

Preferential Disruption of Prefrontal GABAergic Function by Nanomolar Concentrations of the $\alpha 7$ nACh Negative Modulator Kynurenic Acid

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Increased concentrations of kynurenic acid (KYNA) in the prefrontal cortex (PFC) are thought to contribute to the development of cognitive deficits observed in schizophrenia. Although this view is consistent with preclinical studies showing a negative impact of prefrontal KYNA elevation on executive function, the mechanism underlying such a disruption remains unclear. Here, we measured changes in local field potential (LFP) responses to ventral hippocampal stimulation *in vivo* and conducted whole-cell patch-clamp recordings in brain slices to reveal how nanomolar concentrations of KYNA alter synaptic transmission in the PFC of male adult rats. Our data show that prefrontal infusions of KYNA attenuated the inhibitory component of PFC LFP responses, a disruption that resulted from local blockade of $\alpha 7$ -nicotinic acetylcholine receptors ($\alpha 7$ nAChR). At the cellular level, we found that the inhibitory action exerted by KYNA in the PFC occurred primarily at local GABAergic synapses through an $\alpha 7$ nAChR-dependent presynaptic mechanism. As a result, the excitatory–inhibitory ratio of synaptic transmission becomes imbalanced in a manner that correlates highly with the level of GABAergic suppression by KYNA. Finally, prefrontal infusion of a GABA_AR positive allosteric modulator was sufficient to overcome the disrupting effect of KYNA and normalized the pattern of LFP inhibition in the PFC. Thus, the preferential inhibitory effect of KYNA on prefrontal GABAergic transmission could contribute to the onset of cognitive deficits observed in schizophrenia because proper GABAergic control of PFC output is one key mechanism for supporting such cortical functions.

Key words: GABA; kynurenic acid; local field potentials; nicotinic receptor; prefrontal cortex; ventral hippocampus

Significance Statement

Brain kynurenic acid (KYNA) is an astrocyte-derived metabolite and its abnormal elevation in the prefrontal cortex (PFC) is thought to impair cognitive functions in individuals with schizophrenia. However, the mechanism underlying the disrupting effect of KYNA remains unclear. Here we found that KYNA biases the excitatory–inhibitory balance of prefrontal synaptic activity toward a state of disinhibition. Such disruption emerges as a result of a preferential suppression of local GABAergic transmission by KYNA via presynaptic inhibition of $\alpha 7$ -nicotinic acetylcholine receptor signaling. Therefore, the degree of GABAergic dysregulation in the PFC could be a clinically relevant contributing factor for the onset of cognitive deficits resulting from abnormal increases of cortical KYNA.

Introduction

The functional maturation of GABAergic circuits in the prefrontal cortex (PFC) is one key mechanism for enabling proper pro-

cessing of ventral hippocampal afferent information (Thomases et al., 2013; Caballero et al., 2014) and for supporting PFC-dependent cognitive functions (Floresco, 2013; Tse et al., 2015). It is thought that a disruption of such GABAergic control in the PFC underlies the onset of cognitive deficits seen in a variety of

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psychiatric disorders including schizophrenia (Tseng et al., 2009; Tse et al., 2015; Caballero et al., 2016). Further understanding of the different synaptic mechanisms that enable proper regulation of GABAergic function in the PFC is therefore a critical step toward gaining insights on how to restore normal cognition in schizophrenia and related psychiatric syndromes (Caballero and Tseng, 2016).

Of special interest in this context is the abnormal elevation of the tryptophan metabolite kynurenic acid (KYNA) in the PFC of individuals with schizophrenia, a biochemical dysregulation that is not secondary to antipsychotic medication (Schwarcz et al., 2001; Ceresoli-Borroni et al., 2006; Sathyaikumara et al., 2011; Larsson et al., 2015). Brain KYNA is an astrocyte-derived metabolite that is present in the mammalian CNS at nanomolar concentrations (Moroni et al., 1988; Turski et al., 1988) and negatively modulates $\alpha 7$ -nicotinic acetylcholine receptor ($\alpha 7$ nAChR) function under physiological conditions (Albuquerque and Schwarcz, 2013). At higher concentrations, KYNA can also function as a competitive inhibitor of the glycine coagonist (glycine-B) site of the NMDA receptor (NMDAR) (Kessler et al., 1989; Parsons et al., 1997). Therefore, the increased PFC levels of KYNA observed in schizophrenia may be clinically relevant and mechanistically linked to the onset of cognitive impairments as a result of deficits in $\alpha 7$ nAChR and/or NMDAR function in cortical circuits (Robbins and Murphy, 2006; Timofeeva and Levin, 2011). Accordingly, studies from animal models converge to indicate that increased cortical levels of KYNA can lead to deficits in executive function (Chess et al., 2007; Akagbosu et al., 2012; Alexander et al., 2012, 2013; Pocivavsek et al., 2012; Pershing et al., 2015; Pershing et al., 2016). However, the precise mechanism underlying the disrupting action of KYNA in cortical circuits remains elusive despite the fact that fluctuations in endogenous KYNA levels in the PFC are known to modulate bidirectionally the extracellular concentrations of glutamate (Konradsson-Geuken et al., 2010; Wu et al., 2010), GABA (Beggiato et al., 2014), dopamine (Pocivavsek et al., 2016; Valentini et al., 2016), and acetylcholine (Zmarowski et al., 2009).

The goal of the present study is to gain insights into how KYNA disrupts cortical circuits by determining the functional impact of elevations of nanomolar concentrations of KYNA in the PFC. We used local field potential (LFP) recordings to assess disruptions in the pattern of PFC response to ventral hippocampal train stimulation (10, 20, and 40 Hz) following PFC infusions of KYNA *in vivo*. This protocol of train stimulation was chosen because of its sensitivity in revealing changes in the relative contribution of excitatory and inhibitory transmission in the PFC (Cass et al., 2013; Thomases et al., 2013). At the cellular level, whole-cell patch-clamp recordings in brain slices were then used to identify the mechanisms by which nanomolar concentrations of KYNA regulate synaptic transmission onto pyramidal output neurons in the PFC.

Materials and Methods

Procedures. All experimental procedures were approved by the Rosalind Franklin University Institutional Animal Care and Use Committee in accordance with National Institutes of Health guidelines. All recordings were conducted from adult (>80-d-old) male Sprague Dawley rats (Harlan Laboratories). Rats were allowed to habituate for at least 7 d upon arrival, group housed (3/cage), and maintained under constant temperature (21–23°C) in a 12/12 h light/dark cycle with food and water available *ad libitum*. All chemicals were purchased from Sigma-Aldrich except for Indiplon, which was obtained from Tocris Bioscience.

In vivo recordings of LFPs in the medial PFC. All LFP recordings were conducted in the medial PFC (prelimbic and infralimbic regions) using a concentric bipolar electrode (SNE-100x 50 mm; Rhodes Medical Instru-

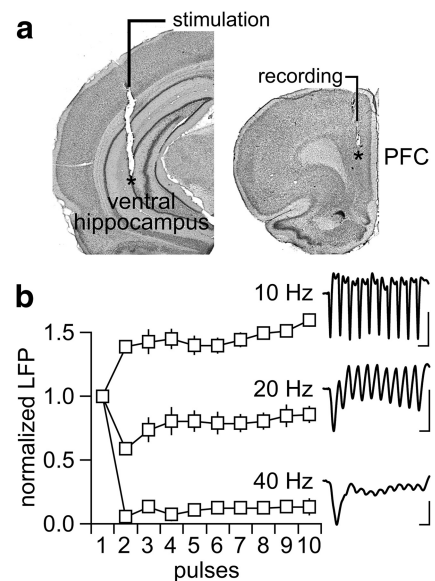


Figure 1. Ventral hippocampal-evoked LFP responses in the adult PFC. *a*, Coronal sections of the ventral hippocampus and PFC showing the anatomical location (asterisks) of the stimulating and recording electrodes, respectively. *b*, Characteristic response pattern of LFP in the adult PFC ($n = 6$) elicited by ventral hippocampal train stimulation at 10, 20, and 40 Hz (calibration: 10 mV/100 ms at 10 Hz; 10 mV/50 ms at 20 Hz; 10 mV/25 ms at 40 Hz).

ments) attached to a 28-gauge cannula to enable local administration of artificial CSF (aCSF)-containing agonists and antagonists as described previously (Thomases et al., 2013). Here, single infusions of 0.8 μ l of aCSF alone (control) or in combination with one of the following neuromodulators were delivered into the PFC at 0.1 μ l/min: (1) KYNA (100–300 nM), (2) methyllycaconitine (MLA; 300 nM), (3) 7-chlorokynurenic acid (7Cl-KYNA; 300 nM), (4) Indiplon (10 μ M), (5) KYNA + Indiplon, or (6) MLA + Indiplon. The chemical composition of the aCSF solution was as follows (in mM): 122.5 NaCl, 3.5 KCl, 25 NaHCO₃, 1 NaH₂PO₄, 2.5 CaCl₂, 1 MgCl₂, 20 glucose, and 1 ascorbic acid, pH 7.40, 295–305 mOsm. For stimulation, another concentric bipolar electrode (NE-100x 50 mm; Rhodes Medical Instruments) was lowered into the ventral hippocampus (Fig. 1) and trains of electrical pulses (300 μ s square pulses at 0.75 mA) were delivered every 15 s through a computer-controlled pulse generator (Master-8; A.M.P.I.). Changes in the pattern of LFP at 10, 20, and 40 Hz were assessed by the amplitude of the evoked LFP as described previously (Thomases et al., 2013). All example traces of LFP shown are averages of six evoked responses.

Whole-cell patch-clamp recordings of IPSCs in the medial PFC. All experimental procedures (brain slicing and patch-clamp recordings) were conducted as described previously (Cass et al., 2014; Flores-Barrera et al., 2014). Briefly, all recordings were conducted from layer V pyramidal neurons of the medial PFC (infralimbic and prelimbic regions; 350- μ m-thick coronal slices) at 33–35°C using a cesium-based internal solution containing 0.1% Neurobiotin (Vector Laboratories) and the following (in mM): 140 CsCl, 10 HEPES, 2 MgCl₂, 5 NaATP, 0.6 NaGTP, and 3 QX-314, pH 7.23–7.28, 280–282 mOsm. The recording aCSF contained 10 μ M CNQX and 50 μ M APV and the following (in mM): 122.5 NaCl, 3.5 KCl, 25 NaHCO₃, 1 NaH₂PO₄, 2.5 CaCl₂, 1 MgCl₂, 20 glucose, and 1 ascorbic acid, pH 7.40–7.43, 295–305 mOsm. Both GABA-A receptor-mediated spontaneous and evoked IPSCs were obtained in voltage-clamp mode (holding potential: –70 mV). Only neurons exhibiting stable baseline activity (i.e., 10 min) were included for analyses. Typically, the mean baseline IPSC frequency obtained from at least two noncontiguous epochs of 60 s each was compared with equivalent measures taken 10 min after bath application of 300 nM KYNA, MLA, or 7Cl-KYNA. The effects of KYNA, MLA, and 7Cl-KYNA were also assessed by measuring changes in the amplitude of electrically evoked IPSCs. Data from the evoked response were collected from another cohort of neurons by means of local stimulation elicited every 10 s using a Teflon-coated

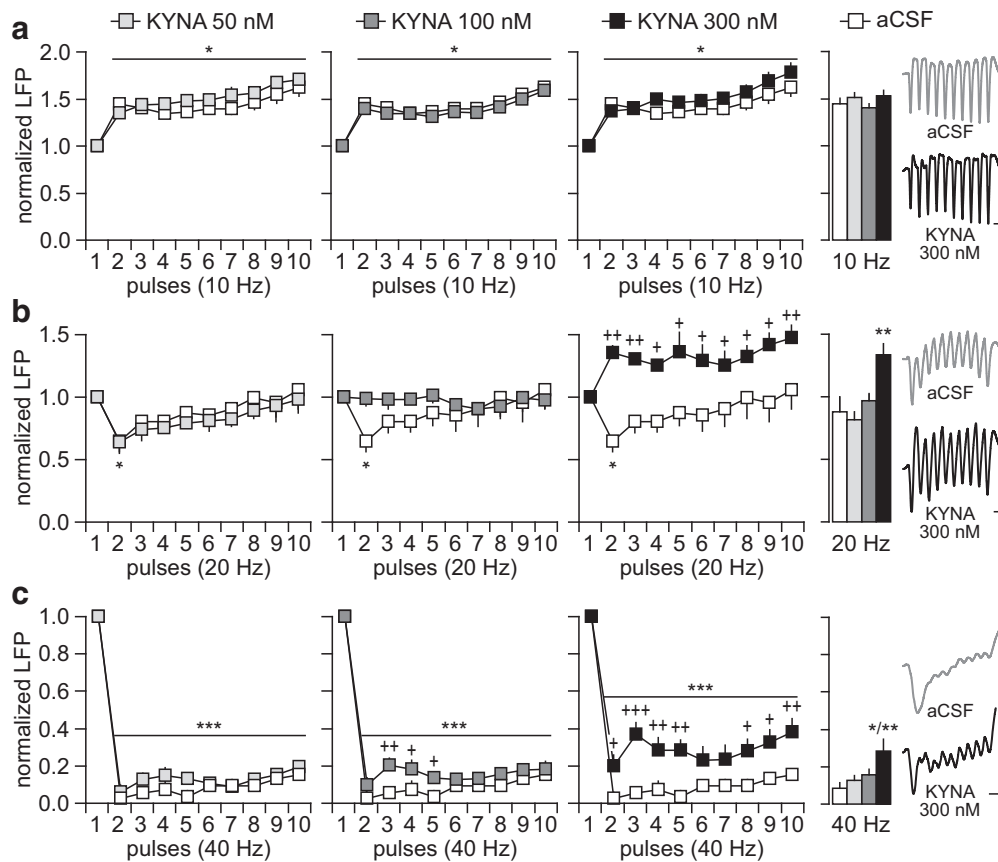


Figure 2. Disruption of prefrontal LFP response by local infusion of KYNA. **a**, PFC infusions of aCSF ($n = 6$) or KYNA (50, 100, and 300 nM; $n = 5–8$ /group) failed to alter the pattern of LFP facilitation resulting from ventral hippocampal train stimulation at 10 Hz. Note the similar magnitude of LFP facilitation across the different treatment groups ($*p < 0.05$ vs first pulse, LSD *post hoc* test after significant ANOVA). Inset traces of LFP show the response pattern elicited by hippocampal stimulation at 10 Hz (calibration: 5 mV/100 ms). **b**, At 20 Hz, PFC infusions of aCSF or 50 nM KYNA resulted in similar patterns of transient LFP inhibition ($*p < 0.05$ vs first pulse, LSD *post hoc* test after significant ANOVA). Such a pattern of LFP inhibition was absent after the infusion of 100 nM KYNA, whereas a facilitation emerged with 300 nM KYNA ($*p < 0.05$ vs first pulse, $+p < 0.05/+p < 0.005$ vs aCSF, LSD *post hoc* test; main effect of treatment $F_{(1,120)} = 57.2, p < 0.0001$, two-way ANOVA). Bar graph summarizes the mean LFP response calculated from pulses 2–10 ($***p < 0.005$ vs any other group, Tukey's *post hoc* test; $F_{(3,20)} = 9.6, p < 0.0005$, one-way ANOVA). Inset traces illustrate the abnormal LFP facilitation at 20 Hz resulting from PFC infusion of 300 nM KYNA (calibration: 5 mV/50 ms). **c**, Stimulation at 40 Hz typically suppresses LFP in the PFC. Both the aCSF and 50 nM KYNA groups showed similar patterns of LFP inhibition ($++p < 0.005/+p < 0.05$ vs aCSF, $***p < 0.0005$ vs first pulse, LSD *post hoc* test after significant ANOVA). However, the magnitude of LFP suppression was markedly attenuated following PFC infusion of 300 nM KYNA ($+++p < 0.0005/+p < 0.005/+p < 0.05$ vs aCSF, $***p < 0.0005$ vs first pulse, LSD *post hoc* test; main effect of treatment, $F_{(1,120)} = 60.7, p < 0.0001$, two-way ANOVA). Bar graph summarizes the mean LFP response calculated from pulses 2–10 ($***p < 0.005$ vs any other group, Tukey's *post hoc* test; $F_{(3,20)} = 7.4, p < 0.002$, one-way ANOVA). Inset traces of 40 Hz-induced LFP illustrate the disruption elicited by 300 nM KYNA (calibration: 5 mV/30 ms).

bipolar electrode placed $\sim 200 \mu\text{m}$ from the cell body along the apical dendritic axis. The stimulation intensity (300 μs square pulses at 50–100 μA range) was chosen from the minimum current required to elicit an IPSC response with $<20\%$ variability in amplitude. For the frequency analysis, relative changes from baseline IPSC amplitude were determined after 10 min bath application of KYNA or MLA.

Whole-cell patch-clamp recordings of excitatory and inhibitory synaptic currents within a single pyramidal neuron in the medial PFC. All recordings were conducted from layer V pyramidal neurons using a low-chloride-based internal solution and an external solution free of glutamate and GABA blockers to enable concurrent acquisition of excitatory and inhibitory synaptic currents at the single-cell level. The low-chloride-based internal solution contained 0.1% Neurobiotin (Vector Laboratories) and the following (in mM): 10 CsCl, 130 Gluconic acid, 10 HEPES, 2 MgCl₂, 5 NaATP, 0.6 NaGTP, and 3 QX-314, pH 7.23–7.28, 280–282 mOsm. As a result, both spontaneous glutamatergic and GABAergic events could be assessed readily by recording the frequency of postsynaptic currents (PSCs) at the -60 mV ($\text{PSC}_{-60 \text{ mV}}$) and $+15 \text{ mV}$ ($\text{PSC}_{+15 \text{ mV}}$) holding potentials, respectively. As described above, only neurons with at least 10 min of stable baseline activity were included for analyses. The frequency of $\text{PSC}_{-60 \text{ mV}}$ and $\text{PSC}_{+15 \text{ mV}}$ events from at least two noncontiguous baseline epochs of 60 s each was compared with equivalent measures taken 10 min after bath application of KYNA, KYNA + MLA, or KYNA + 7Cl-KYNA.

The impact of KYNA was also assessed on locally evoked $\text{PSC}_{-60 \text{ mV}}$ and $\text{PSC}_{+15 \text{ mV}}$ at the single-cell level following the same experimental design used to record glutamatergic and GABAergic events. This dataset was obtained from another cohort of layer V pyramidal neurons by means of local stimulation elicited every 10 s (as described above) and only neurons with at least 15 min of stable baseline recordings were included for analyses. Relative changes of evoked $\text{PSC}_{-60 \text{ mV}}$ and $\text{PSC}_{+15 \text{ mV}}$ amplitude were determined after 10 min bath application of KYNA or KYNA + Indiplon.

Experimental design and statistical analysis. Data were summarized as mean \pm SEM and differences among experimental conditions (within- and between-subjects design) were considered statistically significant at $p < 0.05$. Paired *t* test was used for two-group within-subject comparison involving a single continuous variable (e.g., predrug vs postdrug application), whereas one- and two-way ANOVAs were applied to assess between-subject comparisons along three or more dependent variables (e.g., vehicle vs drug A vs drug B), followed by the appropriate *post hoc* tests (StatSoft).

Results

We first determined how single PFC infusion of KYNA (50–300 nM) affects LFP responses to ventral hippocampal train stimulation at 10, 20, and 40 Hz (Fig. 1). These frequencies of stimulation were

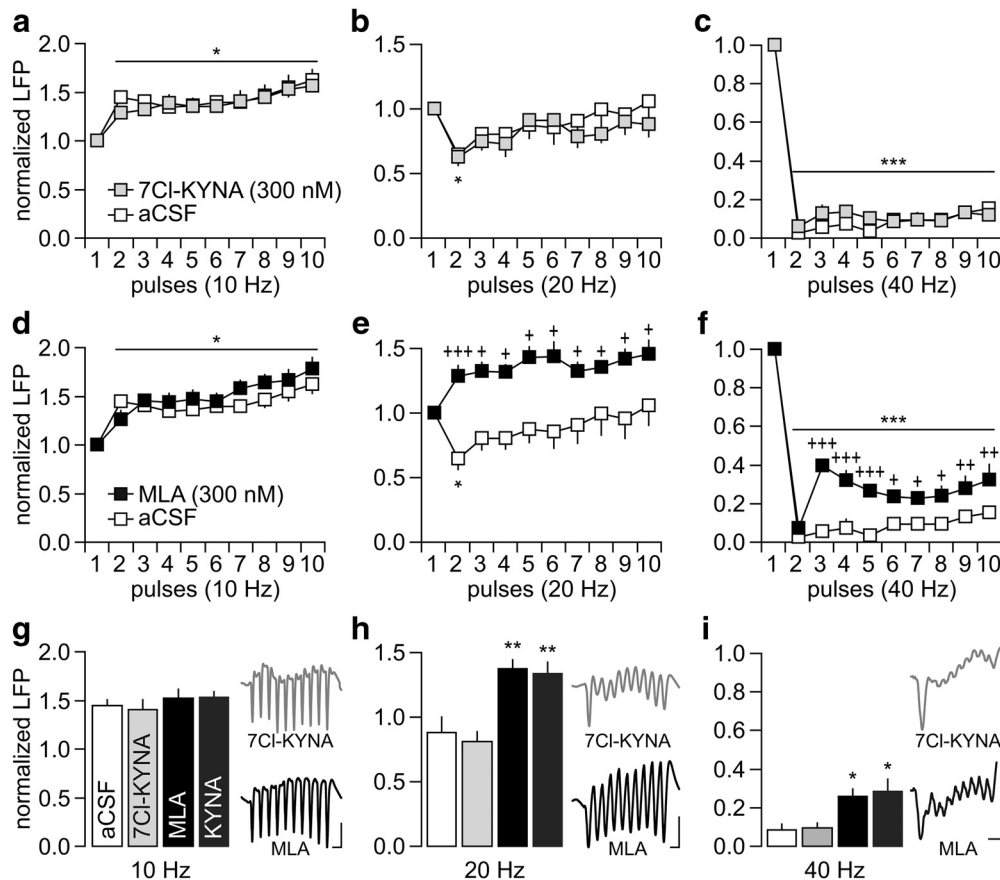


Figure 3. PFC infusion of the $\alpha 7$ nAChR antagonist MLA mimics the frequency-dependent disruption induced by KYNA. **a–c**, Summary of the results obtained after PFC infusion of 300 nM 7CI-KYNA ($n = 5$). Note that the patterns of LFP response to ventral hippocampal stimulation after 7CI-KYNA infusions are indistinguishable from the aCSF controls ($*p < 0.05$ / $***p < 0.0005$ vs first pulse, LSD *post hoc* test after significant ANOVA). **d–f**, In contrast, PFC infusion of 300 nM MLA ($n = 5$) disrupted the LFP response at 20 and 40 Hz without altering the pattern of facilitation at 10 Hz. Two-way ANOVA revealed a main effect of pulse at 10 Hz ($F_{(1,90)} = 10.6, p < 0.0001$) and main effects of treatment at both 20 Hz ($F_{(1,90)} = 54.8, p < 0.0001$) and 40 Hz ($F_{(1,90)} = 95.6, p < 0.0001$). A main effect of pulse ($F_{(9,90)} = 106.9, p < 0.0001$) and treatment \times pulse were also observed at 40 Hz ($F_{(9,90)} = 3.3, p < 0.002$). LSD *post hoc* tests: $***p < 0.0005$ / $*p < 0.05$ versus first pulse, $+++p < 0.0005$ / $++p < 0.005$ / $+p < 0.05$ versus aCSF. **g–i**, Summary of the frequency-dependent LFP changes calculated from pulses 2–10 ($***p < 0.005$ / $*p < 0.05$ vs aCSF or 7CI-KYNA, Tukey's *post hoc* test after significant one-way ANOVA: $F_{(3,20)} = 12.4, p < 0.0001$ at 20 Hz and $F_{(3,20)} = 9.5, p < 0.0005$ at 40 Hz). Inset traces illustrate the pattern of LFP response following PFC infusions of 7CI-KYNA or MLA (calibration: 5 mV/100 ms at 10 Hz; 5 mV/50 ms at 20 Hz; 5 mV/30 ms at 40 Hz).

chosen to reveal changes in the balance of excitation and inhibition in the PFC *in vivo* (Thomases et al., 2013). Relative to aCSF, PFC infusions with 50, 100, and 300 nM KYNA failed to alter the pattern of LFP facilitation observed at 10 Hz (Fig. 2a). This was not the case at 20 Hz. Although the normal pattern of transient inhibition (i.e., second pulse) remained unchanged after PFC infusion of 50 nM KYNA, a marked LFP facilitation emerged when 300 nM KYNA was administered (Fig. 2b). Notably, the 100 nM KYNA concentration was sufficient to block the transient inhibition without causing any LFP facilitation. A similar dose-dependent effect was observed at 40 Hz after PFC infusion of KYNA (Fig. 2c). Typically, ventral hippocampal stimulation at 40 Hz suppresses the amplitude of LFP in the PFC (Thomases et al., 2013). Although the overall pattern of PFC response at 40 Hz remained largely unaffected by KYNA, the magnitude of LFP suppression began to appear slightly diminished at 100 nM. Such attenuation of LFP inhibition became markedly significant after PFC infusion of 300 nM KYNA (Fig. 2c). Together, these results indicate that increasing prefrontal KYNA levels within nanomolar concentrations is sufficient to disrupt PFC processing of afferent drive in a frequency-dependent manner, as revealed by the reduced inhibitory control of ventral hippocampal-evoked LFP responses.

Nanomolar concentrations of KYNA are known to negatively modulate $\alpha 7$ nAChRs (Hilmas et al., 2001) and to block the glycine-B site of the NMDAR (Kessler et al., 1989; Hilmas et al., 2001). Therefore, inhibition of these receptors could contribute to the frequency-dependent disruption of LFP inhibition observed following PFC infusions of KYNA. To begin testing these mechanisms, we first assessed the impact of 7CI-KYNA, which blocks the glycine-B site of the NMDAR without affecting the $\alpha 7$ nAChR (Kemp et al., 1988; Hilmas et al., 2001). Results revealed no apparent changes in the pattern of LFP response after PFC infusions of 300 nM 7CI-KYNA (Fig. 3a–c). The magnitude of LFP facilitation (10 Hz) and suppression (20 and 40 Hz) recorded in the presence of 7CI-KYNA were indistinguishable from aCSF controls. In contrast, PFC infusion of the $\alpha 7$ nAChR antagonist MLA (300 nM) effectively disrupted the pattern of LFP response in a frequency-dependent manner resembling that induced by KYNA (Fig. 3d–f). Similar to the latter, PFC infusion of MLA revealed a pattern of sustained LFP facilitation at 20 Hz (Fig. 3e) and a marked attenuation of LFP suppression at 40 Hz (Fig. 3f). Collectively, these results indicate that the disrupting effects of KYNA in the PFC could result from local blockade of $\alpha 7$ nAChR function (Fig. 3g–i).

GABAergic function in the PFC plays a critical role in mediating the distinctive frequency-dependent pattern of LFP

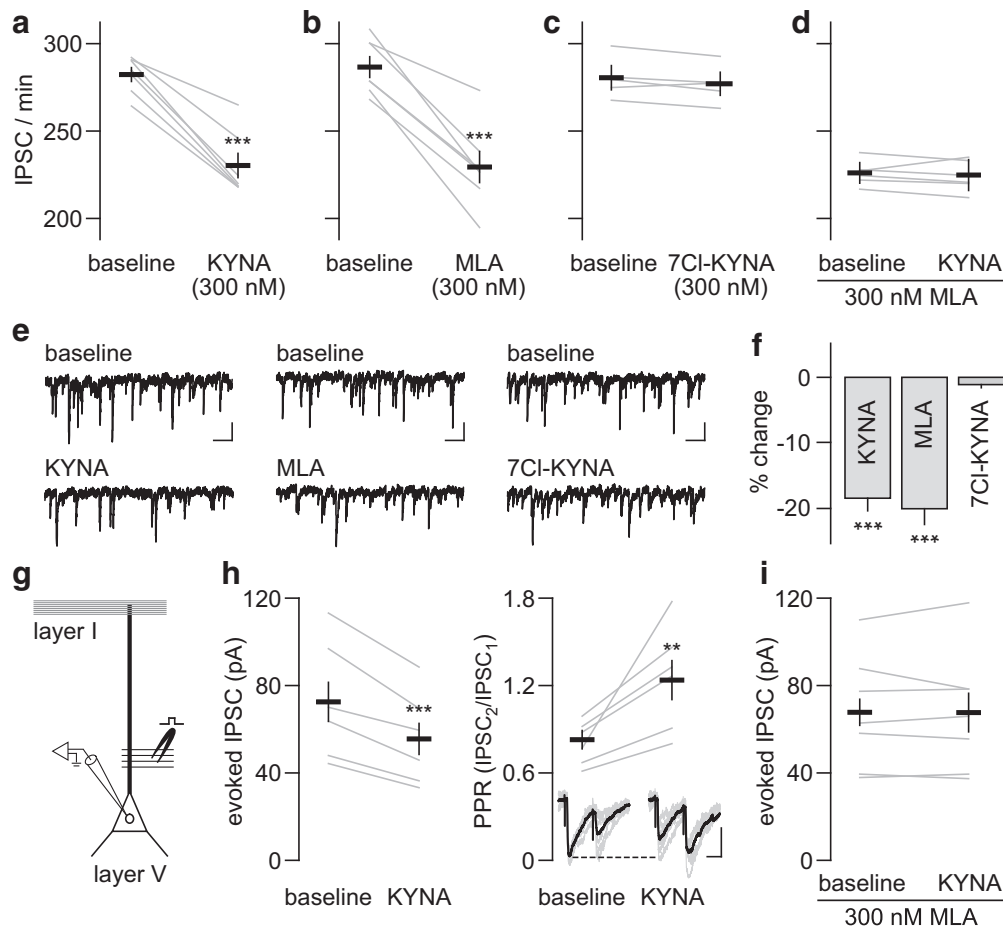


Figure 4. KYNA-induced attenuation of GABAergic transmission in the PFC is occluded by MLA. **a**, Bath application of 300 nM KYNA (10 min, $n = 7$) reduced the frequency of spontaneous IPSCs in layer V pyramidal neurons of the PFC ($***p < 0.0005$ vs baseline, paired t test). **b**, A similar degree of inhibition was observed after bath application of 300 nM MLA (10 min, $n = 7$; $***p < 0.0005$ vs baseline, paired t test). **c**, Conversely, bath application of 300 nM 7CI-KYNA (10 min, $n = 5$) failed to change the frequency of IPSCs. **d**, As expected from **b**, the addition of MLA into the baseline aCSF reduced the frequency of IPSCs. However, bath application of 300 nM KYNA failed to diminish the IPSC frequency further ($n = 6$). **e**, Traces of IPSC recordings illustrating the effects of bath application of KYNA, MLA, and 7CI-KYNA (calibration: 10 pA/0.5 s). **f**, Bar graph summarizing the effects of KYNA, MLA, and 7CI-KYNA on IPSC frequency as a percentage change from baseline ($***p < 0.0005$ vs 7CI-KYNA, Tukey's *post hoc* test; $F_{(2,16)} = 23.6$, $p < 0.0001$, one-way ANOVA). **g**, Recording design used to elicit IPSC in layer V pyramidal neurons by local electrical stimulation ($***p < 0.0005$ vs 7CI-KYNA, Tukey's *post hoc* test; $F_{(2,16)} = 23.6$, $p < 0.0001$, one-way ANOVA). **h**, Bath application of 300 nM KYNA ($n = 6$) elicited a significant attenuation of the evoked IPSC amplitude ($***p < 0.0005$ vs baseline, paired t test) concurrent with an elevation of the IPSC paired-pulse ratio (PPR: $\text{IPSC}_2/\text{IPSC}_1$ at 50 ms intervals; $**p < 0.005$ vs baseline, paired t test). Inset: Traces of evoked IPSC illustrating the inhibitory action of KYNA (calibration: 20 pA/25 ms). **i**, Note that the inhibitory effect of KYNA was no longer apparent when recordings were conducted in the presence of MLA ($n = 7$).

inhibition resulting from ventral hippocampal train stimulation (Thomases et al., 2013). Therefore, it is conceivable that a GABAergic component contributes to the disinhibitory action of KYNA in the PFC. To test this, we conducted whole-cell patch-clamp recordings in PFC brain slices obtained from adult rats to assess the impact of KYNA on GABA_AR-mediated IPSC onto layer V pyramidal neurons. Relative to baseline activity, bath application of 300 nM KYNA significantly reduced the number of spontaneous IPSC events (Fig. 4*a,e*). A comparable level of IPSC suppression was observed after bath application of MLA (Fig. 4*b,e,f*), but not of 7CI-KYNA (Fig. 4*c,e,f*). However, the mean amplitude of spontaneous IPSC events remained unchanged after bath application of KYNA (from 24.9 ± 3.1 pA to 25.1 ± 3.4 pA), MLA (from 24.5 ± 3.2 pA to 24.8 ± 3.6 pA), or 7CI-KYNA (from 24.6 ± 3.8 pA to 25.1 ± 3.5 pA). Notably, the inhibitory effect of KYNA on IPSC frequency was occluded by MLA (Fig. 4*d*). Similarly, bath application of 300 nM KYNA diminished the amplitude of locally evoked IPSC concurrently with a facilitation of the paired-pulse ratio (Fig. 4*g,h*). No further suppression of IPSC amplitude by KYNA was observed when recordings were conducted in the presence of MLA (Fig. 4*i*). Together, these re-

sults indicate that the inhibitory action of KYNA on PFC GABAergic transmission occurs via presynaptic $\alpha 7$ nAChR antagonism, as revealed by the reduction in IPSC frequency and increased paired-pulse ratio accompanying the attenuation of the evoked IPSC amplitude.

In addition to attenuating prefrontal GABAergic function, nanomolar concentrations of KYNA could also diminish the level of glutamatergic drive onto pyramidal output neurons. The balance of excitatory–inhibitory (E–I) synaptic activity within a single pyramidal neuron is expected to remain unaltered if KYNA's inhibition of GABAergic and glutamatergic transmission is comparable. To address this, recordings from layer V pyramidal neurons in the PFC were collected using a protocol that enables the acquisition of GABAergic and glutamatergic synaptic activity within a single cell (Fig. 5*a,b*; see Materials and Methods for details). Bath application of 300 nM KYNA also reduced the frequency of glutamatergic synaptic activity, as determined by the number of spontaneous postsynaptic current events recorded at the -60 mV ($\text{PSC}_{-60 \text{ mV}}$) holding potential (Fig. 5*c*). However, KYNA induced a much greater suppression of GABAergic synaptic activity (i.e., picrotoxin-sensitive $\text{PSC}_{+15 \text{ mV}}$) within the same

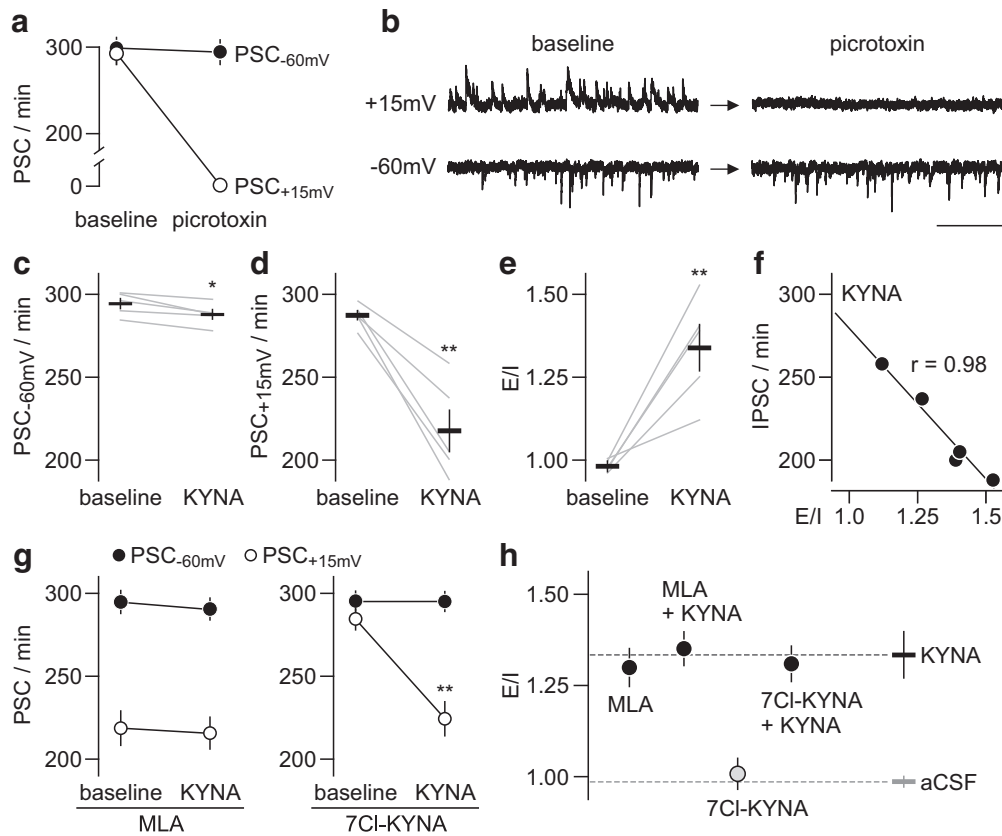


Figure 5. Preferential inhibition of PFC GABAergic over glutamatergic transmission by KYNA. **a**, Concurrent acquisition of inhibitory (PSC_{+15mV}) and excitatory (PSC_{-60mV}) postsynaptic currents at layer V pyramidal neurons in the PFC using low-chloride-based internal solution. Note that bath application of the $GABA_A$ antagonist picrotoxin ($50 \mu M$, 10 min) suppressed PSC_{+15mV} events ($n = 6$; $p < 0.0001$ vs baseline, paired t test) without altering the frequency of synaptic activity recorded at the -60 mV holding potential (PSC_{-60mV}). **b**, Example traces of PSC_{+15mV} and PSC_{-60mV} recorded from the same pyramidal neuron illustrating the effect of picrotoxin shown in **a** (calibration: 20 pA/ 1 s). **c**, KYNA (300 nM, 10 min) exerted a small but significant attenuation of PSC_{-60mV} frequency ($n = 5$; $*p < 0.05$ vs baseline, paired t test). **d**, However, the extent of KYNA-induced synaptic inhibition at PSC_{+15mV} was significantly larger ($n = 5$; $**p < 0.005$ vs baseline, paired t test). **e**, Accordingly, KYNA increases the E–I ratio of synaptic activity in layer V pyramidal neurons ($n = 5$; $**p < 0.005$ vs baseline, paired t test). **f**, Further analysis revealed a significant correlation between the E–I ratio and PSC_{+15mV} (i.e., IPSC) frequency obtained after bath application of KYNA. **g**, Consistent with data shown in Figure 4d, the inclusion of MLA (300 nM, $n = 6$) into the baseline aCSF reduced the frequency of PSC_{+15mV} and occluded the inhibitory effect of KYNA. Conversely, 7Cl-KYNA (300 nM, $n = 6$) failed to alter the frequency of both PSC_{-60mV} and PSC_{+15mV} events. Subsequent bath application of KYNA significantly diminished the number of PSC_{+15mV} ($**p < 0.005$ vs baseline, paired t test) without disrupting the PSC_{-60mV} frequency. **h**, Summary of the E–I ratio data obtained following bath application of KYNA, MLA, and 7Cl-KYNA.

cell (Fig. 5d) such that a major shift toward a higher E–I ratio emerged (Fig. 5e). Further analyses of the data revealed that the magnitude of E–I imbalance elicited by KYNA correlates highly with the level of IPSC (PSC_{+15mV}) frequency suppression (Fig. 5f). A similar inhibitory effect was found with MLA, but not with 7Cl-KYNA (Fig. 5g). Notably, bath application of KYNA failed to further reduce the diminished PSC_{+15mV} frequency elicited by MLA (Fig. 5g). Such an occlusion was not observed when recordings were conducted in the presence of 7Cl-KYNA (Fig. 5g). Together, these results indicate that the E–I imbalance induced by KYNA in the PFC results from a preferential suppression of local GABAergic transmission via presynaptic $\alpha 7nAChR$ antagonism (Fig. 5h).

If the frequency-dependent disruption elicited by KYNA is due to a presynaptic attenuation of GABAergic function, it is anticipated that a postsynaptic facilitation of $GABA_A$ transmission would restore the normal pattern of LFP inhibition in the PFC. To test this hypothesis, we first investigated whether the inclusion of the $GABA_A \alpha 1$ -positive allosteric modulator Indiplon ($10 \mu M$) (Thomases et al., 2013) into the perfusion solution prevents the disrupting effect of KYNA on locally evoked E–I synaptic events using the same protocol as in Figure 5, which enables the acquisition of GABAergic and glutamatergic transmis-

sion (Fig. 6a). Data show that KYNA reduced the amplitude of locally evoked PSC_{+15mV} without altering the PSC_{-60mV} response (Fig. 6b). As a result, the balance of the evoked postsynaptic currents becomes disrupted and a shift toward a higher E–I ratio emerges (Fig. 6c). The inclusion of Indiplon effectively prevented the effects of KYNA at both PSC_{+15mV} amplitude (Fig. 6d) and E–I ratio of the evoked response (Fig. 6e). Next, Indiplon was coinjected with KYNA into the PFC and changes in hippocampal-evoked LFP were compared with those induced by KYNA alone (Fig. 7). Whereas the pattern of LFP response at 10 Hz remained unaffected (Fig. 7a), the abnormal facilitation of LFP at 20 Hz resulting from PFC infusions of KYNA was no longer apparent with the inclusion of Indiplon (Fig. 7b). Similarly, the addition of Indiplon was sufficient to prevent the attenuated LFP suppression induced by KYNA at 40 Hz (Fig. 7c). Indiplon alone does not alter the pattern of LFP response in the PFC. Together, these results provide a mechanistic link between the level of prefrontal GABAergic function and the frequency-dependent LFP disruption elicited by nanomolar concentrations of KYNA in the PFC.

Discussion

The present study showed that local administration of nanomolar concentrations of KYNA into the PFC attenuated the inhibi-

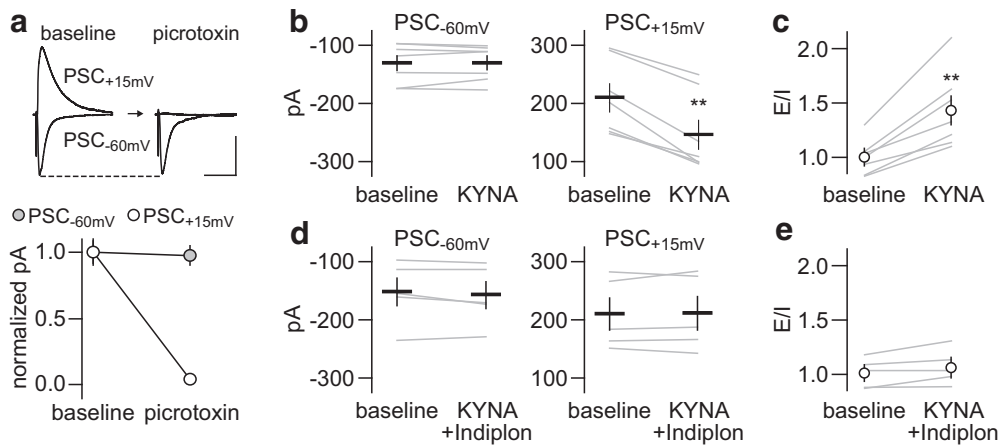


Figure 6. Reversal of KYNA-induced E–I imbalance by Indiplon. **a**, Recording of locally evoked inhibitory (PSC_{+15 mV}) and excitatory (PSC_{-60 mV}) postsynaptic currents at layer V pyramidal neurons using low-chloride-based internal solution. As in Figure 5, bath application of picrotoxin (50 μM, 10 min) suppressed the amplitude of locally evoked PSC_{+15 mV} ($n = 5$; $p < 0.001$ vs baseline, paired t test) without altering the PSC_{-60 mV} response (calibration: 100 pA/100 ms). **b**, KYNA (300 nM, 10 min) failed to reduce the amplitude of locally evoked PSC_{-60 mV}, but diminished the PSC_{+15 mV} amplitude by ~30% ($n = 7$; $**p < 0.001$ vs baseline, paired t test). **c**, As a result, KYNA increases the E–I ratio of the evoked synaptic response ($**p < 0.002$ vs baseline, paired t test), as estimated from the normalized PSC amplitude to baseline. **d**, The addition of Indiplon into the perfusion solution effectively blocked the inhibitory effect of KYNA on locally evoked PSC_{+15 mV} ($n = 5$). **e**, Accordingly, the E–I ratio of evoked postsynaptic currents remained unchanged.

tory component of LFP responses (20–40 Hz) without affecting the pattern of LFP facilitation. This is consistent with data obtained from PFC brain slices revealing an inhibitory action of KYNA, which primarily reduces GABAergic transmission through a presynaptic $\alpha 7$ nAChR-dependent mechanism. Accordingly, PFC infusion of the GABA_A $\alpha 1$ positive allosteric modulator Indiplon effectively prevented the disrupting effect of KYNA and restored the normal pattern of LFP inhibition. Together, these results indicate that local prefrontal GABAergic function is the preferential target of KYNA's inhibitory effect in the PFC.

Brain KYNA is an astrocyte-derived metabolite of the kynurenine pathway of tryptophan degradation and its dysregulation is thought to be implicated in schizophrenia (Plitman et al., 2017). Evidence for such a pathophysiological link arises from studies showing increased levels of KYNA in the brain and CSF (Erhardt et al., 2001; Schwarcz et al., 2001; Linderholm et al., 2012), and abnormal expression and activity of key kynurenine pathway enzymes in the brain (Miller et al., 2004; Sathyasaikumar et al., 2011; Wonodi et al., 2011) of patients with schizophrenia. Certainly, a causal link between relatively modest elevations of cortical KYNA and deficits in executive function has been well documented in preclinical studies (Chess et al., 2007; Akagbosu et al., 2012; Alexander et al., 2012, 2013). At the mechanistic level, KYNA is known to modulate $\alpha 7$ nAChR negatively (Hilmas et al., 2001) and to block the glycine coagonist site of the NMDAR (Parsons et al., 1997). Notably, however, the negative impact of KYNA on PFC-related cognitive functions has been suggested to occur mainly through inhibition of $\alpha 7$ nAChR signaling (Alexander et al., 2012, 2013). This interpretation is consistent with the results of the present study showing that the disrupting effect of KYNA in the PFC is also $\alpha 7$ nAChR dependent, primarily at local GABAergic synapses. Thus, the degree of GABAergic dysregulation in the PFC could be a clinically relevant contributing factor for the onset of cognitive deficits resulting from abnormal increases of cortical KYNA as seen in schizophrenia.

In addition to downregulating GABAergic function and decreasing extracellular GABA levels (Beggiato et al., 2014), nanomolar KYNA also reduces extracellular glutamate levels in the PFC (Konradsson-Geuken et al., 2010; Wu et al., 2010). However, the extent of GABAergic synaptic inhibition by KYNA was

much greater than the effect on glutamatergic synapses. As a result, a disinhibitory imbalance of E–I synaptic activity emerges in the PFC. Accordingly, the frequency-dependent LFP disinhibition elicited by PFC infusion of KYNA is indistinguishable from that induced by the GABA_AR antagonist picrotoxin (Cass et al., 2013; Thomases et al., 2013). The disruption of LFP by KYNA was no longer apparent after strengthening of prefrontal GABA_A $\alpha 1$ R function with Indiplon, indicating that local GABAergic synapses are more sensitive to the inhibitory effect of KYNA. Future studies will determine whether distinct functional expression of $\alpha 7$ nAChR between excitatory and inhibitory synapses contributes to bias the inhibitory action of KYNA onto GABAergic transmission in the PFC. It is also possible that differences in the localization rather than expression per se of $\alpha 7$ nAChR receptors are critical to the observed effects (Frazier et al., 1998).

Proper maturation of prefrontal GABAergic function during adolescence and its control of PFC output are critical for supporting adult cognitive functions such as working memory, decision making, and impulse control (Tse et al., 2015; Caballero et al., 2016). Although massive functional remodeling takes place in the PFC during adolescence (Caballero and Tseng, 2016), it is also the local GABAergic system that renders the PFC labile during this developmental period (Caballero et al., 2016). Therefore, it is possible that persistent elevations of KYNA in the PFC during adolescence may elicit enduring GABAergic dysfunction and disrupt the acquisition of inhibitory control that normally emerges in the adult PFC (Caballero et al., 2016). Elevations of KYNA levels during sensitive periods of perinatal development (Pocivavsek et al., 2012; Alexander et al., 2013; Forrest et al., 2013; Liu et al., 2014; Piser et al., 2014; Pershing et al., 2015, 2016) or adolescence (Akagbosu et al., 2012; DeAngeli et al., 2014; Pershing et al., 2016) may alter the balance of E–I in the PFC and contribute to the onset of cognitive deficits later in life, as seen in schizophrenia and other major psychiatric disorders.

Collectively, the results of the present study suggest a mechanism by which endogenous KYNA could affect PFC-dependent cognitive functions adversely. We showed for the first time that the inhibitory effect of KYNA in the PFC occurs primarily at GABAergic synapses through a presynaptic blockade of $\alpha 7$ nAChR signaling. Although KYNA can also function as a competitive

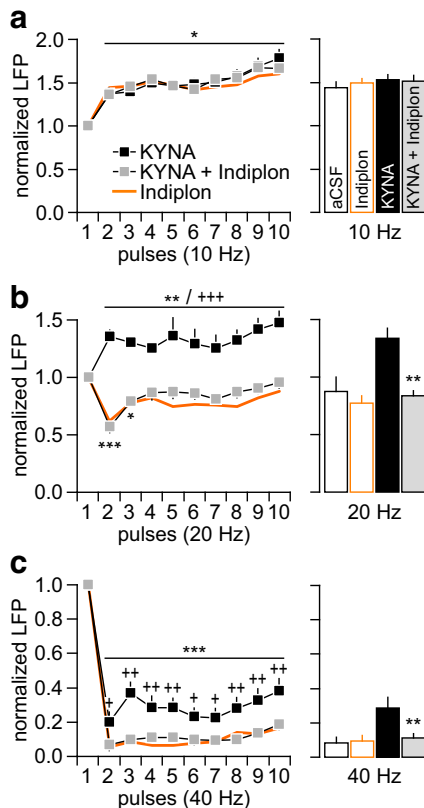


Figure 7. PFC infusion of Indiplon prevents the disrupting effects of KYNA. **a**, Normal pattern of LFP facilitation at 10 Hz remained unaltered by the addition of Indiplon (10 μ M) to the KYNA infusion solution (KYNA + Indiplon: $n = 8$; $*p < 0.05$ vs first pulse, LSD *post hoc* test after significant main effect of pulse, two-way ANOVA). The pattern of LFP facilitation elicited by Indiplon alone ($n = 5$) is included for comparison. Bar graph summarizes the mean LFP response calculated from pulses 2–10. Data from the aCSF group are included for comparison. **b**, At 20 Hz, the abnormal LFP facilitation induced by KYNA is no longer apparent with the addition of Indiplon ($*p < 0.05$ / $**p < 0.005$ / $***p < 0.0005$ vs first pulse, $+++p < 0.0005$ vs KYNA + Indiplon, LSD *post hoc* test; main effect of treatment: $F_{(1,140)} = 230.3$, $p < 0.0001$, two-way ANOVA). Indiplon alone does not affect the normal pattern of LFP suppression at 20 Hz. Bar graph of the mean LFP response (from pulses 2–10) summarizes the reversal effect of Indiplon ($**p < 0.005$ vs KYNA, Tukey's *post hoc* test; $F_{(3,26)} = 14.31$, $p < 0.0001$, one-way ANOVA). **c**, Attenuated LFP suppression at 40 Hz was no longer observed when Indiplon was coadministered with KYNA into the PFC ($***p < 0.0005$ vs first pulse, $+p < 0.05$ / $++p < 0.005$ / $+++p < 0.0005$ vs KYNA + Indiplon, LSD *post hoc* test; main effect of treatment: $F_{(1,140)} = 63.1$, $p < 0.0001$, two-way ANOVA). Indiplon alone does not alter the pattern of LFP suppression at 40 Hz. Bar graph of the mean LFP response (from pulses 2 to 10) summarizes the reversal effect of Indiplon ($**p < 0.005$ vs KYNA, Tukey's *post hoc* test; $F_{(3,26)} = 11.09$, $p < 0.0001$, one-way ANOVA).

inhibitor of NMDAR function via the glycine-B site (Kessler et al., 1989; Parsons et al., 1997), its contribution to disrupting PFC synaptic activity is not apparent because both the pattern of LFP responses and GABAergic transmission remained unaltered after application of the highly selective glycine-B site antagonist 7-Cl-KYNA (Kemp et al., 1988).

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