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The laterodorsal tegmentum contributes to behavioral sensitization to amphetamine

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Abstract

A critical event in the development of behavioral sensitization is a transient increase in excitatory drive to dopamine neurons of the ventral tegmental area (VTA). This is likely to be due, in part, to the ability of drugs of abuse to produce long-term potentiation, expressed as increased AMPA receptor transmission, at excitatory synapses onto VTA dopamine neurons. We investigated the role of the laterodorsal tegmentum (LDT) in behavioral sensitization because LDT neurons provide an important source of excitatory drive to VTA dopamine neurons, through mixed glutamate and cholinergic inputs. To test the role of the LDT in amphetamine sensitization, ibotenic acid or sham lesions of the LDT were performed 1 week before the first of 6 daily amphetamine injections. When challenged with amphetamine 13 days after the last injection, sham rats expressed sensitization of stereotypy and post-stereotypy locomotor hyperactivity, whereas the latter was attenuated by ibotenic acid lesions of the LDT. To determine whether plasticity occurs in the LDT during amphetamine sensitization, we used a previously developed microdialysis assay in which increased ability of AMPA to activate a pathway serves as a marker for long-term potentiation. Two days after discontinuing repeated saline or amphetamine injections, the responsiveness of LDT-VTA neurons to AMPA was determined by microinjecting AMPA (0.4nmol) into the LDT and measuring glutamate efflux in the ipsilateral VTA. Glutamate efflux was transiently increased in both groups but a delayed group difference was apparent with relatively higher glutamate efflux in amphetamine rats 30–60 min after AMPA injection. In parallel experiments, dopamine efflux in the NAc following intra-LDT AMPA declined in saline rats but remained relatively stable in amphetamine rats. Both results suggest relatively greater excitability of the LDT-VTA-NAc pathway after repeated amphetamine treatment. Our results provide the first evidence that neuronal plasticity in the LDT contributes to behavioral sensitization.

Keywords

AMPA receptors; dopamine; long-term potentiation; microdialysis; nucleus accumbens; ventral tegmental area

A critical event in the development of behavioral sensitization is transient activation of dopamine (DA) neurons in the ventral tegmental area (VTA) (Wolf, 1998). This is attributable in part to long term potentiation (LTP) at excitatory synapses in the VTA (White et al., 1995; Zhang et al., 1997; Ungless et al., 2001; Saal et al., 2003), resulting in enhanced

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excitability of VTA DA neurons projecting to the nucleus accumbens (NAc) (Giorgetti et al., 2001). It was initially believed that glutamate inputs involved in these events arose from the prefrontal cortex (PFC) (e.g., Wolf et al., 1995), but it was shown subsequently that PFC neurons do not make excitatory synapses on DA mesoaccumbens neurons (Carr and Sesack, 2000). The laterodorsal tegmentum (LDT) is another candidate for providing excitatory input to DA mesoaccumbens neurons. Neurochemical studies have shown that stimulation of the LDT activates VTA DA neurons via glutamatergic and cholinergic mechanisms, and thus increases DA release in the NAc (e.g., Forster and Blaha, 2000; Forster et al., 2002b). Omelchenko and Sesack (2005) demonstrated that many LDT neurons make excitatory synapses on DA mesoaccumbens neurons, although the LDT-VTA projection is very complex, involving glutamate, acetylcholine and GABA components that differentially innervate VTA neurons comprising the GABA and DA mesoaccumbens and mesoprefrontal systems.

The goal of the present study was to explore the role of the LDT in behavioral sensitization to amphetamine. First, we examined the ability of repeated amphetamine injections to produce behavioral sensitization in rats previously subjected to excitotoxic or sham lesions of the LDT. Second, we were interested in whether repeated amphetamine injections produce LTP-like events in the LDT. Many forms of LTP are expressed as enhancement of the AMPA receptor-mediated component of the postsynaptic response (Kauer et al., 1988). This is now understood to reflect increased insertion of AMPA receptors into synapses during LTP (Malinow and Malenka, 2002). Based on this, we developed a microdialysis assay to detect LTP-like events in the DA mesoaccumbens pathway by applying AMPA to the VTA and measuring DA efflux in the NAc. Intra-VTA AMPA produced greater NAc DA efflux in rats previously treated with amphetamine compared to saline rats, suggesting that repeated amphetamine produced LTP in the VTA (Giorgetti et al., 2001). In the present study, we used a similar microdialysis assay to determine if repeated amphetamine treatment alters the excitability of the LDT-VTA pathway by injecting AMPA into the LDT and measuring glutamate efflux in the VTA and DA efflux in the NAc.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague Dawley rats (Harlan, Indianapolis, IN, USA), weighing between 275–300 grams at the beginning of the experiment, were housed 2–3 per cage. Animals were maintained on a 12:12 light-dark cycle, with food and water available ad libitum. Animals were handled at least 3–4 days prior to experimental procedures. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–213), revised in 1996, and were approved by the Institutional Animal Care and Use Committee of the Rosalind Franklin University of Medicine and Science.

Lesion studies

Rats were anesthetized with Isoflurane (AErrane; Baxter, Deerfield, IL) and placed in a stereotaxic frame. A 1 μ l Hamilton syringe was positioned in the LDT (L $\pm$ 2.5, P $\pm$ 8.8, V $\pm$ 6.0, at an angle of 25°) (Paxinos & Watson, 1997). Ibotenic acid (0.3 μ l of 1mg/0.1ml; Sigma-Aldrich) was delivered bilaterally to create LDT lesions. Sham rats received 0.3 μ l of PBS (pH 7.4). Syringes remained in place for 5 min after the injection. This lesion protocol is adapted from Forster et al. (2002a). Lesions were evaluated by NDAPH diaphorase staining as described previously (Vincent and Kimura, 1992; Inglis et al., 1993) and by Nissl staining.

Behavioral studies of amphetamine sensitization

Seven days after surgery (day 1), rats were placed in photobeam cages. After 30 min, all rats (sham and lesioned) were injected (s.c.) with 2.5 mg/kg amphetamine (dose expressed as free base). Activity was recorded using photobeam cages, as described below, for 4 hrs after amphetamine injection. For the next 5 days (days 2–6), all rats received daily injections of 2.5 mg/kg amphetamine (s.c.) in home cages. The test for sensitization was performed after 13 days of withdrawal (day 19). Rats received 2.5 mg/kg amphetamine in photobeam cages and behavior was measured as on day 1. This amphetamine regimen, developed by Segal and Kuczenski (1987) and used extensively by our laboratory, was selected because it enables excellent discrimination between sensitized and non-sensitized rats because sensitized rats respond to amphetamine with a characteristic biphasic pattern of ambulation counts never seen in naïve rats (see below). To control for effects of LDT lesions on basal activity, additional groups received ibotenic acid or sham lesions of the LDT, followed by 6 daily saline injections and a challenge injection of saline on day 19. Figures include data only from rats with lesions verified by NADPH-diaphorase staining (see Results).

Activity measurements for all experiments described above were performed using photobeam frames and the PAS monitoring system (San Diego Instruments, San Diego, CA). Each frame is 50×30 cm, with three photocells (separated by 10.5 cm) located lengthwise, 3.5 cm above the floor. A standard polyethylene rat cage was set inside each frame and one rat was placed in each cage. All tests were conducted between 10:00 am and 3:00pm. Rats were habituated to photobeam cages for 30 min prior to amphetamine or saline injection. Ambulation counts, which register upon breaking of consecutive photobeams, were used as a measure of horizontal locomotor activity. Sensitized rats display a biphasic pattern (see Fig. 2 for an example), with low ambulation counts during the first hour due to stereotyped behaviors and higher ambulation counts during the second and third hours of the test that reflect post-stereotypy locomotor hyperactivity (Segal and Kuczenski, 1987). To verify that low ambulation counts during the first hour of the test are due to stereotyped behavior, an observer recorded intervals scored positive for stereotyped nose-down in-place sniffing or continuous rearing using criteria described previously (Wolf et al., 1995).

Microdialysis studies

Rats received daily injections of 5.0 mg/kg amphetamine sulfate (i.p.) or saline for 5 days in home cages. Experiments were performed on the second day after discontinuing injections (day 7). This amphetamine regimen differs from the regimen used for behavioral studies, which employs a dose of 2.5 mg/kg amphetamine free base (equivalent to 3.4 mg/kg amphetamine sulfate) and is superior for distinguishing sensitization of stereotypy and post-stereotypy locomotor hyperactivity (see prior section). The 5 mg/kg amphetamine sulfate regimen also produces robust sensitization (Wolf and Jeziorski, 1993). The slightly higher amphetamine dose was originally selected to maximize neurochemical changes and is used here to enable comparison of the present microdialysis results to our prior studies on VTA neurochemistry, all of which used the 5 mg/kg regimen (e.g., Xue et al., 1996; 1998; Giorgetti et al., 2001; 2002). On the day before the microdialysis experiments, rats were anesthetized with Isoflurane (AErrane; Baxter, Deerfield, IL) and placed in a stereotaxic frame. A microdialysis probe guide (BAS, West Lafayette, IN) was implanted unilaterally in the VTA (L +0.5, P –5.8, V –9.0) or the NAc (L +1.0, P +1.7, V –6.0) while a microinjection cannula guide (Small Parts, Miami Lakes, FL, USA) was implanted in the ipsilateral LDT (L +0.8, P –8.8, V –7.0) (Paxinos and Watson, 1997). Placements were verified in Nissl stained sections after completion of the experiment. Rats were placed in polyethylene cages within photobeam frames (see prior section), and tethered to liquid swivels (Instech Laboratories, Plymouth Meeting, PA) that were connected to CMA/100 microinjection pumps (CMA, Stockholm, Sweden). The next morning, microdialysis probes (2 mm active surface; BAS, West Lafayette, IN) were inserted into guides

and perfused with artificial CSF [NaCl 145 mM, KCl 2.7 mM, MgCl₂ 1 mM, CaCl₂ 1.2 mM, and Na₂HPO₄ 2 mM (pH 7.4)] at a rate of 1.5 µl/min for three hours. Six 10 min (15 µl) baseline fractions were collected during the last hour of this period. Then, injection cannulae were inserted into the guides and 0.4 nmol AMPA was injected ipsilaterally into the LDT (0.3 µl/min for 1 min). Injection cannulae remained in place for 5 min after the injection. At least 70 min of additional post-AMPA fractions (10 min each) were collected. Dialysates were stored at -80° for HPLC analysis. Locomotor activity during microdialysis experiments was monitored using the photobeam system described in the previous section. Total photobeam interruptions were measured for 10 min intervals throughout the microdialysis experiment. For some experimental groups, n values for microdialysis data are not equivalent to n values for activity data, due to technical problems with either activity measurements or microdialysis for particular rats.

HPLC Analysis

Glutamate levels in dialysates were analyzed by gradient HPLC with fluorescence detection (Shimadzu Scientific Instruments, Columbia MD) while DA levels were analyzed by isocratic HPLC with electrochemical detection (ESA, Chelmsford, MA, USA). Methods have been described previously (e.g., Giorgetti et al., 2001).

Statistical analysis

Lesion studies: Within sham or lesioned groups, ambulation counts on days 1 and 19 were compared using within-subjects two-way repeated measures ANOVA. Subsequently, within-subjects repeated measures ANOVAs were performed on both the stereotypy (10–60 min) and the post stereotypy locomotor hyperactivity (70–240 min) portions of the data. Microdialysis studies: Two-way repeated measures ANOVA was used to compare saline and amphetamine group, while one-way ANOVA was used to analyze potential changes over time within a group. These analyses were conducted using SPSS 12.0.

RESULTS

Ibotenic acid lesions of the LDT attenuate sensitization of post-stereotypy locomotor hyperactivity

To test the role of the LDT in behavioral sensitization to amphetamine, rats received sham or ibotenic acid lesions of the LDT prior to amphetamine treatment. After completion of experiments, lesions were verified by staining for NADPH diaphorase, a nitric oxide synthase that is an effective histochemical marker for staining cholinergic neurons in the mesopontine tegmentum (Vincent and Kimura, 1992; Semba and Fibiger, 1992; Inglis et al., 1993) and for analyzing lesions of cholinergic neurons in this region (Alderson et al., 2004). Fig. 1 shows NADPH diaphorase staining in the LDT of representative sham and lesioned rats (a and b, respectively), as well as a summary of the extent of most lesions based on NADPH diaphorase and Nissl stained sections (c). Only rats with extensive bilateral cell loss in the LDT and cell sparing in the adjacent pedunculopontine tegmentum (PPT) were included in our analyses. Three rats from each group were excluded based on these criteria. We did not specifically examine the loss of glutamate or GABA neurons in the LDT of lesioned rats. However, the complete or near-complete loss of NADPH-positive cells in both hemispheres of all subjects included in the study, combined with results of Nissl staining, indicates that most of the LDT was destroyed.

Seven days after the lesion, we began a regimen of 6 daily injections of 2.5 mg/kg amphetamine (s.c., dose expressed as free base). The first amphetamine injection was administered in photobeam cages so the behavioral response could be recorded, while injections on days 2–6 occurred in home cages. All rats were challenged with amphetamine in photobeam cages 13

days after the last injection (day 19), because our laboratory has typically observed robust sensitization after a week or two of withdrawal. The development of sensitization was assessed, within each group, by comparing ambulation counts (a measure of horizontal locomotion) on day 1 and day 19. In prior studies using this regimen, we have found that sensitized rats exhibit significantly lower ambulation counts than naïve rats during the first hour of the test due to sensitization of stereotyped behavior, but significantly higher ambulation counts during the second and third hours of the test due to sensitization of post-stereotypy locomotor hyperactivity (Wolf et al., 1995; Li and Wolf, 1997; Li et al., 1997; Li and Wolf, 1999). This biphasic pattern was first characterized by Segal and Kuczenski (1987).

In the present study, comparison of ambulation counts on days 1 and 19 for the sham group revealed significantly lower counts on day 19 during the stereotypy phase (0–60 min) and significantly higher counts during the period of post-stereotypy locomotor hyperactivity phase (70–240 min) (Fig. 2). Thus, sham rats exhibited the biphasic pattern of ambulation counts characteristic of behavioral sensitization to amphetamine. In contrast, lesioned rats did not exhibit a significant difference between days 1 and 19 in ambulation counts during the period of post-stereotypy locomotor activity, indicating that this aspect of sensitization was attenuated by the lesion (Fig. 3). Lesioned rats did exhibit sensitization of stereotypy, indicated by significantly lower ambulation counts during the first hour of the test on day 19 compared to day 1 (Fig. 3). This was confirmed by an observer who recorded the proportion of intervals during the first hour of the test in which rats exhibited two stereotyped behaviors never seen in naïve rats (nose-down in place sniffing and continuous rearing), using methods described in detail elsewhere (Wolf et al., 1995). Lesioned rats exhibited a significantly greater proportion of intervals scored positive for these behaviors on day 19 versus day 1, and the proportion of intervals scored positive on day 19 did not differ between sham and lesioned rats (z-test, $P < 0.05$). These results, combined with ambulation counts presented in Fig. 3, indicate that ibotenic acid lesions of the LDT do not interfere with sensitization of stereotypy but do attenuate sensitization of post-stereotypy locomotor hyperactivity.

Two control groups were included in the experiment. One control group received ibotenic acid lesions of the LDT followed by 6 daily saline injections, and was challenged on day 19 with saline. A second group received sham lesions of the LDT followed by 6 daily saline injections, and was challenged with saline on day 19. Consistent with prior results in intact rats (Forster et al. 2002a), a minimal behavioral response to saline injection was observed on both test days in both groups (data not shown). Collectively, these data indicate that LDT lesions, sham lesions and saline injections did not significantly alter basal locomotor activity.

Repeated amphetamine treatment alters the responsiveness of LDT neurons to AMPA

Having established that the LDT contributes to amphetamine sensitization, we were interested in whether repeated amphetamine produces LTP-like plasticity in the LDT similar to that detected in the VTA. We used a microdialysis assay in which increased responsiveness to AMPA, suggestive of LTP, is detected by injecting AMPA into a cell body region and measuring transmitter output in the nerve terminal region (Giorgetti et al., 2001; see Introduction). In this case, we examined the excitability of the LDT-VTA-NAc pathway by injecting AMPA into the LDT and measuring glutamate efflux in the VTA and DA efflux in the NAc. We compared rats treated with 5 daily injections of saline or 5 mg/kg amphetamine. This regimen produces robust behavioral sensitization and has been consistently used in all of our prior microdialysis studies, although the 2.5 mg/kg free base regimen described above is preferred for monitoring sensitization of stereotypy and post-stereotypy locomotor hyperactivity (see Methods for more discussion). All rats were tested on the second day after discontinuing injections (day 7).

First, we examined the effect of microinjection of AMPA (0.4nmol) ipsilaterally into the LDT on glutamate efflux in the ipsilateral VTA. This concentration of AMPA was selected because it was effective in prior studies at driving AMPA-receptive cell populations (Cornish et al., 1999; Cornish and Kalivas, 2000). As predicted based on electrical stimulation experiments showing that the LDT sends glutamate projections to the VTA (Forster and Blaha, 2000; Foster et al., 2002b), intra-LDT injection of AMPA produced an immediate and transient increase in glutamate efflux in the VTA of both saline and amphetamine treated rats (fraction 4 in Fig. 4). However, in subsequent portions of the experiment, glutamate levels in saline rats remained around baseline levels whereas levels in amphetamine rats were significantly higher (see legend to Figure 4). These results indicate that the responsiveness of LDT neurons to the excitatory effect of AMPA was altered by repeated amphetamine treatment.

If this has functional consequences for activation of DA mesoaccumbens neurons, one might predict that intra-LDT AMPA would produce a more robust activation of DA efflux in the NAc of amphetamine rats compared to saline rats. To test this, AMPA was microinjected ipsilaterally into the LDT as described above and DA efflux was measured using a microdialysis probe in the ipsilateral NAc. To our surprise, saline rats showed a decline in DA levels following intra-LDT injection of AMPA. In contrast, DA levels in amphetamine rats remained relatively stable during the hour following AMPA injection and were therefore significantly higher than those measured in saline rats during this period. Like the glutamate data, this may suggest that there is relatively more excitatory tone in the VTA of amphetamine rats than saline rats during this period (Fig. 5).

Locomotor activity data collected during the microdialysis experiments shown in Figs. 4 and 5 are presented in Figs. 6 and 7, respectively. In each case, the figures show the time course of locomotor activity (Figs. 6a and 7a) and also a comparison of activity counts summed over the 30 min preceding AMPA injection versus the 30 min after AMPA injection (Figs. 6b and 7b). We focused on the first 30 min after AMPA injection because behavioral effects of AMPA microinjection have a rapid onset (Cornish et al. 1999, Cornish and Kalivas 2000). Interestingly, different behavioral responses to intra-LDT injection of AMPA were observed depending on whether the microdialysis probe was located in the VTA (Fig. 6) or the NAc (Fig. 7). With the probe in the VTA, intra-LDT AMPA produced a similar level of locomotor activation in saline and amphetamine groups (Fig. 6). In contrast, with the probe in the NAc, intra-LDT AMPA produced significantly greater locomotor activation in repeated amphetamine rats (Fig. 7). This difference is most evident when comparing summed activity counts during the 30 min before and after AMPA injection (Figs. 6b and 7b). Greater locomotor activation in amphetamine rats with NAc probes (Fig. 7) is consistent with relatively greater DA efflux in the NAc in the amphetamine rats, compared to saline rats, during the same experiment (see Fig. 5).

DISCUSSION

Role of the LDT in circuitry related to behavioral sensitization

Both excitatory and inhibitory inputs are important in regulating the activity of midbrain DA neurons (Overton and Clark, 1997; Diana and Tepper, 2002). The mesopontine tegmentum, which is composed of the rostral PPT and the more caudal LDT, is an important source of both excitatory (glutamate and cholinergic) and inhibitory (GABA) projections to the midbrain (Hallanger and Wainer, 1988; Oakman et al., 1995; Chahara et al., 1995). The PPT preferentially innervates the substantia nigra pars compacta and influences striatal DA release, while the LDT preferentially innervates the VTA and influences NAc DA release (Blaha and Winn, 1993; Blaha et al., 1996; Forster and Blaha, 2000; Miller et al., 2002; Forster et al., 2002a,b; Forster and Blaha, 2003). These projections contribute to reward-related behaviors (Winn et al., 1997; Inglis et al., 2000; Alderson et al., 2004).

There is good evidence that LTP at excitatory synapses onto DA mesoaccumbens neurons in the VTA is a critical step in the development of behavioral sensitization (White et al., 1995; Zhang et al., 1997; Ungless et al., 2001; Saal et al., 2003), but the origin of the glutamate projections involved in this LTP is not known. Recently, Omelchenko and Sesack (2004) showed that about 50% of the pathway from the LDT to the VTA forms asymmetric synapses, presumably excitatory, that target DA mesoaccumbens neurons but not GABA mesoaccumbens neurons. Furthermore, they showed that the LDT sends excitatory projections to DA and GABA mesoprefrontal neurons of the VTA, as well as inhibitory inputs that preferentially innervate DA and GABA mesoprefrontal neurons and GABA but not DA mesoaccumbens neurons. Taken together with earlier work (Carr and Sesack, 2000), it appears that the LDT is most important in providing excitatory drive to DA mesoaccumbens neurons, while both LDT and PFC provide excitatory drive to DA mesoprefrontal cells. Thus, the LDT, rather than the PFC, is currently the best candidate for providing the glutamate inputs to DA mesoaccumbens neurons that are important for initiating behavioral sensitization. The present study examined the role of the LDT in behavioral sensitization from two perspectives. First, we performed excitotoxic lesions of the LDT to determine if it contributed to behavioral sensitization to amphetamine. Second, we investigated the possibility that LTP-like events associated with amphetamine sensitization occur in the LDT as well as in the VTA.

LDT lesions interfere with behavioral sensitization to amphetamine

Moderate doses of amphetamine, such as the dose used in this study, produce an initial stereotypy phase followed by a period of post-stereotypy locomotor hyperactivity. Both phases sensitize in response to repeated injections of amphetamine (Segal and Kuczenski, 1987), but sensitization of stereotypy and post-stereotypy locomotor hyperactivity are dissociable both genetically and with respect to the timing of their development and recovery (Leith and Kuczenski, 1982). In prior studies, we have found that lesions of prefrontal cortex or basolateral amygdala also produce dissociation of these components of sensitization (Wolf et al., 1995). The present study revealed another example of dissociation, as ibotenic acid lesions of the LDT did not alter sensitization of stereotypy, but attenuated sensitization of post-stereotypy locomotor hyperactivity. This resembles a “mild” version of the deficit observed after ibotenic acid lesions of the prefrontal cortex, which completely prevent the development of sensitization of post-stereotypy locomotor hyperactivity (Wolf et al., 1995). The prefrontal cortex projects to the mesopontine tegmentum (which in turn projects to the midbrain; see above), and it has been proposed that this indirect route to the midbrain enables the prefrontal cortex to exert excitatory effects on midbrain DA neurons (Lokwan et al., 1999). Based on the fact that we observed selective effects on post-stereotypy locomotor hyperactivity following lesions of both the prefrontal cortex (Wolf et al., 1995) and the LDT (present study), it seems possible that a PFC-LDT-VTA pathway contributes to the development of sensitization of post-stereotypy locomotor hyperactivity in response to repeated amphetamine administration. However, other pathways must also be important, since LDT lesions did not completely block sensitization of post-stereotypy locomotor hyperactivity.

An earlier study reported that ibotenic acid lesions of the LDT attenuated sensitization of stereotypy but not locomotor activity in response to repeated amphetamine injections (Laviolette et al., 2000). The design was very different from the present study. In particular, locomotor activity was monitored for only 60 min after amphetamine injection. This is not long enough to detect the post-stereotypy hyperactivity measured in the present experiments. In a study of the effect of LDT lesions on the acute response to amphetamine, Forster et al. (2002a) found that lesioned rats exhibit more intense stereotyped behaviors and higher NAc DA levels in response to d-amphetamine than sham rats (Forster et al., 2002).

The effects of excitotoxic lesions of the PPT have been more extensively studied. The results implicate the PPT in learning related to amphetamine-induced reinforcement (Olmstead and Franklin, 1994; Alderson et al., 2004) and stimulus-reward learning in general (e.g., Inglis et al., 2000). With respect to its role in motor activation produced by acute amphetamine, PPT lesions increase the intensity of stereotyped behavior but do not alter horizontal activity (Inglis et al., 1994; Olmstead and Franklin, 1994; Allen and Winn, 1995). Enhanced stereotypy is accompanied by an increased striatal DA response to amphetamine (Miller et al., 2002). Only one study has examined the effect of ibotenic acid lesions of the PPT on the development of behavioral sensitization to amphetamine (Alderson et al., 2003). Sensitization of horizontal locomotor activity over the course of 7 injections of 1.5 mg/kg amphetamine was attenuated in the lesioned group, while stereotypy scores over the test sessions did not differ between sham and lesioned groups. This is perhaps surprising in light of the relationship between the PPT and the nigrostriatal DA system (see above), but it suggests that the PPT and the LDT may play complementary roles in the sensitization of locomotor hyperactivity. This may be one reason that lesions of the LDT produce only a modest attenuation of sensitization.

Repeated amphetamine alters the responsiveness of LDT neurons to AMPA

The second goal of this study was to determine if LDT neurons in amphetamine sensitized rats show enhanced responsiveness to AMPA, indicative of LTP. This was studied with a microdialysis assay previously used to demonstrate an LTP-like increase in responsiveness to AMPA in the VTA of amphetamine-sensitized rats (Giorgetti et al., 2001).

Two parallel microdialysis experiments were conducted. The first set of experiments was designed to assess the effect of repeated amphetamine administration on the responsiveness to AMPA of LDT neurons sending glutamate projections to the VTA. AMPA was microinjected into the LDT and glutamate efflux was measured in the ipsilateral VTA. The second set of experiments was designed to determine if amphetamine-induced changes in the LDT-VTA pathway influenced the output of VTA DA neurons projecting to the NAc. In these experiments, AMPA was injected into the LDT and DA was measured in the ipsilateral NAc. We compared rats treated with saline or amphetamine for 5 days. Microdialysis was conducted 2 days after the last injection, because sensitization-related adaptations in the VTA are transient and thus detected only during the first several days after discontinuing drug administration (e.g., Zhang et al., 1997).

Prior studies have shown that electrical stimulation of the LDT increased the activity of mesoaccumbens DA neurons through a mechanism requiring activation of glutamate and cholinergic receptors in the VTA (Forster and Blaha, 2000; Forster et al., 2002b). Based on this, we predicted that intra-LDT AMPA would increase glutamate efflux in the ipsilateral VTA and DA efflux in the ipsilateral NAc, at least in the saline control group. While we did observe a transient increase in VTA glutamate efflux in saline rats (Fig. 4), we did not observe a corresponding increase in DA levels in the NAc. In fact, DA levels in the NAc declined after injection of AMPA into the LDT (Fig. 5). The decline was small (~30%) and some of it may be attributable to the gradual decrease in DA levels sometimes observed over the course of microdialysis experiments. Another possibility is that intra-LDT AMPA preferentially activates GABA-containing projections from the LDT to VTA, inhibiting mesoaccumbens DA neurons. However, this is not consistent with evidence that inhibitory pathways from LDT to VTA terminate on GABA but not DA mesoaccumbens neurons (Omelchenko and Sesack, 2004).

Regardless of the explanation for declining DA levels in the saline group, the most important point is that a different response was seen in the amphetamine group. While these rats also showed a transient increase in glutamate efflux after intra-LDT AMPA, they exhibited relatively higher glutamate efflux 30–60 min after AMPA injection compared to saline controls

(fractions 7–9; Fig. 4). Similarly, while NAc DA efflux declined in saline rats after AMPA injection, it remained more stable in the amphetamine group (Fig. 5). Both effects suggest relatively greater excitability in the LDT of amphetamine rats (see below). In amphetamine rats, it is possible that relatively greater VTA glutamate efflux during fractions 7–9 underlies relatively greater NAc DA efflux during the same period. However, as glutamate efflux did not differ between groups immediately after AMPA injection, this cannot explain relatively greater NAc DA efflux in amphetamine rats during this earlier period (fractions 4–6).

Interestingly, injection of AMPA into the LDT produced greater behavioral activation in the amphetamine rats, compared to saline rats, in microdialysis experiments with the probe in the NAc (Fig. 7). The augmented behavioral response in the amphetamine group was confined to the first 30 min after AMPA injection. It could be related to relatively greater DA efflux during this same period in amphetamine rats relative to saline rats (Fig. 5). Surprisingly, amphetamine rats did not demonstrate an augmented behavioral response when the microdialysis probe was in the VTA (Fig. 6). We speculate that the augmented behavioral response to intra-LDT AMPA in the amphetamine group (apparent in Fig. 7) depends on the integrity of neurotransmission in the LDT-VTA-NAc pathway, which might be compromised by the presence of a microdialysis probe in the VTA [explaining absence of this response in Fig. 6, where the probe is in the VTA]. The VTA, which is very small relative to the NAc, is more likely to be functionally compromised by the presence of a microdialysis probe.

Taken together, these results suggest that repeated amphetamine administration enhances the response of LDT neurons to AMPA receptor activation, leading to a state favoring relatively greater activation of mesoaccumbens DA neurons. We speculate that this contributes to the induction of behavioral sensitization, based on evidence that induction requires increased excitatory drive to DA mesoaccumbens neurons (see above). Although our findings do not fit a simple model for LDT regulation of DA mesoaccumbens neurons, they provide the first evidence that drugs of abuse can produce plasticity in the LDT. Many additional studies are required to determine if the plasticity detected by our microdialysis assay is really attributable to LTP, to identify the subset of LDT neurons that exhibit this plasticity, and to fully characterize its consequences for subtypes of VTA projection neurons. However, in light of high variability evident in our graphs, despite our best efforts, it appears that our microdialysis assay is useful for identifying potential sites of drug-induced plasticity in an intact animal preparation, but does not have the sensitivity required for characterization of underlying mechanisms. Further characterization will require electrophysiological methods.

Conclusions

Previous studies showed that LTP at excitatory synapses onto DA mesoaccumbens neurons is important for behavioral sensitization, but the origin of the glutamate pathways involved in this LTP is not known. The LDT is a good candidate because it sends excitatory projections to DA mesoaccumbens neurons. We found that excitotoxic lesions of the LDT attenuate sensitization of post-stereotypy locomotor hyperactivity in response to repeated amphetamine injections, supporting a role for LDT projections in the induction process. In addition, the responsiveness of LDT neurons to AMPA is altered after repeated amphetamine administration in a manner suggesting a shift towards relatively greater excitability in the LDT. This effect, combined with a LTP-like effect in the VTA (above), would be expected to dramatically increase excitability in the LDT-VTA-NAc pathway. We speculate that both effects contribute to the development of behavioral sensitization, explaining the ability of LDT lesions to attenuate sensitization.

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Abbreviations

AMPA, α -amino-3-hydroxy-5-methylisoxazole-4-propionate; DA, dopamine; LDT, laterodorsal tegmentum; LTP, long-term potentiation; NAc, nucleus accumbens; PPT, pedunculopontine tegmentum; VTA, ventral tegmental area.

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Fig 1.

Lesions were verified by staining brain sections for NADPH diaphorase, which stains cholinergic neurons in the mesopontine tegmentum (see Results). **a.** Representative brain section from a sham-lesioned control rat. **b.** Representative brain section from a rat with an ibotenic acid lesion of the LDT. **c.** Schematic drawing summarizing the extent of the LDT lesions in rats included in our analysis. The central light gray region illustrates the primary area of damage in all lesioned rats as determined by NADPH diaphorase and Nissl staining. The adjacent darker region medial to the central region and the adjacent hatched region lateral to the central region represent additional areas affected in 25–50% of the rats. Atlas figure is –8.80mm from Bregma (modified from Paxinos and Watson, 1997). Brain sections (**a**, **b**) are at approximately the same level. Only rats with extensive bilateral cell loss in the LDT and cell sparing in the adjacent PPT were included in our analyses. PPT staining is not shown in these sections because the PPT is slightly rostral (between –7.0 and –8.0 mm from Bregma) to the LDT and therefore PPT and LDT staining are not optimal in the same sections.

Fig 2.

Rats with sham lesions of the LDT exhibit sensitization of stereotypy and post-stereotypy locomotor hyperactivity. Rats ($n=8$) received sham lesions of the LDT 7 days before the start of 6 daily injections of 2.5 mg/kg amphetamine (s.c.). Ambulation counts were recorded on day 1 and again after 13 days of withdrawal (day 19). Within-subjects two-way repeated measures ANOVA comparing days 1 and 19 indicated significant sensitization of stereotypy [10–60 min; $F(5,35) = 3.37$, $p < 0.05$] and post-stereotypy locomotor hyperactivity [70–240 min; $F(17,119) = 2.54$, $p < 0.05$].

Fig 3.

Ibotenic acid lesions of the LDT attenuate sensitization of post-stereotypy locomotor hyperactivity but do not alter sensitization of stereotypy. Rats ($n=7$) received ibotenic acid lesions of the LDT 7 days before the start of 6 daily injections of 2.5 mg/kg amphetamine (s.c.). Ambulation counts were recorded on day 1 and again after 13 days of withdrawal (day 19). Within-subjects two-way repeated measures ANOVA comparing days 1 and 19 indicated significant sensitization of stereotypy [10–60 min; $F(5,30) = 2.91$, $p < 0.05$] but not post-stereotypy locomotor hyperactivity [70–240 min; $F(17,102) = 1.81$, NS].

Fig 4.

Repeated amphetamine injections alter the responsiveness of the LDT-VTA pathway to AMPA, measured as glutamate efflux in the VTA. Rats received 5 daily injections of saline (n=8) or 5 mg/kg amphetamine (n=8) and were tested 2 days after the last injection. Microinjection of 0.4nmol AMPA into the LDT at the end of fraction 3 produced a transient increase in VTA glutamate efflux in both the saline and amphetamine groups [$F(2,21) = 4.76$, $p < 0.05$ and $F(2,21) = 1.96$, $p < 0.05$, respectively; one-way ANOVAs including last baseline and first 2 fractions immediately following AMPA]. However, amphetamine and saline rats differed in the overall temporal response to AMPA, indicated by a two-way repeated measures ANOVA over the hour following AMPA injection (fractions 3–9) [$F(1,14) = 5.94$, $p < 0.05$]. A significant difference between groups was also indicated by two-way repeated measures ANOVA on fractions 7–9 [$F(1,14) = 7.07$, $p < 0.05$] and fractions 7–10 [$F(1,14) = 6.48$, $p < 0.05$].

Fig 5.

Repeated amphetamine injections alter the responsiveness of the LDT-VTA-NAc pathway to AMPA, measured as DA efflux in the NAc. Rats received 5 daily injections of saline (n=6) or 5 mg/kg amphetamine (n=8) and were tested 2 days after the last injection. Microinjection of 0.4nmol AMPA into the LDT at the end of fraction 3 produced a different pattern of DA efflux in the NAc of amphetamine-treated rats compared to saline controls, indicated by a repeated measures ANOVA of the hour following drug administration (fractions 3–9) that revealed a significant difference between groups [$F(1,12) = 5.03$, $p < 0.05$].

Fig 6.

Behavioral data from the microdialysis experiment shown in Fig. 4. All rats received 5 daily injections of either saline or 5 mg/kg amphetamine. Two days after the last injection, AMPA was injected into the LDT and glutamate was measured in the VTA. With the microdialysis probe in the VTA, intra-LDT AMPA produced similar behavioral activation in repeated saline and amphetamine rats. **a.** Activity during the experiment measured as total photobeam interruptions. Repeated measures ANOVA indicated no significant difference between the groups [$F(1,12) = 1.03$, $p = 0.33$]. **b.** Summation of photobeam interruptions during the 30 min prior to AMPA injection (fractions 1–3 in Fig. 6a; black bars) and the 30 min after AMPA

injection (fractions 4–6 in Fig. 6a; white bars). * $P < 0.05$ versus the respective pre-AMPA interval (Tukey test).

Fig 7.

Behavioral data from the microdialysis experiment shown in Fig. 5. All rats received 5 daily injections of either saline or 5 mg/kg amphetamine. Two days after the last injection, AMPA was injected into the LDT and DA was measured in the NAc. With the microdialysis probe in the NAc, intra-LDT AMPA produced greater behavioral activation in the amphetamine group.

a. Activity during the experiment measured as total photobeam interruptions. Repeated measures ANOVA indicated a significant difference between the groups [$F(1,15) = 5.69$, $P < 0.05$]. **b:** Summation of photobeam interruptions during the 30 min prior to AMPA injection (fractions 1–3 in Fig. 7a; black bars) and the 30 min after AMPA injection (fractions 4–6 in Fig. 7a; white bars). * $P < 0.05$ versus the respective pre-AMPA interval (Tukey test).