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ORIGINAL INVESTIGATION

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Repeated stimulation of L-type calcium channels in the rat ventral tegmental area mimics the initiation of behavioral sensitization to cocaine

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Abstract *Rationale:* A substantial body of evidence indicates that ion flux through L-type calcium channels and *N*-methyl-D-aspartate (NMDA) receptors contributes to behavioral sensitization to cocaine. *Objectives:* The following experiments were designed to evaluate the role of calcium influx through L-type calcium channels or NMDA receptors in the ventral tegmental area (VTA) in the initiation of behavioral sensitization to cocaine. *Methods*: The L-type calcium channel agonist BayK 8644, the glutamate agonist NMDA, or vehicle was microinjected into the VTA on 3 consecutive days. Following a 2-week withdrawal period, all rats received a challenge injection of cocaine (15 mg/kg, i.p.) in order to assess potential cross-sensitization with the NMDA or BayK 8644 pretreatments. *Results:* Repeated intra-VTA microinjections of BayK 8644, but not NMDA, resulted in an augmentation of the behavioral response to cocaine. *Conclusions:* These results indicate that calcium influx through L-type calcium channels produces neurophysiological adaptations that mimic those resulting from intermittent exposure to cocaine.

Key words NMDA · BayK 8644 · Psychostimulant · Drug abuse

Introduction

Behavioral sensitization is a progressive and enduring augmentation of locomotor and stereotyped behavior in rats resulting from intermittent psychostimulant administration. It has been suggested that the neurophysiological adaptations underlying behavioral sensitization influence the motivational properties of both pharmacological and natural reinforcers. The results of a growing number of self-administration experiments suggest that psychostimulant sensitization increases the reinforcing efficacy of this drug class. Rats sensitized to psychostimulants subsequently self-administer subthreshold doses of cocaine or amphetamine (Horger et al. 1990; Vezina et al. 1999) and display a higher break point for cocaine or amphetamine self-administration using a progressive ratio schedule of reinforcement (Mendrek et al. 1998; Lorrain et al. 2000). A recent study also demonstrated that sensitization to amphetamine facilitated sexual behavior among male rats, indicating that amphetamine can cross-sensitize to a natural behavior (Fiorino and Phillips 1999). Collectively, these results suggest that the neurophysiological adaptations associated with behavioral sensitization enhance the reinforcing efficacy of both pharmacological and natural reinforcers.

The initiation of behavioral sensitization takes place primarily in the ventral tegmental area (VTA). Repeated microinjections of amphetamine into the VTA, but not into the nucleus accumbens, result in an augmented behavioral response to a subsequent systemic or intra-accumbal amphetamine injection (Kalivas and Weber 1988; Perugini and Vezina 1994; Cador et al. 1995; Bjijou et al. 1996). An ample body of research indicates that L-type calcium channels and *N*-methyl-D-aspartate (NMDA) receptors play an important role in the initiation of behavioral sensitization to psychostimulants. Systemic injections of L-type calcium channel antagonists (Karler et al. 1991b; Reimer and Martin-Iverson 1994) or selective NMDA receptor antagonists (Karler et al. 1989, 1990, 1994; Kalivas and Alesdatter 1993; Stewart and Druhan 1993; Wolf and Jeziorski 1993; Ida et al. 1995; Li and Wolf 1999; Li et al. 1999) block the initiation of behavioral sensitization to cocaine or amphetamine. Moreover, microinjection of the NMDA antagonist, MK-801, directly into the VTA blocks the initiation of cocaine sensitization (Kalivas and Alesdatter 1993). Taken together, these results suggest that L-type calcium channels and NMDA receptors located on dopamine

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cells in the VTA play a critical role in the initiation of behavioral sensitization to psychostimulants.

The present series of experiments was designed to assess the ability of L-type calcium channel and NMDA agonists to mimic the initiation of behavioral sensitization. The L-type calcium channel agonist, BayK 8644, or NMDA was microinjected directly into the VTA for 3 consecutive days. Following a 2-week withdrawal period, all rats received a challenge injection of cocaine and behavioral activity was monitored. The results indicate cross-sensitization between BayK 8644 and cocaine, while microinjections of NMDA did not influence the subsequent behavioral response to cocaine.

Materials and methods

Animals

Male Sprague-Dawley rats weighing 251–275 g were obtained from Taconic Farms (Germantown, N.Y., USA). Rats were individually housed with food and water available ad libitum. A 12/12 h light/dark cycle was used with lights on at 7:00 a.m. All experimental procedures were performed in the afternoon (between 1:00 and 5:00 p.m.).

Procedure

The protocols for the behavior experiments, which are summarized in Table 1, are based on previous results indicating that repeated daily microinjections of amphetamine (Perugini and Vezina 1994; Bjijou et al. 1996; Vezina 1996) or SKF-38393 (Pierce et al. 1996) into the VTA/substantia nigra result in a sensitized behavioral response to a subsequent systemic injection of a psychostimulant. A 14-day withdrawal period was imposed between the repeated microinjections and the cocaine challenge injection. The use of a withdrawal period is based on previous research in which the insertion of 14 or more days of withdrawal between the repeated drug treatment and a subsequent psychostimulant challenge injection resulted in a more robust sensitization of the behavioral response (Kolta et al. 1985; Kalivas and Duffy 1993a; Paulson and Robinson 1995).

Surgery

The rats were anesthetized with sodium pentobarbital (50 mg/kg) and mounted in a stereotaxic apparatus. Guide cannulae (12 mm, 24 gauge) were implanted bilaterally 1 mm dorsal to the VTA (–5.5 A/P, ±1.0 M/L, –7.0 D/V relative to bregma; Paxinos and Watson 1997) and cemented in place by affixing dental acrylic to three stainless steel screws secured in the skull. Following surgery the rats were allowed to recover for 3–5 days prior to the start of the microinjection regimen.

Microinjections

The rats initially were habituated to the photocell apparatus (AccuScan Instruments, Columbus, Ohio, USA) for 3 h. On the day of experimentation the rats were rehabituated to the behavior boxes for 1 h. Following this adaptation period the obturators were removed from the guide cannulae and replaced by injection needles (33 gauge stainless steel) that extended 1 mm below the tips of the guide cannulae into the VTA. Bilateral infusions of BayK 8644 (10 or 30 nmol/0.5 µl), NMDA (0.68 or 0.068 nmol/0.5 µl), or vehicle (100% DMSO or 0.9% sterile saline, respectively) were made over 60 s in a volume of 0.5 µl/side.

Table 1 Protocols for the behavioral experiments. On days 1–3 the microinjection cannulae were lowered into the ventral tegmental area (VTA) and one of the following substances was infused (0.5 µl over 1 min): *N*-methyl-D-aspartate (NMDA; 0.68 or 0.068 nmol/0.5 µl per side), BayK 8644 (10 or 30 nmol/0.5 µl per side) or vehicle. Fourteen days after the last of the three daily microinjections (i.e., on day 17) the rats were injected with 15 mg/kg cocaine (i.p.). Every daily procedure was preceded by a 60-min exposure to the behavioral apparatus and was followed by a 120-min period in which behavioral activity was monitored

Days 1–3	Day 17
NMDA–cocaine cross-sensitization	
NMDA microinjection (0.68 or 0.068 nmol/0.5 µl)	Cocaine (15 mg/kg, i.p.)
Vehicle microinjection (0.5 µl 0.9% saline)	Cocaine (15 mg/kg, i.p.)
BayK 8644–cocaine cross-sensitization	
BayK 8644 microinjection (10 or 30 nmol/0.5 µl)	Cocaine (15 mg/kg, i.p.)
Vehicle microinjection (0.5 µl 100% DMSO)	Cocaine (15 mg/kg, i.p.)

The injectors were left in place for another 30 s to allow the compound to diffuse from the site of injection, and then removed. The rats were returned to the photocell boxes and behavior was monitored for a 2-h period. On the day of the cocaine challenge the rats were habituated to the boxes for 1 h followed by a systemic injection of cocaine (15 mg/kg, i.p.); cocaine-induced behavioral activity was monitored for 2 h.

Apparatus

The AccuScan activity monitors generate several measures of motor activity. For the purposes of this study, we present measures of locomotion and stereotyped behaviors. Using the AccuScan system, the most accurate measure of locomotion is distance traveled, which is expressed in centimeters. Rodents administered psychostimulants also display a number of repetitive behaviors including head bobbing and grooming. These behaviors are quantified as stereotypy counts, which the AccuScan system defines as the animal breaking the same photocell beam or set of beams repeatedly. Previous experiments were performed to insure a strong positive correlation between the cocaine-induced locomotion and stereotypy ratings made by experienced human observers and those obtained with the photocell-based AccuScan activity monitors (Pierce and Kalivas 1998).

Histology

Upon completion of the behavior experiments the rats received an overdose of sodium pentobarbital (100 mg/kg, i.p.) and were perfused intracardially with 0.9% saline followed by 10% formalin. The brain was removed and coronal sections (100 µm) were taken at the level of the VTA with a vibratome (Technical Products International, St. Louis, Mo., USA). The sections were mounted on gelatin-coated slides and stained with cresyl violet. An individual unaware of the behavioral responses determined cannulae placements as well as potential microinjection-induced neurotoxicity.

Drugs

Cocaine was a gift from the National Institute on Drug Abuse (Rockville, Md., USA) and was dissolved in 0.9% saline. NMDA

was obtained from RBI (Natick, Mass., USA) and dissolved in 0.9% sterile saline. BayK 8644 was purchased from Calbiochem (La Jolla, Calif., USA) and dissolved in 100% DMSO.

Results

Location of microinjection sites in the VTA

The schematic brain sections depicted in Fig. 1 are from the atlas of Paxinos and Watson (1997). The filled circles represent the placement of the tips of the microinjection cannulae from the experiments graphed in Figs. 2, 3, 4, 5. Some of the cannulae tips were located at the border between the VTA and the medial substantia nigra, the ventral medial lemniscus, and the ventral red nucleus. The numbers indicate millimeters from bregma.

The histologies also were checked closely for microinjection-induced neurotoxicity. If cell death and gliosis other than that typically observed following a cannulation was noted, the data from that animal was removed from the data analysis. Two rats were removed from the data analysis due to apparent BayK 8644-induced neurotoxicity. No cell death was observed in any of the rats comprising the NMDA, saline, or DMSO groups.

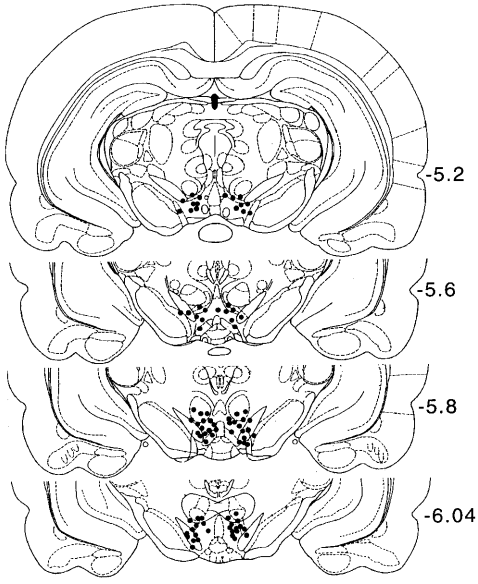

Fig. 1 Location of microinjection sites in the ventral tegmental area (VTA). The *filled circles* represent the placement of the tips of the microinjection cannulae. Note that some of the cannula tips were located on the border between the VTA and the medial substantia nigra, the ventral medial lemniscus, and the ventral red nucleus. The schematic brain sections are from the atlas of Paxinos and Watson (1997). The *numbers* indicate millimeters from bregma

Repeated microinjections of NMDA into the VTA had no influence on the behavioral effects induced by a subsequent challenge injection of cocaine

The data summarized in Fig. 2a indicate that repeated intra-VTA microinjections of 0.68 nmol NMDA produced a trend toward a progressive augmentation in stereotypy. The data were analyzed with a mixed factors analysis of variance (ANOVA). The between-subjects factor was saline or drug treatment and the within-subjects factor was treatment day. This analysis revealed no significant main effects or interactions. However, the behavioral response elicited by 0.68 nmol NMDA on day 3 was substantially greater than observed on day 1. The time courses of the stereotypy counts from the saline and 0.68 nmol NMDA groups on day 3 are summarized in Fig. 2b. These data were analyzed with a mixed factors ANOVA (repeated measure over time), which revealed a significant main effect of time [$F(11,165)=9.51$, $P<0.0001$], and a significant drug×time interaction [$F(11,165)=1.83$, $P<0.05$]. Pairwise comparisons (Fisher's LSD) showed a significant difference between the NMDA and saline groups at the 10-min time point. Following a 2-week withdrawal period, all rats were injected with 15 mg/kg cocaine. The total stereotypy counts recorded over the 2-h period post-cocaine are summarized in Fig. 2c. These data were analyzed with a one-way ANOVA, which revealed no main effect of drug treatment. The time course of the saline and 0.68 nmol NMDA data from day 17 are graphed in Fig. 2d. A mixed factors ANOVA (repeated measures over time) performed on these data revealed no significant main effects or interactions. The data obtained on day 17 indicate that pretreatment with NMDA did not influence the stereotypy counts produced by a subsequent challenge injection of cocaine.

The distance traveled data summarized in Fig. 3 were analyzed using the same statistics as the stereotypy data summarized above. The analysis of the data shown in Fig. 3a revealed no significant main effects or interactions. Analysis of the time course data from day 3 (see Fig. 3b) revealed a significant main effect of time [$F(11,165)=12.88$, $P<0.0001$]. Analysis of the total distance traveled following cocaine on day 17 showed no significant main effect of drug treatment (see Fig. 3c). Analysis of the time course data from the saline and 0.68 nmol NMDA groups on day 17 (see Fig. 3d) revealed a significant main effect of time [$F(11,165)=8.26$, $P<0.0001$]; there were no other significant main effects or interactions. For the NMDA/saline experiments, there were seven to ten rats per group.

Repeated microinjections of BayK 8644 into the VTA augmented the behavioral effects of a subsequent challenge injection of cocaine

The data summarized in Fig. 4a indicate that repeated intra-VTA microinjections of BayK 8644 did not induce stereotypy (although there was a trend toward a BayK 8644-induced increase in stereotypy counts on day 3). The data were analyzed with a mixed factors ANOVA. The between-subjects factor was vehicle or

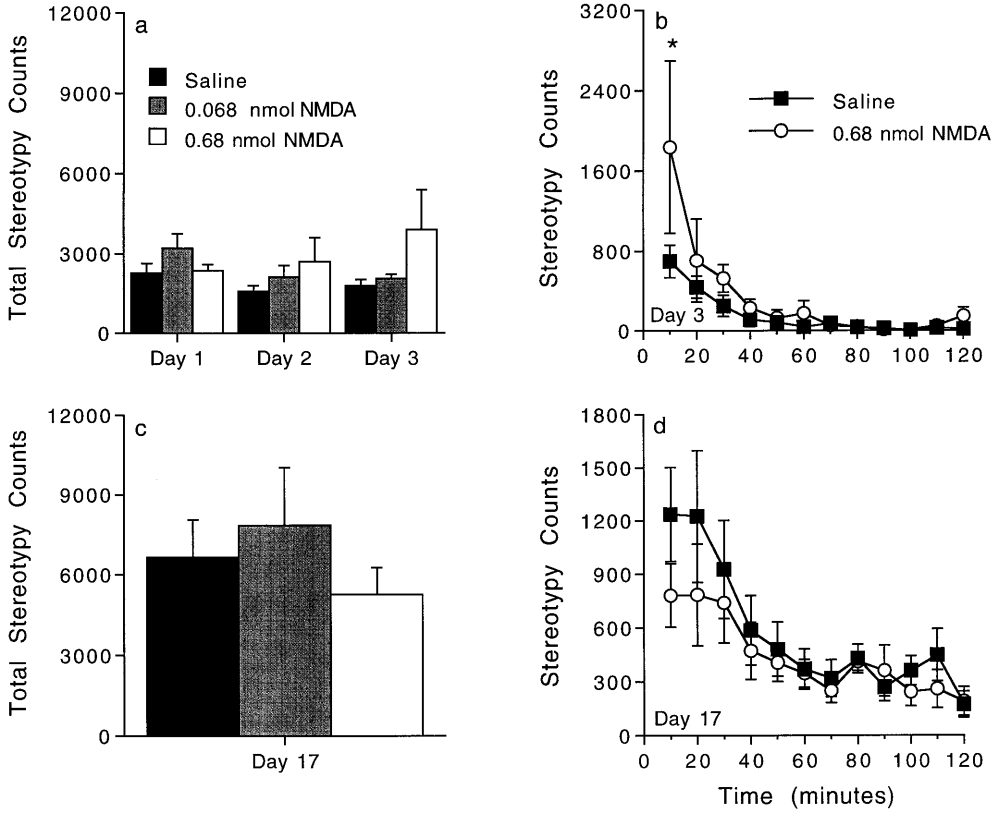

Fig. 2a–d Repeated microinjections of *N*-methyl-D-aspartate (NMDA) into the VTA did not influence the stereotypy counts induced by a subsequent challenge injection of cocaine. On days 1–3 the rats were bilaterally microinjected with sterile saline or NMDA (0.68 or 0.068 nmol/0.5 µl). After a 2-week withdrawal period (i.e., on day 17) all rats received a challenge injection of cocaine (15 mg/kg, i.p.) and behavioral activity was monitored. **a** Total stereotypy counts recorded over the 120-min period following the microinjection of NMDA or saline. Note that there was a trend toward an increased behavioral effect of 0.68 nmol NMDA relative to the saline group on day 3. **b** Timecourse of the stereotypy counts from the 0.68 nmol NMDA and saline groups recorded on day 3. The data are presented as the 120 min behavioral response (stereotypy counts) divided into 10-min blocks. Note that the behavioral effect of 0.68 nmol NMDA was greater than that of saline 10 min after microinjection. The *asterisk* represents a significant difference from saline at that time point (Fisher's LSD, $P<0.05$). **c** The total stereotypy counts recorded following a systemic challenge injection of cocaine on day 17. Note that there were no differences in cocaine-induced behavioral hyperactivity in either NMDA group relative to saline. **d** Timecourse of stereotypy counts induced by cocaine in the 0.68 nmol NMDA and saline groups on day 17. The data are divided into 10-min blocks. In **a–d** the data are presented as mean (± SEM). For the experiments summarized in Figs. 2 and 3, there were seven to ten rats per group

drug treatment and the within-subjects factor was treatment day. This analysis revealed no significant main effects or interactions. The time course of the stereotypy counts from the vehicle and 30 nmol BayK 8644 groups on day 3 is summarized in Fig. 4b. These data were analyzed with a mixed factors ANOVA (repeated measure over time), which showed a significant main effect of time [$F(11,132)=25.57$, $P<0.0001$] and a significant drug×time interaction [$F(11,132)=4.53$, $P<0.0001$]. Pairwise comparisons (Fisher's LSD) revealed a significant difference between the BayK and vehicle groups at the 10-min time point. Following a 2-week withdrawal period, all rats were injected with 15 mg/kg cocaine. The total stereotypy counts recorded over the 2-h period post-cocaine are summarized in Fig. 4c. These data were analyzed with a one-way ANOVA, which did not reveal a significant main effect of drug treatment [$F(2,16)=2.78$, $P<0.092$]. However, subsequent pairwise comparisons (Fisher's LSD) revealed a significant difference between the vehicle and 30 nmol BayK 8644 groups. The time course of the vehicle and 30 nmol BayK 8644 data from day 17 are shown in Fig. 4d. A mixed factors ANOVA (repeated measures over time) revealed a significant main effect of time [$F(11,132)=8.35$, $P<0.0001$] and a significant drug×time interaction [$F(11,132)=2.07$, $P<0.027$]. Post hoc analyses using Fisher's LSD demonstrated a significant difference between the vehicle and 30 nmol BayK 8644 groups at the 10- and 20-min time points. The data obtained on day 17 indicate that pretreatment with BayK 8644 resulted in a significant augmentation in the stereotypy counts produced by a subsequent challenge injection of cocaine.

The distance traveled data summarized in Fig. 5 were analyzed using the same statistics as the stereotypy data summarized above. The analysis of the data shown in Fig. 5a revealed no significant main effects or interactions. Analysis of the time course data from day 3 (see Fig. 5b) revealed a significant main effect of time [$F(11,132)=24.98$, $P<0.0001$] and a significant drug×time interaction [$F(11,132)=4.25$, $P<0.0001$]. Subsequent pairwise compari-

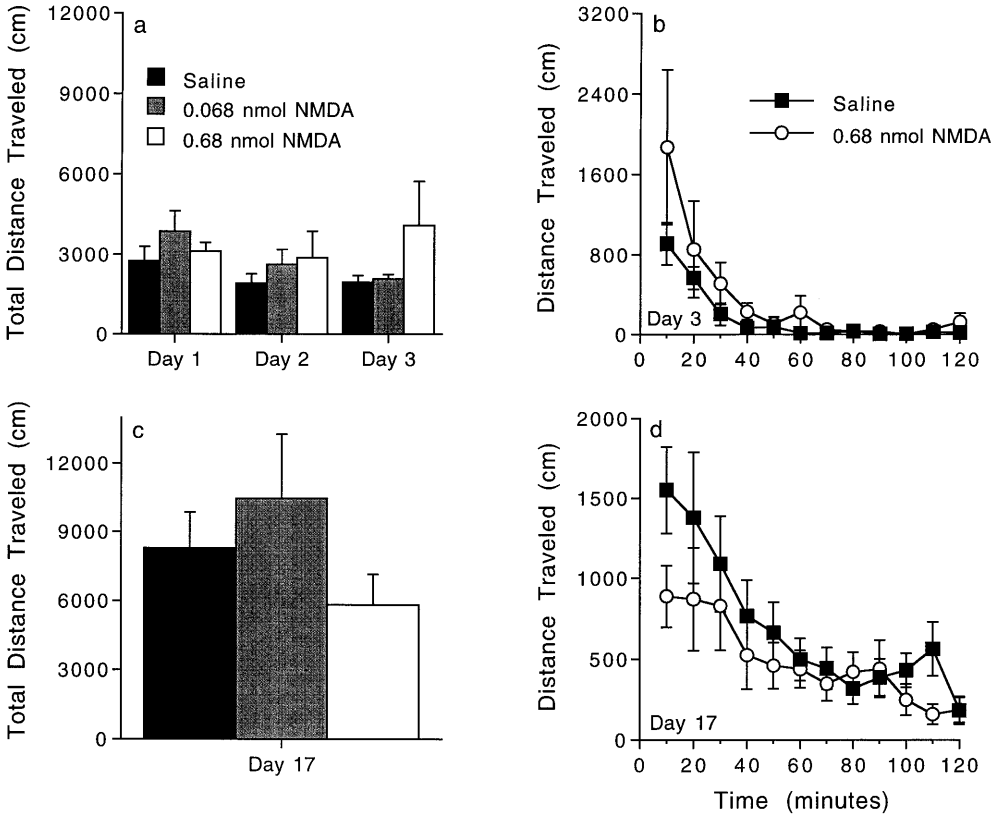

Fig. 3a–d Repeated intra-VTA microinjections of NMDA had no influence on the distance traveled (in cm) induced by a challenge injection of 15 mg/kg cocaine (i.p.). The experimental design was the same as described in Fig. 2. **a** Total distance traveled measured over the 120-min period on days 1–3 following intra-VTA microinjection of NMDA or saline. **b** Timecourse of the distance traveled after microinjection of saline or 0.68 nmol NMDA on day 3. The data are presented as the 120 min behavioral response (distance traveled) divided into 10-min blocks. **c** The total distance traveled recorded following a systemic injection of cocaine on day 17. **d** Timecourse of the distance traveled after a cocaine challenge in the 0.68 nmol NMDA and saline groups from day 17. The data are divided into 10-min blocks. In **a–d** the data are presented as mean (± SEM)

sons showed that the behavioral responses of the vehicle and BayK 8644 groups differed significantly at the 10- and 20-min time points. As shown in Fig. 5c, analysis of the total distance traveled following cocaine on day 17 showed only a marginally significant main effect [$F(2,16)=3.26$, $P<0.065$]. However, pairwise comparisons (using Fisher's LSD) revealed a significant difference between the vehicle and 30 nmol BayK 8644 groups. Analysis of the time course data from the vehicle and 30 nmol BayK 8644 groups on day 17 (see Fig. 5d) revealed a significant main effect of drug treatment [$F(1,132)=4.84$, $P<0.048$] and time [$F(11,132)=14.51$, $P<0.0001$] as well as a significant drug×time interaction [$F(11,132)=2.46$, $P<0.008$]. Post hoc analyses with Fisher's LSD showed a significant difference between the vehicle and BayK 8644 groups at the 10-, 20-, 30-, and 40-min time points. For the BayK 8644/DMSO experiments, there were five to nine rats per group.

Discussion

The results reported here indicate that repeated microinjections of the L-type calcium channel agonist BayK 8644 into the VTA mimicked the initiation of behavioral sensitization to cocaine. Thus, pretreatment with BayK 8644 resulted in an augmented behavioral response to an ensuing cocaine challenge injection. The present data also showed that repeated intra-VTA microinjections of NMDA had no influence on the subsequent behavioral response to cocaine.

NMDA receptors and behavioral sensitization

The NMDA receptor plays a central role in many of the models describing the neurophysiological alterations associated with the initiation of psychostimulant behavioral sensitization. One of these models proposes that enhanced glutamate release and NMDA receptor stimulation in the VTA resulting from repeated psychostimulant injections is a consequence of increased stimulation of D1 dopamine receptors on glutamatergic terminals in this nucleus (for review see Kalivas 1995). A wealth of data support this model, including the following: (1) repeated psychostimulant injections result in increases in both dopamine (Kalivas and Duffy 1993b) and glutamate (Kalivas and Duffy 1995, 1998; Xue et al. 1996; Wolf and Xue 1998, 1999) in the VTA, (2) behavioral sensitization is blocked by intra-VTA microinjections of D1 antagonists (Stewart and Vezina 1989; Vezina 1996) and sensitization is mimicked by intra-VTA microinjections of a D1 agonist (Pierce et al. 1996), and (3) the initiation of behavioral sensitization is impaired by NMDA receptor antagonists (Karler et al. 1989, 1990, 1994; Kalivas and Alesdatter 1993; Stewart and Druhan 1993; Wolf and Jeziorski 1993; Ida et al. 1995; Li and Wolf 1999; Li et al. 1999). It should be noted, however, that stimulation of presynaptic D1 receptors *decreases* glutamate transmission in the nucleus accumbens (Pennartz et al. 1992; Nicola et al. 1996). In addition, consistent with the present results, recent evidence indicates that repeated microinjections of NMDA into the VTA do not cross-

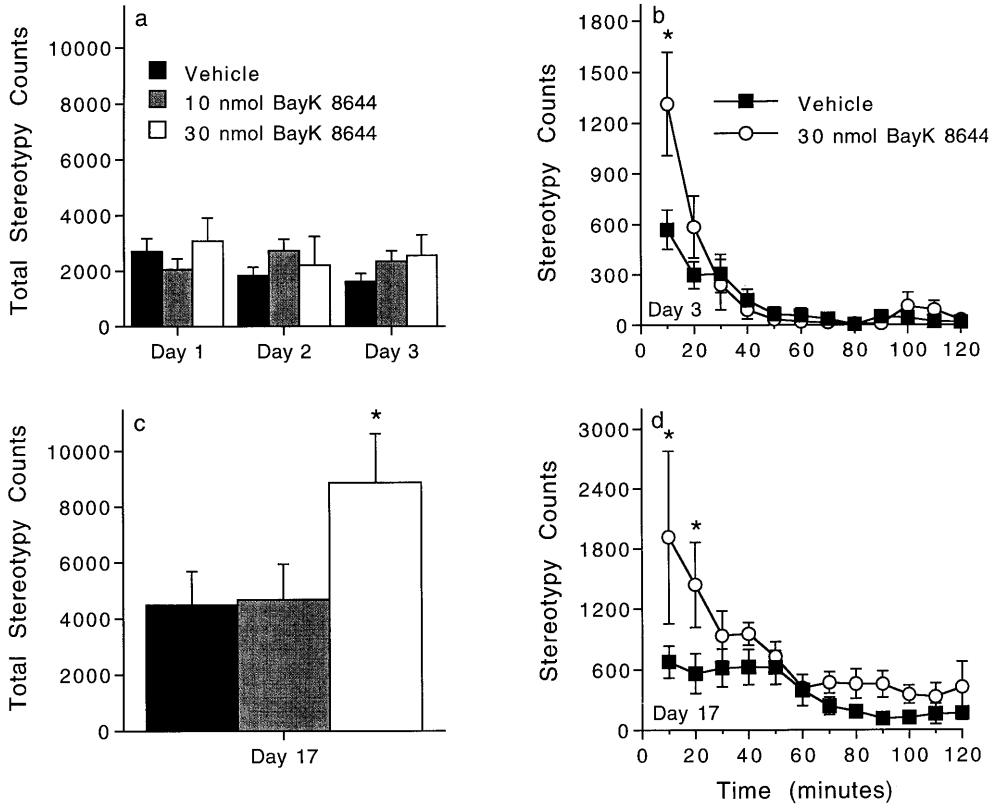

Fig. 4a–d Repeated microinjections of the L-type calcium channel agonist, BayK 8644, into the VTA enhanced the stereotypy counts produced by a subsequent challenge injection of cocaine. On days 1–3 the rats received bilateral microinjections of vehicle or BayK 8644 (10 or 30 nmol/0.5 µl) into the VTA. After a 2-week withdrawal period (i.e., on day 17) all rats received an injection of cocaine (15 mg/kg, i.p.) and stereotypy counts were monitored. **a** Total stereotypy counts induced by intra-VTA microinjection of either dose of BayK 8644 or vehicle. **b** Timecourse of the stereotypy counts recorded after microinjection of 30 nmol BayK 8644 or vehicle on day 3. The data are presented as the 120 min behavioral response (stereotypy counts) divided into 10-min blocks. Note that the stereotypy counts produced by 30 nmol BayK 8644 were greater than those of vehicle 10 min after microinjection. **c** The total stereotypy counts recorded following a systemic challenge injection of cocaine on day 17. Note that there was a significant increase in behavioral activity in the 30 nmol BayK 8644 group relative to the vehicle group. The *asterisk* represents a significant difference from vehicle (Fisher's LSD, $P<0.05$). **d** Timecourse of the stereotypy counts recorded after cocaine in the 30 nmol BayK 8644 and vehicle groups from day 17. The data are divided into 10-min blocks. Note that the stereotypy counts from the 30 nmol BayK 8644 group were greater than vehicle 10 and 20 min after the cocaine injection. The *asterisks* in **b** and **d** represent significant differences from vehicle at that time point (Fisher's LSD, $P<0.05$). In **a–d** the data are presented as mean (± SEM). There were five to nine rats per group in the experiments outlined in Figs. 4 and 5

sensitize with cocaine (Schenk and Partridge 1997), suggesting that NMDA receptor stimulation in the VTA may be necessary, but not sufficient, to promote sensitization to psychostimulants. It is possible, however, that there are substantial differences between the NMDA receptor stimulation produced by sensitization-induced glutamate release in the VTA and the intra-VTA microinjections of NMDA used here and by Schenk and Partridge (1997). For example, microinjections of NMDA into the VTA may result in rapid receptor desensitization (Krupp et al. 1998), which may not occur with the endogenous glutamate released in response to repeated psychostimulant injections. It also is possible that the NMDA treatment regimen used in the present study and by Schenk and Partridge (1997) may not have been sufficiently aggressive or the doses of NMDA were too low, although neither of these explanations seems likely. The 3-day treatment regimen used in the present experiment was modeled after studies in which sensitization to a psychostimulant challenge injection was observed following three microinjections of amphetamine (Perugini and Vezina 1994; Vezina 1996) or SKF-38393 (Pierce et al. 1996) into the VTA. In addition, similar to the present findings, Schenk and Partridge (1997) reported that higher doses of NMDA produced sensitization to the behavioral activating effects of NMDA itself, which may be due to an enhancement in dopamine release in the nucleus accumbens resulting from alterations in NMDA-induced burst firing of dopamine cells in the VTA (Suaud-Chagny et al. 1992; Karreman et al. 1996).

L-type calcium channels and behavioral sensitization

The finding that repeated treatment with an L-type calcium channel agonist cross-sensitizes with cocaine is consistent with results indicating that L-type calcium channel antagonists impair the initiation of behavioral sensitization

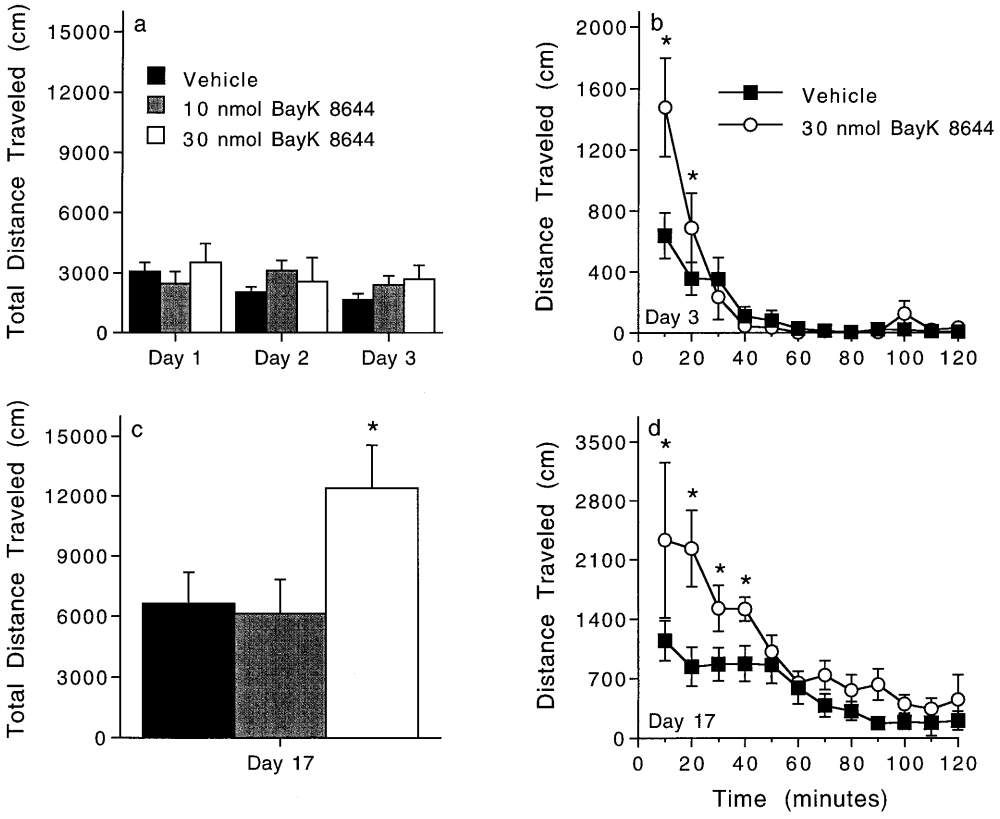

Fig. 5a–d Repeated microinjections of BayK 8644 into the VTA resulted in sensitization of the distance traveled (in cm) produced by a subsequent challenge injection of cocaine. The experimental design was the same as described in Fig. 4. **a** Total distance traveled measured over the 120-min recording period following microinjection of BayK 8644 or vehicle on days 1–3. **b** Timecourse of the distance traveled after microinjection in the 30 nmol BayK 8644 and vehicle groups on day 3. The data are presented as the 120 min behavioral response (distance traveled) divided into 10-min blocks. Note that the behavioral effect of 30 nmol BayK 8644 was greater than that of vehicle 10 and 20 min after microinjection. **c** The total distance traveled on day 17 following a systemic injection of cocaine. Note that there was a significant increase in the distance traveled produced by cocaine in the 30 nmol BayK 8644 group relative to the vehicle group. The *asterisk* represents a significant difference from vehicle (Fisher's LSD, $P<0.05$). **d** Timecourse of the distance traveled after a cocaine injection in the 30 nmol BayK 8644 and vehicle groups from day 17. The data are divided into 10-min blocks. Note that the enhanced behavioral effect in the 30 nmol BayK 8644 group as compared to the vehicle group persisted for the first 40 min following the cocaine injection. The *asterisks* in **b** and **d** represent significant differences from vehicle at that time point (Fisher's LSD, $P<0.05$). In **a–d** the data are presented as mean (± SEM)

to both cocaine and amphetamine (Karler et al. 1991b; Reimer and Martin-Iverson 1994). It seems likely that L-type calcium channel stimulation associated with behavioral sensitization results from glutamate-induced membrane depolarization. As reviewed above, there is enhanced glutamate release in the VTA during the initiation of behavioral sensitization (Kalivas and Duffy 1995, 1998; Xue et al. 1996; Wolf and Xue 1998, 1999). This would result in depolarization of dopamine cells in the VTA via stimulation of either NMDA or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate receptors, both of which have been linked to the initiation of psychostimulant sensitization (Karler et al. 1991a; Li et al. 1997, 1999; Jackson et al. 1998; Wolf 1998).

Activation of L-type calcium channels may be critical for the stimulation of second messenger systems, such as calmodulin and calmodulin-dependent kinases, that are thought to play a role in psychostimulant sensitization (Roberts-Lewis et al. 1986; Gnegy et al. 1991; Pierce and Kalivas 1997; Pierce et al. 1998; Kantor et al. 1999). Relative to other voltage-gated calcium channels, the L-type channel deactivates more slowly in response to membrane depolarization (Dunlap et al. 1995; Jones 1998). The more sustained increase in intracellular calcium produced by L-type channels may lead to prolonged activation and, eventually, sustained autophosphorylation of calcium-mediated second messengers such as calcium/calmodulin-dependent protein kinase II (CaM-KII). CaM-KII has been proposed as a candidate molecule for the long-term storage of information due to its ability to remain phosphorylated in the absence of calcium/calmodulin (Lisman 1994). This calcium-independent kinase activity is sustained by the multiple catalytic subunits of the CaM-KII holoenzyme, which rephosphorylate adjacent subunits that are inactivated by phosphatases and phosphorylate new subunits that are added during protein turnover (Miller and Kennedy 1986; Lisman 1994). Increases in intracellular CaM-KII also influence the activity of L-type calcium channels in that CaM-KII augments the frequency and length of opening of these calcium channels (Dzhura et al. 2000).

Previous studies suggest that an increase in CaM-KII activity plays an important role in the expression of behavioral sensitization (Pierce and Kalivas 1997; Pierce et al. 1998; Kantor et al. 1999). Thus, inhibition of CaM-KII activity in the nucleus accumbens impairs both the long-term expression of cocaine-induced behavioral sensitization and the enhanced dopamine release in this structure observed following repeated cocaine injections (Pierce and Kalivas 1997; Pierce et al. 1998), suggesting that kinases stimulated by calcium/calmodulin contribute to the long-term expression of behavioral sensitization. While the mechanisms underlying this increase in kinase activity are unclear, it is possible that sustained phosphorylation of an enzyme such as CaM-KII in mesoaccumbens dopaminergic neurons following repeated psychostimulant exposure is initiated by calcium influx through L-type calcium channels. This increase in CaM-KII activity may contribute to the increase in dopamine synthesis observed following repeated psychostimulant injections (Vrana et al. 1993; Masserano et al. 1996; Zhang and Angulo 1996). Consistent with this hypothesis, calcium influx through L-type calcium channels has been shown to increase catecholamine synthesis via the activation of CaM-KII (McCullough and Westfall 1996).

Conclusions

The findings reported here indicate that repeated activation of L-type calcium channels in the VTA result in neuroplasticity that is similar to the neuronal alterations produced by repeated injections of cocaine. That is, repeated injections of an L-type calcium channel agonist cross-sensitize with a subsequent cocaine injection. Interestingly, repeated microinjections of NMDA into the VTA did not influence the subsequent behavioral response to cocaine, suggesting that NMDA receptor stimulation alone may not be sufficient to promote the changes in dopamine neuronal function that are observed following repeated psychostimulant injections. It is possible that AMPA and NMDA glutamate receptor stimulation in the VTA during the initiation of behavioral sensitization is important only to the extent that it results in membrane depolarization and activation of L-type calcium channels. Activation of L-type calcium channels could, in turn, lead to the stimulation of second messengers that are associated with neuronal plasticity. CaM-KII is particularly suited for this role due to its ability to remain phosphorylated in the absence of calcium or calmodulin as well as the fact that CaM-KII augments L-type calcium currents through a positive feedback mechanism. Thus, a transient increase in intracellular calcium in response to repeated psychostimulant injections could result in persistent changes in the phosphorylation state of CaM-KII, which may contribute to the initiation and/or long-term maintenance of psychostimulant sensitization.

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