Frequency-dependent gating of feedforward inhibition in thalamofrontal synapses

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Thalamic recruitment of feedforward inhibition is known to enhance the fidelity of the receptive field by limiting the temporal window during which cortical neurons integrate excitatory inputs. Feedforward inhibition driven by the mediodorsal nucleus of the thalamus (MD) has been observed, but its physiological function and regulation remain unknown. Accumulating evidence suggests that elevated neuronal activity in the prefrontal cortex is required for short-term storage of information. Furthermore, the elevated neuronal activity is supported by the reciprocal connectivity between the MD and the medial prefrontal cortex (mPFC). Therefore, detailed knowledge about the synaptic connections during high-frequency activity is critical for understanding the mechanism of short-term memory. In this study, we examined how feedforward inhibition of thalamofrontal connectivity is modulated by activity frequency. We observed greater short-term synaptic depression during disynaptic inhibition than in thalamic excitatory synapses during high-frequency activity. The strength of feedforward inhibition became weaker as the stimulation continued, which, in turn, enhanced the range of firing jitter in a frequency-dependent manner. We postulated that
this phenomenon was primarily due to the increased failure rate of evoking action potentials in parvalbumin-expressing inhibitory neurons. These findings suggest that the MD-mPFC pathway is dynamically regulated by an excitatory-inhibitory balance in an activity-dependent manner. During low-frequency activity, excessive excitation is inhibited, and firing is restricted to a limited temporal range by the strong feedforward inhibition. However, during high-frequency activity, such as during short-term memory, the activity can be transferred in a broader temporal range due to the decreased feedforward inhibition.

Keywords
thalamofrontal, feedforward inhibition, mediodorsal nucleus of the thalamus, dorsal anterior cingulate cortex, short-term memory
Introduction

The activity patterns of inhibitory neurons play a critical role in sculpting cortical network dynamics. Thalamic excitatory inputs diverge on both excitatory and inhibitory cortical neurons, generating disynaptic feedforward inhibition. Despite the fact that thalamic efferent inputs on parvalbumin-expressing (PV) interneurons are bifurcated from the same set of axons, their pre- and postsynaptic mechanisms tend to be stronger than those on principal neurons [1–3]. Furthermore, the connection probability of GABAergic interneurons is remarkably higher than that of pyramidal neurons [4–7]. Thereby, feedforward inhibition dominates the excitatory responses and limits the temporal window for integration of excitatory thalamic inputs (hereafter referred to as the “integration window”) [8,9]. It has been well accepted that feedforward inhibition sharpens spatial and temporal discrimination of sensory information [2,10,11]. In the medial prefrontal cortex (mPFC), the mediodorsal thalamic nucleus (MD) drives feedforward inhibition in the dorsal anterior cingulate cortex (dACC) via local parvalbumin-expressing GABAergic neurons [12]. The increased and sustained neural activity of the mPFC has been widely believed to be the substrate of short-term memory [13,14]. Studies in monkeys and rodents have demonstrated that functional interaction with the reciprocally connected MD is critical for maintaining working memory [15–17]. Specifically, interrupting this interaction caused coincident increases in firing in the MD, reduced reverberant activity of the PFC, and reduced performance of short-term memory-dependent tasks [13,15,16,18]. In many of these experiments, recorded units in the mPFC as well as in the MD during the delay period of the tasks exhibited high-frequency firing, often over 10 Hz [17,19,20].
Although feedforward inhibition mediated by cortical PV neurons has been described previously [12], the mechanism underlying the modulation of feedforward inhibition during high-frequency activity has never been examined. In this study, we examined how feedforward inhibition in MD-dACC connectivity is modulated during high-frequency activity with whole-cell recordings with optogenetic stimulation to better understand how feedforward inhibition is modulated during short-term memory.
Methods

Animals

Genetically modified mouse lines were purchased from Jackson Laboratories and bred in-house. The PV-Cre mouse line (B6;129P2-Pval^tm1(cre)Arbr/J; JAX stock #008069) was used to target PV-expressing interneurons. The PV-Cre mouse line was bred with Ai9 mice (B6.Cg-Gt(Rosa)26Sor^tm9(CAG-tdTomato)Hze/J; JAX stock #007909) to identify PV neurons. PV interneurons of the PV:Cre/Ai9 line express robust tdTomato fluorescence following Cre-mediated recombination. While maintaining a live colony, both PV-Cre and Ai9 transgenic lines were bred as homozygotes. Mice were housed under a 12-hour light-dark cycle with freely available food and water. Only male mice were used to avoid the potential effect of the estrus cycle. All care procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Korea Brain Research Institute (M2-IACUC-19-00040).

Viral vectors and stereotactic surgeries

Animals were anesthetized with ketamine (100 mg/kg) supplemented with dexmedetomidine hydrochloride (0.4 mg/kg) by intraperitoneal injection and positioned in a stereotaxic injection frame (Kopf instruments). Ketoprofen (5 mg/kg) was subcutaneously injected for anti-inflammation. During the surgery, responses to pedal withdrawal reflex stimuli were absent. Virus injection was conducted followed by a stereotaxic surgical procedure.

An EF1a-driven, Cre-dependent, humanized channel rhodopsin (hChR2) H134R mutant fused to enhanced yellow fluorescent protein (eYFP) for optogenetic activation (Addgene # 20298-AAV1) and hSyn-Cre (Addgene # 105553-AAV1) was transduced.
by adeno-associated virus serotype 1 (AAV1). Between postnatal days 40–50, the mixture (50:50) of AAV1-double floxed-H134R and AAV1-Cre was unilaterally injected into the MD of the PV:Cre/Ai9 mouse. To derive PV-induced iPSCs in the PFC, AAV1-double floxed-H134R was bilaterally injected into the PV-Cre mouse PFC. Approximately 70 nL of virus solution ($10^{12}$ virus particles/mL) was delivered with a glass micropipette (Drummond Scientific) through a small skull window (1–2 mm²). To avoid leakage into surrounding brain areas, we left the injection pipettes in the brain for 6 min after injection before slowly withdrawing them. The injections were performed using the following stereotaxic coordinates: anterior-posterior -1.58 mm/medial-lateral ±0.30 mm/dorsal-ventral -3.10 mm (MD coordinates from the bregma) and anterior-posterior 1.75 mm/medial-lateral ±0.30 mm/dorsal-ventral -1.00 mm (PFC coordinates from the bregma). During all surgical procedures, the animals were kept on a heating pad in an isolated cage and were brought back to their home cages upon regaining movement. For optimal virus expression, all mice were euthanized at least 3 weeks after surgery.

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Mice aged 9–10 postnatal weeks were euthanized by exposure to CO$_2$ followed by decapitation. The brains were quickly and carefully removed in ice-cold dissection solution (in mM): 25 sodium bicarbonate (NaHCO$_3$), 1.25 sodium monophosphate (NaH$_2$PO$_4$), 25 D-Glucose, 2.5 KCl, 7 MgSO$_4$·6H$_2$O, 0.5 CaCl$_2$, 110 choline chloride, 11.61 (+) sodium-L-ascorbate, and 3 sodium pyruvate. The measured osmotic concentration was between 320–330 mOsm. Acute 300-μm thick brain slices were prepared via coronal sections with a vibratome (Leica VT1200S) in ice-cold dissection
solution. The composition of artificial cerebrospinal fluid (aCSF) was as follows (in mM): 119 NaCl, 2.5 KCl, 1 MgSO$_4$·7H$_2$O, 26 sodium bicarbonate (NaHCO$_3$), 1.25 sodium monophosphate (NaH$_2$PO$_4$), 20 D-glucose, 0.4 L-ascorbic acid, 2 CaCl$_2$, and 2 pyruvic acid. The measured osmotic concentration was between 305–310 mOsm. After 30 min of recovery time in warmed aCSF (32 °C), slices were transferred to room temperature. For each mouse, PFC slices were prepared first and then slices around the MD were prepared to ensure that slices of the injection sites were obtained. Mice were excluded from data analysis whenever the virus expression overflowed outside the MD. The dACC L2/3 pyramidal neurons and PV interneurons were recorded either by voltage clamping or current clamping for the following procedures. PV neurons were discerned visually and electrophysiologically by measurements of intrinsic properties. PV interneurons exhibited higher firing rates with little adaptation and a lower AP threshold. Recordings were performed with patch pipettes (3.5–4 MΩ) filled with an internal solution that consisted of the following components (in mM): 20 KCl, 125 K-gluconate, 10 HEPES, 4 NaCl, 0.5 EGTA, 4 ATP, 0.3 Tris-GTP, and 10 phosphocreatine with a pH adjusted to 7.20 with KOH. The measured osmotic concentration was between 307–314 mOsm. Recordings were performed at room temperature.

Optogenetic stimulation was applied with a 1 ms light pulse with a 470 nm laser source; the light was guided with an optic fiber placed within 1 mm from the recorded neurons. The total power of the laser measured at the tip of the fiber by Power Meter (Thorlabs) was ~5 mW/mm$^2$.

We measured the resting potential of all neurons in current-clamp mode immediately after rupture of the neuronal membrane. Input resistance was determined by measuring
the voltage change in response to a small hyperpolarizing current pulse (5 pA, 50 ms) at resting potential. Spike threshold was acquired by 20 pA step increments of current injection and determined as the point at which the first AP was evoked. Series resistance was observed throughout the entire experiment and was not compensated. Cells with series resistances over 20 MΩ were excluded.

All solutions were kept saturated with 95% O₂ and 5% CO₂. Acute slices were continuously perfused with aCSF at room temperature. All data were sampled at 20 kHz by the EPC-10 amplifier (HEKA Elektronik) with Patchmaster software (HEKA Elektronik) and further analyzed by MATLAB (Mathworks).

**Drug application**

The following drugs were perfused in aCSF: 100 µM AP5 ((2R)-amino-5-phosphonovaleric acid, an NMDA receptor antagonist, Tocris; IC₅₀ = ~50 µM), 10 µM CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, an AMPA/kainate receptor antagonist, Tocris; IC₅₀ = 1.5 µM), 2 µM bicuculline (a GABA_A receptor antagonist, Sigma-Aldrich; IC₅₀ = 2 µM), 0.5 µM TTX (tetrodotoxin, a Na⁺ channel blocker, Abcam; IC₅₀ < 10 nM), and 100 µM 4-AP (4-aminopyridine, a Kv1 channel blocker, Tocris; IC₅₀ = 147 µM). All drugs were perfused throughout the experimental protocol and washed out for at least 30 min after the end of the protocol.

**Statistics**

Data analysis was performed using MATLAB (Mathworks), and statistics were acquired by GraphPad Prism 6.0 (GraphPad Software). Data are presented as the means (standard deviation) unless otherwise noted. Parametric or non-parametric tests were
employed according to the normality tests. Statistical analyses were performed using a two-tailed Student’s t-test for comparison of two groups. For comparisons across more than two groups, data were analyzed using one-way ANOVA followed by Tukey’s post hoc analysis to correct for multiple comparisons. For data with non-normal distribution, the non-parametric Mann-Whitney or Wilcoxon signed-rank tests were used. P value < 0.05 was considered significant.
Results

To selectively examine the thalamofrontal synapses and recruited local inhibitory inputs, we transduced the MD with adeno-associated virus (AAV)-channelrhodopsin-2 (ChR2) (Fig. 1). As previously described [12], optogenetic stimulation delivered to thalamofrontal axons with a 470-nm laser evoked both excitatory and inhibitory synaptic currents on pyramidal neurons in the dACC L2/3. We observed a large depolarizing inward current near the reversal potential of chloride (Fig. 1A). When the membrane voltage (Vm) was clamped near 0 mV, we observed a hyperpolarizing outward current which was sensitive to the GABA\(_A\) receptor antagonist bicuculline (2 µM), suggesting that the current was an inhibitory synaptic current, i.e., an IPSC (Fig. 1A). The IPSCs were also completely blocked by the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline (CNQX, 10 µM), indicating that the observed IPSCs were not directly from the MD but from local inhibitory neurons excited by the MD [12]. To further confirm that the IPSCs were current from disynaptically connected interneurons, we compared the onset latency of IPSCs with that of excitatory postsynaptic currents (EPSCs, Fig. 1A, right). As expected, the onset of the IPSCs appeared significantly delayed (EPSCs: 7.06 ± 0.62 ms; IPSCs: 10.58 ± 0.96 ms; 17 cells, P = 0.0008, paired t-test; Fig. 1B). The observed delay was 3.52 ± 0.86 ms and corroborated well with the synaptic delays described in previous studies [12,21]. We concluded that feedforward inhibition is driven by activation of MD axons in the dACC.

We then examined how the temporal window of excitation is modulated by high-frequency MD activity. We voltage-clamped neurons at -30 mV and observed a series of optogenetic stimulus-evoked biphasic inward and outward currents (Fig. 1C). We
defined the integration window as the temporal duration of the net inward current in this condition (Fig. 1D) [2,22]. The measured integration window upon a low-frequency stimulus was approximately 6.02 ± 0.88 ms (Mean ± range). Upon high-frequency (5 Hz or 10 Hz) MD activity, the width of the integration window increased significantly (Fig. 1F–G). The normalized length of the integration window gradually increased as the stimulation continued (Fig. 1F–G). The length of the integration window upon the fifth stimulus was approximately five-fold wider than that of the first window (5 Hz: 6.45 ± 1.37 ms vs 30.01 ± 13.14 ms, 9 cells; 10 Hz: 5.58 ± 1.18 ms vs 21.33 ± 8.43 ms, 9 cells, mean ± range).

Figure 1. The thalamofrontal integration window increases at high frequency. (A) Representative traces of the synaptic current. An EPSC measured at 0 mV (outward
black trace) and an IPSC in the presence of a GABA$_A$ receptor antagonist (2 µM bicuculline, green trace). An EPSC measured at -70 mV (inward black trace) and an EPSC in the presence of 10 µM CNQX (orange trace). An NMDA channel-dependent current was ruled out by 100 µM (2R)-amino-5-phosphonopentanoate (APV) throughout the experiment. Magnified EPSC and IPSC traces around the onset of the synaptic currents (inset). Each inflection point of the EPSC and IPSC was defined as the onset and used to calculate the onset latency. (B) The onset latency of the thalamofrontal IPSC on pyramidal cells was longer than that of the EPSC (17 cells, ***p=0.0008, paired t-test, parametric). (C) Example trace of EPSC/IPSC complex sequence at -30 mV with 5 Hz optogenetic thalamofrontal stimulation. (D) An example trace showing how the integration window (IW) was measured, namely, as the duration of the net inward current in EPSC-IPSC sequences. (E) An EPSC-IPSC sequence with and without 2 µM bicuculline (Vhold = -30 mV, red). (F−G) The normalized length of the IW at 5 Hz (F) (9 cells, *P$_{stim2}$=0.023, **P$_{stim3}$=0.0039, *P$_{stim4}$=0.012, **P$_{stim5}$=0.0078, paired t-test, non-parametric) and 10 Hz (G) (9 cells, *P$_{stim2}$=0.016, **P$_{stim3}$=0.0078, **P$_{stim4}$=0.0039, **P$_{stim5}$=0.0078, paired t-test, non-parametric).

Weakened feedforward inhibition during high frequency predicts greater temporal variability of action potential (AP) generation. We measured AP jitter between the stimulus and AP peaks with whole-cell voltage recordings and optogenetic stimulation of MD axons (Fig. 2). As expected, we observed greater AP jitter during high-frequency activity of MD axons. The firing ranges of the frequencies were initially 22.48 (16.42) ms at 0.1 Hz, 17.59 (18.47) ms at 5 Hz, and 15.92 (8.30) ms at 10 Hz each. After five
consecutive stimulations, the ranges became much broader with high-frequency activity (13.96 (6.02) ms at 0.1 Hz, 33.66 (16.81) ms at 5 Hz, and 37.56 (23.38) ms at 10 Hz).

Figure 2. The dACC L2/3 pyramidal spike output jitters along with high-frequency stimulation. (A–C) Superimposed traces of voltage changes due to thalamofrontal optogenetic stimulation at 0.1 Hz (A, 9 cells), 5 Hz (B, 53 cells), and 10 Hz (C, 48 cells). Light stimulations (1 ms) were shown as a blue vertical line. (D) The time-to-peak of optogenetically evoked APs after the first and fifth stimulation at the indicated frequencies. The stimulation points were shown as a blue horizontal line.
These results demonstrate that feedforward inhibition, and thereby the integration window of dACC neurons, are dynamically regulated in a frequency-dependent manner. Next, we examined the circuit mechanisms by which the integration window is selectively widened in the presence of high-frequency MD activity. Lengthening of the integration window can occur by the facilitation of EPSCs [23] and/or depression of IPSCs [2]. The first possibility is unlikely because MD-to-mPFC synapses, similar to other thalamocortical synapses, are known to have a high probability of neurotransmitter release and tend to be depressed by high-frequency stimulation [24]. Therefore, we focused on the possibility of reduced feedforward inhibition [2,8]. To test this idea, we compared the changes in thalamofrontal EPSCs and feedforward IPSCs during high-frequency activity (Fig. 3). As previously shown, MD-dACC synapses were depressed strongly during high-frequency activity [24]. However, the indirect inputs via cortical inhibitory neurons showed a remarkably faster short-term depression [25] (Fig. 3B–C). The amplitude of IPSCs was depressed to 0.10 ± 0.04 and 0.10 ± 0.02 of the first amplitude by five consecutive stimuli at 5 Hz and 10 Hz, respectively (5 cells, mean ± standard error), whereas the amplitude of EPSCs was depressed to 0.37 ± 0.10 and 0.37 ± 0.10 of the initial current by five consecutive stimuli at 5 Hz and 10 Hz, respectively (7 cells, mean ± standard error).

The reduced feedforward IPSCs accompanied by widened integration windows can be explained by the failure or delayed onset of APs in the inhibitory neurons. In other words, the strong short-term depression of the MD-driven synaptic current during tetanic stimulation could lengthen the time needed or even fail to evoke AP(s) in the
cortical inhibitory neurons. We first examined this possibility by comparing the onset of EPSCs and IPSCs during a series of stimulations (Fig. 3D–E). Supporting the idea of delayed AP onsets, we found that the temporal differences between the onsets of the EPSCs and the feedforward IPSCs were more pronounced with consecutive stimulations.

Figure 3. Feedforward IPSCs depress faster than EPSCs. (A) Sample traces of an EPSC and IPSC in dACC L2/3 pyramidal neurons. (B–C) Normalized amplitudes of an EPSC and IPSC at 5 Hz (B) and 10 Hz (C) (7 and 5 cells were recorded for the EPSC and IPSC, respectively; 5 Hz P_{stim2}=0.35, P_{stim3}=0.28, P_{stim4}=0.073, *P_{stim5}=0.047, unpaired t-test, parametric; 10 Hz P_{stim2}=0.17, P_{stim3}=0.10, *P_{stim4}=0.050, *P_{stim5}=0.032, unpaired t-
Onset latencies of EPSC and IPSC after consecutive stimulations at 5 Hz (D) and 10 Hz (E) (17 and 8 cells were recorded for the EPSC and IPSC, respectively; EPSC 5 Hz, $P_{\text{stim2}}=0.080$, $*P_{\text{stim3}}=0.013$, $****P_{\text{stim4}}<0.0001$, $\text{**P}_{\text{stim5}}=0.0011$, unpaired t-test, parametric; EPSC 10 Hz, $P_{\text{stim2}}=0.40$, $***P_{\text{stim3}}=0.0008$, $**P_{\text{stim4}}=0.0038$, $**P_{\text{stim5}}=0.0082$, unpaired t-test, parametric; IPSC 5 Hz, $***P_{\text{stim2}}=0.0007$, $***P_{\text{stim3}}=0.0002$, $****P_{\text{stim4}}<0.0001$, $***P_{\text{stim5}}=0.0007$, unpaired t-test, parametric; IPSC 10 Hz, $P_{\text{stim2}}=0.015$, $****P_{\text{stim3}}<0.0001$, $****P_{\text{stim4}}<0.0001$, $***P_{\text{stim5}}=0.0001$, unpaired t-test, parametric; P value between EPSC and IPSC groups, 5 Hz, $#P_{\text{stim2}}=0.013$, $#P_{\text{stim3}}=0.017$, $P_{\text{stim4}}=0.13$, $P_{\text{stim5}}=0.020$, unpaired t-test, parametric; P value between EPSC and IPSC groups, 10 Hz, $P_{\text{stim2}}=0.26$, $##P_{\text{stim3}}=0.0038$, $P_{\text{stim4}}=0.069$, $##P_{\text{stim5}}=0.0044$, unpaired t-test, parametric).

We tested two possibilities to elucidate the stronger depression of feedforward IPSCs.

We first examined the short-term plasticity of PV-pyramidal synapses. To selectively evoke APs in cortical PV neurons, we expressed channelrhodopsin-2 on PV neurons by introducing AAV-double floxed-ChR2-EYFP (Addgene #20298) to PV-Cre mouse lines. Upon 5 Hz tetanic stimulation of PV-pyramidal synapses, synaptic depression of IPSCs of MD-pyramidal synapses was indistinguishable from feedforward inhibition derived from MD stimulation on pyramidal neurons in PV-tdTomato mice (3 cells, 0.28 ± 0.15 of the initial plateau amplitude at 5 Hz; Fig. 4A–C, mean ± standard error). This result indicates that the short-term synaptic plasticity of PV-pyramidal synapses alone accounts for the extraordinarily fast synaptic depression of feedforward inhibition.
Previous studies have shown that PV neurons receive greater and more frequent responses from thalamic inputs [1–3] and mediate feedforward inhibition [12,26]. Therefore, it is probable that MD inputs on PV synapses depress too fast to evoke reliable APs in PV neurons during tetanic stimulation. To test this, we identified and recorded voltage changes specifically from PV neurons during the high-frequency activity of MD synapses in the PV-TdTomato mouse line. In response to high-frequency optogenetic stimulation, the probability of successful AP generation by activation of MD axons dropped dramatically (Fig. 4D). The observed failure in AP generation was not due to ChR2 inactivation, as optogenetic 10 Hz stimulation evoked immediate and reliable APs in ChR2-expressing MD neurons in our experimental condition (data not shown). Therefore, we concluded that, during the high-frequency activity of the MD, thalamofrontal synapses depress rapidly enough to fail in recruiting feedforward inhibition.
Figure 4. Short-term plasticity of PV-IPSCs and thalamofrontal EPSCs on PV neurons in the dACC L2/3. (A) Schematic image of the experiment. IPSCs derived from local PV activity were measured in pyramidal neurons in the dACC. (B) An example trace of a PV-derived IPSC in the presence of APV and CNQX (green) and after bicuculline (gray) (Vhold = -70 mV). Light stimulations (1 ms) were shown as a blue vertical line. (C) PV-IPSCs are depressed similarly to the feedforward disynaptic IPSCs evoked by MD stimulation at 5 Hz (3 cells in 3 mice, P_{stim2}=0.51, P_{stim3}=0.33, P_{stim4}=0.44, P_{stim5}=0.26, paired t-test, non-parametric). Standard deviation is depicted as the shaded area. (D) Schematic image of a thalamofrontal EPSC in PV neurons. (E–F) Action potential peak time in PV and pyramidal neurons at 5 Hz (E) and 10 Hz (F) is shown as
a horizontal bar (9 cells). **(G)** Normalized EPSC amplitude on PV and pyramidal neurons at 5 Hz (4 PV cells, 6 Pyr cells, $P_{\text{stim}2}=0.72$, $P_{\text{stim}3}=0.69$, $P_{\text{stim}4}=0.94$, $P_{\text{stim}5}=0.79$, unpaired t-test, non-parametric). Standard deviation is depicted as the shaded area. **(H)** Spike probability of PV neurons at 5 Hz and 10 Hz (9 cells).
The current findings indicate that feedforward inhibition decreases during high-frequency stimulation, and thereby, the integration of excitation can be dynamically regulated during high-frequency MD activity, such as during short-term memory. During high-frequency activity, we observed frequent failure in excitation transfer from MD to PV neurons in the dACC and, in turn, rapidly decreased feedforward inhibition. The temporal window of integration was widened accordingly, which increases the probability of integrating thalamofrontal excitatory inputs. Frequency-dependent gating of the feedforward inhibition, and thus regulation of the integration window, is well established in primary sensory cortices in vitro and in vivo [1,2,27,28]. However, physiological consequences of the broadened integration window could be drastically different. Considering that thalamofrontal excitatory synapses undergo rapid depression during high-frequency MD activity [24], frequency-dependent switching off of feedforward inhibition can be critical for continuous transfer of MD activity toward the PFC. In other words, during the maintenance of short-term memory, in which high-frequency MD activity must be transferred to the PFC [16], widening of the integration window can be critical for maintaining the activity loop between the MD and PFC.

Many uncertainties remain concerning the role of feedforward inhibition in the function of the PFC. In the sensory cortices, intra-cortical inhibitory synaptic transmission plays an important role in the construction of response selectivity. Upon inhibition, a significant reduction in the selectivity of neuronal responses to sensory stimuli such as orientation-selectivity and direction-selectivity was reported [29–35].
Projection from MD drives disynaptic feedforward inhibition in the PFC as well [12], and blocking the feedforward inhibition leads to a significant alteration of the spatial selectivity of the neurons during working memory tasks [36]. However, in a considerable minority of PFC neurons, iontophoretically applied bicuculline unmasked new spatial tunings [36]. This result suggests that GABAergic transmission plays critical roles not only in the construction but also in switching of the response selectivity of PFC neurons. Although additional studies are required to test the impact of reduced feedforward inhibition on the spatial tuning of the PFC neurons, on/off switching of the response selectivity could occur during high-frequency MD-PFC activity, as seen in short-term memory. If this is the case, it is tempting to hypothesize that the switching of response selectivity by reduced feedforward inhibition might force different populations of neurons to activate at different time intervals during short-term memory tasks [37].

Fine regulation of the MD-mPFC connection strength is critical for normal function of the PFC. A subtle decrease in MD inputs reduced the functional synchrony of the MD and mPFC as well as cognitive functions [17,38], and lesions of the MD recapitulated the cognitive impairments caused by PFC dysfunction, including loss of short-term memory [39,40]. Altered functional connectivity between the MD and PFC was reported in patients with short-term memory deficits [41,42]. Furthermore, in patients with schizophrenia, decreased volume [43], number of neurons [44], and activity during short-term memory tasks were observed in the MD [45]. Deteriorating alteration of feedforward inhibition in the PFC as a possible etiology of schizophrenia would be an interesting subject for future studies. Supporting this possibility, deficient
output from PV neurons was proposed [46] based on the decreased density of GABAergic neurons [47] without a decrease in the number of total neurons in cases of schizophrenia [48]. Altered transcriptional regulation of GABA_A receptor subtypes [49] and decreased expression of glutamate decarboxylase, a synthesizing enzyme for GABA [50], in schizophrenic patients also support the possible engagement of feedforward inhibition.

Additional studies that directly measure the strength of feedforward inhibition during short-term memory tasks are required to prove the physiological significance of feedforward inhibition in the thalamofrontal circuit. In fact, the pattern of short-term depression on PV neurons can be assumed to be more moderate in vivo primarily due to ongoing neuronal activity and lower calcium concentration [51,52]. The ongoing activity in vivo results in partially depressed synapses to begin with, and thus the short-term depression will be less pronounced compared to the initial amplitude [53,54]. Moreover, the spontaneous network activity enhances the replenishment of the readily releasable pool (RRP) of synaptic vesicles [55]. However, the reduced short-term depression due to ongoing activity is only a relative measure, and does not delay the depletion of RRP. It is demonstrated that the MD-PV synapses in the mPFC are strongly depressed even in the presence of low calcium concentrations [24]. Furthermore, failure in excitation transfer occurred at as low as 5 Hz, which is a relatively low activity frequency compared to the firing rate observed in MD during the maintenance of short-term memory [17,19,20].
In summary, our study suggests that frequency-dependent on/off switching of feedforward inhibition serves as an active gating mechanism of the activity loop between the MD and mPFC, and thus finely controls the maintenance of short-term memory.
References

1. Cruikshank SJ, Lewis TJ, Connors BW. Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex. Nat Neurosci. 2007;10:462–8.

2. Gabernet L, Jadhav S, Feldman D, Carandini M, Scanziani M. Somatosensory Integration Controlled by Dynamic Thalamocortical Feed-Forward Inhibition. Neuron. 2005;48:315–27.

3. Hull C, Isaacson JS, Scanziani M. Postsynaptic mechanisms govern the differential excitation of cortical neurons by thalamic inputs. J Neurosci. 2009;

4. Yoshimura Y, Callaway EM. Fine-scale specificity of cortical networks depends on inhibitory cell type and connectivity. Nat Neurosci. 2005;

5. Fino E, Yuste R. Dense inhibitory connectivity in neocortex. Neuron. 2011;

6. Packer AM, Yuste R. Dense, unspecific connectivity of neocortical parvalbumin-positive interneurons: A canonical microcircuit for inhibition? J Neurosci. 2011;

7. Nigro MJ, Hashikawa-Yamasaki Y, Rudy B. Diversity and connectivity of layer 5 somatostatin-expressing interneurons in the mouse barrel cortex. J Neurosci. 2018;

8. Swadlow HA. Fast-spike Interneurons and Feedforward Inhibition in Awake Sensory Neocortex. Cereb Cortex [Internet]. 2003;13:25–32. Available from: https://academic.oup.com/cercor/article/13/1/25/354638

9. Agmon A, Connors BW. Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. Neuroscience. 1991;

10. Pouille F, Scanziani M. Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. Science (80- ). 2001;293:1159–63.
11. Li LY, Ji XY, Liang F, Li YT, Xiao Z, Tao HW, et al. A Feedforward Inhibitory Circuit Mediates Lateral Refinement of Sensory Representation in Upper Layer 2/3 of Mouse Primary Auditory Cortex. J Neurosci. 2014;
12. Delevich K, Tucciarone J, Huang ZJ, Li B. The Mediodorsal Thalamus Drives Feedforward Inhibition in the Anterior Cingulate Cortex via Parvalbumin Interneurons. J Neurosci [Internet]. 2015;35:5743–53. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4565-14.2015
13. Fuster JM, Alexander GE. Neuron activity related to short-term memory. Science (80- ) [Internet]. 1971;173:652–4. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.173.3997.652
14. Funahashi S, Bruce CJ, Goldman-Rakic PS. Mnemonic coding of visual space in the monkey’s dorsolateral prefrontal cortex. J Neurophysiol. 1989;
15. Schmitt LI, Wimmer RD, Nakajima M, Happ M, Mofakham S, Halassa MM. Thalamic amplification of cortical connectivity sustains attentional control. Nature. 2017;545:219–23.
16. Bolkan SS, Stujenske JM, Parnaudeau S, Spellman TJ, Rauffenbart C, Abbas AI, et al. Thalamic projections sustain prefrontal activity during working memory maintenance. Nat Neurosci. 2017;20:987–96.
17. Parnaudeau S, O’Neill PK, Bolkan SS, Ward RD, Abbas AI, Roth BL, et al. Inhibition of Mediodorsal Thalamus Disrupts Thalamofrontal Connectivity and Cognition. Neuron. 2013;
18. Parnaudeau S, Bolkan SS, Kellendonk C. The Mediodorsal Thalamus: An Essential Partner of the Prefrontal Cortex for Cognition. Biol Psychiatry. 2018;83:648–56.
19. Baeg EH, Kim YB, Huh K, Mook-Jung I, Kim HT, Jung MW. Dynamics of population code for working memory in the prefrontal cortex. Neuron. 2003;
20. Alexander GE, Fuster JM. Firing Changes in Cells of the Nucleus Medialis Dorsalis associated with delayed Response Behavior. Brain Res [Internet]. 1973;61:79–91. Available from: https://www.ncbi.nlm.nih.gov/pubmed/4204130/
21. Kanichay RT, Silver RA. Synaptic and Cellular Properties of the Feedforward Inhibitory Circuit within the Input Layer of the Cerebellar Cortex. J Neurosci [Internet]. 2008;28:8955–67. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.5469-07.2008
22. Kruglikov I, Rudy B. Perisomatic GABA Release and Thalamocortical Integration onto Neocortical Excitatory Cells Are Regulated by Neuromodulators. Neuron [Internet]. 2008;58:911–24. Available from: https://www.ncbi.nlm.nih.gov/pubmed/18579081
23. Crandall SR, Cruikshank SJ, Connors BW. A Corticothalamic Switch: Controlling the Thalamus with Dynamic Synapses. Neuron. 2015;86:768–82.
24. Yoon JY, Lee HR, Ho W-K, Lee S-H. Disparities in Short-Term Depression Among Prefrontal Cortex Synapses Sustain Persistent Activity in a Balanced Network. Cereb Cortex. 2019;
25. Collins DP, Anastasiades PG, Marlin JJ, Carter AG. Reciprocal Circuits Linking the Prefrontal Cortex with Dorsal and Ventral Thalamic Nuclei. Neuron [Internet]. Elsevier Inc.; 2018;98:366-379.e4. Available from: https://doi.org/10.1016/j.neuron.2018.03.024
26. Rotaru DC, Barrionuevo G, Sesack SR. Mediodorsal thalamic afferents to layer III of the rat prefrontal cortex: Synaptic relationships to subclasses of interneurons. J Comp Neurol. 2005;

27. Heiss JE, Katz Y, Ganmor E, Lampl I. Shift in the balance between excitation and inhibition during sensory adaptation of S1 neurons. J Neurosci. 2008;

28. Higley MJ, Contreras D. Frequency adaptation modulates spatial integration of sensory responses in the rat whisker system. J Neurophysiol. 2007;

29. Jones EG. Gabaergic neurons and their role in cortical plasticity in primates. Cereb Cortex. 1993;

30. Sato H, Katsuyama N, Tamura H, Hata Y, Tsumoto T. Mechanisms underlying orientation selectivity of neurons in the primary visual cortex of the macaque. J Physiol. 1996;

31. Murthy A, Humphrey AL. Inhibitory contributions to spatiotemporal receptive-field structure and direction selectivity in simple cells of cat area 17. J Neurophysiol. 1999;

32. Eysel UT, Crook JM, Machemer HF. GABA-induced remote inactivation reveals cross-orientation inhibition in the cat striate cortex. Exp Brain Res. 1990;

33. Alloway KD, Rosenthal P, Burton H. Quantitative measurements of receptive field changes during antagonism of GABAergic transmission in primary somatosensory cortex of cats. Exp Brain Res. 1989;

34. Alloway KD, Burton H. Differential effects of GABA and bicuculline on rapidly- and slowly-adapting neurons in primary somatosensory cortex of primates. Exp Brain Res. 1991;
35. Kyriazi HT, Carvell GE, Brumberg JC, Simons DJ. Effects of baclofen and phaclofen on receptive field properties of rat whisker barrel neurons. Brain Res. 1996;

36. Rao SG, Williams G V., Goldman-Rakic PS. Destruction and creation of spatial tuning by disinhibition: GABA(A) blockade of prefrontal cortical neurons engaged by working memory. J Neurosci. 2000;

37. Constantini dis C, Williams G V., Goldman-Rakic PS. A role for inhibition in shaping the temporal flow of information in prefrontal cortex. Nat Neurosci. 2002;

38. Parnaudeau S, Taylor K, Bolkas SS, Ward RD, Balsam PD, Kellendonk C. Mediodorsal thalamus hypofunction impairs flexible goal-directed behavior. Biol Psychiatry. 2015;

39. Browning PGF, Chakraborty S, Mitchell AS. Evidence for mediodorsal thalamus and prefrontal cortex interactions during cognition in macaques. Cereb Cortex. 2015;

40. Bailey KR, Mair RG. Lesions of specific and nonspecific thalamic nuclei affect prefrontal cortex-dependent aspects of spatial working memory. Behav Neurosci. 2005;

41. Minzenberg MJ, Laird AR, Thelen S, Carter CS, Glahn DC. Meta-analysis of 41 functional neuroimaging studies of executive function in schizophrenia. Arch Gen Psychiatry. 2009;

42. Mitelman SA, Byne W, Kemether EM, Hazlett EA, Buchsbaum MS. Metabolic disconnection between the mediodorsal nucleus of the thalamus and cortical brodmann’s areas of the left hemisphere in schizophrenia. Am J Psychiatry. 2005;

43. Byne W, Hazlett EA, Buchsbaum MS, Kemether E. The thalamus and schizophrenia: current status of research. Acta Neuropathol. 2009.

28
44. Popken GJ, Bunney WE, Potkin SG, Jones EG. Subnucleus-specific loss of neurons in medial thalamus of schizophrenics. Proc Natl Acad Sci U S A. 2000;

45. Andrews J, Wang L, Csernansky JG, Gado MH, Barch DM. Abnormalities of thalamic activation and cognition in schizophrenia. Am J Psychiatry. 2006;

46. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. Nat. Rev. Neurosci. 2005.

47. Woo TU, Whitehead RE, Melchitzky DS, Lewis DA. A subclass of prefrontal γ-aminobutyric acid axon terminals are selectively altered in schizophrenia. Proc Natl Acad Sci U S A. 1998;

48. Selemon LD, Rajkowska G, Goldman-Rakic PS. Abnormally High Neuronal Density in the Schizophrenic Cortex: A Morphometric Analysis of Prefrontal Area 9 and Occipital Area 17. Arch Gen Psychiatry. 1995;

49. Huntsman MM, Tran B Van, Potkin SG, Bunney WE, Jones EG. Altered ratios of alternatively spliced long and short γ2 subunit mRNAs of the γ-amino butyrate type A receptor in prefrontal cortex of schizophrenics. Proc Natl Acad Sci U S A. 1998;

50. Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE, et al. Gene Expression for Glutamic Acid Decarboxylase is Reduced without Loss of Neurons in Prefrontal Cortex of Schizophrenics. Arch Gen Psychiatry. 1995;

51. Borst JGG. The low synaptic release probability in vivo. Trends Neurosci. Elsevier Ltd; 2010;33:259–66.

52. Jones HC, Keep RF. Brain fluid calcium concentration and response to acute hypercalcaemia during development in the rat. J Physiol. 1988;
53. Reig R, Gallego R, Nowak LG, Sanchez-Vives M V. Impact of cortical network activity on short-term synaptic depression. Cereb Cortex [Internet]. 2006;16:688–95. Available from: https://academic.oup.com/cercor/article/16/5/688/276950

54. Boudreau CE. Short-Term Depression in Thalamocortical Synapses of Cat Primary Visual Cortex. J Neurosci [Internet]. 2005;25:7179–90. Available from: http://www.jneurosci.org/cgi/pmidlookup?view=long&pmid=16079400

55. Neher E, Sakaba T. Multiple Roles of Calcium Ions in the Regulation of Neurotransmitter Release. Neuron. 2008.
Abbreviations

aCSF: Artificial cerebrospinal fluid
AHP: After-hyperpolarization
AP: Action potential
dACC: Dorsal anterior cingulate cortex
EPSC: Excitatory postsynaptic current
IC\textsubscript{50}: Half maximal inhibitory concentration
IPSC: Inhibitory postsynaptic current
IW: Integration window
MD: Mediodorsal thalamic nucleus
mPFC: Medial prefrontal cortex
PSC: Postsynaptic current
PV: Parvalbumin
TTX: Tetrodotoxin
V\text{hold}: Holding membrane potential

Availability of data and materials

The data used in our study are available from the authors on reasonable request

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Authors’ contribution

JML: data collection, analyses and manuscript drafting, JHC: participation in the study design and instrumentation; JCR: project design, manuscript finalization. All authors read and approved the final manuscript.

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Ethical declaration

- Ethics approval

All the experimental procedures and caring procedures for mice involved in this study were approved by the Institutional Animal Care and Use Committee of the Korea Brain Research Institute (approval number M2-1ACUC-19-00040).

- Consent for publication

Not applicable.

- Competing interests

The authors declare that they have no competing interests.