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Dual Role of Medial A10 Dopamine Neurons in Affective Encoding

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Increasing evidence suggests that the activation of medial A10 neurons mediates positive affective encoding. However, little is known about the functions of the inhibition of midbrain dopamine neurons. Here we show evidence suggesting that the inhibition of medial A10 neurons mediates a negative affective state, leading to negative affective encoding, whereas blunting the activation of medial A10 neurons disrupts positive affective encoding involving food reward. We used a microinjection procedure, in which the D_2 dopamine receptor agonist quinpirole was administered into the cell body region of the dopamine neurons, a procedure that reduces dopamine cell firing. Microinjections of quinpirole into the posteromedial ventral tegmental area, but not its more lateral counterparts, led to conditioned place aversion. Quinpirole administration to this site also decreased food intake and basal dopamine concentration in the ventromedial striatum, a major projection area of medial A10 neurons. In addition, moderate quinpirole doses that did not lead to conditioned place aversion or disrupt food intake abolished food-conditioned place preference, suggesting that blunting dopamine impulse activity in response to food reward disrupts positive affective encoding in associated external stimuli. Our data support the hypothesis that activation of medial A10 dopamine neurons mediates a positive affective state, leading to positive affective encoding, while their inhibition mediates a negative affective state, leading to negative affective encoding. Together with previous findings, we propose that medial A10 neurons are an important component of the mechanism via which animals learn to avoid negative incentive stimuli.
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INTRODUCTION

Midbrain dopamine neurons localized adjacent to the brain's midline appear to play an important role in stimulus-incentive learning, in which an external stimulus that occurs closely in time and contingently with an incentive stimulus (eg food, drug administration, or any conditioned stimulus) acquires the same incentive-motivational (or affective) properties as those possessed by the incentive stimulus (Bolles, 1972; Bindra, 1978). This hypothesis is supported by converging evidence from electrophysiological, anatomical, and behavioral investigations.

Electrophysiological work, which has characterized how dopamine neurons respond to reward-related events, provides important insight on these neurons' functional roles. Recent data suggest that the impulse activities of midbrain dopamine neurons encode discrepancies between the affective event that is predicted and the affective event that actually occurs (Schultz *et al*, 1995, 1997; Montague *et al*, 1996). Importantly, such discrepancies (or reward prediction errors) have long been used to model associative learning including stimulus-incentive learning (Rescorla and Wagner, 1972; Mackintosh, 1975; Pearce and Hall, 1980; Sutton and Barto, 1981). However, to fully understand dopamine's functions, it is important to make a distinction between what dopaminergic signals reflect and how various target regions use such signals in adaptive behavior. Indeed, while dopamine neurons respond to reward-related events similarly regardless of their locations in A8, A9, and A10, dopaminergic signals have different functional consequences depending on their target region. Of those neurons projecting to the striatum, medial A10 neurons preferentially project to the ventromedial striatum, including medial accumbens shell, lateral A10 neurons to the ventrolateral striatum, including accumbens core (Ikemoto, 2007), and A9 neurons to the dorsal striatum (Fallon and Moore, 1978). Psychopharmacological and lesion data suggest that these projections are involved in different functions such as stimulus-outcome, response-outcome, and stimulus-response learning (Everitt and Robbins, 2005; Yin and

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Knowlton, 2006; Ikemoto, 2007). In particular, dopamine neurons projecting from the medial A10 to the ventromedial striatum appear to be important for the affective component of stimulus-outcome learning or stimulus-incentive learning.

Activation of medial A10 neurons appears to elicit positive affective states, which lead to stimulus-positive incentive learning (or positive affective encoding; Ikemoto, 2007). Local administration of psychomotor stimulants such as amphetamine or cocaine into the ventral striatum, or administration of drugs like carbachol or opiates into the A10 area, increases dopamine concentration in the ventral striatum (Carboni *et al*, 1989; Devine *et al*, 1993; Westerink *et al*, 1996) and somatomotor activity (Ikemoto, 2002; Zangen *et al*, 2002; Ikemoto *et al*, 2003) and leads to conditioned place preference (Carr and White, 1986; Ikemoto and Wise, 2002; Zangen *et al*, 2002; Ikemoto, 2003). Conditioned place preference has been shown to depend on stimulus-positive incentive learning (Perks and Clifton, 1997; Yin and Knowlton, 2002). In addition, rats learn to lever-press for administration of cocaine or amphetamine into the ventromedial striatum (Ikemoto, 2003; Ikemoto *et al*, 2005), and various other drugs into medial A10 (Ikemoto and Wise, 2002; Zangen *et al*, 2002, 2006; Rodd *et al*, 2004; Ikemoto *et al*, 2006). Further, blockade of dopamine receptors or lesions of dopaminergic terminals in the ventromedial striatum, but not the ventrolateral striatum, disrupts the establishment of conditioned place preference induced by systemic administration of cocaine, amphetamine, nicotine, or morphine (Sellings and Clarke, 2003; Fenu *et al*, 2006; Sellings *et al*, 2006a, b; Spina *et al*, 2006).

Little is known about the functions of dopamine neuron inhibition. Midbrain dopamine neurons appear to be actively inhibited by aversive stimuli including noxious stimuli (Ungless *et al*, 2004), the absence of expected food rewards (or reward omission) (Waelti *et al*, 2001; Tobler *et al*, 2003; Matsumoto and Hikosaka, 2007), drug withdrawal (Diana *et al*, 1993), conditioned stimuli associated with lithium-induced sickness (Mark *et al*, 1991), or reward omission (Waelti *et al*, 2001; Tobler *et al*, 2003; Matsumoto and Hikosaka, 2007). These data are consistent with the idea that dopamine neuron inhibition encodes prediction errors involving negative incentive stimuli. If excitatory signals of medial A10 neurons are used to create a positive affective state, leading to stimulus-positive incentive learning, then the inhibitory signals of medial A10 neurons may elicit a negative affective state, leading to stimulus-negative incentive learning. This hypothesis is consistent with the finding that pairing external stimuli with high-dose injections of D_1 receptor antagonists into the nucleus accumbens leads to conditioned aversion for paired stimuli (Shippenberg *et al*, 1991). In addition, conditioned place aversion results from systemic or intraventricular administration of high doses of dopamine receptor antagonists (Shippenberg *et al*, 1991) or a dopamine release inhibitor (Calcagnetti and Schechter, 1991; Schechter and Meechan, 1994).

The aims of the present study were to determine whether the inhibition of dopamine neurons leads to conditioned aversion and whether this effect is selective to medial A10, but not lateral A10 or A9, neurons. In addition, we examined whether moderate disruption of dopamine neuron activity leads to deficit in stimulus-positive incentive learning involving food. We tested these using pharmacological tools that stimulate somatodendritic autoreceptors and, in turn, inhibit basal firings of midbrain dopamine neurons (Aghajanian and Bunney, 1977; Beckstead *et al*, 2004).

MATERIALS AND METHODS

Subjects

A total of 236 male Wistar rats (280–350 g at the time of surgery; Harlan, Dublin, VA) were used. They were housed individually after surgery and maintained under a reversed 12 h light/dark cycle (lights on at 9 p.m.). Colony and experimental rooms were kept at a constant temperature (21°C). Water and laboratory chow (Zeigler Rodent NIH-07 22.5–5, Zeigler Bros Inc., Gardners, PA) were freely available. Starting 7 days after surgery, however, each rat received a daily ration of 13 g chow, and this regimen was maintained till the end of the experiments. The experiments began after 5 days on this feeding regimen; the rats received food during or after daily experimental procedures. These procedures were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse Intramural Research Program and were in accordance with the guidelines of National Institutes of Health.

Surgery

Under sodium pentobarbital (31 mg/kg, i.p.) and chloral hydrate (142 mg/kg, i.p.) anesthesia, all rats were implanted with bilateral 24-gauge guide cannulas for microinjection in the ventral midbrain. Some rats were also implanted with a unilateral guide cannula for microdialysis probes (CMA/Microdialysis, North Chelmsford, MA) in the ventromedial striatum (medial nucleus accumbens shell). Guide tips for microinjection and microdialysis ended 1.0 and 2.0 mm, respectively, above the target sites. We studied two regions within the ventral tegmental area (VTA) because of previous functional studies (Ikemoto *et al*, 1997, 1998; Carlezon *et al*, 2000; Rodd-Henricks *et al*, 2000; Ikemoto and Wise, 2002; Zangen *et al*, 2002) that divided the VTA into the anterior and posterior portions. The present study divided the VTA into anterolateral and posteromedial portions, because recent neuron tracer data suggest that the cell bodies of dopaminergic neurons projecting to the ventral striatum distribute from the posteromedial to anterolateral dimensions at an approximate 45° angle to the anteroposterior axis (Ikemoto, 2007). In other words, the majority of neurons in the posteromedial VTA project to the ventromedial striatum, while the majority of anterolateral VTA neurons project to the ventrolateral striatum. Accordingly, guide cannulas for medial and lateral A10 injections were aimed at the posteromedial and anterolateral VTA, respectively. The coordinates were 5.8 mm posterior to bregma, 2.0 mm lateral to the midline, and 8.1 mm ventral to the skull surface with a 10° angle toward midline for the posteromedial VTA; 4.9 posterior, 2.3 lateral, and 7.9 ventral with a 10° angle for the anterolateral VTA; 4.8 posterior, 3.5 lateral, and 7.8 ventral with a 10° angle for the substantia nigra; 2.0 anterior, 1.2 lateral, and 5.3 ventral with a 0° angle for the ventromedial striatum. These surgeries were

performed with the 'flat-skull' method, in which bregma and lambda were set at the same dorsal-ventral level. The experiments described below commenced 12 days after surgery.

Chemical Substances and Microinjection Procedure

The dopamine D_2 agonist quinpirole hydrochloride and the antagonist *S*(−)-raclopride(+)-tartrate salt were purchased from Sigma (St Louis, MO) and were dissolved in artificial cerebrospinal fluid (aCSF) consisting of 148 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, and 0.85 mM $MgCl_2$ (pH adjusted to 6.5–7.8). One of the following doses and combinations of quinpirole and raclopride in a volume of 0.3 μl (per hemisphere) was injected into the ventral midbrain of each rat: 0.03, 0.1, 0.3, 1, and 3 μg quinpirole (per hemisphere) and the doses of quinpirole 0.3 and 3 μg mixed with 3 μg raclopride.

Injection cannulas, connected to 10-μl Hamilton syringes with polyethylene tubes, were bilaterally inserted into the guide cannulas. The rats were left unrestrained inside a 30-cm diameter cylinder, while a syringe pump delivered a volume of 0.3 μl into the target regions over 60 s. The injection cannulas were removed after an additional period of 30 s. Immediately after the microinjections, the rats were placed in the test chambers.

Place Conditioning, Food Intake, Somatomotor Activity, and Injection Treatments

Each of four place conditioning chambers consisted of two compartments ($21 \times 21 \times 28\,cm^3$) and an area ($21 \times 21 \times 12\,cm^3$) connecting the compartments; a guillotine door separated each compartment from the connecting area. Each compartment was equipped with four pairs of photocells, used to assess time spent and somatomotor activity in the compartment. One compartment differed from the other by wall color (black *vs* white), floor type (net *vs* grid), and lighting; the amount of light was modulated in each compartment so that the rats would not prefer one compartment to the other prior to place conditioning. In session 1, each rat was placed in the place-conditioning chamber for 15 min without any injections or food reward; the rat had access to both compartments and the time spent in each compartment was recorded. Place preference scores were obtained by subtracting the time spent in the compartment that would be associated with no food from the time spent in the compartment that would be associated with food. In sessions 2–5, each rat was confined in one of the two compartments for 30 min per session; one contained 13 g of the laboratory chow and the other contained no food. Rats were placed in the compartment containing food immediately after microinjections, whereas they were placed in the compartment containing no food when no injections were given. Their general activity levels were assessed by counting numbers of beam interruptions in each compartment. The amount of food consumed in the compartment was measured after each food session. Left-over food from the session was then placed in the home cage. The pairings of one compartment with food and the other with no food were alternatively repeated twice over four sessions. In session 6, each rat was placed in the chamber without any injections or food; the rat had access to both compartments and the time spent in each compartment was recorded for 15 min. Sessions were separated by 24 h. The order of these treatments and the assignment of the two compartments for treatments were counterbalanced among rats in each group.

Microdialysis Procedure

Concentrations of extracellular dopamine in the medial accumbens shell (a major target region of medial A10) were determined before and after food access as a function of VTA injections. Rats were food-restricted as described above and placed in a dialysis chamber ($40 \times 40 \times 30\,cm^3$) in which aCSF was perfused overnight through a dialysis probe. Then, four consecutive dialysate samples were collected in 15-min bins to determine basal levels of dopamine. Each rat then received bilateral injections of vehicle, 0.3 or 3 μg quinpirole into posteromedial VTA. Immediately after the injections, 13 g of food was placed in the chamber, and four additional dialysate samples were collected. A group of rats that received 3 μg quinpirole into posteromedial VTA did not receive food immediately after injections. For this group, two dialysate samples were collected before their receiving food, and then four additional samples were collected.

Dopamine was measured with HPLC coupled to the Coulochem II Detector (model 5200; ESA, Chelmsford, MA) with a dual-electrode microdialysis cell and an ESA model 501 data station. Samples were injected manually onto the column (3 μm particle size, 3 × 150 mm; Analytical MD-150; ESA). The mobile phase for dopamine separation consisted of 75 mM NaH_2PO_4, 1.5 mM N-1-octanesulfonic acid, 10 μM EDTA, and 8% acetonitrile (pH 3.0 adjusted with H_3PO_4). Dopamine was quantified on both reducing (−250 mV) and oxidizing (350 mV) electrodes. The limit of detection for dopamine was ∼5 fmol per injection.

Histology

When each rat completed the experimental procedure, it was deeply anesthetized with a mixture of sodium pentobarbital (31 mg/kg) and chloral hydrate (142 mg/kg) and its brain was removed and placed in a 10% formalin solution. Coronal sections (40 μm) at the microinjection site and microdialysis site were cut with a cryostat. Sections were subsequently stained with cresyl violet. The placements of the injection cannulas and dialysis probes were confirmed by microscopic examination.

Statistical Analyses

Details of the statistical analyses are provided in 'Results' section and figure legends. Generally, ANOVAs were used followed by appropriate *post hoc* tests. For food intake and somatomotor activity, mean scores of each rat from two sessions, taken after the microinjections, were processed for one-way between-subjects ANOVAs with six doses. Dialysis data were analyzed using raw values (nM), but not percent values.

RESULTS

Figure 1 depicts the injection sites of the rats examined for food place conditioning described in Figure 2. Each rat received bilateral injections into one of three sites: posteromedial VTA (medial A10), anterolateral VTA (lateral A10), or substantia nigra (A9). Regardless of the injection site, quinpirole injections reduced somatomotor activity (Figure 2a). This observation was confirmed by significant main dose effects (one-way between-subjects ANOVAs with six doses), $F_{5,54} = 2.92$, $P < 0.05$; $F_{5,53} = 8.227$, $P < 0.0001$; and $F_{5,36} = 6.55$, $P < 0.0005$ for the posteromedial VTA, anterolateral VTA, and substantia nigra, respectively. It should be noted that motor activity levels in the other compartment, following no microinjection, did not differ among different dose groups receiving injections in any of the injection sites (data not shown). In addition, quinpirole injections were generally not effective in reducing the amount of food consumed (Figure 2b). An exception is that the highest dose (3 μg) of quinpirole injected into the posteromedial VTA reliably decreased food intake as revealed by a significant main effect of one-way ANOVA with six doses ($F_{5,54} = 3.12$, $P < 0.05$), followed by a Dunnett's *post hoc* test.

Figure 2c shows place preference scores between before and after food conditioning as a function of quinpirole dose. Before conditioning, rats did not show reliable preference for one compartment over the other. After conditioning, those that received vehicle injections spent significantly more time in the compartment paired with food. Marked disruption of place conditioning was found following injections of quinpirole into the posteromedial VTA. Medium doses (0.1 and 0.3 μg) of quinpirole into the posteromedial VTA abolished food-conditioned place preference (Supplementary Figure S1a). Higher doses (3 μg) of quinpirole injected into the posteromedial VTA led to conditioned place aversion (Supplementary Figure S1b). These observations were confirmed by two-way mixed ANOVAs with dose (between-subjects factor; 6) and conditioning (within-subjects factor; before *vs* after). Injections into the posteromedial VTA had a significant dose-by-conditioning interaction ($F_{5,54} = 4.79$, $P < 0.01$). To evaluate significant difference in place preference score between before and after conditioning at each dose, Bonferroni-corrected *post hoc* *t*-tests (six comparisons) were conducted. Significant differences were found at 0 and 3 μg, but not at other, in-between doses. Quinpirole injections into the anterolateral VTA or substantia nigra resulted in significant main conditioning effects ($F_{1,53} = 10.18$, $P < 0.005$ and $F_{1,36} = 51.86$, $P < 0.0001$, respectively), but not in a significant main dose effect or dose-by-conditioning interaction.

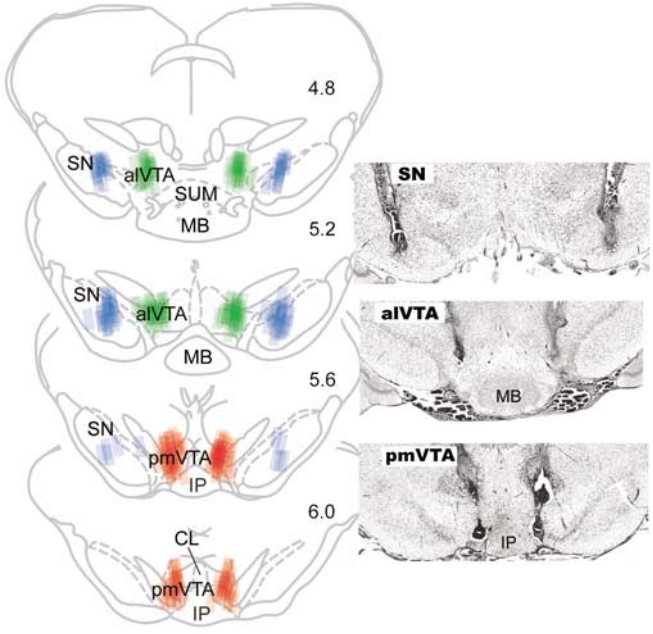

Figure 1 Microinjection sites. The drawings (adapted from Paxinos and Watson, 1997) on the left depict injection sites from which the data shown in Figure 2 are generated. Placements for the posteromedial ventral tegmental area (pmVTA), anterolateral ventral tegmental area (alVTA), and substantia nigra (SN) are shown with transparent rectangles colored red, green, and blue, respectively. The photomicrographs on the right show representative cannula tracks leading into the three regions. Additional abbreviations: CL, central linear nucleus raphe; IP, interpeduncular nucleus; MB, mammillary body.

Additional experiments were conducted to help interpret the effects of quinpirole injections into the posteromedial VTA. The rats in the following experiments were treated exactly the same as those in the above experiment except that they had no food in either compartment during conditioning. The data involving the 0.3 μg dose in the experiment described above are consistent with the explanation that this dose disrupted stimulus-incentive learning involving food, because the dose completely disrupted the establishment of food-conditioned place preference, while it did not affect food intake. However, an alternative explanation is that administration of this dose may have been aversive, an effect that could counteract the positive affective state induced by food reward. Pairing a compartment containing no food with injections of 0.3 μg quinpirole did not lead to conditioned place aversion or preference ($t_9 = 0.74$; Figure 3). In addition, injections of the 3 μg dose into the posteromedial VTA in the above experiment led to conditioned place aversion. The simplest explanation is that the administration of this dose elicited a negative affective state, thereby leading to conditioned place aversion and reduced food intake. However, the aversive effect of this dose could have resulted from an interaction with food intake; quinpirole injections may have altered perception of food, leading to an aversive effect. Again, pairing a compartment containing no food with injections of 3 μg quinpirole led to conditioned aversion to the paired compartment ($t_{10} = 2.48$, $P < 0.05$).

The following two experiments support the hypothesis that the effects of posteromedial VTA quinpirole on place conditioning were mediated by dopamine neurons. First, we examined whether the effects of quinpirole on place conditioning were reversed by coadministration of the dopamine D_2 receptor antagonist raclopride (3 μg). As shown in Figure 4a, coadministration of raclopride with quinpirole reversed the effects of quinpirole on place conditioning. This observation was confirmed by a three-way mixed ANOVA on place preference data including those shown in Figure 1c, with raclopride treatment (between-subjects factor; 0 and 3 μg), quinpirole treatment

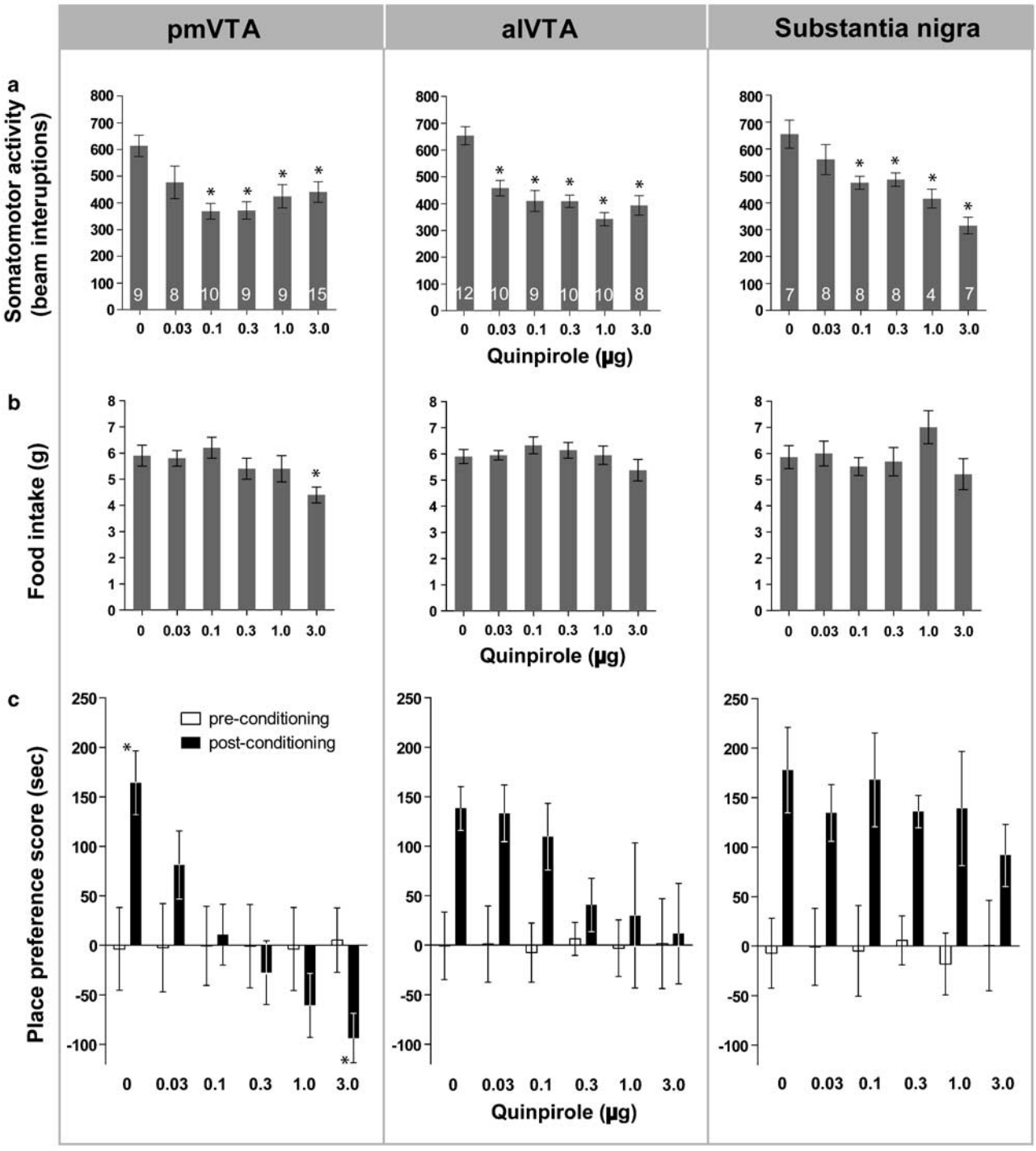

Figure 2 Effects of quinpirole injections into the posteromedial ventral tegmental area (VTA), anterolateral VTA, and substantia nigra on activity, food intake and place conditioning. (a) Activity levels (numbers of beam interruptions) were assessed in the food compartment during two 30-min sessions, following injection treatments. The number of rats used in each group is indicated in the bars. Data are means ± SEM. *$P<0.05$, compared to vehicle value by a Dunnett's *post hoc* test. (b) The amounts of food consumed for 30 min after microinjections were assessed. Data are means ± SEM. *$P<0.05$, compared to vehicle value by a Dunnett's *post hoc* test. (c) Place preference scores were derived by subtracting the time spent in the compartment paired with no food from the time spent in the compartment paired with food. Data are means ± SEM. *$P<0.05$, compared between pre- and postconditioning values.

(between-subjects factor; 0, 0.3, and 3 µg), and conditioning (within-subjects factor; before and after). A significant three-way interaction between raclopride, quinpirole, and conditioning was found ($F_{2,52}=4.50$, $P<0.05$; Figure 4a). It should be noted that injections of raclopride alone into the posteromedial VTA appeared to have an effect. Raclopride injections alone tended to disrupt food-conditioned place preference, as suggested by a large error bar at 0 µg quinpirole dose. Injections of raclopride alone, without quinpirole, reliably decreased food intake (Figure 4b).

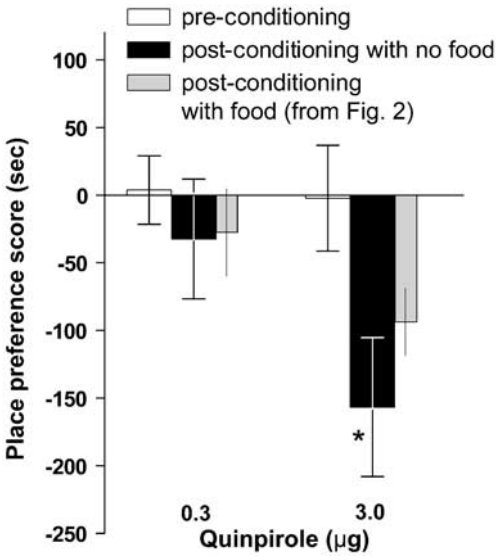

Figure 3 Effects of 0.3 or 3 μg quinpirole injections into the posteromedial ventral tegmental area (VTA) on place conditioning without food. Rats were treated exactly the same as those in the experiment described in Figure 2 except that they did not have access to food in the compartments. The numbers of rats receiving 0.3 and 3 μg quinpirole were 10 and 12, respectively. Shaded bars indicate the data presented in Figure 2 for comparison. Data are means ± SEM. $^*P<0.05$, compared to preconditioning value.

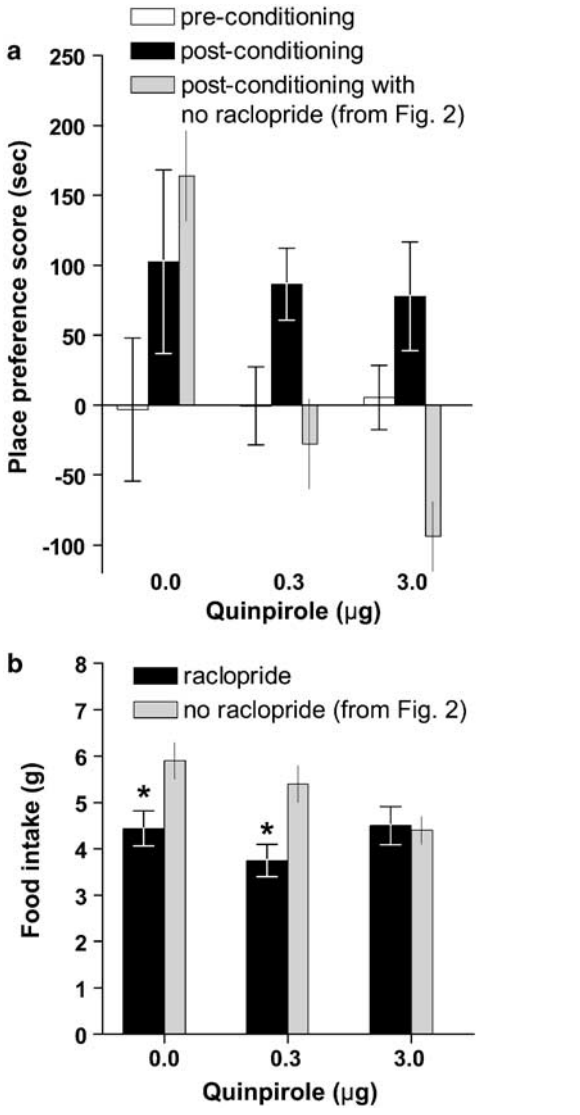

Figure 4 Effects of coadministration of raclopride into the posteromedial ventral tegmental area (VTA) on place conditioning (a) and food intake (b). Rats were treated exactly the same as those in the experiment described in Figure 2 except that they received raclopride in addition to quinpirole. The numbers of raclopride rats receiving 0, 0.3, and 3 μg quinpirole were 8, 8, and 9, respectively. Shaded bars indicate the data presented in Figure 2 for comparison. Data are means ± SEM. $^*P<0.05$, compared values between injections containing raclopride and no raclopride.

Interestingly, the combination of raclopride and the high dose of quinpirole did not have an additive effect on food intake. These observations on food intake were confirmed by a significant interaction between raclopride (0 and 3 μg) and quinpirole (0, 0.3, and 3 μg) in a two-way ANOVA on food intake ($F_{2,50}=4.66$, $P<0.05$).

A microdialysis experiment was conducted not only to confirm that the quinpirole treatments modulated the activity of dopamine neurons, but also to determine how the quinpirole treatments affected extracellular levels of dopamine in the ventromedial striatum. The access to food reward after vehicle injections into the posteromedial VTA significantly increased extracellular dopamine levels in the ventromedial striatum by 75% over basal levels (Figure 5a). The injection of 0.3 μg quinpirole, however, prevented dopamine levels from increasing. On the other hand, injections of the high dose (3 μg) of quinpirole significantly decreased extracellular levels of dopamine. These observations were confirmed by a three-way mixed ANOVA on dopamine concentration with quinpirole dose (between-subjects factor; 0, 0.3, and 3 μg), food access (within-subjects factor; before and after), and block (within-subjects factor; 4). A significant interaction between quinpirole dose and food access was found ($F_{2,20}=14.39$, $P<0.0005$). Consistent with the above findings, injections of the high dose (3 μg) decreased the amount of food consumed, whereas injections of the medium dose (0.3 μg) did not reliably decrease food intake (Figure 5b). This observation was confirmed by a significant dose effect on food intake ($F_{2,20}=5.01$, $P<0.05$) by a one-way ANOVA followed by a Newman–Keuls *post hoc* test.

Because dialysis data obtained with the highest dose of quinpirole (3 μg) were confounded with the presentation of food, an additional experiment was conducted to determine if injections of 3 μg quinpirole alone without food reduce dopamine concentration in the ventromedial striatum lower than basal concentration. Injections of 3 μg quinpirole into the posteromedial VTA significantly decreased dopamine concentration as indicated by a significant main effect in phase (basal, postinjection without food, and postinjection with food; $F_{2,8}=10.28$, $P<0.01$) in a two-way within-subjects ANOVA with phase (3) and block (two 15-min blocks within each phase), followed by a Newman–Keuls test (Figure 6). The access to food, which became available 30 min after the injections, did not significantly reverse decreased dopamine concentration induced by quinpirole injections. These rats consumed the mean food amount of

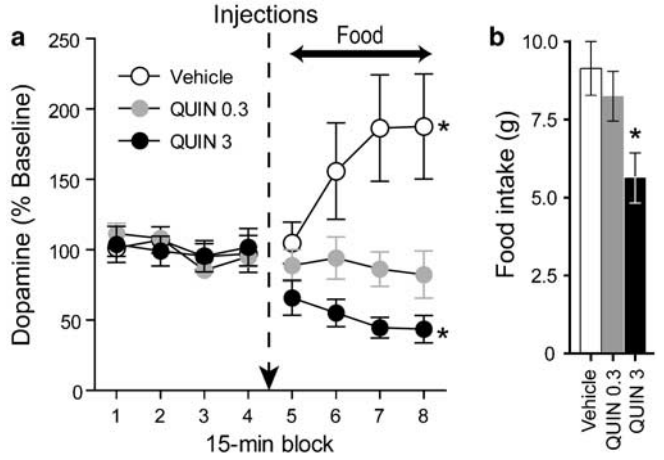

Figure 5 Effects of quinpirole injections into the posteromedial ventral tegmental area (VTA) on extracellular dopamine concentration in the ventromedial striatum (medial nucleus accumbens shell) and food intake. (a) Concentrations of dopamine from dialysate samples collected every 15 min are shown before and after food access as a function of quinpirole dose. The numbers of rats receiving 0, 0.3, and 3 μg quinpirole were 7, 8, and 8, respectively. Data are mean percents ± SEM of basal levels (100% = the mean of four basal values). *$P<0.05$, significant difference between before and after food access with time block collapsed together (Newman–Keuls *post hoc* test). (b) The amounts of food consumed during the 60 min after microinjections are shown. Data are means ± SEM. *$P<0.05$, compared to vehicle value by a Newman–Keuls *post hoc* test.

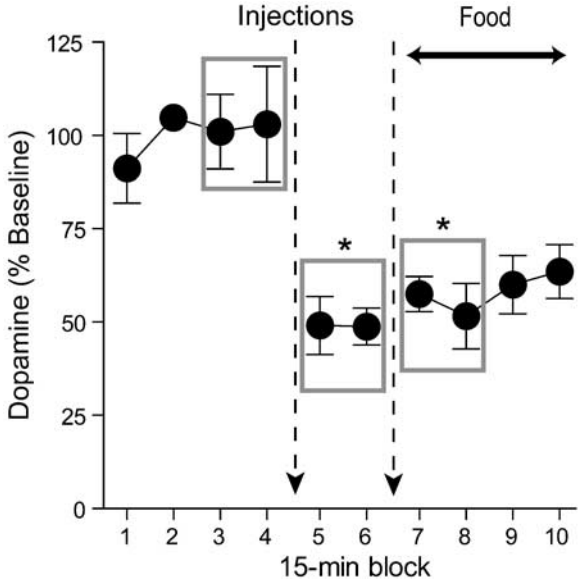

Figure 6 Effects of quinpirole injections into the posteromedial ventral tegmental area (VTA) on extracellular dopamine concentration in the ventromedial striatum. Concentrations of dopamine from dialysate samples collected every 15 min are shown before and after injections of 3 μg quinpirole and food access. Data ($N=5$) are mean percent ± SEM of basal levels (100% = the mean of four basal values). An analysis of variance (ANOVA) was conduced for blocks 3–8 with three phases (basal, postinjection without food, and postinjection with food). *$P<0.01$, significantly different from the basal phase with block collapsed together.

5.4 g (0.9, SEM) in 1-h food access started 30 min after injections. This value is comparable to the amount consumed following 3 μg injections in the above-described experiment.

DISCUSSION

We argue that the inhibition of dopamine neurons has active functional consequences. Specifically, our findings support the hypothesis that the inhibition of medial A10 neurons elicits a negative affective state, leading to negative affective encoding. In addition, blunting the activation of medial A10 neurons appears to disrupt the induction of a positive affective state involving food reward, leading to no affective encoding. In the following subsections, we first argue that medial A10 neurons rather than their lateral counterparts are closely involved in stimulus-incentive learning, and discuss how medial A10 neurons mediate stimulus-incentive learning. We then discuss the implications of our findings on food intake and aversive control of behavior, followed by their clinical implications.

Medial A10 Neurons are More Important for Stimulus-Incentive Learning than their Lateral Counterparts

Place preference scores were altered by lower doses of quinpirole injections into the posteromedial VTA than into its lateral counterparts, suggesting that quinpirole acts in the vicinity of the medial A10. It is also likely that injections of quinpirole into the anterolateral VTA or substantia nigra influenced dopamine neuron activity, since they decreased motor activity counts as effectively as injections into the posteromedial VTA. Although the electrophysiological observation that dopamine neurons across the mediolateral axis (A10, A9, and A8) respond similarly to reward-related events, these data, along with many previous findings, suggest that dopaminergic signals have different functional consequences depending on their target region. As we argue in the subsequent subsections, dopaminergic signals emitted by medial A10 neurons appear to be used for altering affective states, leading to stimulus-incentive learning. Although the present data do not shed new light on the roles played by lateral A10 and A9 neurons, previous findings suggest that they are important for other types of associative learning, including stimulus-response and action-outcome learning (Everitt and Robbins, 2005; Yin and Knowlton, 2006; Ikemoto, 2007).

How do Medial A10 Neurons Mediate Stimulus-Incentive Learning?

Converging evidence suggests that tonic activation of medial A10 neurons projecting to the ventromedial striatum elicits a positive affective state, leading to stimulus-positive incentive learning that associates external stimuli with an induced internal positive state (for an extensive review, see Section 4 of Ikemoto, 2007). Our present findings support this hypothesis. Medium doses of quinpirole, including 0.3 μg, prevented the establishment of food-conditioned place preference but did not reduce food intake, suggesting that the treatment effects on conditioning were not due to the lack of food intake. In addition, the 0.3 μg dose prevented tonic elevation of dopamine concentration in the ventromedial striatum following food reward. It should be noted that conditioned place preference not only depends on affective memories but also relational (or declarative) memories, which encode spatial relationships

among external stimuli (eg compartments and food) and factual events (eg food intake in one of the compartments). While it may seem possible that medium doses of quinpirole into the medial A10 area impaired relational encoding, this hypothesis is not a viable one because the high dose (3 μg) of quinpirole injected into the same site led to conditioned place aversion, an effect that depends on relational memories.

The findings from the highest quinpirole dose support the hypothesis that tonic inhibition of medial A10 neurons elicits a negative affective state, which then leads to stimulus-negative incentive learning that associates external stimuli with an induced internal negative state. Pairing external stimuli with injections of the high 3 μg dose of quinpirole into the posteromedial VTA, in the presence of either food or no food, led to conditioned place aversion to paired stimuli. In addition, the finding that the administration of this dose into the medial A10 decreased food intake in hungry rats supports the interpretation that this treatment elicited a negative affective state. Further, with or without the presence of food, this manipulation significantly decreased extracellular dopamine in the ventromedial striatum. Thus, the inhibition of medial A10 neurons appeared to elicit a negative affective state, leading to negative affective encoding. This understanding is consistent with the previous finding that the blockade of accumbens dopamine receptors in opiate-dependent rats elicits somatic withdrawal symptoms (Harris and Aston-Jones, 1994) as well as the aforementioned finding that administration of high doses of dopamine antagonists into the nucleus accumbens leads to conditioned place aversion (Shippenberg *et al*, 1991).

Previous findings suggest that accumbens dopamine is involved in memory consolidation. Studies have shown that post-training injections of dopamine receptor agonists and antagonists into the nucleus accumbens, respectively, facilitate and disrupt associative learning involving both positive and negative incentive stimuli (Setlow and McGaugh, 1998; Dalley *et al*, 2005; LaLumiere *et al*, 2005). Our data suggest that rats can learn conditioned place aversion under severe inhibition of medial A10 neurons, bringing into question the idea that dopamine, at least in the terminal regions of these neurons, is required for or facilitates learning *per se*. Additional information is needed to understand how dopamine modulates learning and memory.

Implications on Food Intake

Increases in dopaminergic transmission or impulse activity after access to food do not appear to be important for regulating food intake. The administration of medium doses of quinpirole, including 0.3 μg, into the posteromedial VTA abolished increases in dopamine concentration induced by food, but had no detectable effect on food intake. However, food intake decreased after administration of the highest dose of quinpirole, which decreased dopamine concentration to below-baseline levels. Moreover, food intake decreased slightly after injections of a high dose of raclopride into the posteromedial VTA. Taken together, medial A10 neurons appear to participate in food intake in a subtle or indirect manner. These data are consistent with the hypothesis that mesolimbic dopamine neurons play more important roles in reward-seeking than reward-consummatory processes (Ikemoto and Panksepp, 1996, 1999; Baldo and Kelley, 2007).

Implications on Aversive Control of Behavior

Part of what makes aversive stimuli aversive may be their capacity to inhibit medial A10 neurons. Previous data suggest that dopamine neurons are inhibited by aversive stimuli, including foot shock, tail pinch, whole-body restraint, lithium-induced sickness, and conditioned stimuli predicting such aversive stimuli. Electrophysiological data (Mirenowicz and Schultz, 1996) suggest that aversive stimuli inhibit firing of midbrain dopamine neurons (for a review, see Ungless, 2004); unequivocal biochemical markers have confirmed that the neurons inhibited by noxious stimuli are dopaminergic (Ungless *et al*, 2004). In light of our finding that pharmacological inhibition of medial A10 neurons leads to conditioned aversion, the role of phasic inhibitions of medial A10 neurons in response to aversive stimuli may be to elicit negative affective states, leading to stimulus-negative incentive learning.

In addition, the omission of expected rewards or the stimulus predicting reward omission triggers phasic inhibitions of dopamine neurons and leads to conditioned suppression of responding in instrumental discrimination learning (Waelti *et al*, 2001; Tobler *et al*, 2003; Matsumoto and Hikosaka, 2007). Indeed, behavioral work has long suggested that animals display similar affective reactions to the omission of expected rewards as to the presentation of noxious stimuli (Amsel, 1958; Wagner, 1969). In light of our data, we argue that stimuli predicting reward omission may encode negative affect via inhibitions of medial A10 neurons, a component of the mechanisms that enable animals not to respond upon the presentation of such stimuli. Recent data suggest that major inhibitory inputs to the midbrain dopamine neurons come from lateral habenula neurons that are activated by aversive events (Matsumoto and Hikosaka, 2007).

Paradoxically, negative incentive stimuli not only decrease but also increase extracellular dopamine concentrations in the ventral striatum (Mark *et al*, 1991; Puglisi-Allegra *et al*, 1991; Diana *et al*, 1993; Young *et al*, 1993; Kalivas and Duffy, 1995; Bassareo *et al*, 2002). In addition, pharmacological disruption of dopaminergic activity such as blockade of dopamine receptors prevents the formation of conditioned responding induced by aversive stimuli (for a review, see Salamone, 1994). These data cast potential doubt on the notion that the activation of dopamine neurons mediates positive affective states and then positive affective encoding. Therefore, it is important to consider how the activation of dopaminergic systems triggered by aversive stimuli relates to the activation triggered by positive incentive stimuli. Aversive situations appear to be more complicated than appetitive situations, recruiting multiple motivational processes (Masterson and Crawford, 1982; Gray, 1987). Ikemoto and Panksepp (1999) suggested that increased accumbens dopamine transmission in response to aversive events is important for learning about the relationships between external stimuli and 'safety' from aversive events, ie a form of stimulus-positive incentive

learning. In light of our data, there may be two types of dopamine-mediated avoidance behaviors: one mediated by inhibition of dopamine systems (withdrawal-type avoidance) and the other mediated by activation of dopamine systems (approach-type avoidance). We speculate that inhibition of medial A10 neurons in response to aversive events is involved in stimulus-negative incentive learning and conditioned withdrawal, while their activation is involved in avoidance learning that associates external stimuli with safety from aversive stimuli and conditioned approach.

This hypothesis is not mutually exclusive with one pharmacological explanation. Disruption of aversive learning by inactivation of the dopamine is explained if pharmacological manipulations, such as administration of dopamine receptor antagonists into the nucleus accumbens or D_2 agonists into the VTA, blunt not only phasic activation dopamine signals for stimulus-positive incentive learning, but also phasic inhibitory dopamine signals for stimulus-negative incentive learning. Microinjections of quinpirole into the VTA lead to the disruption of second-order associative learning between neutral stimuli and conditioned stimuli previously paired with foot shock (Nader and LeDoux, 1999). This effect does not appear to result from secondary effects of the aversive state induced by intra-VTA quinpirole, because these experiments employed a medium quinpirole dose range (0.1–1.0 μg), which did not induce conditioned place aversion (but did disrupt food-conditioned place preference) in our study. Similarly, low-dose injections (25–50 ng per side) of SCH23390 into the ventromedial striatum disrupt stimulus-negative incentive learning between lithium-induced sickness and taste stimuli associated with sickness (Fenu *et al*, 2001), whereas higher dose injections (500–1000 ng per side) of SCH23390 into the nucleus accumbens are needed to lead to conditioned place aversion (Shippenberg *et al*, 1991). Therefore, if lower doses of quinpirole administered into the VTA or lower doses of SCH23390 administered into the ventromedial striatum blunt phasic inhibitory signals of dopamine neurons in response to aversive stimuli, this explains why such pharmacological manipulations are so effective in disrupting behavior controlled by aversive stimuli.

Clinical Implications

The loss of dopaminergic neurons in the ventral midbrain leads to devastating movement disorders including Parkinson's disease. Intriguingly, electrophysiological findings suggest that midbrain dopamine neurons respond to incentive stimuli rather than movement (Schultz, 2002). Many investigators now believe that these neurons play an important role in motor learning in relation to external events (Hikosaka, 1991; Wickens *et al*, 2003; Graybiel, 2005). In light of our data, patients with the loss of medial A10 dopamine neurons, which may be prevalent in Parkinson's patients (Uhl, 1985), may have affective encoding deficits in which external stimuli no longer acquire appropriate motivational signals for initiating movements. Therefore, the loss of medial A10 neurons could contribute to movement-related disorders. This idea fits with the suggestion that motivational signals processed by the ventromedial portion of the basal ganglia interact with motor signals processed by the dorsolateral counterpart for adaptive behavior (Nauta and Domesick, 1978; Mogenson *et al*, 1980).

In addition, the therapeutic effects of psychoactive drug treatments for mood disorders and schizophrenia emerge gradually over days and weeks, despite the fact that these drugs exert full pharmacological effects only hours after administration. If it is correct to assume that a set of brain mechanisms including medial A10 neurons mediates affective encoding, the delay in improvement of affective symptoms following administration of psychoactive drugs partly reflects the time needed for individuals to re-acquire adequate affective codes in their central representation of external stimuli and events, which, in turn, control their affect, thoughts, and behavior.

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DISCLOSURE/CONFLICT OF INTEREST

The authors disclose that they have no conflict of interest.

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