HHS Public Access

Author manuscript

Neuroscience. Author manuscript; available in PMC 2017 March 24.

Published in final edited form as:

Neuroscience. 2016 March 24; 318: 134–142. doi:10.1016/j.neuroscience.2016.01.017.

Glycine blocks long-term potentiation of GABAergic synapses in the ventral tegmental area

Yan-Zhong Guan[1,2] and **Jiang-Hong Ye**[1]

[1]Department of Anesthesiology, Pharmacology and Physiology, Rutgers, the State University of New Jersey, New Jersey Medical School, Newark, New Jersey, USA

[2]Department of Physiology, Mudanjiang Medical University, Mudanjiang, China

Abstract

The mesocorticolimbic dopamine system, originating in the ventral tegmental area (VTA) are normally constrained by GABA-mediated synaptic inhibition. Accumulating evidence indicates that long-term potentiation of GABAergic synapses (LTP_{GABA}) in VTA dopamine neurons plays an important role in the actions of drugs of abuse, including ethanol.

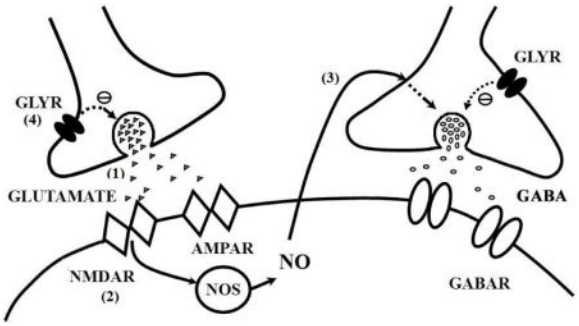
We previously showed that a single infusion of glycine into the VTA of rats strongly reduces ethanol intake for 24 hours. In the current study, we examined the effect of glycine on the electrophysiological activities of putative dopamine VTA neurons in midbrain slices from ethanol-naïve rats. We report here that a 15 min exposure to 10 μM glycine prevented trains of high frequency stimulation from producing LTP_{GABA}, which was rescued by the glycine receptor antagonist strychnine. Glycine also concentration-dependently decreased the frequency of spontaneous excitatory postsynaptic currents. By contrast, glycine pretreatment did not prevent potentiation of inhibitory postsynaptic currents during a continuous exposure to the nitric oxide donor, SNAP (S-nitroso-N-acetylpenicillamine), or a brief exposure to 10 μM glycine and 10 μM NMDA (N-methyl-D-aspartate), an agonist of NMDA-type glutamate receptors. Thus, the blockade of LTP_{GABA} by glycine is probably resulted from suppressing glutamate release by activating the glycine receptors on the glutamatergic terminals. This effect of glycine may contribute to the reduction in ethanol intake induced by intra-VTA glycine observed *in vivo*.

Graphical abstract

*Address correspondence to Jiang-Hong Ye, M.D., Department of Anesthesiology, Rutgers, the state University of New Jersey, New Jersey Medical School, 185 South Orange Avenue, Newark, New Jersey 07103; ye@njms.rutgers.edu; and Yan-Zhong Guan, MD, PhD, Department of Physiology, Mudanjiang Medical University, Mudanjiang, China. md-guan@163.com.

Disclosure

The authors declare no conflict of interest.



Keywords

Synaptic plasticity; mesolimbic system; EPSCs; Long-term potentiation; GABA

INTRODUCTION

The mesocorticolimbic dopaminergic system, originating in the ventral tegmental area (VTA), plays an important role in alcohol use disorders (Gilpin and Koob, 2008). VTA dopamine neurons are normally constrained by powerful GABA-mediated synaptic inhibition (Johnson and North, 1992, Tepper et al., 1998). Both $GABA_A$ and $GABA_B$ receptor subtypes have been identified within the VTA. Activation of $GABA_A$ receptor ($GABA_AR$) on GABAergic interneurons increases VTA dopamine neuron activity through disinhibition. Conversely, GABAergic neurons decreases spike output of dopamine neurons via $GABA_B$ receptors on dopamine neurons.

GABAergic signaling in the VTA has been linked to ethanol drinking behaviors. Intra-VTA administration of $GABA_A$ receptor ($GABA_AR$) antagonists decreases ethanol intake in rodents (Nowak et al., 1998, Melon and Boehm, 2011). Importantly, VTA GABA neurons become hyperexcitable during ethanol withdrawal (Gallegos et al., 1999). Interestingly, rodents readily self-administer $GABA_AR$ antagonists into the VTA (Ikemoto et al., 1997, Gavello-Baudy et al., 2008) and this blockade of $GABA_ARs$ in the VTA increases dopamine levels in the nucleus accumbens (Ikemoto et al., 1997), producing robust rewarding effects (Laviolette and van der Kooy, 2001). Changes in synaptic strength are important for persistent pathologies such as drug addiction. In particular, accumulating evidence indicates that long-term potentiation of GABAergic synapses (LTP_{GABA}) in VTA dopamine neurons increases or decreases in response to the actions of drugs of abuse, including ethanol (Nugent et al., 2007, Nugent et al., 2009, Guan and Ye, 2010).

Like GABA, glycine is also a major inhibitory neurotransmitter in the CNS. Functional glycine receptors (GlyRs) are found throughout the mammalian CNS, including the mesocorticolimbic dopamine system (Zheng and Johnson, 2001, Ye et al., 2002, Lewis and O'Donnell, 2003, Wang et al., 2005). Our previous *in vitro* electrophysiological data indicate that GlyRs exist on the GABAergic terminals, which make synapses on VTA dopamine neurons, and activation of these GlyRs reduces GABAergic transmission and increases VTA dopamine neuron activity (Ye et al., 2004). *In vivo* studies have shown that GlyRs in the mesolimbic dopamine system are involved in ethanol drinking behaviors.

Activation of GlyRs in the nucleus accumbens substantially decreases ethanol intake (Molander et al., 2005, Molander and Soderpalm, 2005b, a). Remarkably, we have shown that a single intra-VTA infusion of glycine substantially reduces ethanol intake for 24 hours in rats, in three different drinking models: intermittent access, continuous access, and operant self-administration (Li et al., 2012). However, the underlying neuronal basis has not been well explored. Glycine could alter the activity of dopamine neurons by activating the GlyRs on the GABAergic terminals, which make synapses on VTA dopamine neurons (Ye et al., 2004). However, given that extracellular glycine is probably removed rapidly by reuptake, additional mechanisms must be involved.

In an attempt to understand the mechanisms underlying the long-lasting effect of intra-VTA perfusion of glycine on ethanol drinking, we examined glycine's effects on the electrophysiological activities of putative dopamine neurons in the VTA in midbrain slices from ethanol-naïve rats.

EXPERIMENTAL PROCEDURES

Animals

The experiments were carried out on Sprague–Dawley rats, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of the Rutgers, the State University of New Jersey, New Jersey Medical School.

Chemicals and applications

DNQX (6, 7-dinitroquinoxaline-2, 3-dione), gabazine (SR-95531), glycine, NMDA (N-Methyl-D-aspartic acid), SNAP (S-nitroso-N-acetylpenicillamine), strychnine, and all common salts were obtained from Sigma-Aldrich Chemical Company (St Louis, MO). Drugs were added to the superfusate at final concentrations. Chemicals were applied to the recorded neurons at the stated concentrations through bath perfusion.

Brain slice preparation

The midbrain slices were prepared as described previously (Guan and Ye, 2010). Briefly, Sprague–Dawley rats (21–35 d old) were anesthetized using ketamine/xylazine and then sacrificed. Coronal midbrain slices (200–250 mm thick) were cut using a VF-200 slicer (Precisionary Instruments, Greenville, NC) and prepared in ice-cold glycerol-based artificial cerebrospinal fluid (aCSF) containing (in mM): 250 glycerol, 1.6 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose, and saturated with 95%O_2/5%CO_2 (carbogen) (Ye et al., 2006). Slices were allowed to recover for at least 1 h in a holding chamber at 32 ° in carbogen-saturated regular aCSF, which had the same composition as glycerol-based aCSF, except that glycerol was replaced by 125 mM NaCl.

Electrophysiological recording

Electrical signals were obtained with a MultiClamp 700 A amplifier (Molecular Devices Co., Union City, CA, USA), and pCLAMP software (Molecular Devices Co.). Data were filtered at 1 kHz and sampled at 5 kHz, stored on a computer, and analyzed offline. The

amplitudes of IPSCs were calculated by taking the mean of a 2–4 ms window around the peak and comparing it with the mean of a 2–8 ms window immediately before the stimulation artifact, using Clampfit (Molecular Devices).

Patch pipettes (4–6 MΩ) for recording IPSCs contained (in mM): 125 KCl, 2.8 NaCl, 2 $MgCl_2$, 0.6 EGTA, 10 HEPES, 2 Mg ATP-Na and 0.2 GTP-Na; and for EPSCs contained (in mM): 140 Cs-methanesulfonate, 5 KCl, 2 $MgCl_2$, 10 HEPES, 2 MgATP, 0.2 NaGTP, pH 7.2. The pH was adjusted to 7.2 with Tris base, and osmolality to 300 mOsmol/l with sucrose. A single slice was transferred to a 0.4 ml recording chamber, where a platinum ring held it down. Throughout the experiments, the bath was continually perfused with warm (32°C) carbogenated aCSF (1.5–2.0 ml/min).

Under infrared video microscopy (E600FN; Nikon Corporation, Tokyo, Japan), the VTA was identified medial to the accessory optic tract and lateral to the fasciculus retroflexus. Currents were recorded using whole-cell mode. Experiments were carried out only after series resistance had stabilized. Series resistance and input resistance were monitored continuously online with a −4 mV hyperpolarizing step (50 ms), which was given after every afferent stimulus, and experiments were discarded if these values changed by 20% during the experiment. Putative dopamine neurons in the current study were identified by the presence of a large Ih current (Johnson and North, 1992) that was assayed immediately after break-in, using a series of incremental 10 mV hyperpolarizing steps from a holding potential of −50 mV. Specifically, if the steady-state h-current was greater than 60 pA during a step from −50 to −100 mV, the neuron was considered a dopamine neuron. A recent study showed that expression of Ih alone is not sufficient to identify dopamine cells unequivocally (Margolis et al., 2006). Therefore, in each set of our experiments, a subset of the neurons recorded from and reported here are possibly non-dopaminergic neurons (Nugent et al., 2009).

$GABA_A$R-mediated IPSCs were stimulated at 0.1 Hz using a bipolar stainless steel-stimulating electrode placed 200–400 μm away from the recording site in the VTA. As previously described (Guan and Ye, 2010), LTP_{GABA} was induced by stimulating afferents at 100 Hz for 1 s, and the train was repeated twice, 20 s apart (high-frequency stimulation; HFS). After recording the baseline currents, during the drug application and washout, synaptic stimulation was stopped and the recorded neuron was taken from voltage-clamp into bridge mode. The HFS trains were also delivered under bridge mode.

Statistics

Data are presented, as means ± SEM. Significance was determined using a Student's unpaired t-test with a significance level of $P < 0.05$. Levels of LTP are reported as averaged IPSC amplitudes for 5 min just before LTP induction compared with averaged IPSC amplitudes during the 5 min period from 20 to 25 min after HFS. Paired-pulse ratios (PPR=IPSC2/IPSC1) (50 ms inter-stimulus interval) were measured over 5 min epochs of 30 IPSCs each, as previously described (Guan and Ye, 2010, Guan et al., 2012).

RESULTS

Glycine blocks LTP_{GABA} in VTA-dopamine neurons in brain slices via the activation of strychnine-sensitive glycine receptors

Currents evoked by electrical stimuli were recorded from putative dopamine neurons of the VTA in midbrain slices in the presence of DNQX (10 μM), an AMPA receptor antagonist, and at a holding potential of −70 mV. These currents were blocked by 10 μM gabazine, a $GABA_A$ receptor ($GABA_AR$) antagonist (not illustrated), indicating that they were IPSCs mediated by $GABA_ARs$. The amplitude of IPSCs was greatly increased and maintained after high frequency stimulation (HFS, see Method). Thus, HFS induced long-term potentiation of IPSC (LTP_{GABA}, Fig. 1A), in keeping with previous reports (Nugent et al., 2007, Guan and Ye, 2010). LTP_{GABA} was associated with a decrease in the paired pulse ratio (PPR; Fig. 1a, insets), suggesting that LTP_{GABA} is maintained by persistently increased GABA release, which is probably caused by high-frequency firing of the presynaptic GABAergic afferents (Nugent et al., 2007, Guan and Ye, 2010).

We then examined the effect of glycine on LTP_{GABA}. Glycine (10 μM) was bath perfused for 15 min and then washed out for 6 min before HFS. HFS failed to induce LTP_{GABA} under such conditions (Fig. 1B and C). In fifteen experiments, in the absence of glycine, the averaged peak amplitude of IPSC (IPSC1) elicited by the first stimulus of the paired pulse measured 20–25 min after HFS was 198.25 ± 1.37 % of pre-HFS values (Fig. 1C, filled circles). After exposure to glycine, it was 101.43 ± 2.18 % of pre-HFS values (Fig. 1C, open circles). Glycine also reversed the changes in PPR (in aCSF, PPR = 0.68 ± 0.15 of pre-HFS values; in glycine, PPR = 1.06 ± 0.18 of pre-HFS values, $P < 0.05$; Fig. 1D). To determine whether the effects of glycine were mediated by strychnine sensitive GlyRs, strychnine (1 μM) was bath applied for 5 min before the application of the mixture containing strychnine (1 μM) and glycine (10 μM) for 15 min. HFS was delivered 6 min after washout of the mixture. Under these experimental conditions, the cells exhibited normal LTP_{GABA} (Fig. 1E). In ten experiments, the averaged peak amplitude of IPSC1 measured 20–25 min after HFS was 196.29 ± 10.47 % of pre-HFS values in cells exposed to glycine plus strychnine (Fig. 1F, filled circles) vs 100.33 ± 4.26% of pre-HFS values in glycine alone (Fig. 1F, open circles). The PPR was 0.70 ± 0.10 of pre-HFS values in cells exposed to glycine plus strychnine. These results suggest that glycine-induced suppression of LTP_{GABA} was mediated by strychnine-sensitive GlyRs, which prevents HFS-induced increase of GABA release.

Glycine prevents LTP_{GABA} upstream of NMDARs in the heterosynaptic pathway

LTP_{GABA} is characterized as heterosynaptic plasticity, initiated by glutamate release onto NMDARs on the postsynaptic dopamine neurons. There is previous evidence that nitric oxide (NO) is produced in dopamine neurons and released extracellularly to enhance GABA release from GABAergic terminals, and application of the NO donor, SNAP, rapidly enhances GABAergic IPSCs (Nugent et al., 2007). In the current study, we perfused SNAP (400 μM) in the aCSF in the presence of glycine (10 μM) throughout recording. Thirty minutes after SNAP application, eIPSC amplitudes potentiated to 147.98 ± 4.26% of pre-

SNAP values (n = 11) (Fig. 2). These data indicate that glycine blocks LTP_{GABA} in the upstream of nitric oxide synthase (NOS) in dopamine neurons.

We next tested whether glycine's prevention of LTP_{GABA} involves NMDARs in VTA-dopamine neurons. To inhibit evoked IPSCs, we first bath applied glycine (10 μM, for 5 min), and then to activate NMDARs, we bath applied a mixture containing 10 μM NMDA and 10 μM glycine, for 10 min. After washout, IPSCs recovered to an enhanced level (Fig. 3). Eighty minutes after washout, eIPSC amplitude was 142.14±6.22 % (n =5) of pre-treatment values. Bath application of glycine (10 μM) alone did not produce potentiation (not illustrated). These data support previous observations that NMDAR activation alone (without HFS) is sufficient to induce LTP_{GABA} (Nugent et al., 2007).

Glycine decreases the frequency of spontaneous EPSCs in VTA-dopamine neurons

The above result showed that glycine could not inhibit LTP_{GABA} that was induced by NMDAR activation; glycine's inhibition of LTP_{GABA} thus may result from targeting upstream events of NMDARs (e.g. glutamate release). To test this possibility, we examined the effects of glycine on spontaneous EPSCs (sEPSCs) in VTA-dopamine neurons. All sEPSCs were recorded in the presence of 10 μM gabazine and at a holding potential of −70 mV with Cs-methanesulfonate based pipette solution. These events were eliminated by DNQX (20 μM, data not show), indicating that they are AMPAR-mediated EPSCs. As illustrated in Fig. 4, glycine (10 μM) decreased sEPSC frequency, and the inhibition was blocked by strychnine (1 μM). Glycine-induced inhibition of sEPSC frequency depended on its concentration. Specifically, glycine (1, 3 and 10 μM) decreased sEPSC frequency by 9.15 ± 11.05%, 26.31 ± 7.42 %, and 51.38 ± 7.19% (*$p < 0.05$, n = 15, **$p<0.01$ (Fig. 4)), respectively, without a significant effect on sEPSC amplitude (7.24 ± 9.10%, 8.18 ± 11.27%, 11.41 ± 14.27%, respectively, all $p > 0.05$, n = 15).

DISCUSSION

Our major finding is that a short (15 minute) exposure to 10 μM glycine prevented trains of HFS from producing LTP_{GABA} on VTA-dopamine neurons in midbrain slices of rats, which was paralleled with the reduction of glutamate release. By contrast, glycine pretreatment did not prevent potentiation of eIPSCs during a continuous exposure to the NO donor SNAP. Similarly, glycine pretreatment did not prevent an increase of IPSC amplitude in response to a brief exposure to 10 μM NMDA and 10 μM glycine. The effects of glycine could conceivably be mediated by strychnine-sensitive GlyRs, because co-application of glycine and strychnine failed to affect LTP_{GABA}. Our data suggest that glycine-induced change of LTP_{GABA} may contribute to the reduction of ethanol drinking by intra-VTA infusion of glycine observed *in vivo* (Li et al., 2012).

Glycine blocks LTP_{GABA} in VTA-dopamine neurons by preventing the increase of GABA release induced by high frequency stimulation

LTP_{GABA} is known to be produced by a heterosynaptic mechanism, requiring the activation of postsynaptic NMDARs at glutamate synapses, resulting from increased GABA release at neighboring inhibitory nerve terminals (Nugent et al., 2007). We recorded HFS-induced

LTP_{GABA} on VTA-dopamine neurons and found that LTP_{GABA} was coupled to a change in PPR, an electrophysiological parameter typically used to identify changes in probability of transmitter release (Nugent et al., 2007, Guan and Ye, 2010). An increased presynaptic GABA release in response to HFS was indicated by the significant reduction in the mean PPR of GABAergic synaptic currents associated with LTP_{GABA} (Nugent et al., 2007, Guan and Ye, 2010), arguing against a potential role of increased sensitivity of postsynaptic $GABA_A$Rs in this process. In the present study, we demonstrated that bath perfusion of 10 μM glycine blocks the change of PPR induced by HFS, indicating that glycine prevents the increase in presynaptic GABA release induced by HFS.

Our previous *in vivo* study showed that a single intra-VTA infusion of glycine (5–10 μM, 500 μl/side) reduces alcohol drinking for 24 hours, and the effect is mediated by strychnine-sensitive GlyRs (Li et al., 2012). We speculate that this glycine may quickly disappear by reuptake, and the activation of GlyRs must trigger a process that outlasts acute receptor activation. In an attempt to mimic the *in vivo* experimental conditions, we applied HFS 6 mins after wash-out of glycine. Future studies are necessary to determine how long it takes the system to recover from glycine-induced suppression of LTP_{GABA}, and whether the results are different if glycine were continually present.

Glycine blocks LTP_{GABA} by reducing presynaptic glutamate release

Remarkably, glycine-induced blockade of LTP_{GABA} is prevented by strychnine, indicating that glycine's effect is mediated by strychnine-sensitive GlyRs, instead of the NMDARs, where it is a co-agonist. The strychnine-sensitive GlyR conducts Cl^-. In adult mammals, it is inhibitory because it enhances Cl^- influx (Werman et al., 1968, Nicoll et al., 1990, Kuhse et al., 1995). Previous studies have shown that GlyRs exist in the VTA (Ye et al., 1998, Ye, 2000, Zheng and Johnson, 2001). Notably, in the VTA, functional GlyRs are not only found in the postsynaptic membrane, but also in the presynaptic membrane, including the GABAergic terminals, which make synapses onto VTA-dopamine neurons. Activation of these receptors modulates the release of transmitters and the activity of VTA-dopamine neurons (Ye et al., 2004). Glycine at 3 and 10 μM substantially suppressed sEPSC frequency but not their amplitude (Fig. 4), suggesting that strychnine-sensitive GlyRs exist on the glutamatergic terminals. By activating these GlyRs, glycine reduces glutamate release, and thus the activation of NMDARs, resulting in the prevention of LTP_{GABA}. These data also indicate that the GlyRs on the glutamatergic terminals are very sensitive to glycine, in keeping with our previous report that GlyRs on the GABAergic terminals are very sensitive to glycine (Ye et al., 2004). Specifically, whereas the presynaptic GlyRs have an EC_{50} of ~1.5 μM (Ye et al., 2004), which is much lower than that for the postsynaptic GlyRs, which have an EC_{50} of ~25 μM (Ye, 2000). Currently, it remains unclear why the GlyRs on presynaptic axons in the VTA are more sensitive to glycine than GlyRs on DA neurons, although receptor density, subunit composition, and structure difference may play a role. In the current study, we did not investigate the mechanisms of glycine-induced reduction of glutamate release. However, previous studies have shown that calcium channels are involved in glycine-induced changes of GABAergic IPSCs in the VTA (Ye et al., 2004), and in glycine-induced changes of glutamatergic EPSCs in other CNS areas (Turecek and Trussell, 2001).

The sources of endogenous GlyR agonists in the VTA are unclear. Glycine-releasing neurons are sparse in rostral areas of the brain (Danglot et al., 2004), including the VTA. However, glycine could be released by glial cells (Legendre, 2001). In human cerebrospinal fluid and in the frontal cortex, free glycine is normally at levels between 7 to 10 μM, however, higher levels (18 to 28 μM) have been detected in the cerebellum (Ferraro and Hare, 1985) (Matsui et al., 1995) (Fabricius et al., 1993). The extracellular concentration of glycine is regulated by at least two glycine transporters (GLYT1 and GLTY2). These GLYTs are likely to play a role both at inhibitory glycinergic synapses, and at glutamatergic synapses (Ascher, 1990); (Attwell et al., 1993).

The current study demonstrated that glycine blockage of LTP_{GABA} is paralleled with the inhibition of sEPSCs. These data support the idea that presynaptic glutamate release is necessary for the induction of LTP_{GABA} (Nugent et al., 2007). By contrast, glycine failed to inhibit LTP_{GABA} either induced by a short co-application of NMDA and glycine, or by the continuous perfusion of the NO donor SNAP. Previous studies by Kauer's group have shown that NO, a retrograde signal generated by an NMDAR-mediated rise in postsynaptic Ca^{2+} acting at a presynaptic site, is produced in dopamine neurons and released extracellularly to enhance GABA release from GABAergic terminals (Nugent et al., 2007). These investigators also showed that NO scavengers (Hemoglobin and 2-pheyl-4, 4, 5, 5,-tetramethylimidazoline-1-oxyl 3-oxide) prevent HFS-induced LTP_{GABA}. On the basis of their findings, our results suggest that glycine is probably acting upstream of NMDAR and NO production. We propose that glycine inhibition of LTP_{GABA} is mainly resulted from its inhibition of glutamate release, since the induction of LTP_{GABA} requires the activation of NMDARs (Nugent et al., 2007). By activating the GlyRs on the GABAergic terminals on VTA-dopamine neurons, glycine could directly suppress GABAergic IPSCs (Ye et al., 2004).

Interestingly, 10 μM glycine did not alter the eIPSC amplitude as shown in Fig. 1B, but decreased it as seen in Fig. 3. The mechanism underlying the difference is unclear, but may be resulted from the difference in the conditions when the data were obtained. In Fig. 1, glycine was applied for 15 min and washed out for 6 min before HFS. During this period (at least 21 min), we did not record IPSCs since our focus was on the changes of IPSCs after HFS, therefore it is not clear whether IPSCs have been changed or not. Conversely, in Fig. 3, a 5 min exposure to 10 μM glycine decreased IPSCs, which is consistent with our previous report, that activation of the GlyRs on the GABAergic terminals reduces GABA release (Ye et al., 2004).

According to the model circuit, LTP_{GABA} should decrease activity of dopamine neurons and the release of dopamine. The hypo-dopamine state has been associated with the motivation for ethanol drinking (Gonzales et al., 2004, Chen et al., 2011). Interestingly, as described earlier, GABAA antagonists are rewarding at the same time as they reduce ethanol intake. The underlying mechanism needed to be determined. The rewarding effect of GABAA antagonists may be resulted from the ability of these agents to increase activity of dopamine neurons and dopamine levels in the target areas. Higher dopamine levels in the nucleus accumbens may cause rewarding effects. Under these conditions, the animals would not need to drink as much ethanol as that in normal conditions to achieve the wanted effect,

thereby resulting in reduced ethanol consumption. Although the current study demonstrates that glycine blocks LTP_{GABA} on dopamine neurons in the VTA of ethanol-naïve rats, it remains to be determined whether glycine has the same effect on these neurons from rats chronically exposed to ethanol. The effects of glycine on GABA transmission under such conditions may have been changed since GABAergic transmission has been changed after chronic ethanol exposure (Gallegos et al., 1999). Additional limitation of current study is that we used 11 mM glucose in aCSF that is far beyond the physiological level of 2.5 mM. Future study using 2.5 mM glucose is needed to determine whether this will affect the outcome.

Collectively, our data support an important role for glutamate release in the maintenance of heterosynaptic efficacy in the VTA. A reduction in glutamate release by activation of strychnine sensitive GlyRs on glutamate terminals in VTA-dopamine neurons triggers a rapid decrease in heterosynaptic efficacy (GABAergic synapse). Together, the current study reports a previous unrecognized effect of glycine on the LTP_{GABA} recorded from VTA-dopamine neurons. These data provide new insight into glycine's effects on brain reward pathways and could lead to the development of new therapies against alcohol use disorders.

Acknowledgments

This work was made possible by NIH grants (AA01694; AA 021657; AA 022292, JHY), New Jersey Health foundation (PC109-13, JHY), and National Natural Science Foundation of China (grant 81371463, YZG); Graduate innovative research programs of Mudanjiang Medical University, China. (No. 2014YJSCX-04 & YJSCX2012-380HLJ, YZG)

Abbreviations

DNQX	(6, 7-dinitroquinoxaline-2, 3-dione)
eIPSC	evoked inhibitory postsynaptic current
GABA	(gamma - aminobutyric acid)
GABAAR	GABAA receptor
GlyR	glycine receptor
HFS	high frequency stimulation
IPSC	inhibitory postsynaptic current
LTP_{GABA}	long-term potentiation of GABA-mediated synapses
NMDA	(N-methyl-D-aspartate)
NO	nitric oxide
PPR	paired pulse ratio
sEPSC	Spontaneous excitatory postsynaptic current
SNAP	(S-nitroso-N-acetylpenicillamine)
VTA	ventral tegmental area

References

Ascher P. Measuring and controlling the extracellular glycine concentration at the NMDA receptor level. Adv Exp Med Biol. 1990; 268:13–16. [PubMed: 2150149]

Attwell D, Barbour B, Szatkowski M. Nonvesicular release of neurotransmitter. Neuron. 1993; 11:401–407. [PubMed: 8104430]

Chen G, Cuzon Carlson VC, Wang J, Beck A, Heinz A, Ron D, Lovinger DM, Buck KJ. Striatal involvement in human alcoholism and alcohol consumption, and withdrawal in animal models. Alcohol Clin Exp Res. 2011; 35:1739–1748. [PubMed: 21615425]

Danglot L, Rostaing P, Triller A, Bessis A. Morphologically identified glycinergic synapses in the hippocampus. Mol Cell Neurosci. 2004; 27:394–403. [PubMed: 15555918]

Fabricius M, Jensen LH, Lauritzen M. Microdialysis of interstitial amino acids during spreading depression and anoxic depolarization in rat neocortex. Brain Res. 1993; 612:61–69. [PubMed: 8330214]

Ferraro TN, Hare TA. Free and conjugated amino acids in human CSF: influence of age and sex. Brain Res. 1985; 338:53–60. [PubMed: 4027591]

Gallegos RA, Lee RS, Criado JR, Henriksen SJ, Steffensen SC. Adaptive responses of gamma-aminobutyric acid neurons in the ventral tegmental area to chronic ethanol. J Pharmacol Exp Ther. 1999; 291:1045–1053. [PubMed: 10565823]

Gavello-Baudy S, Le Merrer J, Decorte L, David V, Cazala P. Self-administration of the GABAA agonist muscimol into the medial septum: dependence on dopaminergic mechanisms. Psychopharmacology (Berl). 2008; 201:219–228. [PubMed: 18670763]

Gilpin NW, Koob GF. Neurobiology of alcohol dependence: focus on motivational mechanisms. Alcohol Res Health. 2008; 31:185–195. [PubMed: 19881886]

Gonzales RA, Job MO, Doyon WM. The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. Pharmacol Ther. 2004; 103:121–146. [PubMed: 15369680]

Guan Y, Xiao C, Krnjevic K, Xie G, Zuo W, Ye JH. GABAergic actions mediate opposite ethanol effects on dopaminergic neurons in the anterior and posterior ventral tegmental area. J Pharmacol Exp Ther. 2012; 341:33–42. [PubMed: 22209891]

Guan YZ, Ye JH. Ethanol blocks long-term potentiation of GABAergic synapses in the ventral tegmental area involving mu-opioid receptors. Neuropsychopharmacology. 2010; 35:1841–1849. [PubMed: 20393452]

Ikemoto S, Kohl RR, McBride WJ. GABA(A) receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. J Neurochem. 1997; 69:137–143. [PubMed: 9202304]

Johnson SW, North RA. Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J Physiol. 1992; 450:455–468. [PubMed: 1331427]

Kuhse J, Betz H, Kirsch J. The inhibitory glycine receptor: architecture, synaptic localization and molecular pathology of a postsynaptic ion-channel complex. Curr Opin Neurobiol. 1995; 5:318–323. [PubMed: 7580154]

Laviolette SR, van der Kooy D. GABA(A) receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems. Eur J Neurosci. 2001; 13:1009–1015. [PubMed: 11264674]

Legendre P. The glycinergic inhibitory synapse. Cell Mol Life Sci. 2001; 58:760–793. [PubMed: 11437237]

Lewis B, O'Donnell P. Blockade of the GlyT1 glycine transporter prolongs response to VTA stimulation in nucleus accumbens neurons. Ann N Y Acad Sci. 2003; 1003:431–434. [PubMed: 14684480]

Li J, Nie H, Bian W, Dave V, Janak PH, Ye JH. Microinjection of glycine into the ventral tegmental area selectively decreases ethanol consumption. J Pharmacol Exp Ther. 2012; 341:196–204. [PubMed: 22238211]

Margolis EB, Lock H, Chefer VI, Shippenberg TS, Hjelmstad GO, Fields HL. Kappa opioids selectively control dopaminergic neurons projecting to the prefrontal cortex. Proc Natl Acad Sci U S A. 2006; 103:2938–2942. [PubMed: 16477003]

Matsui T, Sekiguchi M, Hashimoto A, Tomita U, Nishikawa T, Wada K. Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. J Neurochem. 1995; 65:454–458. [PubMed: 7790891]

Melon LC, Boehm SL 2nd. GABAA receptors in the posterior, but not anterior, ventral tegmental area mediate Ro15-4513-induced attenuation of binge-like ethanol consumption in C57BL/6J female mice. Behav Brain Res. 2011; 220:230–237. [PubMed: 21320533]

Molander A, Lof E, Stomberg R, Ericson M, Soderpalm B. Involvement of accumbal glycine receptors in the regulation of voluntary ethanol intake in the rat. Alcohol Clin Exp Res. 2005; 29:38–45. [PubMed: 15654289]

Molander A, Soderpalm B. Accumbal strychnine-sensitive glycine receptors: an access point for ethanol to the brain reward system. Alcohol Clin Exp Res. 2005a; 29:27–37. [PubMed: 15654288]

Molander A, Soderpalm B. Glycine receptors regulate dopamine release in the rat nucleus accumbens. Alcohol Clin Exp Res. 2005b; 29:17–26. [PubMed: 15654287]

Nicoll RA, Malenka RC, Kauer JA. Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. Physiol Rev. 1990; 70:513–565. [PubMed: 1690904]

Nowak KL, McBride WJ, Lumeng L, Li TK, Murphy JM. Blocking GABA(A) receptors in the anterior ventral tegmental area attenuates ethanol intake of the alcohol-preferring P rat. Psychopharmacology (Berl). 1998; 139:108–116. [PubMed: 9768548]

Nugent FS, Niehaus JL, Kauer JA. PKG and PKA signaling in LTP at GABAergic synapses. Neuropsychopharmacology. 2009; 34:1829–1842. [PubMed: 19194373]

Nugent FS, Penick EC, Kauer JA. Opioids block long-term potentiation of inhibitory synapses. Nature. 2007; 446:1086–1090. [PubMed: 17460674]

Tepper JM, Paladini CA, Celada P. GABAergic control of the firing pattern of substantia nigra dopaminergic neurons. Adv Pharmacol. 1998; 42:694–699. [PubMed: 9327994]

Turecek R, Trussell LO. Presynaptic glycine receptors enhance transmitter release at a mammalian central synapse. Nature. 2001; 411:587–590. [PubMed: 11385573]

Wang F, Xiao C, Ye JH. Taurine activates excitatory non-synaptic glycine receptors on dopamine neurones in ventral tegmental area of young rats. J Physiol. 2005; 565:503–516. [PubMed: 15817633]

Werman R, Davidoff RA, Aprison MH. Inhibitory of glycine on spinal neurons in the cat. J Neurophysiol. 1968; 31:81–95. [PubMed: 4384497]

Ye J. Physiology and pharmacology of native glycine receptors in developing rat ventral tegmental area neurons. Brain Res. 2000; 862:74–82. [PubMed: 10799671]

Ye JH, Ren J, Liu PL, McArdle JJ. Glycine-activated chloride currents of neurons freshly isolated from the ventral tegmental area of rats. Brain Res. 1998; 796:53–62. [PubMed: 9689454]

Ye JH, Tao L, Zhu L, Krnjevic K, McArdle JJ. Decay of ethanol-induced suppression of glycine-activated current of ventral tegmental area neurons. Neuropharmacology. 2002; 43:788–798. [PubMed: 12367623]

Ye JH, Wang F, Krnjevic K, Wang W, Xiong ZG, Zhang J. Presynaptic glycine receptors on GABAergic terminals facilitate discharge of dopaminergic neurons in ventral tegmental area. J Neurosci. 2004; 24:8961–8974. [PubMed: 15483115]

Ye JH, Zhang J, Xiao C, Kong JQ. Patch-clamp studies in the CNS illustrate a simple new method for obtaining viable neurons in rat brain slices: glycerol replacement of NaCl protects CNS neurons. J Neurosci Methods. 2006; 158:251–259. [PubMed: 16842860]

Zheng F, Johnson SW. Glycine receptor-mediated inhibition of dopamine and non-dopamine neurons of the rat ventral tegmental area in vitro. Brain Res. 2001; 919:313–317. [PubMed: 11701143]

Highlights

- A transit exposure to 10 μM glycine prevents trains of HFS from producing LTP_{GABA}
- Glycine does not prevent potentiation of IPSCs induced by the NO donor SNAP
- Glycine does not prevent potentiation of evoked IPSCs induced by NMDA and glycine
- Glycine lowers the frequency of spontaneous EPSCs in the putative dopamine neurons
- Glycine inhibits EPSCs by activating strychnine-sensitive glycine receptors

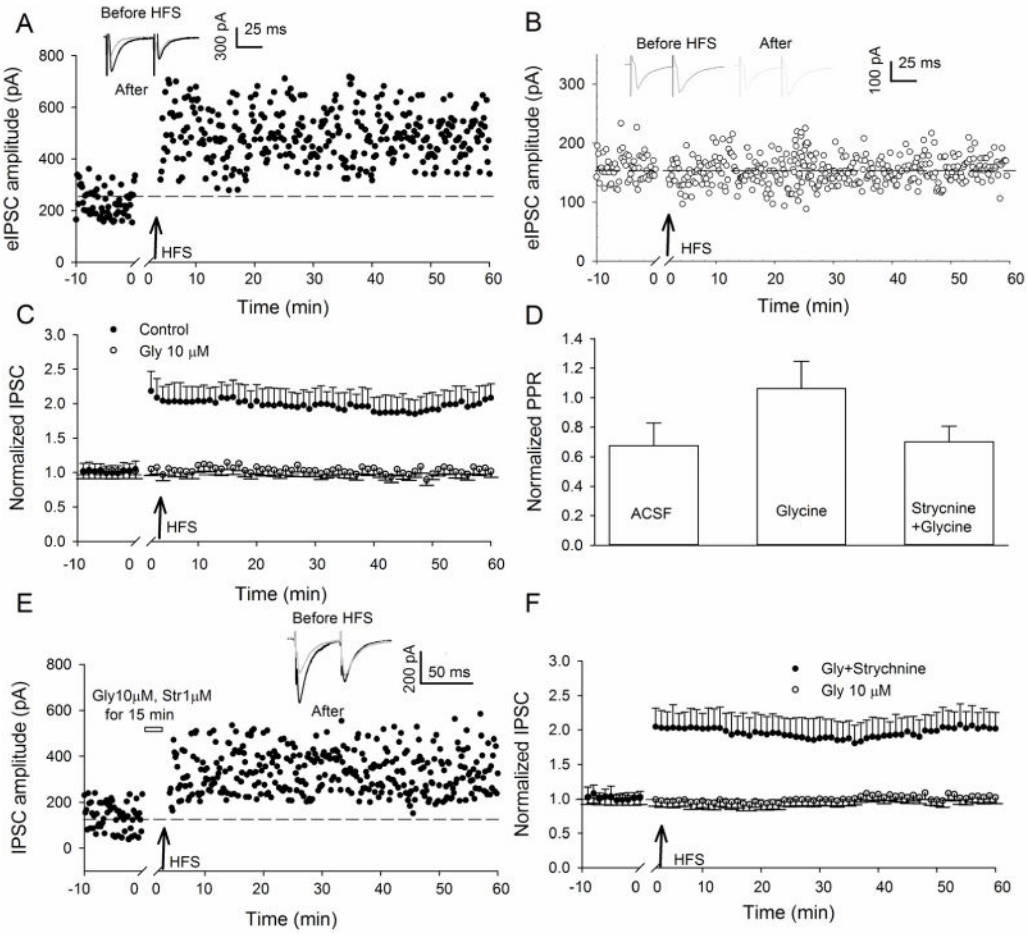


Fig. 1. Glycine blocks LTP_{GABA} in VTA-dopamine neurons in brain slices via the activation of strychnine sensitive glycine receptors

A, LTP_{GABA} recorded in a putative dopamine neuron with the whole-cell mode. High-frequency stimulation (HFS) was delivered at the point indicated by the arrow. Inset: averaged evoked IPSCs (eIPSCs) recorded before (gray) and 20 min after HFS (black). In this and all other figures, 10 consecutive IPSCs from each condition were averaged for illustration. Calibration for insets: 25 ms, 300 pA. The dotted line in this and other figures is an approximation of the mean response before HFS. B, glycine blocks LTPGABA. After recording the baseline eIPSCs in normal aCSF for 10 min, glycine (Gly, 10 μM) was bath applied for 15 min and then washed out (for 6 min) before the delivery of HFS. Inset: averaged IPSCs before (gray) and 20 min after HFS (black). C, Average of 15 experiments from dopamine neurons (Control LTPGABA, filled circles; Gly, open circles). D, Normalized PPR at 20 min after HFS under different experimental conditions. The PPR at 20 min was normalized to the pre-HFS PPR values. (*p <0.05, Student's t-test for PPR after HFS compared with pre-HFS values). E, Strychnine reversed the inhibition of LTPGABA by glycine. After recording the baseline IPSCs in normal aCSF for 10 min, strychnine (1 μM) was bath applied for 5 min followed by application of the mixed cocktail of strychnine (1 μM) and glycine (10 μM) for 15 min. HFS was then delivered 6 min after washout of the mixture (at the arrow). Inset: averaged IPSCs before (gray) and 20 min after HFS (black). F,

Average of 10 experiments from dopamine neurons (glycine alone, open circles; strychnine + glycine, filled circles).

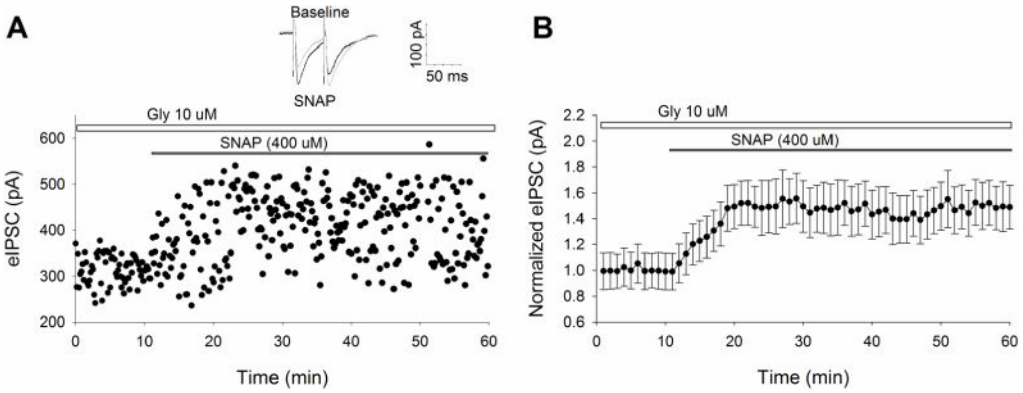


Fig. 2. Glycine (10 μM) could not block SNAP-induced potentiation of GABAergic IPSCs

A, Single experiment illustrating the lack of effect of glycine on SNAP-induced potentiation of GABAergic IPSCs. SNAP (400 μM), an NO donor, potentiated IPSCs during consistent glycine (10 μM) bath-application. Inset: averaged IPSCs recorded before and after 10 min in SNAP. B, average of experiments from 11 cells (147.98±4.26% of pre-SNAP values, n=11).

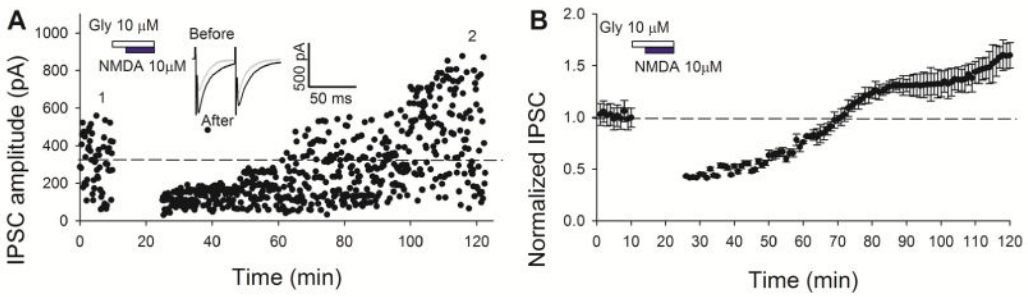


Fig. 3. Glycine could not block LTPGABA induced by NMDA

A, Glycine did not block LTP_{GABA} induced by activation of NMDA receptors in a dopamine neuron. After the baseline eIPSCs were recorded under voltage clamp and held at −70 mV, the recording was switched to current clamp mode. Glycine (10 μM) was bath-applied for 5 min, then a mixture containing glycine (10 μM) and NMDA (10 μM) was perfused for 10 min. Two minutes later, the mixture was washed out and the cell was again voltage clamped. DNQX (10 μM) was present throughout the recording. Inset: averaged IPSCs before (grey) and 80 min after washout of NMDA and glycine (black). B, Averaged data from five experiments using the protocol outlined in (A). IPSC amplitudes after washout of glycine were 142±6 % of pre-HFS values.

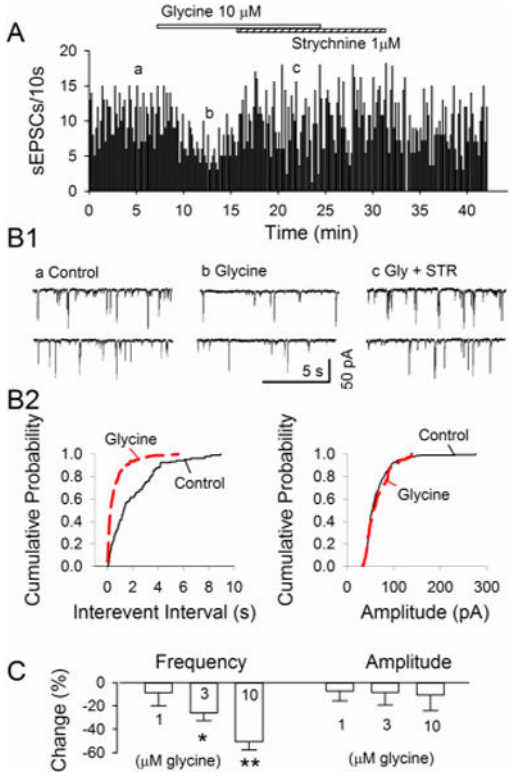


Fig. 4. Glycine, via activation of strychnine-sensitive receptor, decreases the frequency of spontaneous EPSCs (sEPSCs) recorded in VTA-dopamine neurons

A, Time course of glycine's effects on sEPSC frequency in a dopamine neuron in the absence and presence of strychnine (1 μM). B1, Representative sEPSCs recorded at the time points indicated in A. B2, left panel, the cumulative probability of sEPSC frequency of VTA-dopamine neurons before and after glycine ($p < 0.05$, Kolmogorov-Smirnov (K-S) test comparing with and without glycine). B2, right panel, the amplitude of sEPSCs did not differ among tests before and after glycine ($p > 0.3$, bootstrap). C, Summary of the dose-response relationship of glycine's effects on sEPSC frequency and amplitude from 6–13 neurons. (*$p < 0.05$, **$p < 0.01$, paired t test for application of glycine vs. pre-glycine control.)

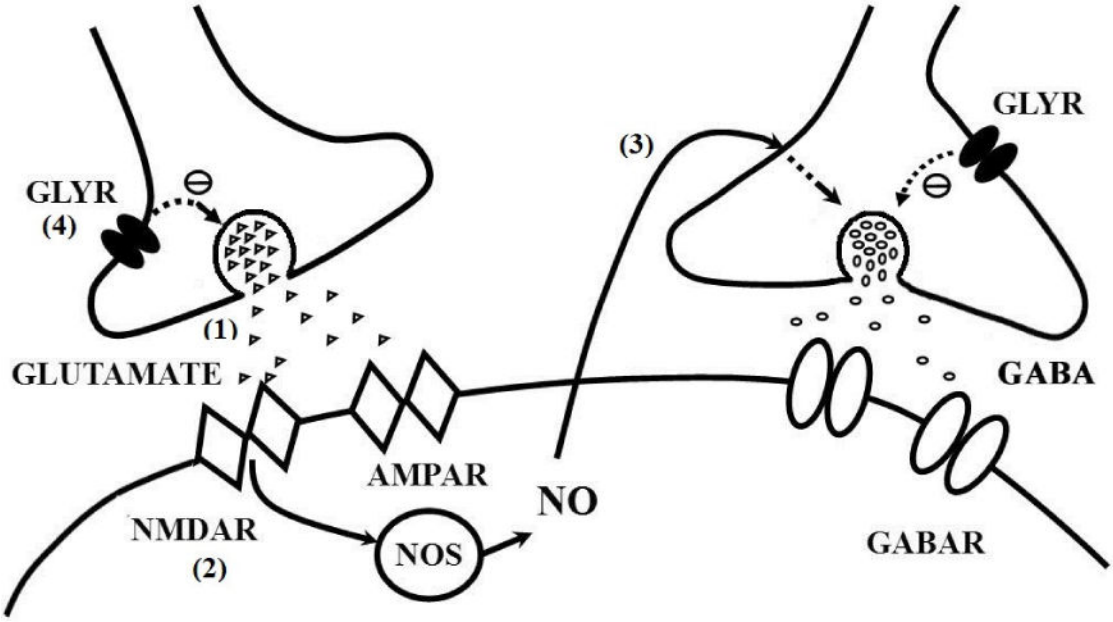


Fig. 5. Schematic diagram for glycine induced inhibition of LTP_{GABA}

In the absence of glycine, (1) HFS activates glutamate release from neurons innervating dopamine neurons; (2) NMDA receptor activation stimulates an influx of calcium into the dopamine neuron, which stimulates NO production; (3) NO diffuses to GABA neurons innervating the dopamine neuron to produce LTP_{GABA}. In the presence of glycine, (4) Strychnine sensitive glycine receptors on the glutamatergic nerve terminals innervating the dopamine neuron are activated to suppress release of glutamate; HFS no longer increases NO production within the dopamine neuron; LTP_{GABA} does not occur.