High frequency stimulation-induced plasticity in the prelimbic cortex of rats emerges during adolescent development and is associated with an increase in dopamine receptor function

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Abstract

Recent studies in rats suggest that high frequency stimulation (HFS) in the ventral hippocampus induces long-term depression (LTD) in the deep layer of the medial prefrontal cortex (mPFC), but only after the prefrontal GABA system has sufficiently developed during early- to mid-adolescence. It is not clear whether this LTD is specific to the hippocampus-mPFC circuit or is instead an intrinsic regulatory mechanism for the developed mPFC neuro-network. The potential mechanisms underlying this HFS-induced LTD are also largely unknown. In the current study, naïve male Sprague Dawley rats were sacrificed during peri-adolescence or young adulthood for *in vitro* extracellular recording to determine if HFS delivered in the prelimbic cortex (PLC) would induce LTD in an age-dependent manner and if dopamine receptors are involved in the expression of this LTD. We found four trains of stimulation at 50 Hz induced an LTD in the PFC of adult, but not peri-adolescent, rats. This LTD required intact GABA_A receptor functioning and could also be blocked by dopamine D₁ or D₂ receptor antagonists. Bath application of selective D₁ or D₂ receptor agonists produced a significant facilitation or suppression in the field potential, respectively, and these effects were only observed in the adult PLC. Furthermore, neither D₁ nor D₂ stimulation prior to HFS was able to facilitate LTD in the peri-adolescent PLC. Together, these results suggest dopamine receptor functionality in the PLC increases during adolescent development and it plays an important role in this late-maturing form of plasticity.

Keywords

adolescence; prefrontal cortex; GABA; plasticity;

1. Introduction

A large body of evidence, mostly from rodent models, suggests that the GABAergic system in the medial prefrontal cortex (mPFC) undergoes significant functional remodeling as individuals mature through adolescence into young adulthood (Erickson et al., 2002; Hashimoto et al., 2009; Fung et al., 2010; Caballero et al., 2014 a,b; Gonzalez-Burgos et al., 2015; Miyamae et al., 2017). One notable developmental change in prefrontal GABAergic cells is an increase in glutamatergic drive onto fast-spiking interneurons (FSIs), which results in an increased frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in the FSIs of adults compared to peri-adolescents (Caballero et al., 2014a). Accordingly, it was also demonstrated that the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) recorded in glutamatergic pyramidal cells in the adult mPFC was increased compared to those in adolescents (Caballero et al., 2014a). Meanwhile, the total number of GABAergic cells in the PFC does not change significantly from peri-adolescence to adulthood (Caballero et al., 2014a; Willing and Juraska, 2015), suggesting that prefrontal GABAergic interneurons may increase their synaptic influence on pyramidal cells and thereby become integrated into the mPFC neural network where they provide critically important inhibitory regulation (Caballero et al., 2016). Consistent with this hypothesis, a series of *in vivo* studies demonstrated some aspects of GABAergic modulation of mPFC neuronal activity emerge only after mid- to late-adolescence. Specifically, male rats that were ≥ 45 days old exhibited a long-term depression (LTD)-like change in the evoked field potential in the deep layer of the mPFC following high frequency stimulation (HFS; four trains at 50 Hz) delivered in the ventral hippocampus (Cass et al., 2013; Caballero et al. 2014b; Thomases et al., 2104). In contrast, identical HFS delivered in the basolateral amygdala induced long-term potentiation (LTP) in the deep layer of mPFC that was independent of the age of the rat

(Caballero et al., 2014b; Thomases et al., 2104). This ability of HFS in the ventral hippocampus to induce LTD in the mPFC may reflect a unique characteristic of the hippocampus-mPFC pathway, but it also may indicate that this late-emerging GABAergic modulation is an intrinsic property of the local network within the mPFC. A primary goal of the current study was to investigate the latter possibility by determining if a GABA-sensitive LTD could be induced *in vitro* by HFS within the mPFC of rats sacrificed at pre- and post-adolescent stages of development.

The second aim of the current study was to investigate the role of dopamine receptors in the expression of HFS-induced LTD in the mPFC. Early studies using juvenile rats showed that dopamine plays a prominent role in regulating inhibitory tone in the mPFC (Seamans et al., 2001, 2004), partially through altering the excitability of interneurons (Gorelova et al., 2002; Trantham-Davidson et al., 2004). Later, it was found that there are significant changes in the expression of PFC dopamine receptors throughout adolescent development (Andersen et al., 2000; Brenhouse et al., 2008; Naneix et al., 2012) and accordingly, the dopaminergic regulation of inhibition has been shown to be influenced by age (Paul and Cox, 2013). The delayed maturation of dopamine's function in the mPFC is also consistent with the finding that prefrontal GABAergic cells increase their responsiveness to dopamine receptor stimulation during adolescent development. For example, after rats are 50 days old, non-FSIs become responsive to dopamine D₁ receptor stimulation, whereas FSIs become responsive to D₂ receptor-mediated increases in excitability (Tseng and O'Donnell, 2007a). Notably, the timing of these functional changes in dopamine's effects on interneurons is similar to when HFS-induced LTD emerges in the mPFC (Caballero et al. 2014b). Thus, we hypothesized that the ontogeny of HFS-induced LTD involves functional changes in both D₁ and D₂ receptors from peri-adolescence to

adulthood such that the absence of LTD in the peri-adolescent mPFC is associated with a relatively reduced function of dopamine receptors.

2. Materials and Methods

2.1 Subjects

A total of 48 male Sprague-Dawley rats, which were offspring of breeders maintained in our facility, were used in these experiments. Rats from individual litters were weaned on postnatal day (P) 22. They were housed with same-sex littermates in groups of 2-3 per cage. Approximately half of the subjects were housed individually for about 24 hours before being sacrificed for recordings because their cagemates had been removed for recordings on the previous day. These cases were equally distributed across all experimental conditions. All rats were kept on a 12-h light/dark cycle (lights on at 0800 h) in a temperature-controlled room with food and water available *ad libitum*. All procedures were performed during the light period of the cycle, were consistent with the 'Principals of Laboratory Animal Care' (NIH Publication no. 85-23) and were approved by the IACUC at the University of Illinois, Urbana-Champaign, USA.

2.2 Brain slices preparation

Rats were assigned to be sacrificed for collection of brain slices when they were within the age ranges targeted for this study: P35-P45, P45-P55, P55-P65, P65-80, and P95-135. The mean (+ SEM) age at sacrifice for each of these groups, which were chosen based on previous studies of functional developmental of plasticity in the mPFC (Caballero et al., 2014b), was 37.4 ± 1.09 , 49.0 ± 0.91 , 59.5 ± 1.76 , 71.7 ± 1.2 , and 117 ± 6.92 days, respectively. On the day they were sacrificed, rats were deeply anesthetized with 55 mg/kg of sodium pentobarbital and decapitated. The brain was removed quickly and chilled in ice-cold, oxygenated slicing medium

containing (in mM): 2.50 KCl, 1.25 NaH₂PO₄, 10.0 MgCl₂, 0.50 CaCl₂, 26.0 NaHCO₃, 11.0 glucose, and 234 sucrose. Tissue slices (450 µm thickness) containing the prelimbic cortex (PLC) were then cut with a vibrating tissue slicer (Pelco EasiSlicer, Ted Pella INC.) in the coronal orientation and transferred to a holding chamber in artificial cerebrospinal fluid (aCSF) containing (in mM): 126 NaCl, 2.50 KCl, 26.0 NaHCO₃, 2.00 CaCl₂, 1.25 MgCl₂, 1.25 NaH₂PO₄, and 10.0 glucose). Slices were kept in the aCSF gassed with 95% O₂/5% CO₂ first at 32°C for 20 min and then room temperature for 1 h before recordings.

2.3 Field potential recording

Brain slices were transferred to a recording chamber (Harvard apparatus, Holliston, MA) and submerged under a thin film of aCSF (30-31°C) gassed with 95% O₂/5% CO₂. A dissecting stereoscope was used to identify specific regions of the slice and to aid in the placement of electrodes. Extracellular signals were obtained by using low resistance (300–600 kΩ insulated tungsten electrodes (FHC, Inc., Bowdoinham, ME) placed in the deep layer of PLC. Extracellular stimuli were delivered through a concentric bipolar tungsten electrode (FHC, Inc., Bowdoinham, ME) placed in either layer II/III or the deep layer of the PLC in line with the recording electrode as shown in the schematics (Fig. 1 and Fig. 2). Stimuli consisted of either single testing pulses (100 µs duration, 50-350 pA at 0.067 Hz) or 4 trains of HFS (100 pulses at 50 Hz per train, inter-train interval = 10 s). The intensity of the test pulse was adjusted to 50% of the intensity required to produce the maximum synaptic response. HFS trains or ligands were applied after a stable baseline response was obtained for at least 10 min. Ligands, which included bicuculline methiodide (BMI), SCH23390, sulpiride, DNQX, SKF38393, quinpirole or tetrodotoxin (TTX; all obtained from Tocris, Minneapolis, MN), were diluted in dimethyl sulfoxide (DMSO) or deionized water as stock solutions (10-40 mM) and kept at -20 °C before

use. For all the drug applications, 5-20 μ l stock solution or vehicle (DMSO or deionized water) was diluted in 200 ml aCSF gassed with 95% O₂/5% CO₂ (30-31°C) to the desired concentration (indicated in the figures or legends) and bath applied to the slice throughout the recording or for 10 min (indicated in the figures or legends). Recorded signals were amplified (1,000X), filtered (low pass =10 kHz), sampled at 20kHz and digitally stored.

2.4 Data analysis

The slope of the first 1-2 ms of the field excitatory postsynaptic potential (fEPSP) was averaged in 1-min bins. For each slice, the averaged slope was then normalized to the mean slope of its 10-min baseline. All data are presented as mean $\pm$ SEM. Statistical tests consisted of one- and two-way ANOVA (drug $\times$ age) with Tukey post hoc tests performed where appropriate.

3. Results

Field potential recordings were obtained in a total of 125 slices obtained from 48 male rats. A schematic diagram showing the approximate location of stimulating and recording electrodes is shown in Fig. 1. Single stimuli delivered in the superficial layer of the PLC typically produced one deflection in cells recorded from the deep layer of the PLC (Fig. 1A). Bath application of the AMPA receptor antagonist DNQX completely blocked this response, suggesting that this component is primarily mediated by an AMPA receptor-dependent postsynaptic response. Occasionally, we observed an additional deflection following single stimuli (Fig. 1B), which was resistant to DNQX. Further application of 1 μ M TTX completely blocked this early component, suggesting that the DNQX-insensitive deflection is a presynaptic fiber volley (Wilson and Cox, 2007). The fiber volley was not included in the analysis because it

did not appear in all the recordings and only the slope of the DNQX-sensitive component was used for analyses.

3.1 Developmental changes in HFS-induced, GABA-sensitive LTD in the PLC

As shown in Fig. 2, the effect of HFS on the fEPSP slope in the deep layer of the PLC was age-dependent. Following four trains of HFS delivered in the superficial layer, we found a sustained decrease of fEPSP slope in rats from groups older than P55 (Fig 1D-F). This was confirmed by significant one-way ANOVAs for the P65-80 and P95-135 groups ($F_{49,235} = 10.8$, $p < 0.001$ and $F_{49,261} = 3.19$, $p < 0.001$, respectively). In contrast, this same stimulation in younger rats failed to significantly change the fEPSP slope compared to baseline (Fig 1A-C). These effects were compared between groups by obtaining the mean fEPSP slope from the 30 to 40 min period following HFS (Fig. 1G). One-way ANOVA revealed a significant main effect of group ($F_{4,22} = 6.81$, $p = 0.001$). Post-hoc analysis suggested that the mean fEPSP slope in young adult rats ($\geq P65$) was significantly lower than that in peri-adolescent rats ($\leq P55$). Slices obtained from rats between P55 and P65 also exhibited a mean reduction in fEPSP slope to about ~80% of baseline, but this was not statistically significant.

To test whether the LTD we observed in the deep layer PLC of rats $\geq P55$ was the result of specifically stimulating deep layer afferents originating from the superficial layers of the PLC, we investigated the effects of applying HFS within the deep layer in slices from young adult rats ($\geq P65$). With electrodes positioned as schematically depicted in Fig. 3, we found that HFS failed to induce LTD and instead produced a transient increase in the fEPSP slope (Fig. 3B). However, one-way ANOVA of the time course data suggested no significant differences in the fEPSP slope across all time points ($F_{49,236} = 1.01$, $p > 0.05$). The mean fEPSP slope from 16-20

min and 46-50 min, along with baseline, is shown in Fig. 3C. One-way-ANOVA with repeated measures suggested no significant group difference ($F_{2,10}=2.44$, $p > 0.05$).

To test if delayed development of GABAergic signaling was involved in age-dependent differences in HFS-induced plasticity in the PLC, we studied the effects of inhibiting GABA signaling during recordings. Following applications of vehicle, we observed similar age-dependent differences in HFS-induced plasticity as we had seen previously (Fig. 4). Specifically, there was a significant decrease in fEPSP slope in slices from young adults (P65-P80; Fig. 4C), but no significant change in those from peri-adolescent rats (P35-P45; Fig. 4B). However, HFS delivered in the presence of the GABA_A receptor antagonist BMI (1 μ M) not only eliminated the LTD of the fEPSP, but led to a significant increase in the fEPSP slope in rats that were $\geq$ P65. There continued to be no significant effect of HFS in slices from peri-adolescent rats. Analysis of the mean slope of the fEPSP from 30-40 min post-HFS (Fig. 4D) revealed a significant main effect of drug ($F_{1,20} = 20.4$, $p < 0.001$) and a drug by age interaction ($F_{1,20} = 8.60$, $p = 0.008$). Post-hoc analysis suggested a significant effect of BMI only in the young adult group.

3.2 Potential role of dopamine in HFS-induced LTD

To determine if dopamine receptor activation was required for the HFS-induced LTD observed in young adult rats, we applied dopamine receptor antagonists to slices for the duration of recordings (Fig. 5). As shown in Fig. 5B, we found that HFS failed to produce a lasting decrease in the fEPSP slope with the presence of either the D₁ (SCH 23390; 10 μ M) or the D₂ receptor antagonist (sulpiride; 10 μ M). Analysis of the mean slope of the fEPSP from 30-40 min post-HFS with one-way ANOVA revealed a significant effect of group ($F_{2,13} = 18.61$, $p <$

0.001). Post-hoc comparisons suggested that both drug groups were significantly different from control (Fig. 5C).

We next determined if application of dopamine receptor agonists alone was sufficient to produce long-term changes in the fEPSP and if agonist-induced changes were age-dependent (Fig. 6). As shown in Fig. 6B, fEPSP recordings from both peri-adolescent and young adult brain slices remained stable and relatively unchanged following vehicle exposure. Following application of a D₁ agonist (SKF38393; 10 μ M), the fEPSP increased significantly in slices from young adult rats. There was also a small increase in the fEPSP from peri-adolescent rats, but this change was not significantly different from baseline (Fig. 6C). The D₂-like receptor agonist quinpirole (1 μ M) also had a small, but non-significant effect on the fEPSP in peri-adolescent rats. In slices from young adult rats, quinpirole (1 μ M) led to a significant decrease in the fEPSP (Fig. 6D) that was similar to the decrease observed in this age group following HFS. Analysis of the mean slope of the fEPSP from 30-40 min post-HFS (Fig. 6E) revealed a significant main effect of drug ($F_{2,23} = 50.1$, $p < 0.001$) and a drug by age interaction ($F_{2,23} = 13.7$, $p < 0.001$). Post-hoc tests suggested that within the young adult group, both the D₁ and D₂-like receptor agonist conditions were significantly different from vehicle. Within each drug condition, there was also a significant difference between the young adult and peri-adolescent groups.

To further elucidate the role of dopamine receptors in HFS-induced LTD, we next determined if their activation prior to delivery of the stimulating pulse would impact HFS-induced LTD. Vehicle exposure just prior to HFS delivery had no impact on the plasticity induced in slices from young adult rats (Fig. 7A). Stimulation of D₁ receptors increased the fEPSP in slices from young adult, but not peri-adolescent rats as we observed in our prior experiment (cf. Fig. 6B), but subsequent application of HFS reversed this effect and returned the

fEPSP to just below 100% of its baseline prior to drug application (Fig. 7B). Stimulation of D₂-like receptors (Fig. 7C) decreased the fEPSP in slices from both peri-adolescent and young adult rats to a similar extent that we had seen in our prior experiment (cf. Fig. 6C). Following HFS, the fEPSP recorded from young adult rats decreased further below baseline while the fEPSP recorded from peri-adolescents was relatively stable. Two-way ANOVA of the mean slope of the fEPSP 30-40 min post-HFS (Fig. 7D) revealed a significant main effect of drug ($F_{2,25} = 13.6$, $p < 0.001$) and age ($F_{1,25} = 25.0$, $p < 0.001$). Post-hoc tests showed that only the D₁ agonist significantly altered HFS-induced changes in the fEPSP and this was the case only in slices from young adult rats.

4. Discussion

Using *in vitro* extracellular recordings, the current study revealed that GABA-sensitive plasticity in the mPFC emerged in the late peri-adolescent period in male rats. Specifically, HFS delivered in the superficial layer of the PLC produced a persistent decrease in evoked field potential in the deep layer PLC in rats > P55 and this decrease was blocked by a GABA_A receptor antagonist. We further demonstrated that dopamine signaling was involved in the expression and, potentially, in the ontogeny of HFS-induced LTD in the PLC. This is evidenced by the findings that antagonism of either D₁ or D₂ receptors prevented the expression of HFS-induced LTD in the adult PLC and that both D₁ and D₂-like receptors in the peri-adolescent PLC were less responsive to their respective agonists when compared to the adult group. Together, these results indicate that a functional up-regulation in prefrontal dopamine receptors may be an important factor enabling a late-maturing GABA system to regulate the mPFC neural network.

As mentioned above, findings by Caballero and colleagues suggest a GABA-related developmental alteration in the deep layer cells that respond to hippocampal input (Caballero et al., 2014b). Besides afferent innervation from subcortical regions, deep layer cells in the mPFC also receive projections from the superficial layers (Yang et al., 1996). Several early investigations have demonstrated that under certain conditions, such as when GABA_A receptors are blocked and 3 μ M dopamine is bath-applied for 40 min prior to stimulation, HFS will induce long-term plastic change in glutamtergic transmission between the superficial and the deep layer in the juvenile or adult mPFC (Otani et al., 2003; Huang et al., 2004; Kolomiets et al., 2009; Sheynikhovich et al., 2013). However, GABA may play a prominent role in modulating HFS-related activity in the mPFC and significant developmental changes are likely to occur to this modulation during adolescence (Caballero et al., 2014b; Caballero et al., 2016; Caballero and Tseng, 2016). To test this hypothesis, we kept endogenous GABA transmission intact and compared the effect of HFS on the field potential across age. We found that HFS delivered within the PLC indeed produced an LTD and this effect was apparent only in rats > P55. We further confirmed that the expression of LTD was sensitive to the antagonism of GABA_A receptors, just as was demonstrated for the LTD observed in the hippocampus-mPFC circuit (Caballero et al., 2014b). In fact, with GABA_A receptors blocked, HFS from the hippocampus (Caballero et al., 2014b) or delivered in the superficial layer produces an LTP in the deep layer cells in the adult mPFC. Collectively, these data suggest that as the mPFC matures, cells in the deep layer become more responsive to HFS-induced plasticity and the net result of LTD may be due to a greater increase in inhibition relative to excitation. Such a significant shift in the excitation-inhibition balance may alter how information is processed in the mPFC network and

since it emerges when animals are approaching adulthood, this GABA-related function may be one of the limiting factors for the mPFC to fully mature (Caballero and Tseng., 2016).

The up-regulation in the GABA tone that may underly the ontogeny of HFS-induced LTD could be explained by a series of changes in interneurons and pyramidal cells during late-adolescence. GABAergic cells in the mPFC are generally categorized as FSIs and non-FSIs based on their electrophysiological properties. According to several studies, functional up-regulation after P45 in male rats seems to occur preferentially in FSIs that express parvalbumin (PV) protein (Caballero et al., 2013; Caballero et al., 2014a; Tseng and O'Donnell, 2007a). The FSIs increase in number and upregulate their level of PV protein expression, as well as become more responsive to glutamate (Caballero et al., 2013). Since FSIs are believed to provide a significant GABA influence in the mPFC, especially in layer V (Naka and Adesnik, 2016), these changes likely result in a gain in inhibitory tone in the mPFC during late peri-adolescence (Caballero et al., 2016). At the same time, pyramidal cells become more sensitive to GABA input during periadolescent development (Datta et al., 2015). Specifically, there is an increase in the mRNA of the α_1 subunit-containing GABA_A receptor in pyramidal cells and these receptors selectively respond to synaptic input from PV-containing cells (Klausberger et al., 2002). Meanwhile, the expression of α_5 -containing GABA_A receptors in pyramidal cells decreases progressively from adolescence to adulthood and these receptors are found in the apical dendrites responding to input from non-FSIs (Datta et al., 2015). These changes in α_1 - and α_5 -containing GABA_A receptors are suggested to shift the inhibitory tone in pyramidal cells from dendritic to a more robust perisomatic locus during periadolescent development (Datta et al., 2015). Accordingly, analysis of sIPSCs in pyramidal cells shows that these inhibitory currents tend to have a faster decay time in the adult compared to the peri-adolescent mPFC (Hashimoto et al.,

2009). Thus, as the mPFC matures, there is a potential increase in the maximum number of inhibitory currents that could occur in pyramidal cells at a given time. In addition, the expression of extrasynaptic GABA_A receptors that contain δ subunits increases significantly in layer V pyramidal cells from adolescence to adulthood and this may provide additional sensitivity to ambient GABA for tonic inhibition (Datta et al., 2015; Zheleznova et al., 2009). Together, these developmental changes in the interneuron-pyramidal cell interaction are predicted to lead to novel patterns of inhibitory modulation in the mPFC (Caballero and Tseng., 2016). Consistent with this notion, the current study and others (Caballero et al., 2014b) demonstrate that HFS triggers a prominent GABAergic modulation of population neural activity in the mPFC that only emerges in the relatively late stage of adolescent development. Future studies are required to reveal the potential functional impact of this LTD on the mPFC network.

In addition to development of GABA signaling, functional changes in dopamine receptors are also likely involved in the ontogeny of the HFS-induced LTD. As mentioned above, dopamine plays an important role in regulating inhibition in the mPFC (Seamans and Yang, 2004) and there are numerous developmental changes in dopamine receptors that may influence GABA functions (Paul and Cox, 2013; Tseng and O'Donnell, 2007a). Notably, peri-adolescent alterations in dopamine signaling by cocaine exposure has been shown to abolish the HFS-induced LTD in the ventral hippocampus-mPFC circuit in adulthood (Cass et al., 2013). To investigate the relationship between dopamine signaling and the HFS-induced LTD we observed, we first demonstrated that either D₁ or D₂ receptor antagonist prevented the expression of LTD. Notably, the moderate concentrations of SCH23390 and sulpiride used in the current study have been commonly seen or comparable to what have been used in other *in vitro* electrophysiological investigations (Banks et al., 2005; Paul and Cox., 2013; Seamans and Yang 2004; Xu and Yao,

2010) and would be unlikely to compromise the selectivity of these antagonists. Thus, both D₁- and D₂ dopamine receptors are necessary for HFS to induce LTD in the PLC.

Early investigations found that high concentration (>50 μ M) of dopamine or dopamine releaser applied to the mPFC slice suppresses fEPSP and produces an LTD-like response (Law-Tho et al., 1995; Mair and Kauer, 2007). In this regard, the HFS-induced LTD may be explained by dopamine's effect on the fEPSP and the absence of LTD in the adolescent mPFC may be due to a reduced dopamine receptor function. To test this hypothesis, we examined the effect of D₁ and D₂/D₄ agonists on the field potential in the adolescent and adult mPFC. These receptors have been suggested to play an important role in modulating both interneuron and pyramidal cell activity (Onn et al., 2006; Zhong and Yan, 2016) and their affinity to respective agonist increases during adolescence (Tarazi and Baldessarini, 2000). Moreover, we chose quinpirole to stimulate D₂/D₄ receptors so our results could be compared directly to several early findings showing developmental changes in the effect of this drug on evoked EPSP (Tseng and O'Donnell, 2007b) and interneuron excitability (Tseng and O'Donnell, 2007a) in the mPFC. We found that quinpirole produced a persistent decrease in the field potential that seemed to mimic an LTD while SKF38393 led to a significant increase in the field potential. In contrast, neither of these drugs produced significant changes in brain slices from rats sacrificed between P35 and P45. These results support our hypothesis that there is a significant functional increase in dopamine receptors after P45, which may be a contributing factor for the ontogeny of the HFS-induced LTD. However, the effects of D₁ and D₂/D₄ stimulation that we observed in the adult mPFC appear contradictory to our finding that both D₁ and D₂ antagonist blocked the expression of LTD, especially because the D₁ agonist led to an increase in the field potential that is opposite to an LTD.

It is notable that approximately 75-80% of D₁, D₂ and D₄ receptors in the mPFC are located on pyramidal cells (Santana et al., 2009; Almeida and Mengod, 2010). Thus, it is likely that the effects we observed of the dopamine agonists were primarily driven by the actions of these drugs on pyramidal cells. In fact, the effects of these agonists on the field potential, as well as the age-related changes we observed, are consistent with previous studies showing dopamine's effect on excitatory currents or potentials in pyramidal cells. For example, in rats $\geq$ P45, selective activation of D₁ receptors was found to increase NMDA receptor-mediated depolarization in the deep layer pyramidal cells in the mPFC (Tseng and O'Donnell, 2005; Flores-Barrera et al., 2014). Quinpirole was found to decrease the AMPA receptor-mediated EPSP in deep layer pyramidal cells and this effect was more pronounced in rats older than P50 (Tseng and O'Donnell, 2007b). In this regard, the expression of HFS-induced LTD may not mainly rely on dopamine's action on the pyramidal cells. This notion is supported by our receptor occlusion results. In the adult mPFC, HFS was able to substantially decrease the field potential after 10 min application of either SKF38393 or quinpirole. Together, our results indicate that the HFS-induced LTD is unlikely to be caused by global activation of dopamine receptors in the mPFC, or more specifically in pyramidal cells, but may be relying more on dopamine-mediated changes in interneurons. This hypothesis is consistent with developmental changes in the dopamine release pools in the mPFC. It is known that dopamine cells in the ventral tegmental area project directly onto superficial and deep layers in the mPFC and contact both pyramidal cells and interneurons (Benes et al., 1993; Sesack et al., 1995). During peri-adolescent development, there is a significant increase in the percentage of GABA cells that have dopamine varicosities nearby as well as the number of dopamine varicosity near a single GABA cell (Benes et al., 1996). Therefore, it is possible that dopamine release following HFS may have

an increased impact on interneurons in adulthood comparing to peri-adolescence, resulting in an overall suppression in synaptic transmission.

Based on the available evidence from the literature, we speculate that the HFS-induced LTD is an augmented inhibition that masks potential changes in excitation rather than a mere reduction in excitation. The putative enhanced inhibition is due to, at least partially, D₁- and D₂-like receptor mediated facilitation in the GABAergic output (Tseng and O'Donnell, 2007a). This hypothesized mechanism is based on the following. First, it is well established that dopamine elevates inhibitory tone in adult mPFC and the increase in inhibitory function could last over 30-40 min (Seamans et al., 2001; Kroner and Lavin, 2012; Paul et al., 2013; Kang et al, 2016). This prolonged action of dopamine on inhibition seems consistent with the post-HFS changes found in the current study. Second, non-FSIs and FSIs start to respond to the excitatory effect of D₁ and D₂/D₄ agonists, respectively, after P50 (Tseng and O'Donnell, 2007a). The timing when these additional dopamine-mediated inhibitory influences start to appear in the mPFC is close to when HFS-induced LTD is possible. Lastly, the enhanced inhibition following HFS is not inconsistent with the potential increase in excitation that may likely co-exist at a limited set of synapses and can not be detected in the populational responses in the current study (Laroche et al., 2000). The potentially increased glutamate transmission may provide further drive on nearby interneurons and the net effect is a reduction in the populational activity. An alternative explanation is that the adult-level GABA inhibition limits the post-synaptic depolarization following HFS, resulting in a low Ca²⁺ rise that is known to lead to LTD (Hirsch and Crepel, 1992; Collingridge et al., 2010). The absence of LTD in peri-adolescent PLC could be due to a relatively weak and immature GABA tone that is unable to attenuate the HFS-induced depolarization. Future studies are required to directly test these hypotheses.

Another noteworthy finding in the current study is that the HFS delivered in the deep layer did not produce significant changes in the cells that are also in the the deep layer. Because the deep-layer pyramidal cells in the mPFC also receive intra-layer glutamate influence, we sought to determine if there is also a GABA-sensitive LTD regulates intra-layer high frequency transmission. However, it seems that the GABA-sensitive LTD in the deep layer is elicited specifically by high frequency superficial layer input. In contrast to our results, a recent *in vitro* study showed that the HFS delivered in the superficial layer in the mPFC produced an LTP in the same layer (Konstantoudaki et al., 2018) and such LTP was insensitive to GABA_A receptor antagonist. These differential expressions of synaptic plasticity could be explained by the cellular arrangement in the mPFC. For example, the intra-superficial layer LTP could be mediated by the horizontal “long range” glutamate projections between pyramidal cells specifically found in layer 2/3 (Kritzer and Goldman-Rakic, 1995). In contrast, layer V pyramidal cells and surrounding interneurons are hypothesized to be organized in aggregates that resemble the structure of columns (as in non-human primates) or barrels (as in rodents) in other neocortexes (Naka and Adesnik, 2016). Activity in layer V pyramidal cells readily recruits surrounding interneurons, which in turn elicits inhibition on nearby excitatory and inhibitory cells (Naka and Adesnik, 2016). This arrangement may be important for confining activity in a given neural aggregate or column to prevent unnecessary influences on neighboring columns. In this regard, the lack of significant changes following HFS delivered in the deep layer could be due to the inter-column inhibition that did not allow signal propagation between columns. However, it is not clear if we would have seen any significant changes in the field potential when the electrode placement had been further manipulated. Future investigations are required to determine the cellular and molecular mechanisms underlying this LTD.

In conclusion, our results reveal a GABA-sensitive regulation in the deep layer mPFC that emerges during late-adolescence and is activated by HFS, which is in keeping with several *in vivo* findings (Caballero et al., 2014b; Cass et al., 2013). While the functional significance of this GABA-regulation requires further investigation, our results suggest that it is expressed when the HFS also becomes able to produce LTP in the mPFC. Perhaps as suggested earlier, this inhibitory function may serve as an “anti-LTP” or a threshold control mechanism to prevent too much LTP saturating in the mPFC network (Laroche et al., 2000). With a focus on the developmental changes in mPFC, the current study further demonstrates an increase in the dopamine receptors that may play an important role in the maturation of the HFS-induced LTD. Given the well-studied dopamine-GABA interplay, this HFS-induced LTD may also serve to regulate the signal-to-noise level in the deep layer output cells (Seamans et al., 2001; 2004; Caballero and Tseng, 2016). Furthermore, the much delayed maturation of dopamine and GABA system revealed in the current study indicates a potential window of vulnerability (Cass et al., 2013; Thomases et al., 2014) in the late-adolescence when humans start to experiment with drugs that have high potential in disrupting the normal development of these neurotransmitter systems (Gulley and Juraska, 2013).

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Figure Legends

Figure 1. Schematic diagram of stimulating and recording electrode placements in the prelimbic cortex (PLC) and sample traces showing the components of the fEPSP that were analyzed.

Single testing pulses (100 μ s duration; 50-350 pA at 0.067 Hz) produced either single (**A**) or double (**B**) deflections that could be blocked by DNQX (10 μ M) or DNQX (10 μ M) + TTX (1 μ M). Calibration axes: 50 μ V for the ordinate; 50 ms for the abscissa in A, 20 ms for the abscissa in B.

Figure 2. Age-dependent differences in deep layer PLC plasticity following HFS delivered in superficial layer PLC. (**A-E**) Effects of HFS delivered in layer 2/3 on the fEPSP slope recorded in layer 5/6 from rats in the indicated age range ($n = 5-6$ slices obtained from 4 rats/age group). The shaded vertical bar indicates the HFS period. **F.** Mean fEPSP response during the 30-40 min period following HFS for each age group. $**p < 0.001$ vs. P35-P45; $*p < 0.05$ vs. P35-P45; $^{\#}p < 0.05$ vs. P45-P55.

Figure 3. The effect of intra-laminar HFS on the evoked field potential in the deep layer PLC from young adult rats (P65-P80, $n = 6$ slices from 4 rats). **A.** Schematic diagram of the location for stimulating and recording electrodes, which were placed in layer 5/6 approximately 400-450 μ m apart. **B.** Effects of HFS (period indicated by shaded vertical bar) on fEPSPs. **C.** Mean fEPSP response during the 10-min baseline and the relatively early (16-20 min) and late (46-50 min) periods following HFS.

Figure 4. The effect of GABA_A antagonist bicuculline (BMI) on HFS-induced plasticity in the PLC of peri-adolescent (P35-P45) and young adult rats (P65-P80). **A.** Representative traces

sampled before (Pre-drug) and after (Baseline) application of 1 μ M BMI or vehicle (VEH) and following HFS (Post-HFS; calibration: 50 μ V, 20 ms). **B-C.** Time course of the fEPSP slope before and after HFS (shaded vertical bar) in peri-adolescent and young adult rats (n=5-6 slices from 3 rats per age group). Slices were exposed to BMI or VEH for the duration of the recording shown. **D.** Mean fEPSP response from the 30-40 min period post-HFS. * $p < 0.05$ vs. P35-P45 VEH; ^{##} $p < 0.001$ vs. P65-P80 VEH.

Figure 5. The role of dopamine D₁ and D₂ receptors in HFS-induced plasticity in the PLC of young adult rats (P65-P80). **A.** Representative traces sampled before application of vehicle (VEH), the D₁ antagonist SCH23390 (10 μ M) or the D₂ antagonist sulpiride (10 μ M), which are labeled Pre-drug, after their application (Baseline), and following HFS (Post-HFS; calibration: 50 μ V, 20 ms). **B.** Time course of the fEPSP slope before and after HFS (shaded vertical bar) in young adult rats (n = 6 slices from 3 rats per age group. The time course shown includes the Baseline and Post-HFS periods (i.e., slices were exposed to VEH or a dopamine receptor antagonist for the duration of the recording shown). **C.** Summary of the mean fEPSP response from the 30-40 min period post-HFS. * $p < 0.05$ vs VEH; ** $p < 0.001$ vs VEH.

Figure 6. Age-dependent effect of dopamine D₁ and D₂ receptors agonists on evoked fEPSP in the PLC of peri-adolescent (P35-P45) and young adult (P65-P80) rats. **A.** Representative traces sampled before and after application of VEH or drug (Pre-drug and Post-drug, respectively; calibration: 50 μ V, 20 ms). **(B-D)** Time course of the fEPSP slope before and after application of VEH, the D₁ agonist SKF38393 (10 μ M) or the D₂ agonist quipirole (1 μ M). The 10-min application period is indicated by the horizontal bar (n = 4-6 slices from 3 rats per group). **E.**

Summary of the mean fEPSP response from the 30-40 min period after VEH or drug application.

****** $p < 0.001$ vs VEH within age group; **#** $p < 0.05$ vs P35-P45 within drug condition.

Figure 7. Age-dependent effect of dopamine D₁ and D₂ receptors agonist paired with HFS on evoked fEPSP in the PLC (n = 4-6 slices from 3 rats per group). (**A-C**). Time course of the fEPSP slope before and after application of VEH, the D₁ agonist SKF38393 (10 μ M) or the D₂ agonist quipirole (1 μ M). The 10-min application period is indicated by the horizontal bar. The shaded vertical bar indicates the HFS period. **D**. Summary of the mean fEPSP response from the 30-40 min period post-HFS. ****** $p < 0.01$ vs VEH within age group; **##** $p < 0.01$ vs P35-45 within drug condition.

Figure 1

Figure 2

Figure 3

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Figure 7