induced devaluation of the natural reward, they need not be intact to respond to this information, at least not in consummatory behavior. Indeed, the essential nature of the substrate appears to become evident in contrast, in classical conditioning, and in ingestion when a shift in reward parameters calls for a shift in behavior in the presence of reward-associated exteroceptive/contextual cues.

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