

High-Frequency Afferent Stimulation Induces Long-Term Potentiation of Field Potentials in the Ventral Tegmental Area

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Excitatory synapses on dopamine neurons in the VTA can undergo both long-term potentiation and depression. Additionally, drug-induced plasticity has been found at VTA synapses, and is proposed to play a role in reward-related learning and addiction by modifying dopamine cell firing. LTP at these synapses is difficult to generate experimentally in that it requires an undisturbed intracellular milieu and is often small in magnitude. Here, we demonstrate the induction of LTP as a property of evoked field potentials within the VTA. Excitatory field potentials were recorded extracellularly from VTA neurons in acute horizontal midbrain slices. Using extracellular and intracellular recording techniques, we found that evoked field potentials originate within the VTA itself and are largely composed of AMPA receptor-mediated EPSPs and action potentials triggered by activation of glutamatergic synapses on both dopamine and GABA neurons. High-frequency afferent stimulation (HFS) induced LTP of the field potential. The induction of this LTP was blocked by application of the NMDAR antagonist, d-APV, prior to HFS. As reported previously, glutamatergic synapses on GABA neurons did not express LTP while those on dopamine neurons did. We conclude that the potentiation of glutamatergic synapses on dopamine neurons is a major contributor to NMDA receptor-dependent LTP of the field potential. Field potential recordings may provide a convenient approach to explore the basic electrophysiological properties of VTA neurons and the development of addiction-related processes in this brain region.

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INTRODUCTION

The ventral tegmental area (VTA) is essential in processing the reinforcing properties of both natural rewards and addictive drugs (Hyman and Malenka, 2001). Sensitization to drugs of abuse has been proposed as a model underlying aspects of addiction (Hyman *et al*, 2006), and the VTA is the gateway for initiation of behavioral sensitization and can contribute to relapse (Kauer, 2004).

The VTA contains two types of neurons. Dopamine neurons make up the majority of the population while GABA neurons comprise up to 35% of the total VTA neurons (Swanson, 1982; Oades and Halliday, 1987; Grace and Onn, 1989; Johnson and North, 1992; Cameron *et al*, 1997). Both neuron types project to the nucleus accumbens and to the prefrontal cortex, although GABA neurons in the VTA also act locally to inhibit VTA dopamine neurons (Carr and Sesack, 2000a; Svingos *et al*, 2001). Excitatory afferents to the VTA include direct glutamatergic inputs

from prefrontal cortex, laterodorsal tegmental nucleus, lateral hypothalamus, and bed nucleus of the stria terminalis (Semba and Fibiger, 1992; Garzon *et al*, 1999; Carr and Sesack, 2000b; Georges and Aston-Jones, 2001). Stimulation of excitatory afferents in the VTA activates both postsynaptic AMPA receptors and NMDA receptors. GABAergic inputs from the nucleus accumbens and the ventral pallidum, as well as from local GABA neurons inhibit dopamine (and possibly non-dopamine) VTA neurons through GABA_A and GABA_B receptors (Johnson and North, 1992; Bonci and Williams, 1996; Fiorillo and Williams, 1998; Bonci and Malenka, 1999).

One possible mechanism underlying the experience- or drug-dependent reorganization of neural circuitry is a change in synaptic strength (ie synaptic plasticity; LTP, and LTD) (Hyman and Malenka, 2001). NMDA receptor-dependent LTP has been demonstrated at excitatory synapses on midbrain dopamine neurons (Bonci and Malenka, 1999; Overton *et al*, 1999; Mansvelder and McGehee, 2000; Liu *et al*, 2005). The demonstration of synaptic plasticity at excitatory synapses in mesolimbic dopaminergic structures, as well as the correlative evidence for changes in glutamate receptor expression and single unit responses to glutamate after *in vivo* exposure to drugs of abuse, support a role for synaptic plasticity at excitatory

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synapses in the development of addiction (Hyman and Malenka, 2001; Zhang *et al*, 1997; Carlezon and Nestler, 2002; Thomas and Malenka, 2003).

For decades, extracellular field potential recording has provided a technically easy and relatively uncomplicated method to assess synaptic transmission and plasticity in different regions of the brain. The greatest advantage of extracellular recordings when compared with intracellular recordings is that stable healthy extracellular recordings can be made over several hours. This is desirable for experiments analyzing synaptic plasticity where long-lasting stable changes will be observed. Moreover, field potential recordings provide the possibility of using older animals for drug addiction studies in the VTA. Technical limitations of the *in vitro* system have thus far limited nearly all studies to very young rats and mice; visualized patch clamp recordings become quite difficult after 3–4 weeks of age. This is particularly relevant as sensitization is less robust and less long lasting at these ages, indicating that examining the cellular physiology of older animals is likely to add information on the persistence of synaptic changes (Kauer, 2004). The great majority of studies on the physiology and pharmacology of the VTA have utilized intracellular microelectrode or patch clamp recordings. Recently, however, extracellular field potential recordings have been made from the VTA in horizontal brain slices (Zheng *et al*, 2006). Using 6-hydroxydopamine lesions, it was shown that VTA field potentials are largely generated by the postsynaptic responses of dopamine neurons. Here we have taken advantage of this recording method to study LTP induction at excitatory synapses. Before determining whether drugs of abuse elicit any form of plasticity that can be detected in field potentials, it is necessary to address the more straightforward question of whether LTP can be observed as a change in VTA field potentials. Here we report that field potential recordings from VTA slices exhibit LTP. Further studies will be directed toward an assessment of drug-related plasticity at VTA synapses using this electrophysiological technique.

MATERIALS AND METHODS

Preparation of Brain Slices

Basic methods were described previously (Jones *et al*, 2000). Sprague–Dawley rats (17–62 days old) were anesthetized using halothane and quickly decapitated. The brain was rapidly removed into ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 21.4 NaHCO₃, 2.5 KCl, 1.2 NaH₂PO₄, 2.4 CaCl₂, 1.2 MgSO₄, 11.1 dextrose, with either 75 mM sucrose or 0.4 ascorbic acid added. These solutions were saturated with 95% O₂ and 5% CO₂ (pH 7.4). Horizontal midbrain slices (400 μm thick) were prepared, stored for at least 1 h at room temperature in either sucrose-containing ACSF or at 35°C in ascorbic acid-containing ACSF, and transferred to an interface recording chamber where the slice rested on a strip of filter paper saturated with ACSF (28–32°C), with warmed, humidified CO₂/O₂ blown over the surface. To record field potentials from midbrain slices prepared from 55 to 62-day-old rats, we cut thinner slices (300 μm) to improve the visibility of VTA landmarks in this more myelinated tissue.

Electrophysiological Recording

Midbrain slices were continuously perfused with ACSF (without sucrose or ascorbic acid) at 28–32°C at a flow rate of 0.5 ml/min. In some experiments 100 μM picrotoxin was added to the ACSF to block GABA_A receptors (see below). Extracellular recordings were made from VTA neurons using broken back microelectrodes with a tip resistance of ~1 MΩ and filled with 2 M NaCl. Sharp microelectrodes for intracellular recordings had resistances between 100 and 200 MΩ and were filled with 2 M KCl or 2 M CsMeSO₄ in some experiments. For intracellular recordings, an *I_h* current was used to distinguish dopamine from GABA neurons. If the *I_h* current during a step from –50 to –100 mV was present, the neuron was considered a dopamine neuron; if not, the cell was classified as a GABA neuron. While the absence of *I_h* reliably predicts that a VTA neuron is non-dopaminergic, *I_h* is also present in a group of VTA neurons, which are not dopaminergic (Margolis *et al*, 2006).

Field potentials or EPSPs were stimulated at 0.1 Hz (100 μs pulse) using a bipolar stainless-steel stimulating electrode placed 200–500 μm rostral to the recording site in the VTA. LTP was induced by stimulating afferents at 100 Hz for 1 s; this train was repeated twice 20 s apart (high-frequency stimulation (HFS)). In intracellular recording experiments, LTP induction was attempted in both dopamine and GABA neurons using the same protocol (HFS) during direct depolarizing current injection (0.4–4 nA during each 1 s train). In all LTP experiments, picrotoxin (100 μM) was included in the ACSF to facilitate NMDA receptor-dependent LTP.

Analysis

Results are expressed as mean ± SEM. Significant changes between experimental conditions were determined using a Student's *t*-test with significance level of *p* < 0.05. Levels of LTP are reported as averaged EPSP or field potential amplitudes for a 5 min time period just before the LTP induction compared with averaged EPSP or field potential amplitudes during the 10 min period from 30 to 40 min after HFS.

Drug Application

The following drugs were used in this study: the GABA_A receptor antagonist, picrotoxin (100 μM); the GABA_B agonist, baclofen (30 μM); the AMPA receptor antagonist, DNQX (10 μM); the NMDA receptor antagonist, D-APV (50 μM); and the sodium channel blocker, tetrodotoxin (TTX, 1 μM). All drugs were added directly to the ACSF perfusing the slice chamber at known concentrations. D-APV was added for at least 15 min before HFS. Drug effects were assessed by averaging field potential amplitudes for 5 min during the peak response and comparing this value with 5 min of averaged data before drug application. In experiments reported in Figure 1, DNQX was focally applied through a puffer pipette positioned close to the recording electrode and above the slice. The solution contained fast green so that the pressure-ejected solution clearly marked the slice. Only experiments in which the drug remained

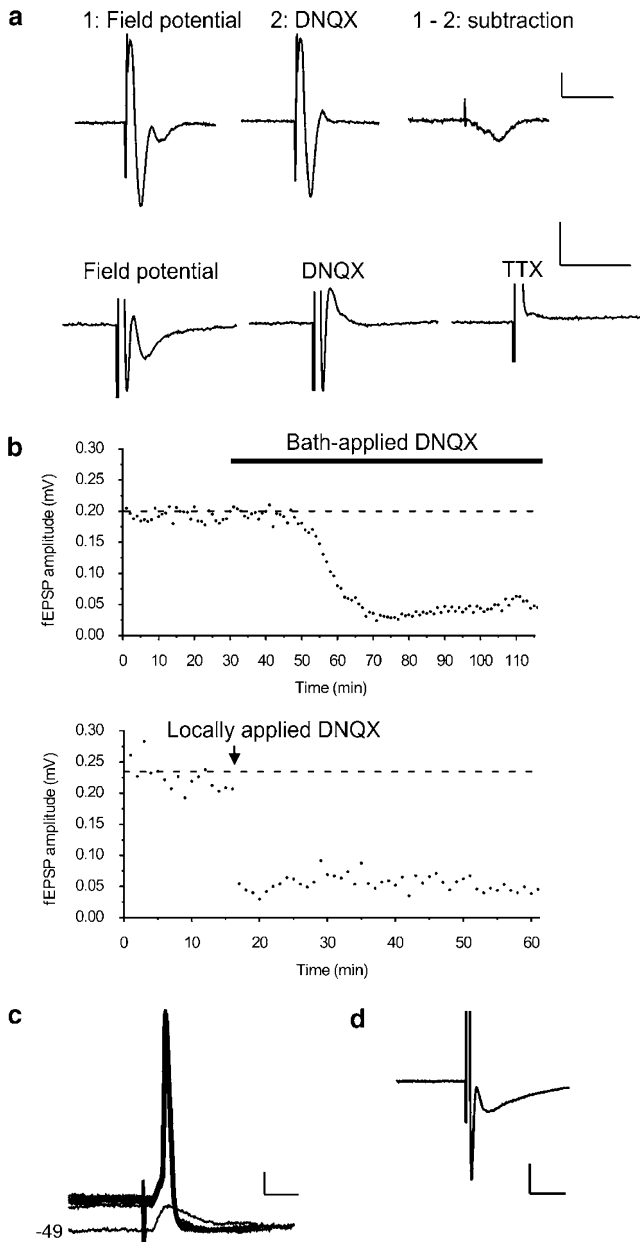


Figure 1 VTA field potentials are mediated by AMPAR-mediated EPSPs and postsynaptic action potentials. (a) Extracellularly recorded field potentials obtained from a VTA slice. Upper panel: Local application of 1 mM DNQX restricted within the VTA abolished the late synaptic component of the field potential (sample traces in first row). Digital subtraction of the field potential with and without DNQX reveals the AMPAR-mediated component of the field potential. Lower panel: in a second slice, bath application of 10 μ M DNQX also abolished the late synaptic component of field potentials; application of the Na⁺ channel blocker, TTX (1 μ M), completely eliminated the fast initial fiber volley component of field potentials (sample traces in second row). For each example, 10 consecutive potentials are averaged. Calibration: 0.25 mV, 10 ms. (b) Bath application of 10 μ M DNQX (top panel) or local application of 1 mM DNQX (bottom panel) blocked the synaptic component of the field potential. (c) Intracellular recordings from VTA neurons revealed that these neurons had resting potentials near threshold, and that synaptic stimulation often elicited an action potential (10 superimposed sweeps of evoked responses from one individual neuron, using a stimulus strength comparable to those used in field potential experiments). Calibration: 5 mV, 10 ms. (d) Extracellularly recorded field potential from the VTA in a brain slice from a 60-day-old rat. Ten consecutive potentials are averaged. Calibration: 0.25 mV, 10 ms.

within the boundaries of the VTA were reported. Salts and all other drugs were obtained from Sigma-Research Biochemicals International.

RESULTS

Characterization of Evoked Field Potentials in the VTA

Extracellular field potentials were both recorded and reliably evoked by electrical stimulation within the VTA (see also Zheng *et al*, 2006). We used identifiable structures (eg the medial terminal nucleus of the accessory optic tract (MT) and substantia nigra) as landmarks in these slices to position the electrodes inside the VTA, generally medial and slightly caudal to the MT (Paxinos and Watson, 1986). We also recorded field potentials using a submerged chamber and found that field potentials can be recorded in this chamber type as well. A major advantage of the interface chamber is that fluid shunting is minimized, which increases the size of the recorded extracellular field potential. In the interface chamber, these potentials in the VTA can be nearly a millivolt in amplitude, whereas in the submerged chamber, they are usually much smaller (<200 μ V).

The evoked local field potential has two components: the initial fast component (the action potential or fiber volley component) and the later synaptic component, which we will refer to as the field potential (Figure 1a). The synaptic component of the field potential is completely eliminated by bath application of the AMPAR antagonist, DNQX (10 μ M, Figure 1a and b). Since field potentials can be recorded at some distance from current sources, we also applied DNQX locally around the recording site. We found that even when DNQX application was restricted to the VTA, the field potential was entirely blocked ($n = 3$; Figure 1a and b). These data strongly suggest that the field potential depends upon activation of AMPA receptors within the VTA. The fast component was unaffected by DNQX application, but was abolished by bath application of the sodium channel blocker, tetrodotoxin (TTX, 1 μ M) (Figure 1a). This finding suggests that this component represents the fiber volley generated by antidromic and orthodromic action potentials originating at the stimulation site.

The synaptic component of the field potential was largely blocked by DNQX. This may have resulted from the combination of two effects—block of both the AMPAR-mediated EPSP and EPSP-triggered action potentials. Intracellular recordings showed that under our recording/stimulating conditions, many individual neurons fire action potentials in response to the stimulus (Figure 1c). Thus, the field potential we record most likely represents combined EPSPs and EPSP-driven action potentials.

To determine whether it is possible to record field potentials from older animals, we next tried recording from midbrain slices prepared from 55 to 62-day-old rats. We were able to stimulate extracellularly recorded field potentials similar to those seen in slices from younger animals, and that ranged from 100 to 500 μ V (Figure 1d, $n = 4$). Picrotoxin (100 μ M) reliably increased the field potential amplitude ($n = 3$). Slices from these animals are far less translucent due to increased myelination, and we

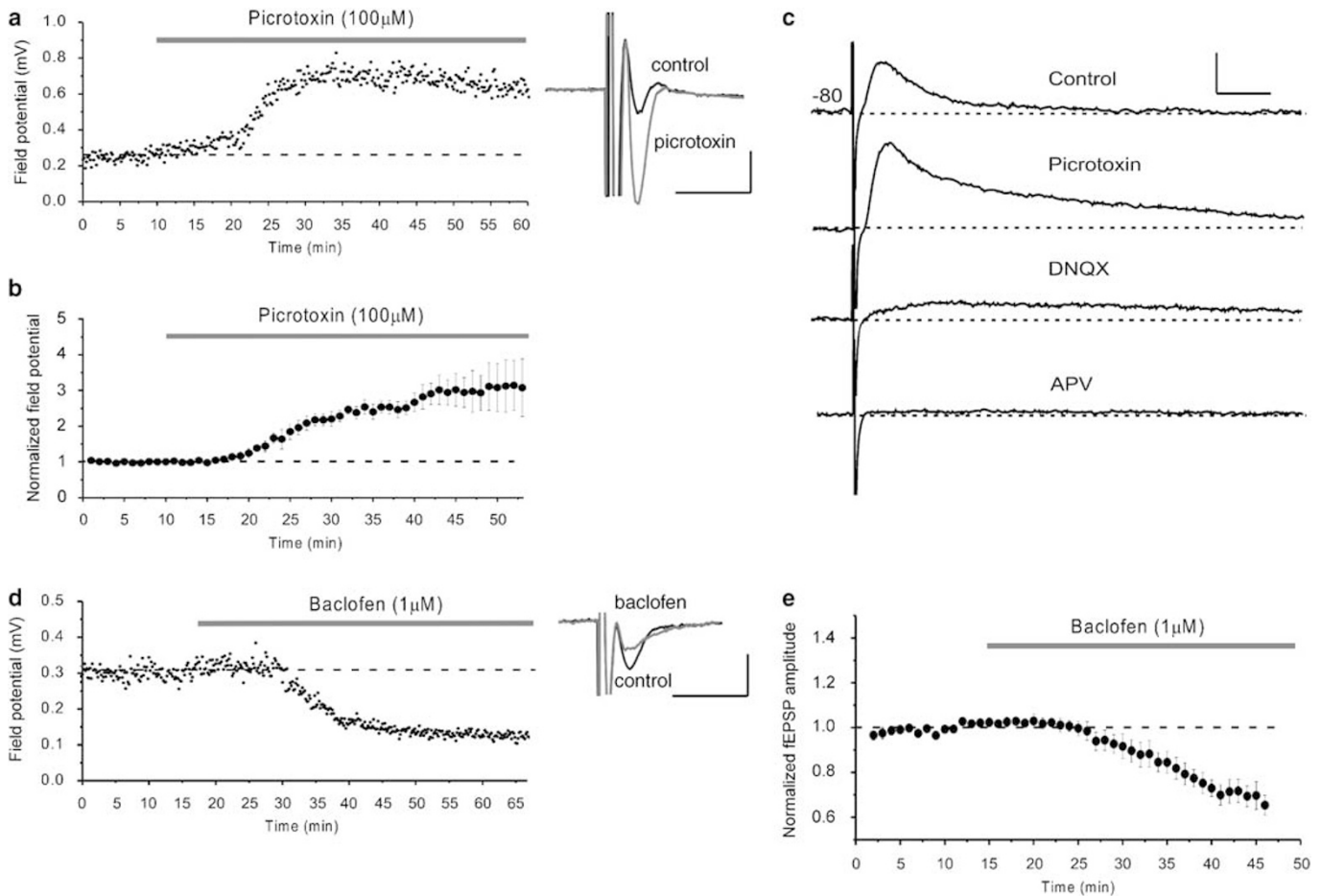


Figure 2 Extracellular field potentials consist of both excitatory and inhibitory synaptic components. (a) A single experiment with the GABA_AR antagonist, picrotoxin (100 μM). Inset: 10 averaged field potentials before (black) and 25 min after drug application (gray). Calibration: 0.25 mV, 10 ms. (b) Average of four experiments with picrotoxin (100 μM). Picrotoxin strongly enhanced the amplitude of field potentials recorded in the VTA ($252 \pm 3\%$ of predrug values). (c) Sample synaptic potentials recorded with an intracellular sharp microelectrode from a dopamine neuron held at -80 mV. Synaptic potentials have components mediated by GABA_AR and glutamate receptors. Top trace: control PSP. Second trace: picrotoxin blocked GABA_AR-mediated IPSP, leaving AMPAR and NMDAR-mediated synaptic potentials (EPSPs). Third trace: 10 μM DNQX blocked much of the remaining synaptic potential. Bottom trace: the remaining postsynaptic potential was abolished by d-APV (50 μM), indicating that it was NMDAR-dependent. Calibration: 5 mV, 10 ms. Baclofen depresses the amplitude of field potentials. (d) A single experiment showing that the GABA_BR agonist, baclofen (1 μM) reduces the VTA field potential. Inset: averaged field potentials before (black) and 25 min after drug application (gray). Calibration: 0.25 mV, 10 ms. (e) Average of five experiments with baclofen ($69 \pm 1\%$ of predrug values).

thus cut thinner slices (300 μM) to improve the visibility of VTA landmarks. Although we have not further characterized the extracellularly recorded field potentials in mature rats, our results indicate that this recording method has the potential for use at many ages.

We expected that evoked field potentials recorded in picrotoxin-free ACSF might contain a GABAergic component, representing current flow through GABA_A receptors at inhibitory synapses. These inhibitory synaptic responses may occur at a similar latency to the excitatory synaptic potentials, potentially reducing the actual amplitude of the field potentials. As predicted, bath application of the GABA_AR antagonist, picrotoxin (100 μM), dramatically increased the amplitude of the evoked field potential (Figure 2a and b). To test whether the enhancing effect of picrotoxin on the size of the field potential results from blockade of the evoked GABA_A IPSPs, we made intracellular recordings from VTA neurons with CsMeSO₄-filled electrodes while holding the cells at -80 mV. In agreement with

previous findings (Johnson and North, 1992), focal stimulation within the VTA evoked a mixed synaptic potential. Bath application of picrotoxin blocked the IPSP, which increased the amplitude of the depolarizing excitatory potential (EPSP). Even at this quite hyperpolarized potential, the EPSP is mediated by both AMPA and NMDA receptor-mediated potentials (Figure 2c; see also Johnson and North, 1992). These data suggest that the enhancing effect of picrotoxin on field potentials is due to the elimination of overlapping IPSPs. The complete block of the field potential by DNQX in Figure 1 also suggests that the GABA_AR-mediated IPSP does not contribute to the extracellularly recorded field potential, or perhaps that most of the IPSP is driven by stimulus-activated glutamatergic afferents onto local GABA neurons. Since picrotoxin blocks GABAergic inhibition, which controls the membrane potential of neurons, picrotoxin most likely facilitates the excitability of VTA neurons, promoting an increase in action potential firing.

In addition to AMPA, NMDA, and GABA_A receptor-mediated synaptic potentials, GABA_B receptors contribute to a late hyperpolarizing synaptic potential in dopamine neurons (Johnson and North, 1992; Cameron and Williams, 1993; Shoji *et al*, 1999). We tested the effect of baclofen, the GABA_BR agonist on field potential amplitude. Baclofen caused a 30% depression in the field potential amplitude (Figure 2d–e). However, the effect of baclofen on the field potential could involve two distinct populations of GABA_B receptors, located not only on postsynaptic cells but also on presynaptic glutamatergic terminals. GABA_B receptors on dopamine neurons will hyperpolarize these cells, thus reducing their likelihood of firing action potentials (and therefore reducing field potential amplitude) (Mueller and Brodie, 1989); GABA_B receptors have been suggested to be present only on glutamatergic afferents on GABA neurons but not dopamine neurons (Bonci and Malenka,

1999). Thus, a decrease in the field potential with baclofen could also reflect a selective depression of EPSPs on GABA cells.

High-Frequency Afferent Stimulation can Induce NMDA Receptor-Dependent LTP of Field Potential

Previously, synaptic plasticity of glutamatergic inputs onto VTA neurons has been analyzed using perforated patch clamp recordings (Bonci and Malenka, 1999) or using sharp microelectrodes (Overton *et al*, 1999). We recorded intracellularly using sharp microelectrodes and confirmed these previous findings. LTP induction was attempted in both dopamine and GABA neurons using HFS during direct depolarizing current injection. We found that glutamatergic synapses on dopamine neurons expressed LTP but synapses on GABA cells did not (Figure 3a–c).

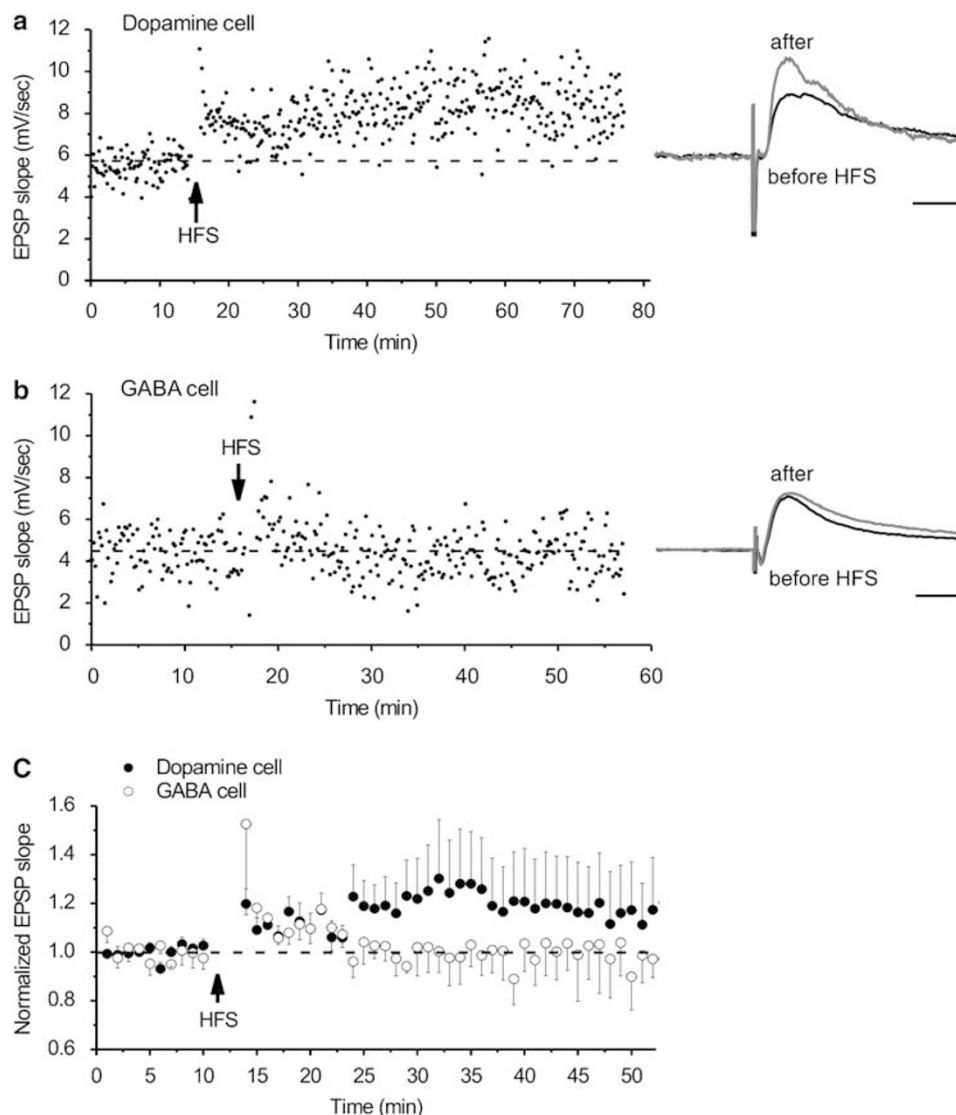


Figure 3 Excitatory synapses on dopamine cells express LTP while those on GABA cells do not. (a) Intracellular recording from a dopamine neuron exhibiting LTP after HFS (arrow). Inset: averaged EPSPs before (black) and 25 min after HFS (gray). Calibration: 5 mV, 10 ms. (b) Intracellular recording from a GABA neuron that did not express LTP after HFS. Inset: averaged EPSPs before (black) and 25 min after HFS (gray). Calibration: 5 mV, 10 ms. (c) Averaged experiments from dopamine and GABA neurons in response to HFS (dopamine neurons, filled symbols: $127 \pm 2.4\%$ of pre-HFS values, $p < 0.002$, $n = 10$; GABA neurons, open symbols: $97 \pm 2.5\%$ of pre-HFS values, $p = 0.587$, $n = 4$).

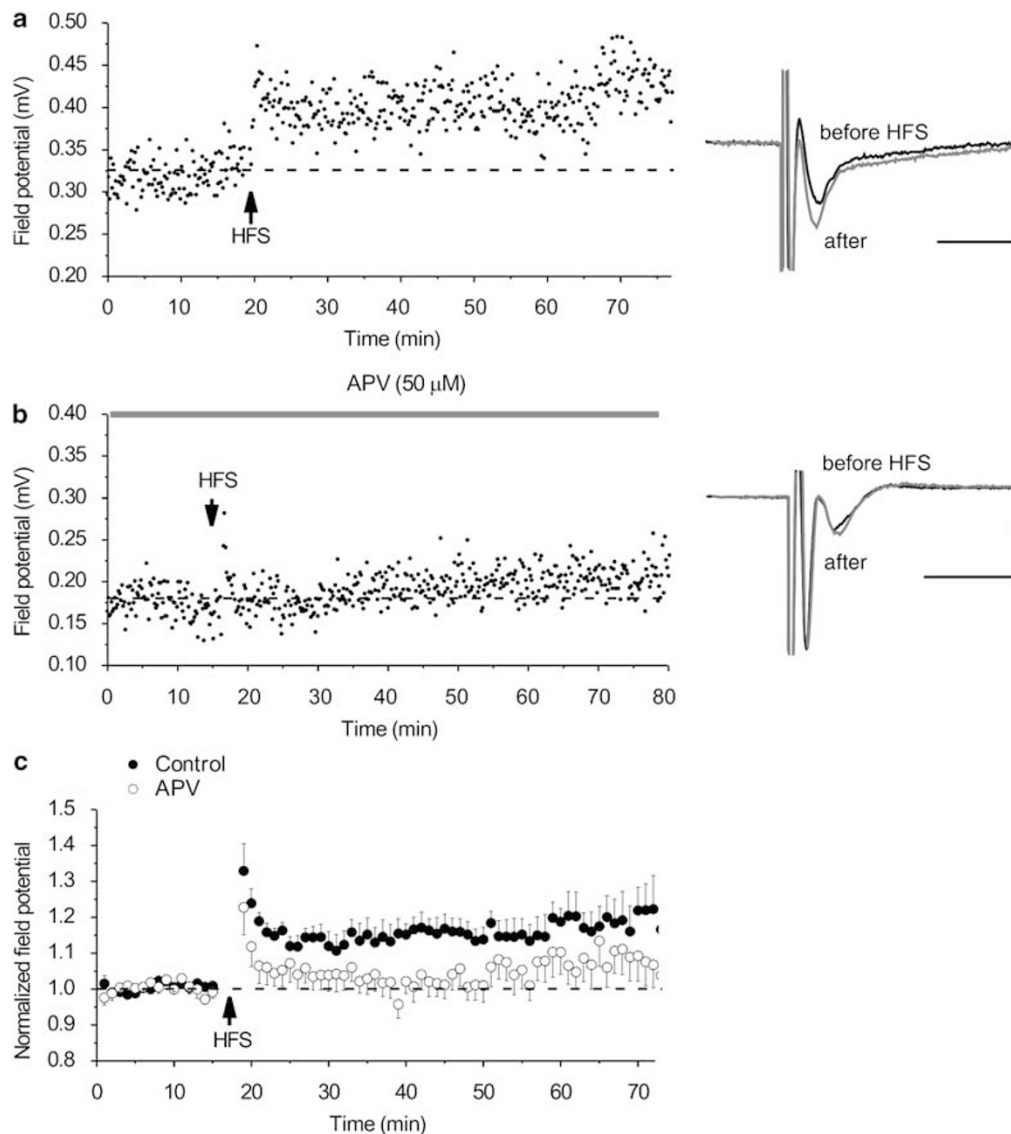


Figure 4 Induction of NMDAR-dependent LTP of VTA field potentials by HFS. (a) Single experiment exhibiting LTP of the field potential by HFS. Inset: averaged field potentials before (black) and 25 min after HFS (gray). Calibration: 0.25 mV, 10 ms. (b) Single experiment exhibiting the block of field potential LTP by d-APV (50 μ M). Inset: averaged field potentials before (black) and 25 min after HFS (gray). Calibration: 0.25 mV, 10 ms. (c) Averaged experiments with and without d-APV in response to HFS. d-APV (50 μ M) was present for at least 15 min before HFS (control LTP, filled symbols: 116 ± 0.67 of pre-HFS values, $p < 0.00002$, $n = 15$; APV experiments, open symbols: 101 ± 1.3 of pre-HFS values, $p = 0.168$, $n = 9$).

Owing to the relative ease of making field potential recordings, we wanted to ascertain whether we could observe LTP at excitatory synapses using field potential recordings. HFS of glutamatergic afferents induced LTP. This is the first demonstration of LTP of field potentials of the VTA. In these experiments we observed LTP in 7 of 15 slices (Figure 4a and c). Given that our intracellular recordings showed no LTP of synapses on GABA neurons, these findings suggest that LTP of the field potential represents the potentiated glutamatergic synapses on dopamine neurons. If the LTP of the field potential is due to LTP of glutamatergic synapses on dopamine neurons, then the underlying molecular mechanism for induction of this LTP should be the same. LTP triggered by synaptic stimulation in slices as well as that triggered by drugs of

abuse *in vivo* is blocked by NMDAR antagonists (Bonci and Malenka, 1999; Overton *et al*, 1999; Ungless *et al*, 2001). We therefore tested whether the NMDA receptor blocker, d-APV, prevented LTP of the field potential. As expected, d-APV (50 μ M) blocked the induction of field potential LTP (Figure 4b and c) suggesting that the induction of field potential LTP is also NMDA receptor-dependent. These data further support the idea that the LTP of the field potential represents plasticity that occurs at synapses on dopamine neurons. Although both cell types contribute to field potentials, the different basic properties of excitatory synapses onto these neurons can be detected in field potentials and this will provide the possibility of studying plasticity of synapses onto individual neuronal populations.

DISCUSSION

In the present study, we have demonstrated that excitatory field potentials can be evoked in the VTA in brain slices. We report that the field potential is comprised of both synaptic potentials and postsynaptic action potentials. Furthermore, NMDA receptor-dependent LTP of the field potential can be induced in the VTA by high-frequency afferent stimulation. This is the first demonstration of LTP of field potentials in the VTA. The ability to record LTP using this relatively simple electrophysiological approach will provide a useful tool for assessing drug-related plasticity.

VTA Field Potentials are Comprised of AMPA Receptor-Mediated EPSPs and Action Potentials in Local Neurons

Consistent with the recent report of Zheng *et al* (2006), VTA field potentials could be consistently evoked in the present study. The diffuse distribution of cells and their dendritic arborizations in the VTA, unlike that of highly laminar structures such as the hippocampus, gives rise to a much smaller evoked field potential (0.1–1 mV). The synaptic component of the field potential is completely eliminated by the local application of DNQX, suggesting that the field potential we record indeed arises from synaptic activity in the VTA itself. Intracellular recording experiments revealed that the recorded field potentials are most likely a mix of AMPA receptor-mediated synaptic potentials and postsynaptic action potentials. Blockade of AMPA receptor-mediated EPSPs by DNQX blocks the generation of postsynaptic action potentials, as it prevents postsynaptic neurons from reaching their threshold.

Bath application of picrotoxin considerably increased the amplitude of evoked field potentials, indicating that evoked GABAergic inhibition masks part of the excitatory field potential. Two mechanisms are expected to contribute to this effect. First, the GABA_AR-mediated IPSPs evoked by local electrical stimulation will hyperpolarize the postsynaptic neuron, overlapping in time, and canceling some of the depolarization elicited by simultaneously evoked EPSPs (Johnson and North, 1992). When picrotoxin removes the IPSP, the EPSP will provide more effective depolarization. Second, removal of GABA_A—mediated inhibition will increase the likelihood of action potential generation, which we believe contributes significantly to our recorded field potential.

Dopamine Neurons are Primarily Responsible for Generating VTA Field Potentials

We also tested the effect on field potential amplitude of baclofen, a GABA_B agonist. Baclofen produces a robust inhibition of firing and a pronounced hyperpolarization of neurons of the VTA (Mueller and Brodie, 1989). We found that baclofen reduced the field potential amplitude by 30%, probably due in part to this postsynaptic hyperpolarization. Additionally, however, excitatory synapses on dopamine neurons are reportedly to be minimally affected by baclofen, while synapses on GABA neurons are significantly depressed by baclofen. These data suggest that part of the reduction of the field potential we recorded in baclofen may

be caused by blocking excitatory synapses onto GABA neurons. This raises an interesting question regarding the relative contribution of dopamine vs GABA cells in generating the local field responses. Excitatory synaptic potentials and action potentials can be evoked in both cell types by focal stimulation inside the VTA. The majority of neurons in the VTA are dopamine neurons; Zheng *et al* (2006) have shown that elimination of VTA dopamine cells by neonatal 6-OHDA administration reduced more than 50% of the evoked field potentials. Taken together, these findings suggest that excitatory synaptic potentials and action potentials in dopamine neurons contribute more to the evoked field potential than those on local GABAergic neurons.

NMDAR-Dependent LTP of Field Potentials Presents a Simpler Model to Study Effects of Addictive Drugs

An exciting question in the drug addiction field concerns the functional significance of drug-induced plasticity at VTA synapses. All addictive drugs increase the levels of synaptic dopamine released at nerve terminals within the NAc (Di Chiara and Imperato, 1988; Koob, 1992; Nestler, 1992). Glutamate controls many aspects of dopamine cell function, including midbrain dopamine cell firing and dopamine release. Thus, is drug-induced release of dopamine within the NAc the functional consequence of LTP at VTA glutamatergic synapses? Several lines of evidence suggest that glutamatergic synaptic plasticity in the VTA rapidly induced by exposure to drugs of abuse may in fact change the firing patterns of dopamine neurons and thus modify dopamine release (Kauer, 2004; Jones and Bonci, 2005).

The development of behavioral sensitization involves an LTP-like process in the VTA. *In vivo* administration of drugs of abuse with different molecular mechanisms of action as well as acute stress do, in fact, elicit LTP at excitatory synapses on VTA dopamine neurons (Bonci and Malenka, 1999; Ungless *et al*, 2001; Saal *et al*, 2003). The response to cocaine and stress have also been shown to require NMDAR activation (Shors and Servatius, 1995; Li *et al*, 1999; Thomas *et al*, 2001; Borgland *et al*, 2004). Our results suggest that extracellular field potential recording represents a viable alternative to studying these key questions using a technically straightforward approach. Furthermore, extracellular field potentials recorded *in vivo* from the VTA are also possible, and may make it possible to link more directly *in vivo* and *in vitro* findings (Peters *et al*, 2004; Dommert *et al*, 2006).

In the present study, we have provided the first demonstration of LTP of excitatory field potentials recorded from VTA neurons using high-frequency stimulation of glutamatergic afferents. While excitatory synapses on dopamine cells undergo LTP, we confirmed that excitatory synapses on GABA cells do not (Bonci and Malenka, 1999). The field potential LTP is NMDA receptor-dependent. If pretreatment with *in vivo* drugs of abuse induces LTP of field potentials, then the *in vitro* induction of field potential LTP is expected to be reduced in slices prepared from these animals. Given the technical difficulty of obtaining LTP of excitatory synapses in the VTA using intracellular recording methods, it is worthwhile to pursue an uncomplicated

methodology (ie the field potential recording procedure) to monitor drug-induced plasticity.

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DISCLOSURE/CONFLICT OF INTEREST

The author(s) declare that, except for income received from my primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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