Behavioural Brain Research 197 (2009) 442–449

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

NMDA receptor antagonism in the ventral tegmental area impairs acquisition of reward-related learning

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ARTICLE INFO

Article history:
Received 1 May 2008
Received in revised form 24 July 2008
Accepted 8 October 2008
Available online 15 October 2008

Keywords:
Reward
Glutamate
AP-5
Motivation
Food
Conditioning

ABSTRACT

Mechanisms underlying reward-related learning presumably involve neural plasticity integrating signals representing unconditioned and conditioned stimuli in regions mediating reward. The ventral tegmental area (VTA) receives such signals and shows synaptic plasticity which is NMDA receptor-dependent. To test the hypothesis that NMDA receptor stimulation in the VTA is necessary for the acquisition of food-reinforced appetitive learning, Long–Evans male rats were prepared with bilateral VTA cannulae and tested in operant chambers with the opportunity to lever press for food for 10 sessions. Animals received microinjections of AP-5 or vehicle immediately before sessions 1–4 and 10. AP-5 impaired acquisition of lever pressing during sessions 1–4 (but not when injected dorsal to the VTA). All groups increased lever pressing across sessions 5–9. On session 10, lever pressing was not affected regardless of treatment. In separate experiments, AP-5 failed to reduce free feeding, food reward or motor activity, suggesting that impairment in acquisition was not due to reduced food motivation or activity. NMDA transmission in the VTA thus appears to be necessary for the acquisition, but not expression, of reward-related learning.

1. Introduction

The neural mechanisms underlying reward-related learning are not fully understood. It is likely that neural plasticity in regions along the brain's reward pathways is involved. Mesocorticolimbic dopamine (DA) plays an important role in mediating the behavioral effects of reward stimuli (for detailed reviews see [1–3]). In addition to being involved in primary reward, mesocorticolimbic DA also is implicated in mediating the motivational effects of conditioned stimuli associated with primary rewards. For instance, presumed midbrain DA neurons (some of which are in the ventral tegmental area [VTA]) in the primate [4–9] and VTA neurons in the rat [30–33] increase firing when animals are presented with conditioned stimuli. Furthermore, the relation between conditioned stimuli and mesocorticolimbic DA appears to be functional. For example, increases in mesolimbic DA can reinstate extinguished lever pressing [10,11] and are observed just prior to reinforced lever presses [12–14]. Thus, it appears that, in reward-related learning, mesocorticolimbic DA activity comes under the control of conditioned stimuli. How this occurs remains to be determined. One possibility is that neural plasticity in the VTA, the source of mesocorticolimbic DA, may be involved.

The VTA receives glutamatergic afferents from almost all structures which project to it, ranging from the brainstem to the forebrain (Geisler et al., 2007), including those known to process environmental stimuli such as frontal cortical areas, amygdala, bed nucleus of the stria terminalis, superior colliculus and others [15,16]. It is conceivable, then, that the VTA receives a glutamate signal that is in some way related to environmental stimuli and which might be involved in the acquisition of reward-related learning. Indeed, some evidence of this exists. Microinjections of NMDA or AMPA receptor antagonists into the VTA can block the development of morphine place preference [17] and, when administered simultaneously, can block the development of cocaine place preference [18]. These studies support the idea that glutamate neurotransmission in the VTA is involved in drug-related learning. Whether or not VTA glutamate is also involved in learning about natural reward, such as food, or in instrumental learning has not been investigated.

One form of neural plasticity long believed to be important for learning [19,20] is long-term potentiation, or LTP. In fact, LTP does occur in VTA DA cells [21,22] and, consistent with reports of LTP in other regions [23], VTA LTP appears to be NMDA receptor-dependent. In general, in cases where NMDA receptor stimulation is involved in LTP, it appears to be critical only for the induction, but not expression, of LTP [23], suggesting that it may be critical for the acquisition of learning, but not for its expression.

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doi:10.1016/j.bbr.2008.10.013

Thus, if reward-related learning involves LTP-like neural plasticity in the VTA then it might be critically dependent on NMDA receptor stimulation in this region. The present study was conducted to test this hypothesis. We predicted that microinjections of AP-5 (2-amino-5-phosphonopentanoate), a competitive NMDA receptor antagonist, into the VTA would impair the acquisition of food-reinforced lever pressing but not impair the expression of this response once learned.

2. Methods

The protocols used in the present experiments were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Queens College Institutional Animal Care and Use Committee.

2.1. Subjects

Subjects consisted of 80 male Long–Evans rats, facility-bred from males and females obtained from Charles River Laboratories (Raleigh, NC), with initial free-feeding weights between 275 and 380 g at the time of surgery. All rats were individually housed and maintained on a 12 h light:12 h dark cycle (lights off 06:00). All experimental sessions were conducted during the dark phase in order to test the rats during their active periods. All animals had unlimited access to food (Purina rat chow) until experimental sessions started, at which time access was restricted to daily rations that maintained their weights at 85% of their free-feeding values.

2.2. Surgical procedure

All animals received an intraperitoneal (IP) injection of atropine sulfate (0.1 ml) and were anesthetized by sodium pentobarbital (65 mg/kg). Stainless steel guide cannulae (0.635 mm outer diameter, 0.3302 mm inner diameter) were bilaterally implanted into the ventral tegmental area using the following coordinates: −5.6 mm caudal to bregma, ±2.0 mm from the midline at a 10° angle toward the midline and −8.3 mm below the surface of the skull [24]. For the dorsal anatomical control group the coordinates were identical except the guide cannulae were lowered to −7.3 mm. The cannulae were secured by dental acrylic anchored to the skull by four stainless steel screws. Obturators (0.3048 mm diameter), extending 1 mm beyond the tip of the cannulae, were inserted at all times except during microinjections.

2.3. Microinjection procedure

A stainless steel injector tube (0.3048 mm outer diameter, 0.1524 mm inner diameter) was inserted into the guide cannula delivering either vehicle or AP-5. The test compound was manually injected over a 30-s period and the injector was kept in place for an additional 60 s after which it was removed and the obturator was replaced. This procedure was repeated on the contralateral side.

2.4. Drugs

AP-5 (Sigma–Aldrich, St. Louis, MO) was dissolved in 0.9% saline before the start of the experiments. Each microinjection was delivered in a volume of 0.5 μl.

2.5. Testing apparatus

2.5.1. Operant chambers

Instrumental conditioning and free-feeding sessions were conducted in operant conditioning chambers measuring 30 cm × 21 cm × 18 cm ($l \times w \times h$). One wall was equipped with two removable levers, two white stimulus lights and a food trough. Each chamber was housed in a ventilated, sound-attenuating box.

2.5.2. Activity monitors

Locomotor activity tests were conducted in activity chambers measuring 40.5 cm × 20.5 cm × 24.5 cm ($l \times w \times h$). Each chamber was equipped with eight photo-emitters positioned along the length of the chamber 6 cm above the floor, each paired directly opposite a photocell. Ambulatory counts were registered when adjacent beams were broken consecutively, and stereotypy counts when adjacent beams were broken repeatedly.

2.6. Instrumental conditioning experiment

Prior to beginning instrumental conditioning all rats were given 20 food pellets (45 mg, Bioserv, Frenchtown, NJ) in their home cages on each of three days. During the instrumental conditioning experiments all rats were exposed to the operant chambers for 10 daily 1-h sessions in which presses on the active lever were reinforced under a fixed ratio 1 (FR1) schedule of reinforcement with one food pellet, accompanied by the illumination of the light above the active lever for 2 s. Presses on the inactive lever produced no consequences. For sessions 1–4 all animals received bilateral microinjections of vehicle ($n = 9$) or AP-5 (0.125, 0.25 or 0.5 μg/0.5 μl, ns = 5, 7 and 6, respectively) into the VTA or 1 mm dorsal to the VTA (vehicle, $n = 6$; 0.5 μg AP-5, $n = 5$) immediately before being placed in the operant chambers. No microinjections were given prior to sessions 5–9, which began two days after session 4. For session 10, all rats received the same treatment as the first four sessions. Presses on both levers and head entries into the food trough were recorded, as well as the time between each active lever press that was followed by a head entry ("food approach latency").

2.7. Free-feeding experiment

Two separate groups of rats were tested for the effects of treatment on food consumption. Operant chamber levers were removed, and approximately 25–30 g of rat chow placed on a mesh grid on the chamber floor. A tray beneath the floor collected chow remains. Animals were placed in the food-loaded chambers for four consecutive daily 60-min sessions. After each session, the rat chow remaining on the floor and in the collection tray was weighed and subtracted from the original weight to determine the amount consumed. Immediately prior to the first three sessions, all rats received bilateral vehicle microinjections. After the third session, rats were assigned to either a vehicle or treatment group based on ranked average chow consumption. Immediately prior to session 4 one group received vehicle ($n = 7$) and the other AP-5 (0.5 μg/0.5 μl, the lowest dose that significantly impaired acquisition of lever pressing; $n = 9$).

2.8. Activity experiment

Two separate groups of rats were tested for ambulatory activity and stereotypy during four consecutive daily 60-min sessions in the activity chambers. Immediately before the first three sessions, all rats were given microinjections of vehicle. Rats were then assigned to either a vehicle ($n = 6$) or treatment (0.5 μg AP-5; $n = 7$) group based on their ranked average activity counts during the first three sessions, and received microinjections before the fourth session.

2.9. Reward devaluation assessment experiment

Two separate groups of rats were tested to assess whether intra-VTA AP-5 treatment reduces the reward value of Bioserv food pellets. After two instrumental conditioning sessions that were terminated after 30 active lever presses or 60 min, any rat that pressed the active lever 30 times during at least one session continued on to the next phase. Rats were randomly assigned to either a vehicle ($n = 6$) or 0.5 μg AP-5 ($n = 7$) group, receiving microinjections prior to being placed in the operant chambers in which levers were absent and 50 food pellets available in the food trough, for 15 min. During the final session, rats were again placed in the operant chambers with access to levers. Pressing the active lever resulted in presentation of the light stimulus. Active and inactive lever presses and food trough head entries were recorded.

2.10. Histology

After the final sessions, all rats were anesthetized with an overdose of sodium pentobarbital, perfused with 0.9% saline followed by 4% formalin, and decapitated. The brains were removed and stored in 4% formalin for at least seven days before being sliced in serial sections and inspected for cannulae implantation and injection sites. All animals included in the data analysis had verified cannulae placements.

2.11. Data analysis

Active lever presses, inactive lever presses, and food trough head entries during each of the 10 sessions were analyzed using separate mixed-design ANOVAs with dose (between groups) and day (repeated measures) as factors. For each set of data, three ANOVAs were conducted, one for sessions 1–4, another for sessions 5–9, and another for sessions 9–10.

A detailed analysis was made of the relation between active lever presses and food trough head entries ("response-reward contingencies"), to assess whether impairment in acquisition could be accounted for by a significantly greater number of initial response-reward latencies being longer (and resulting in reduced reinforcement) than a standard value (as defined below). In order to do so, a number of values were calculated and analyzed for the first 40 active lever presses emitted by the vehicle and 0.5 μg AP-5 groups. The number of active lever presses required to reach the criterion where 20 of these presses were followed by a food trough head entry was calculated, as visual inspection determined that most vehicle rats acquired lever pressing after 20 response-reward contingencies. To further determine the effects of possible treatment-induced stereotypy, the number of active lever press clusters, defined as two or more active lever presses occurring within 1 s of each other, was calculated. To assess possible inter-group differences in latency to encounter or consume a food pellet after an active lever press, the time between active lever press and head entry for the 20 response-reward contingencies was calculated and compared

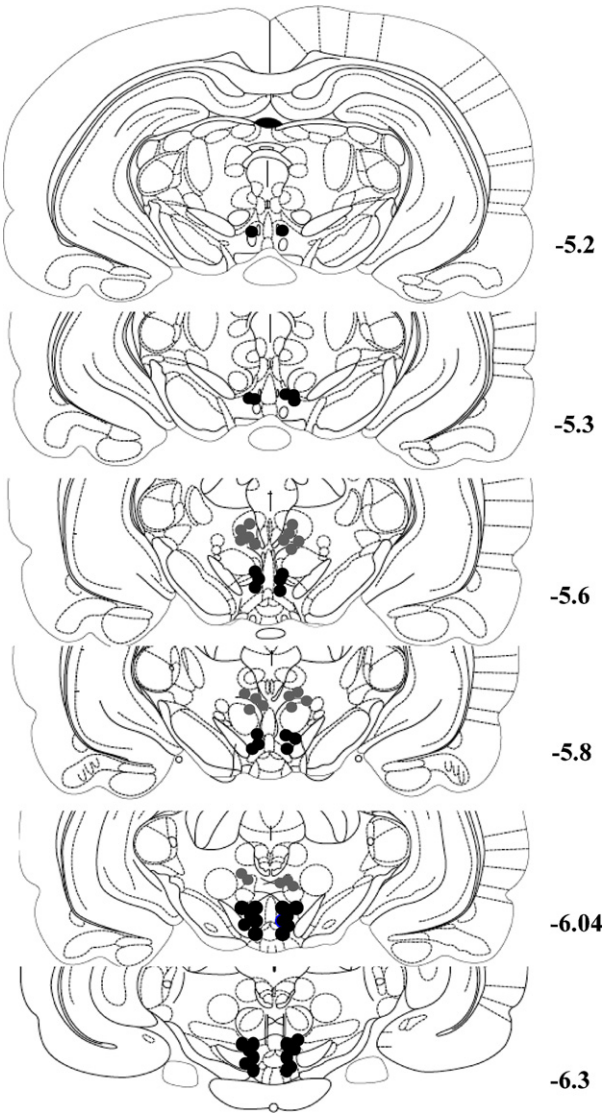

Fig. 1. Histological reconstruction of injection sites adapted from Paxinos and Watson [24]. Black circles represent injections in the VTA group; grey circles represent injections in the dorsal control group. The numbers to the right of each section indicates the distance posterior to the bregma.

between groups. These latencies were then assessed to determine the percentage of response-reward contingencies in both groups which occurred within the standard value for the vehicle group, with this standard value defined as the sum of the average latency and the standard deviation. One-way ANOVAs were conducted to compare group differences on the number of active lever presses required to reach 20 response-reward contingencies, the number of clusters within the first 40 active lever presses, and the percentages of food approach latencies equal to or less than the standard value.

The feeding and activity data from the third and fourth sessions were analyzed using a mixed-design ANOVA with dose and day as factors. Interactions were followed up with tests of simple main effects with ANOVAs using the overall mean error. For the devaluation assessment experiment, the number of active and inactive lever presses in session 4 were compared between groups using separate independent samples *t*-tests. All analyses were conducted using the statistical software package SPSS. Criterion for significance was $p < 0.05$ in all cases.

3. Results

3.1. Histology

Only the data of rats with verified VTA (or dorsal control) placements were included in these results. Most VTA microinjection sites were localized in the caudal portion of the VTA (−5.6 to −6.04 mm posterior to bregma) with some injections occurring in the central portion (−5.2 to −5.3 mm posterior to bregma) (see Fig. 1). Dorsal placements were generally 1 mm above the VTA placements, located just ventral to the red nucleus, from −5.6 to −6.04 mm posterior to bregma.

3.2. Instrumental conditioning experiment

3.2.1. Active lever

The groups receiving vehicle and 0.125 μg AP-5 showed steep, significant increases in lever pressing across days 1–4, the 0.25 μg group showed steep significant increases on days 3 and 4, and the 0.5 μg group failed to show large increases in responding across days (see Fig. 2; a mixed-design ANOVA showed a significant session × dose interaction [$F_{(9,69)} = 2.203$, $p < .05$]). Follow-up tests revealed session effects for the vehicle, 0.125 and 0.25 μg groups [$Fs_{(3,102)} = 14.56$, 8.71, and 10.71, respectively, all $ps < .01$]. During sessions 5–9 all groups except the vehicle showed increases in active lever presses (see Fig. 2; significant session by dose interaction [$F_{(12,92)} = 1.896$, $p < .05$]), with AP-5 groups exhibiting fewer presses (significant dose effect [$F_{(3,23)} = 4.343$, $p < .05$]). Tests of simple effects revealed significant increases in responding across days 5–9 in each of the AP-5 groups ($Fs_{(4,92)} = 3.83$, 9.225 and 7.47, all $ps < .01$ for increasing AP-5 doses, respectively). During session 10 all groups showed similar amounts of active lever pressing when compared to session 9 (Fig. 2; no significant session, dose, or session by dose interaction effects, $ps > .6$).

3.2.2. Inactive lever

During sessions 1–4 all groups showed decreases in lever pressing across days, although this was not significant (see Fig. 3), with AP-5 groups emitting a higher number of inactive lever presses than the vehicle group (significant dose effect [$F_{(4,23)} = 8.075$, $p < .001$]). During sessions 5–9 all groups showed reductions in inactive lever presses across sessions, although this was not a significant effect. During session 10 all groups showed similar amounts of inactive lever pressing when compared to session 9, except for the 0.5 μg group which showed an increase (significant session × dose interaction [$F_{(3,23)} = 4.338$, $p < .05$]; follow-up tests revealed a significant session effect in the 0.5 μg group [$F_{(1,23)} = 7.603$, $p < .05$]).

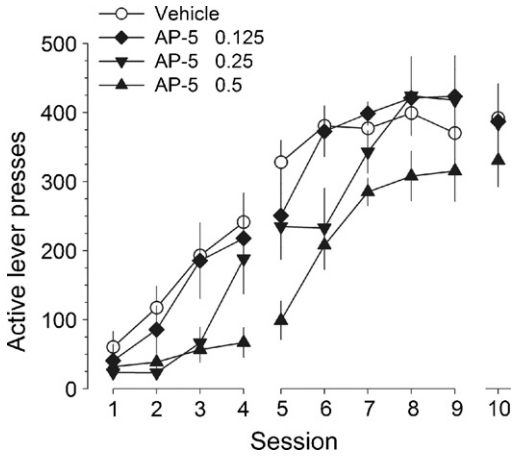

Fig. 2. Mean active lever presses for groups tested in 10 instrumental conditioning sessions. Rats received bilateral intra-VTA microinjections of 0.125, 0.25 or 0.5 μg/0.5 μl of AP-5 ($ns = 5$, 7 and 6, respectively) or 0.9% saline (vehicle; $n = 9$) immediately prior to sessions 1–4 and 10. Vertical bars represent the standard error of the mean (S.E.M.).

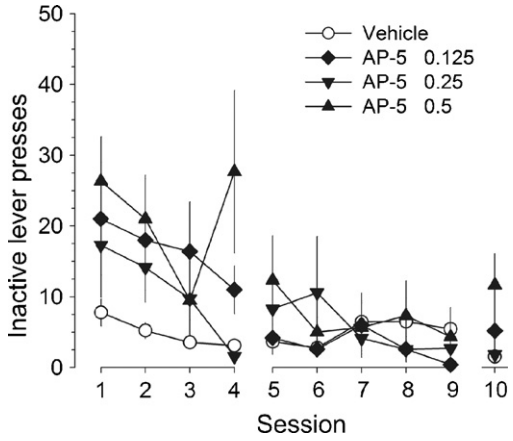

Fig. 3. Mean inactive lever presses for the same groups tested in Fig. 1. Vertical bars represent the standard error of the mean (S.E.M.).

3.2.3. Head entries

During sessions 1–4 all groups receiving AP-5 appeared to emit more head entries than the vehicle group, although the group differences were not significant (see Fig. 4). Head entries showed large within-group variability and generally did not show systematic changes across sessions. During sessions 5–9 the differences among the AP-5 and vehicle groups were not significant (Fig. 4). During session 10 all groups increased their food trough head entries (see Fig. 4; significant session effect [$F_{(1,23)} = 17.47$, $p < .001$]), with the AP-5 groups tending to show larger increases than the vehicle group (multiple t-tests with Bonferroni correction revealed a significant session effect only in the 0.5 μg group [$t(5) = 7.885$, $p < .001$]).

3.2.4. Dorsal anatomical controls

To test the regional specificity of the effects of AP-5 on acquisition of instrumental responding two groups were tested with injections of vehicle ($n = 6$) or 0.5 μg AP-5 ($n = 5$), respectively, in a site 1 mm dorsal to the VTA prior to each of four instrumental responding sessions. In both groups the number of active lever presses rose steeply across the four sessions (see Fig. 5; $F_{(3,27)} = 16.114$, $p < .005$) and were not significantly different from each other.

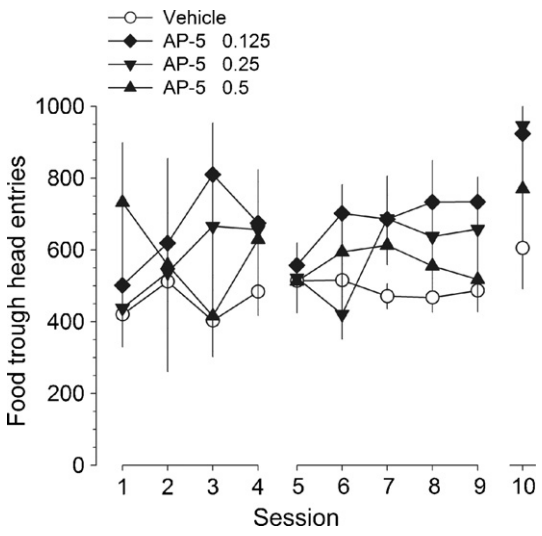

Fig. 4. Mean food trough head entries across 10 instrumental responding sessions for the same groups tested in Fig. 1. Vertical bars represent the standard error of the mean (S.E.M.).

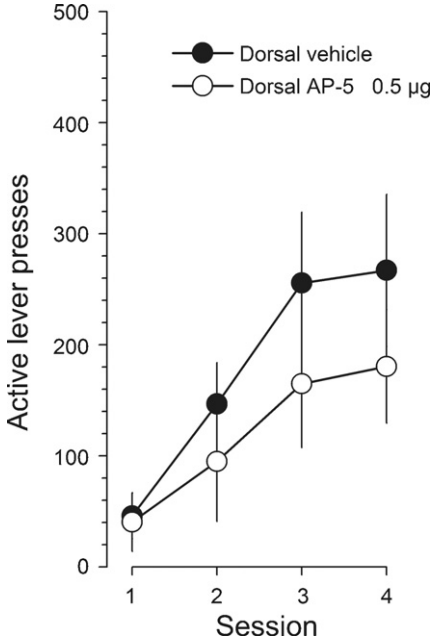

Fig. 5. Mean active lever presses for groups receiving injections of vehicle ($n = 6$) or 0.5 μg AP-5 ($n = 5$) in a site 1 mm dorsal to the VTA site. Injections were made immediately prior to each session. Vertical bars represent the standard error of the mean (S.E.M.).

3.3. Free-feeding experiment

Groups receiving vehicle or 0.5 μg/0.5 μl AP-5 ate the same amount of rat chow on the test session as they did on the baseline session before the test (see Fig. 6).

3.4. Activity testing

The 0.5 μg dose of AP-5 had little effect on ambulatory activity but caused an increase in stereotypy (see Fig. 7; analyses revealed a significant session × dose interaction [$F_{(1,11)} = 8.938$, $p < .05$]).

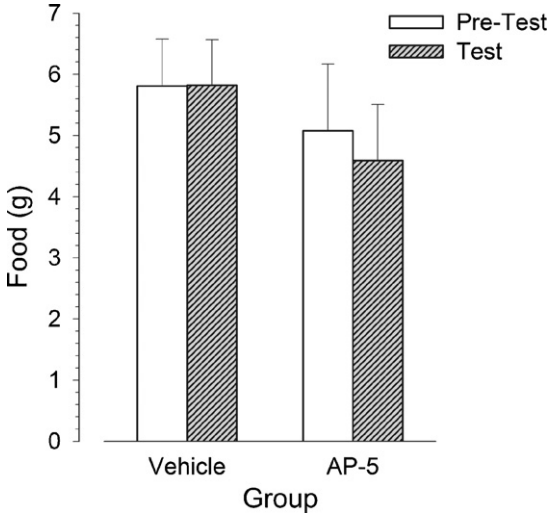

Fig. 6. Mean consumption of rat chow on session 3 of baseline training and session 4 for the vehicle and AP-5 groups. Both groups received bilateral VTA microinjections of vehicle before session 3 and either vehicle ($n = 7$) or 0.5 μg/0.5 μl AP-5 ($n = 9$) before session 4. Vertical bars represent the standard error of the mean (S.E.M.).

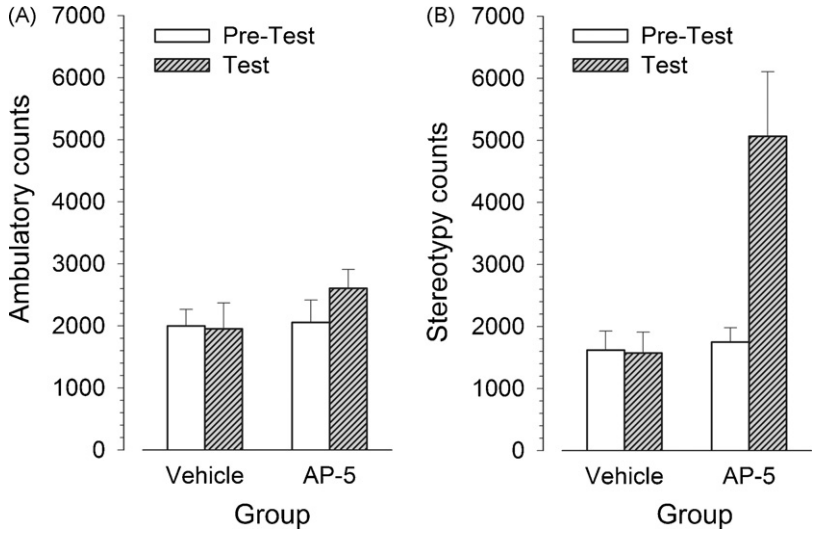

Fig. 7. Mean total number of ambulatory (A) and stereotypy (B) counts on sessions 3 and 4 of activity testing. Both groups received bilateral VTA microinjections of vehicle before session 3 and either vehicle ($n = 6$) or 0.5 μg/0.5 μl AP-5 ($n = 7$) before session 4. Vertical bars represent the standard error of the mean (S.E.M.).

3.5. Response-reward contingency analysis

The possibility that AP-5-induced stereotypy affected latencies between active lever presses and reward receipt, thereby weakening a response-reward contingency, was assessed. Visual inspection of active lever presses followed by head entries (food approach trials) in the vehicle group indicated that food approach latencies declined after the 20th trial, and an analysis of the 0.5 μg AP-5 group revealed that an average of 40 active lever presses occurred before reaching the 20th food approach trial. Of the first 20 food approach trials, the percentage of approach latencies equal to or less than the standard established in the vehicle group was not significantly different between the groups (see Fig. 8A). The number of active lever presses required to reach 20 food approach trials (Fig. 8B) was slightly higher for the AP-5 group, as was the number of clusters within the first 40 active lever presses (Fig. 8C), but neither was significantly different from the vehicle group. Thus, it appears that if AP-5 produced any stereotypy in the operant chambers it did not affect response-reward contingencies.

3.6. Reward devaluation assessment experiment

Following a session in which rats ate operant chamber food pellets after receiving intra-VTA injections of vehicle or 0.5 μg AP-5, the AP-5 group emitted more active lever presses than the vehicle group, although group differences were not significant for active or inactive lever presses (see Fig. 9).

4. Discussion

In the present experiments, the selective competitive NMDA receptor antagonist AP-5 impaired the acquisition of lever pressing

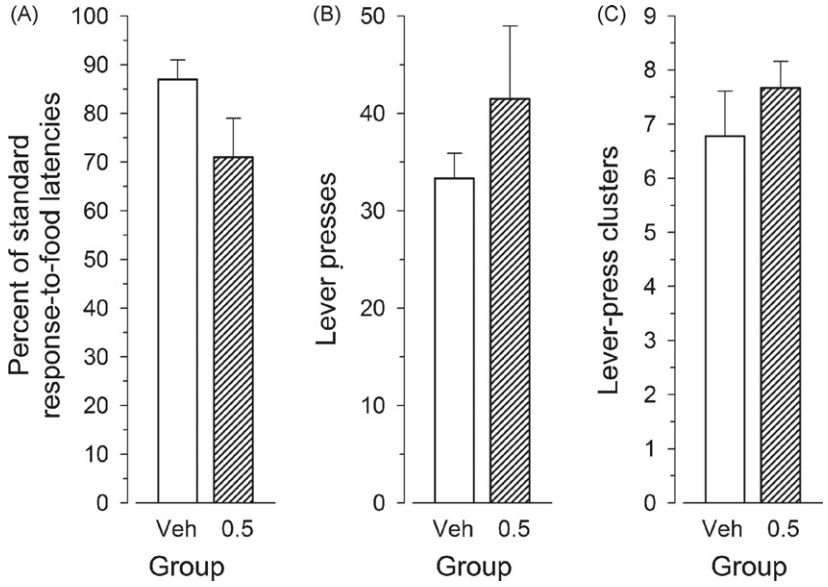

Fig. 8. The relationships between active lever presses and food trough head entries emitted by the animals receiving vehicle or 0.5 μg AP-5 in Fig. 1. (A) The percentage of food approach latencies (time between an active lever press and food trough head entry) that were equal to or less than the standard food approach latency (determined as the mean latency plus one standard deviation for the vehicle group). (B) Mean number of active lever presses accumulated by each group at the time when the criterion of 20 food approach trials (defined as an active lever press followed by a food trough head entry) was reached. (C) Number of clusters of active lever presses by each group within the first 40 active lever presses. Vertical bars represent the standard error of the mean (S.E.M.).

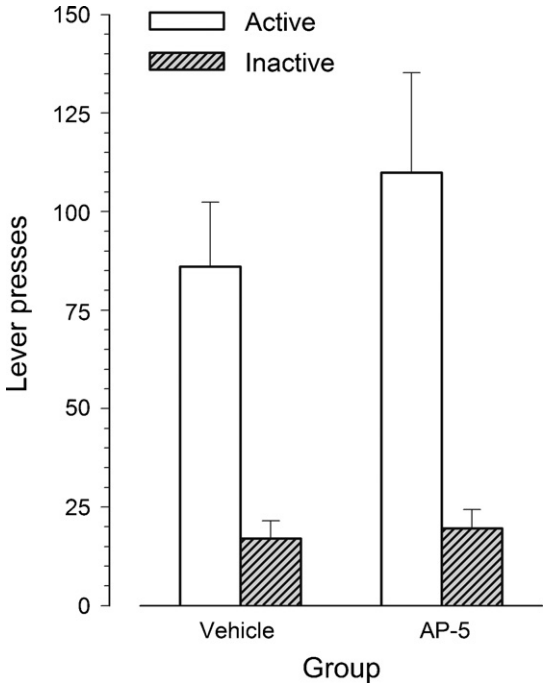

Fig. 9. Mean number of active and inactive lever presses during an extinction session for rats that received either vehicle ($n = 6$) or 0.5 μg AP-5 ($n = 7$) immediately prior to a previous session with free access to 50 operant chamber food pellets. Vertical bars represent the standard error of the mean (S.E.M.).

in a food-reinforced instrumental learning paradigm. When animals were treated with intra-VTA injections of the highest dose of AP-5, lever pressing for food was not acquired, but when they were relieved of this treatment, it was. Injections made 1 mm dorsal to the VTA site did not significantly affect acquisition of the response. These findings support our hypothesis that NMDA neurotransmission in the VTA is necessary for the acquisition of reward-related learning. Furthermore, microinjections of AP-5 into the VTA after lever pressing was learned had no effect, indicating that NMDA receptor stimulation in the VTA is not necessary for the expression of reward-related learning. The impairment in acquisition cannot be accounted for by any global reduction in activity, as AP-5 groups emitted more head entries than the vehicle group during the first four sessions (although group differences were not significant) and pressed the inactive lever more than the vehicle group. This indicates that AP-5 did not impair the abilities to enter the food trough or to press a lever, making it difficult to argue that an inability to perform these responses accounted for the lack of increase in active lever pressing across the first four sessions.

Several of our findings suggest that impaired acquisition of reward-related learning was not likely a result of AP-5 treatment-induced reduction in food reward and motivation. First, when AP-5 was given to rats after acquisition of food-reinforced lever pressing it failed to reduce lever pressing rates, indicating that AP-5-treated animals, similar to those treated with vehicle, could expend as much effort under treatment (an average of 300 lever presses) as when not to gain food reward. Second, AP-5-treated rats consumed operant chamber food pellets during the first four and the final instrumental responding sessions as well as during reward devaluation sessions, indicating that food remained rewarding to these rats when under treatment. Third, in the free-feeding experiment rats treated with intra-VTA AP-5 ate as much rat chow as those treated with vehicle. Altogether, these sets of data indicate that primary food reward and motivation to eat were not affected by treatment. They also suggest that food reward is not dependent on VTA NMDA receptor stimulation.

Control experiments provided further indication that reduced responding in the initial instrumental sessions with AP-5 was likely not due to reduced motoric ability. Effects on motoric activity were assessed in a separate experiment in which AP-5 produced no effects on ambulatory activity and but did increase stereotypy (which may have mainly been accounted for by increased circling behavior which was observed after injections). Whatever stereotypical behavior AP-5 produced, however, did not impact the rats' exposure to stimulus-reward contingencies, as an analysis of lever presses and head entries for the first 20 active lever presses followed by a head entry revealed no significant differences in food approach latencies or response-per-reward rates between vehicle and AP-5-treated animals. Taken together with the increased head entry measures found in the initial experiment, these data all indicate that AP-5 did not impair the animals' abilities to perform the required responses, eliminating this as a possibility for the impairment in the acquisition of the instrumental response.

In recent years mounting evidence suggests that two processes control the acquisition and expression of instrumental learning. During acquisition, responding is largely determined by goal-directed actions and susceptible to manipulations that devalue the reward. After acquisition, responding is largely habit-driven and relatively less susceptible to reward devaluation [25,26]. Thus, it is conceivable that in the present experiments AP-5 selectively impaired acquisition of lever pressing because reward devaluation reduced goal-directed behavior, an effect that would be evident during acquisition but not after. Several sets of data argue against this possibility. First, as mentioned above, AP-5 did not reduce food consumption in any of the tasks. Second, in our food pellet devaluation experiment, animals that consumed food pellets under AP-5 or vehicle treatments during acquisition responded at similar levels during a subsequent extinction test. Because extinction responding represents goal-directed behavior and is indicative of remembered reward (goal) value, this test suggests that food pellet devaluation did not occur in AP-5-treated animals compared to vehicle. Therefore, the selective impairment of acquisition seen here cannot be adequately explained as differential effects of AP-5 on responding that is primarily goal- or habit-driven.

The burst firing of VTA DA cells has been shown to be associated with VTA NMDA neurotransmission [36], suggesting that NMDA blockade here may have impaired acquisition of reward-related learning not by blocking NMDA-dependent plasticity but by reducing DA bursting and subsequent DA release, a mechanism that may be involved in effort exertion. This interpretation hinges on the assumption that, once learned, responding is not dependent on effort expenditure, or other DA-dependent behaviors (i.e., reward, incentive motivation). However, a number of studies indicating that impairment in DA neurotransmission immediately and profoundly reduces responding in well-trained animals [37–39], including rats lever pressing for food under FR1 schedules of reinforcement [40–42], coupled with the absence of reduced responding in session 10 of the present experiment, renders this interpretation not likely.

After eliminating other conceivable explanations (see above) for our findings of impaired acquisition of instrumental responding, the best explanation is that blockade of NMDA receptors in the VTA prevented the animals from forming associations with reward-related stimuli. This suggests that at least some of the neural changes critical for reward-related learning occur in the VTA, and that these neural changes involve NMDA receptor stimulation. It may be that stimulation of NMDA receptors in this region is the route by which signals representing previously neutral environmental stimuli acquire the ability to activate the DA reward

system. In this way, organisms may learn to associate the effects of reward stimuli, including their incentive motivational effects, with the environmental stimuli present when these effects are experienced and this would facilitate reward-related learning. As animals increase their interaction with stimuli associated with incentive motivation, response associations might then be consolidated at other levels of the DA system, in particular the nucleus accumbens and dorsal striatum, where it is thought that stimulus-response associative processes may occur [27–29].

This hypothesis is predicated upon several bodies of evidence. Food-associated stimuli activate midbrain DA cells or VTA cells specifically in non-human primates [4–6,8] and rats [30–33]. This suggests that classically conditioned stimuli functioning as conditioned stimuli [34,35] do so at least partly by acquiring the ability to activate VTA DA cells. Moreover, in animals learning to press a lever for food reward, a reward-associated stimulus is associated with elevations in extracellular DA concentrations in mesolimbic terminals immediately prior to the emission of a lever press [14]. Whether or not the establishment of conditioned mesolimbic DA activation is critical for reward-related learning remains to be determined. However, the possibility is interesting.

Other studies have assessed the involvement of NMDA receptors in reward-related learning. When administered systemically to rats prior to cocaine injections the non-competitive NMDA receptor antagonist MK-801 blocked the development of cocaine conditioned place preference but had no effect when administered before the preference test [43]. When administered into the VTA, NMDA and AMPA receptor antagonists separately blocked the development and together blocked the expression of morphine place preference [17] and the development of cocaine place preference [18]. These studies indicate that blockade of glutamate neurotransmission, including specifically in the VTA, can interfere with acquisition and expression of drug place preference learning. In studies similar to the present one AP-5 administered into the NAcc core [41,44] blocked the acquisition, but not expression, of food-reinforced lever pressing. Thus, it appears that NMDA-dependent synaptic plasticity in both the terminal and cell body regions of the mesolimbic DA system are critical for reward-related learning.

Other neurotransmitters in the VTA may also be involved in reward-related learning. In previous work with a paradigm similar to the one here we found that intra-VTA injections of scopolamine, a muscarinic acetylcholine receptor antagonist, administered before sessions 1–4 significantly reduced lever pressing or consumption of a novel food in a novel environment, but when administered before session 10, did not [45,46]. These studies suggest that muscarinic acetylcholine receptor stimulation in the VTA is necessary for the acquisition, but not expression, of food reward learning. Interestingly, muscarinic receptors are implicated in LTP [47–49], suggesting that such a mechanism underlies the role of VTA acetylcholine in reward-related learning. Our results with scopolamine, together with the present results, suggest that concurrent stimulation of muscarinic acetylcholine and NMDA receptors in the VTA is necessary for food reward learning.

The present findings have implications not only for the understanding of basic mechanisms underlying reward-related learning, but also for the treatment of disorders, such as addiction, that involve this type of learning. Given that drug-taking is similar to other types of reinforced instrumental learning [50,51], it is possible that similar VTA mechanisms operate there too. Thus, further research into the synaptic changes critical for reward-related learning will increase our understanding of brain mechanisms contributing to the development and maintenance of drug-taking habits and lead to improved treatment strategies.

In summary, intra-VTA microinjections of AP-5 reduced or eliminated the daily increases in lever pressing for food seen in vehicle-treated animals. When the injections were made after the response was acquired they had no effect. Furthermore, intra-VTA AP-5 did not reduce food consumption or motoric activity, nor did it selectively reduce goal-directed instrumental behavior. Together, these results suggest that the acquisition, but not expression, of reward-related learning is dependent on NMDA receptor stimulation in the VTA.

Acknowledgments

This research was supported by a CUNY Graduate Center Science Fellowship to M.R.Z. and PSC-CUNY Research Awards to R.R. The authors would like to thank Yaakov Berger, Barry Bornstein, and Danielle Malkusz for their technical assistance.

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