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Serotonin 1B receptors regulate prefrontal function by gating callosal and hippocampal inputs

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SUMMARY

Both the medial prefrontal cortex (mPFC) and serotonin play key roles in anxiety, however, specific mechanisms through which serotonin might act on the mPFC to modulate anxiety-related behavior remain unknown. Here, we use a combination of optogenetics and synaptic physiology to show that serotonin acts presynaptically via 5-HT1B receptors to selectively suppress inputs from the contralateral mPFC and ventral hippocampus (vHPC), while sparing those from mediodorsal thalamus. To elucidate how these actions could potentially regulate prefrontal circuit function, we infused a 5-HT1B agonist into the mPFC of freely behaving mice. Consistent with previous studies that have optogenetically inhibited vHPC-mPFC projections, activating prefrontal 5-HT1B receptors suppressed theta-frequency (4–12 Hz) mPFC activity, and also reduced avoidance of anxiogenic regions in the elevated plus maze. These findings suggest a potential mechanism, linking specific receptors, synapses, patterns of circuit activity, and behavior, through which serotonin may regulate prefrontal circuit function including anxiety-related behaviors.

Graphical Abstract

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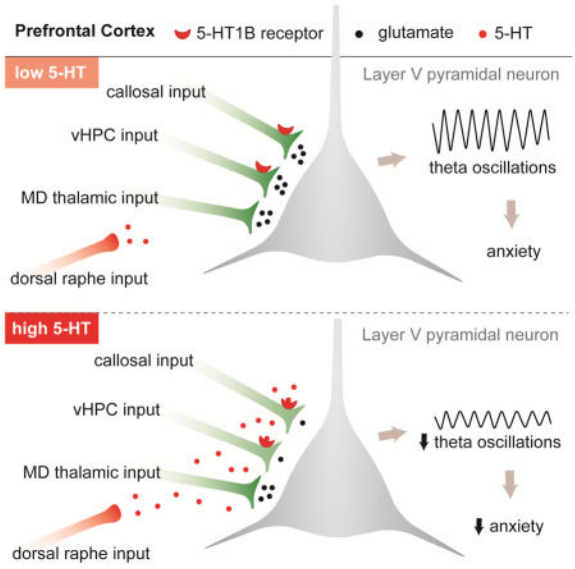
AUTHOR CONTRIBUTIONS

C.K. and V.S.S. designed experiments and wrote the manuscript. C.K. performed all experiments except for the following: S.E.R. performed strontium experiments with wash-in of CP 93129; J.A. measured the postsynaptic effects of the 5-HT1B agonist CP 93129; J.A. also assisted and performed part of the experiments measuring the effects of CP 93129 on LFP recordings in the mPFC; J.I. performed control recordings (no drug) for callosal inputs. C.K. analyzed the data.



Keywords

Serotonin; 5-HT1B receptor; prefrontal cortex; ventral; hippocampus; anxiety; mediodorsal thalamus

INTRODUCTION

The medial prefrontal cortex (mPFC) is an associational cortical region receiving a wide range of inputs from sensory cortices as well as contralateral mPFC, and is connected to limbic areas such as thalamus, hippocampus, and amygdala (Ishikawa and Nakamura, 2006; Miller and Cohen, 2001; Vertes, 2004; Verwer et al., 1997). Serotonin is believed to play an important role in regulating prefrontal function, since mPFC receives dense serotonergic innervation from the raphe nuclei (Groenewegen and Uylings, 2000; Hajós et al., 1998). Nevertheless, specific mechanisms through which serotonin might act on the mPFC to regulate network function remain largely unknown. In mPFC, as well as other forebrain regions, serotonin is generally believed to modulate circuit function through postsynaptic effects (Crino et al., 1990; de Almeida and Mengod, 2008; Santana et al., 2004). The postsynaptic effects of serotonin are mediated in large part by 5-HT1A receptors, such that most prefrontal neurons are inhibited by serotonin (Araneda and Andrade, 1991; Béïque et al., 2004; Zhong and Yan, 2011). However, serotonin can also regulate presynaptic neurotransmitter release. The identity of presynaptically located serotonin receptors remains largely unknown, but has been suggested to comprise 5-HT1A, 5-HT1B, and/or 5-HT2A receptors (Murakoshi et al., 2001; Tanaka and North, 1993; Troca-Marín and Geijo-Barrientos, 2010; Zhou and Hablitz, 1999).

Recent studies suggest that the mPFC may guide anxiety-related behaviors through interactions with the ventral hippocampus (vHPC) (Ciocchi et al., 2015). Specifically, theta-frequency synchronization between these two regions increases in aversive environments (Adhikari et al., 2010). At the level of single neurons, mPFC neurons that encode aspects of

anxiety-related behavior tend to phase-lock to ongoing theta-frequency activity in the hippocampus (Adhikari et al., 2011). This suggests that vHPC inputs to mPFC may transmit theta-frequency activity that encodes anxiety-related signals, and indeed, theta frequency activity in the mPFC seems to actively inhibit the exploration of anxiogenic regions (Adhikari et al., 2010). Conversely, optogenetically suppressing vHPC inputs to mPFC elicits anxiolytic effects by suppressing theta-frequency synchronization between the vHPC and mPFC (Padilla-Coreano et al., 2016). Thus, if serotonin does act presynaptically to control transmitter release, it could potentially gate afferent inputs from limbic regions such as the vHPC to mPFC and thereby regulate behavior.

To explore such a role for serotonin, we first investigated the effects of serotonin receptor activation on callosal, hippocampal, and MD thalamic projections to the mPFC, then explored how these synaptic effects might impact circuit physiology and anxiety-related behavior. We found that by activating presynaptic 5-HT1B receptors, serotonin selectively inhibits callosal and hippocampal inputs to mPFC, and that prefrontal infusion of a 5-HT1B receptor agonist suppresses prefrontal theta oscillations and elicits anxiolytic effects. These findings define – at the levels of synaptic function, *in vivo* electrophysiology, and behavior – a circuit mechanism through which prefrontal 5-HT1B receptors can acutely suppress ventral hippocampal inputs and thereby potentially regulate theta-frequency activity and anxiety-related behavior.

RESULTS

Serotonin suppresses synaptic input to prefrontal pyramidal neurons in a projection-specific manner

To investigate how serotonin affects various inputs to pyramidal neurons in prefrontal cortex, we made whole-cell recordings (voltage-clamped at −70 mV) from layer V pyramidal neurons and examined how serotonin wash-in affected excitatory postsynaptic currents (EPSCs) elicited by optogenetic stimulation of terminals from the contralateral mPFC, vHPC, or MD thalamus (Fig. 1A). We expressed ChR2 in one hemisphere of the mPFC, vHPC, or MD thalamus, and used light flashes to stimulate the terminals. To minimize possible contamination from polysynaptic responses, we measured the EPSC amplitude 1.5 ms after light stimulation (zoomed trace, Fig. 1B). Evoked EPSCs were blocked by application of 1 μM of the voltage-sensitive sodium channel blocker, tetrodotoxin (TTX) (Suppl. Fig. 1S).

We found that 5 min after wash-in, 30 μM serotonin reduced the initial amplitude of EPSCs evoked by optogenetic stimulation of terminals from contralateral mPFC by almost half (46 ± 9% reduction; $p < 0.01$; Fig. 1B). Input from vHPC was similarly reduced by 36 ± 7% ($p < 0.01$; Fig. 1C). However, no effect of serotonin application was observed for EPSCs evoked by stimulation of MD thalamic terminals ($p > 0.05$; Fig. 1D). Thus, serotonin suppresses specific inputs to PFC (Fig. 1E). As a control, we confirmed that this reduction in EPSC amplitude (induced by activation of callosal terminals) was not simply an artifact due to time-dependent effects (Fig. 1F). The suppressing effect of serotonin was also dose dependent (Fig. 1G).

Serotonin suppresses synaptic input through the 5-HT1B receptor

Next, we sought to understand which 5-HT receptors mediate this synaptic suppression. We specifically studied the effects of agonists for 5-HT1A (100 nM 8-OH DPAT), 5-HT1B (300 nM CP 93129), and 5-HT7 (10 nM LP-44) receptors on callosal or vHPC EPSCs. For each experiment, Fig. 2 shows the EPSC amplitude (normalized to the baseline EPSC amplitude) as a function of time with a superimposed control experiment (no drug). We compared the EPSC amplitude during drug application to the average of the amplitude measured before and after drug application. For callosal inputs, the normalized EPSC amplitude during application of the 5-HT1B agonist, CP 93129, was 58 ± 5% of the baseline amplitude. This was significantly smaller than the average of the amplitudes during the pre- and post-drug periods, which was 87 ± 6% of baseline ($p < 0.01$; Fig. 2B). Notably, the reduction observed during CP 93129 application was almost identical to the one observed during application of 3 μM serotonin (pre/post amplitude: 85 ± 4%; amplitude during 5-HT application: 57 ± 6%; $p < 0.05$; Fig. 2A). For vHPC input, the amount of EPSC suppression was again very similar for 3 μM 5-HT (pre/post: 77 ± 4%; 5-HT: 56 ± 8%; $p < 0.05$; Fig. 2E) and the 5-HT1B receptor agonist, CP 93129 (pre/post: 84 ± 3%; CP 93129: 50 ± 4%; $p < 0.001$; Fig. 2F). In control recordings, normalized EPSC amplitudes were 86 ± 4% and 88 ± 7% of baseline for callosal and vHPC EPSCs, respectively (amplitude of callosal EPSCs: $p < 0.001$ for CP 93129 vs. control and $p < 0.01$ for 5-HT vs. control; Fig. 2D and Suppl. Fig. S2E; amplitude of vHPC EPSCs: $p < 0.001$ for CP 93129 vs. control and $p < 0.05$ for 5-HT vs. control; Fig. 2H and Suppl. Fig. S2E). Importantly, the suppression of callosal or hippocampal EPSCs by 5-HT or CP 93129 was reversible with the EPSC amplitudes returning to control levels after 4–5 min of drug washout.

Applying CP 93129 in the presence of the 5-HT1B receptor antagonist, SB 216641 (10 μM for 20 min), blocked the CP 93129-induced suppression of callosal input (EPSC amplitude in SB 216641, pre/post: 83 ± 6%; CP 93129 + SB 216641: 78 ± 3%; n.s.; Fig. 2C). For vHPC input, CP 93129 still caused a slight reduction in EPSC amplitude (SB 216641, pre/post: 73 ± 3%; CP 93129 + SB 216641: 64 ± 5%; $p < 0.01$; Fig. 2G). However, for both callosal and vHPC input, the EPSC suppression induced by CP 93129 alone was significantly larger than that elicited by CP 93129 in the presence of SB 216641 ($p < 0.01$ for callosal input, Fig. 2D; $p < 0.05$ for vHPC input, Fig. 2H).

In contrast to the effects of the 5-HT1B receptor agonist CP 93129, the 5-HT1A and 5-HT7 receptor agonists, 8-OH DPAT and LP-44, respectively, had no consistent reversible effects on EPSC amplitudes elicited by either callosal (Fig. 2D, Suppl. Fig. S2A,B) or vHPC terminal stimulation (Fig. 2H, Suppl. Fig. S2C,D).

Serotonin suppresses synaptic input via presynaptic inhibition

To investigate whether this 5-HT1B receptor-mediated EPSC suppression reflects presynaptic mechanisms, we first measured the paired pulse ratio (PPR) for pairs of EPSCs evoked via optogenetic stimulation of callosal or vHPC terminals using interflash intervals (IFI) of 50 ms (Fig. 3A and F).

For callosal terminals, 3-min wash-in of 5-HT (3 μM) and CP 93129 (300 nM) increased PPR to 118 ± 2% and 117 ± 4% of control, respectively ($p < 0.01$; Fig. 3B and C). This effect could be washed out for CP 93129 (drug versus post-drug: $p < 0.05$). Pre-application of the 5-HT1B receptor antagonist, SB 216641 (10 μM), blocked the PPR increase induced by CP 93129 ($p < 0.05$; Fig. 3D and E). For vHPC terminals, there was a no effect of 3 μM 5-HT application on PPR (Fig. 3G). CP 93129 did significantly increase the PPR compared to the pre-drug period (114 ± 4%; $p < 0.05$; Fig. 3H) and this effect was reversed during drug wash-out (drug versus post-drug: $p < 0.01$). The CP 93129 induced increase in PPR was blocked by pre-application of SB 216641 (Fig. 3I and J).

To more strongly establish a presynaptic locus for the effects of serotonin, we also performed a quantal analysis of synaptic responses by recording asynchronous unitary EPSCs (uEPSC) elicited by optogenetic stimulation of either callosal or vHPC terminals after replacing calcium in the external solution with strontium (Sr^{2+}, 2 mM) (Fig. 3K). Light intensity was adjusted to produce an EPSC of ~200 pA, and analysis of uEPSCs started 50 ms after each flash. uEPSCs from each recorded cell were collected following 120 light stimulations (delivered with a 3 second inter-stimulus interval), both before and following drug wash-in. After washing in serotonin, we observed a reduction in the frequency of uEPSCs following each stimulus delivered to callosal terminals ($p < 0.05$; Fig. 3M), with no change in uEPSC amplitude, rise time, or decay time (Fig. 3L and Suppl. Fig. S3). We observed a similar effect when stimulating terminals from vHPC: serotonin reduced uEPSC frequency ($p < 0.05$; Fig. 3O), but had no effect on uEPSC amplitude or kinetics (Fig. 3N and Suppl. Fig. S3). We confirmed that the selective 5-HT1B receptor agonist CP 93129 (300 nM) elicits a similar, selective reduction in uEPSC frequency by vHPC terminal stimulation (Suppl. Fig. S3).

Local infusion of a 5-HT1B receptor agonist into mPFC reduces innate anxiety

To determine whether this 5-HT1B receptor-mediated inhibition of callosal and hippocampal inputs to mPFC could regulate anxiety-related behavior, we infused the 5-HT1B receptor agonist, CP 93129, into the mPFC of freely moving mice while measuring anxiety-related behavior using the elevated plus maze (EPM). The EPM consists of two open and two closed arms. Time spent in the open arms reflects reduced levels of innate anxiety (Fig. 4A). CP 93129 (4.3 ng in 0.5 μL, 150 nL/min) or saline (0.5 μL) was infused bilaterally into the mPFC through an implanted guide cannula. 1 min after the internal infusion cannula was removed, the animal was placed in the EPM for 15 min. Compared to saline, CP 93129 increased the time mice spent in open arms during the first 5-min of the EPM. There was no difference during the two subsequent 5-min bins (Fig. 4A). We also tested the effect of CP 93129 infusion on behavior in the open field, and no difference in the distance moved was evident for the first 6 min of the test (Fig. 4B). In addition, CP 93129 had a slight tendency to increase time spent in the center of the open field for the first 2 min of the test ($p = 0.14$; Suppl. Fig. S4A).

Local infusion of a 5-HT1B receptor agonist suppresses mPFC theta power

To investigate possible circuit mechanisms linking the 5-HT1B receptor-mediated vHPC suppression to the reduction of anxiety-related avoidance in the EPM, we implanted animals

with integrated guide cannulas/tungsten electrodes and recorded local field potentials (LFP) in mPFC continuously during 10 min infusion of the 5-HT1B receptor agonist, CP 93129 (4.3 ng in 0.5 μL, 50 nL/min) or saline (Fig. 4C), while the animal was in a resting cage. We compared the LFP power at various frequencies for the 4 min periods leading up to the start of infusion, and following the end of infusion (Fig. 4D and E). Compared to saline infusion, infusion of CP 93129 was associated with reduced power (ANOVA on the log-transformed power post-infusion/pre-infusion: treatment: $p < 0.01$; treatment x frequency band interaction: $p < 0.01$). A selective reduction in theta (4–12 Hz) as well as alpha and beta (12–30 Hz) power was evident (Fig. 4D and E; $p < 0.05$).

Finally, we confirmed that in contrast to serotonin itself, 5-HT1B receptor agonist application does not alter the intrinsic properties of layer V pyramidal neurons in mPFC (Suppl. Fig. S4A), suggesting that this reduction in power is due to the suppression of synaptic input.

DISCUSSION

We investigated whether serotonin modulates specific inputs to the mPFC, and if so, whether this might enable specific prefrontal serotonin receptors to regulate anxiety-related behavior. We found that serotonin acts presynaptically via 5-HT1B receptors to suppress callosal and hippocampal inputs, but not those from the MD thalamus. Then we found that activating 5-HT1B receptors within the mPFC increased the exploration of anxiogenic regions of the EPM, while also suppressing mPFC power in the theta, alpha, and beta frequency bands.

Serotonin acts presynaptically through 5-HT1B receptors

For callosal projections, both serotonin and the 5-HT1B receptor agonist, CP 93129, increased the PPR and reduced the frequency of uEPSCs observed in Sr^{2+}, without affecting their amplitude or kinetics, all of which is consistent with a presynaptic site of action. For vHPC terminals, the 5-HT1B receptor agonist, CP 93129, elicited a statistically significant increase in PPR. The effect of serotonin itself on the PPR of vHPC inputs was not statistically significant, but both serotonin and CP 93129 reduced the frequency of uEPSCs elicited by vHPC inputs in Sr^{2+}, without affecting their amplitude or kinetics. Taken together, these effects are consistent with a presynaptic locus for the actions of serotonin.

Serotonin has been shown to reduce excitatory input to layer V visual neurons elicited by local electrical stimulation while increasing the PPR and reducing the frequency of spontaneous excitatory events (Murakoshi et al., 2001). A similar presynaptic effect of serotonin has been observed for excitatory inputs to prefrontal pyramidal neurons elicited via electrical stimulation of the corpus callosum or subcortical white matter (Tanaka and North, 1993; Troca-Marín and Geijo-Barrientos, 2010). Using optogenetics to overcome the limitations of electrical stimulation, which can activate fibers from multiple sources in both anterograde and retrograde directions, we found that serotonin acts presynaptically to suppress callosal and hippocampal inputs to layer V pyramidal neurons, but not those from MD thalamus. The absence of an effect of serotonin on inputs from MD thalamus is in contrast to a study in somatosensory cortex of neonatal mice showing that serotonin reduces excitatory synaptic input elicited by electrical stimulation of the internal capsule (Laurent et

al., 2002). Differences in optogenetic vs. electrical stimulation, brain regions being studied, and developmental stage may explain these discrepancies.

Serotonin targets specific cells and synapses within the mPFC

In the mPFC, postsynaptic serotonin receptors are differentially expressed by various subtypes of layer V pyramidal neurons that project to different targets (Avesar and Gulledge, 2012; Stephens et al., 2014). Thus, serotonin is well poised to dynamically regulate the flow of information both into and out of the mPFC, thereby coordinating its participation in distributed brain networks. Notably, we found that serotonin modulates presynaptic terminals in the mPFC via 5-HT1B receptors, but that these receptors fail to elicit obvious postsynaptic effects, which instead appear to be mediated by 5-HT1A and 5-HT2A receptors. This makes it possible to selectively target these various pre- and postsynaptic effects using specific serotonin receptor agonists or antagonists.

Serotonin regulates the flow of anxiety-related information through the mPFC

The mPFC is well positioned to connect contextual information with appropriate anxiety behavior. A recent study that recorded from mPFC neurons found firing patterns consistent with a model in which these neurons utilize vHPC inputs to guide avoidance behaviors in the EPM (Adhikari et al., 2011). In particular, vHPC projections to the mPFC appear regulate anxiety-related behavior by transmitting theta-frequency (4–12 Hz) activity, since 1) both the firing of mPFC-projecting cells within the vHPC and theta-frequency synchronization between the vHPC and mPFC increase in anxiogenic environments (Adhikari et al., 2010; Ciocchi et al., 2015), and 2) optogenetically suppressing mPFC inputs from vHPC (but not from MD thalamus) decreases open arm avoidance by desynchronizing vHPC and mPFC theta activity (Padilla-Coreano et al., 2016). In this context, our findings that 5-HT1B receptors suppress vHPC input to mPFC, theta-frequency (4–12 Hz) activity in mPFC, and avoidance behaviors in the EPM, suggest that serotonin may suppress the ability of vHPC input in order to drive theta-frequency activity in mPFC and thereby elicit avoidance behaviors. This model, in which serotonin suppresses long-range inputs to the mPFC, is also consistent with a recent study showing enhanced functional connectivity between the mPFC and subcortical networks in a mouse model of serotonin deficiency (Dzirasa et al., 2013).

Of course, the intra-mPFC infusion of CP 93129 may target other 5-HT1B receptors, e.g., possible autoreceptors on serotonergic fibers. We do know that 5-HT1B receptor activation is sufficient to suppress vHPC inputs to mPFC, and that such suppression elicits anxiolytic effects, but we cannot rule out the possibility that other, possibly unknown effects of prefrontal 5-HT1B receptors, contribute as well.

Conclusion

Elucidating the role of prefrontal serotonin in brain network function requires connecting specific serotonin receptors with their cellular and synaptic effects, then understanding how these shape circuit physiology in ways that could impact behavior. Here, we have described a mechanism through which 5-HT1B receptors can presynaptically suppress specific sources of input to the mPFC, as well as a known circuit-level electrophysiological correlate of anxiety (mPFC theta oscillations). Our results are consistent with a model in which the

mPFC integrates inputs from various limbic sources in order to direct affective behavior, and suggest that by acting as a gatekeeper, serotonin may be able to powerfully modulate this process.

EXPERIMENTAL PROCEDURES

Please refer to supporting information for detailed methods. All experiments were conducted in accordance with protocols approved by the Administrative Panels on Laboratory Animal Care at the University of California, San Francisco.

Slice electrophysiology and optogenetics

We expressed hChR2-eYFP in neurons within mPFC, MD thalamus, and vHPC using a previously described AAV5 vector encoding hChR2-eYFP under control of the CaMKIIα promoter (37). After waiting at least 4–6 weeks, we prepared 250 μm coronal slices from 8–12 week-old wild-type C57BL/6 male mice, and made whole cell recordings using patch pipettes filled with a Cs-methanesulfonate based solution from pyramidal neurons in layer V of infralimbic or prelimbic cortex (contralateral to the site of virus injection). We stimulated ChR2 in axon terminals using flashes of 470 nm light delivered to the slice through a 40x objective.

Drug infusion

We implanted stainless steel dual guide cannulas bilaterally into mPFC. One week later, we infused drug through a stainless steel internal cannula projecting 1.5 mm beyond the tip of the guide cannula in a volume of 0.5 μL saline at a rate of 150 nL/min (EPM experiments) or 50 nL/min (LFP recording).

Local field potential recording

50 kΩ impedance tungsten electrodes were attached to the right arm of a dual guide cannula and implanted in mPFC. Two stainless steel screws acting as reference and ground were implanted above cerebellum. The electrode and screws were attached to a head mount using conductive wire and the set-up was cemented down. Mice recovered for one week before recording/infusion sessions, and signal was acquired using an EEG monitoring and acquisition system (Pinnacle Technology).

Behavior

C57BL/6 male mice were kept in a 12-h light/12-h dark cycle with ad libitum access to food and water. The elevated plus maze (EPM) consisted of two open and two closed arms (arm length: 50 cm; width: 6 cm) extending from a central platform, and the open field was 50 x 50 cm. Mice were infused with drug and placed in the center of the EPM and the head and body of the mouse was tracked (ANYmaze software) for 15 min.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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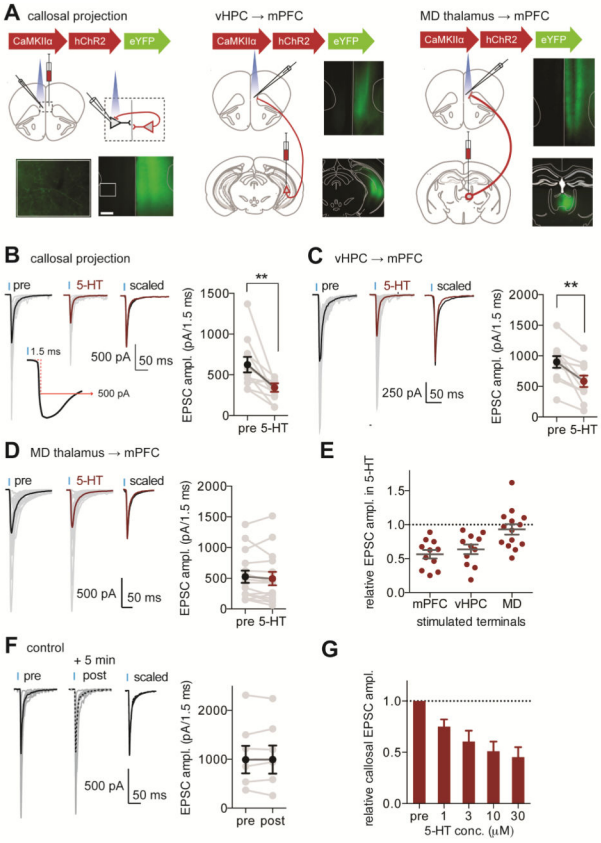

FIGURE 1. Serotonin suppresses afferent input to mPFC layer V pyramidal neurons from the contralateral mPFC and vHPC, but not MD thalamus

A. Experimental design: AAV5-CaMKIIα-hChR2-eYFP was injected unilaterally into the contralateral medial prefrontal cortex (mPFC), the ipsilateral ventral hippocampus (vHPC), or the ipsilateral mediodorsal (MD) thalamus. Whole cell voltage clamp recordings were obtained from layer V pyramidal neurons in mPFC. Postsynaptic currents were evoked by 470 nm light flashes activating ChR2 expressing terminals (red). Low- and high-power images show ChR2-eYFP expression at the injection site and within axon terminals in mPFC (scale bar: 50 μm). **B.** Postsynaptic responses of layer V pyramidal neurons to activation of callosal terminals by single light flashes in voltage clamp. Below, a magnified example trace shows how we measure the amplitude of excitatory postsynaptic currents (EPSC) 1.5 ms after EPSC initiation to minimize possible contamination from polysynaptic responses. Wash-in of 30 μM serotonin (5-HT) reduces the amplitude of evoked EPSCs. Gray traces represent data from individual experiments and black / red traces represent averages across experiments. Scaled responses at baseline (pre) and following 5 min of serotonin wash-in are shown at the right. **C.** 30 μM serotonin (5-HT) reduced the amplitude of EPSCs evoked by vHPC terminal stimulation. **D.** There was no effect of 5-HT on the responses of layer V pyramidal neurons to activation of MD thalamic terminals. **E.** The relative amplitudes of optogenetically evoked EPSCs following 5-HT wash-in for projections from the mPFC, vHPC, or MD thalamus. **F.** Responses to callosal stimulation did not change simply as a function of time. **G.** The relative EPSC amplitude as a function

of serotonin concentration (EPSCs evoked by callosal stimulation). Data are represented as mean ± SEM. *$p < 0.05$; **$p < 0.01$. n = 11 for callosal stimulation; n = 14 for MD thalamus; n = 11 for vHPC; n = 6 for time control; n = 4 for dose-response.

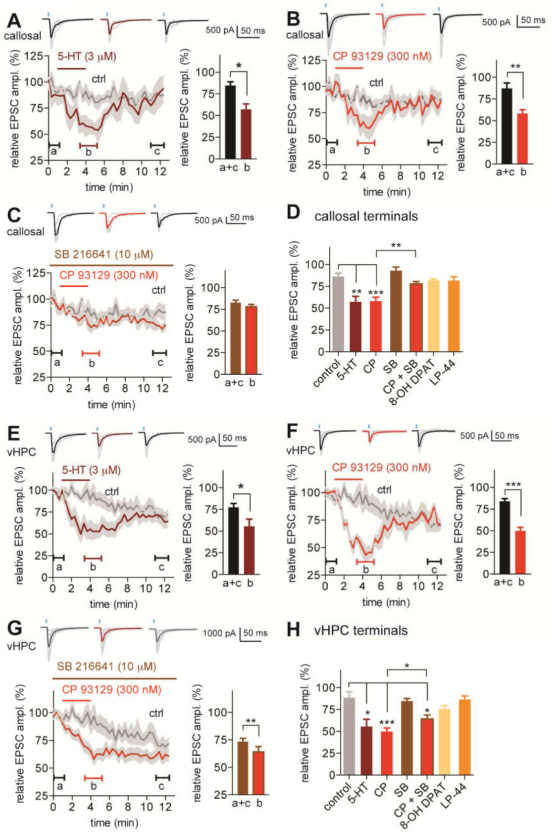

FIGURE 2. Serotonin suppresses callosal and ventral hippocampal input to mPFC through the 5-HT1B receptor

A. Excitatory postsynaptic currents (EPSCs) in mPFC layer V pyramidal cells were recorded in response to optogenetic stimulation of callosal terminals and during 3-min wash-in followed by wash-out of 3 μM 5-HT. Relative EPSC amplitude (normalized by the initial amplitude) is plotted as a function of time (red line). The dark red bar above the trace shows the time of 5-HT application. The labels ‘a’, ‘b’ and ‘c’ below the trace indicate the periods used to calculate EPSC amplitude during the pre-drug period (‘a’), immediately following 5-HT application (‘b’), and the post-drug period (‘c’). The time course of EPSC amplitudes during control recordings (no drug) is superimposed for comparison (gray line, ‘ctrl’). The summary graph (right) shows the average EPSC amplitude during the pre- and post-drug periods (‘a+c’) compared to the period immediately following 5-HT application (‘b’). Top: individual (gray) and mean (black/dark red) traces are shown for pre-drug, drug, and post-drug periods. **B.** Similar to *A*, but shows effects of the 5-HT1B receptor agonist, CP 93129 (300 nM) on callosal EPSC amplitude. **C.** Similar to *A*, but shows that the effect of the 5-HT1B receptor agonist, CP 93129 (300 nM), on EPSC amplitude is blocked in the presence of the 5-HT1B receptor antagonist, SB 216641 (10 μM). **D.** Summary graph comparing callosal EPSC amplitudes during application of each drug combination. Callosal EPSCs were suppressed (relative to control recordings) by 5-HT and CP 93129 (CP), and the effect of CP 93129 was blocked by pre-application of SB 216641 (SB). **E-G.** Similar to *A-C*, but shows effects of 5-HT (E), CP 93129 (F), and CP 93129 + SB 216641 (G) on EPSCs evoked

by optogenetic stimulation of vHPC terminals. **H.** Summary graph comparing the effects of various drugs on vHPC EPSC amplitude. 5-HT and CP 93129 (CP) suppressed EPSC amplitude (relative to control recordings). CP 93129 alone suppressed EPSCs to a larger extent than CP 93129 + SB 216641 (SB). Data are represented as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Callosal input: n = 5 for 5-HT; n = 4 for control; n = 8 for CP 93129; n = 5 for CP 93129 + SB 216641; n= 7 for 8-OH DPAT; n = 5 for LP-44. vHPC input: n = 6 for 5-HT; n = 8 for control; n = 6 for CP 93129; n = 9 for CP 93129 + SB 216641; n = 8 for 8-OH DPAT; n = 11 for LP-44.

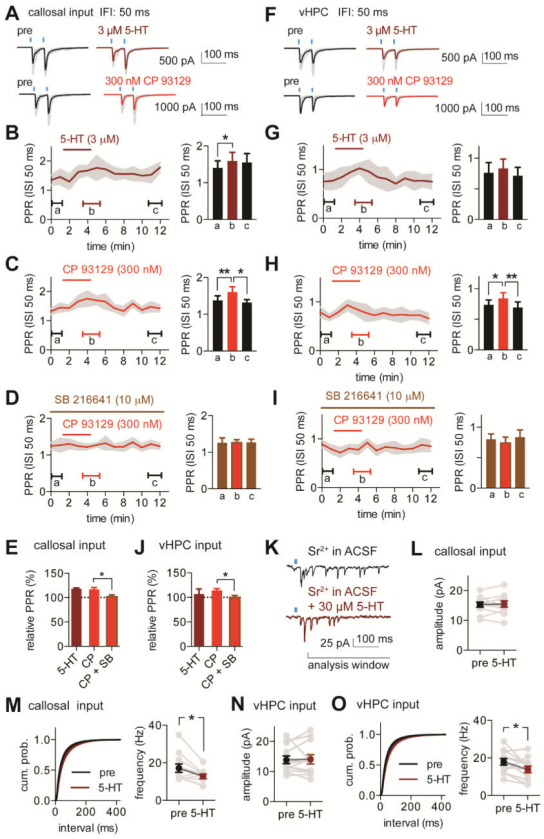

FIGURE 3. 5-HT1B receptors increase the paired pulse ratio and reduce the frequency of asynchronous unitary events elicited by stimulation of callosal or vHPC terminals

A. mPFC pyramidal cell responses to pairs of light flashes activating callosal terminals with interflash intervals (IFI) of 50 ms before and after wash-in of serotonin (5-HT) or the 5-HT1B receptor agonist, CP 93129. Gray traces represent data from individual cells and black / red traces represent averages across experiments. **B.** 3 μM 5-HT increased the PPR of EPSCs evoked by activation of callosal terminals with (IFI: 50 ms). **C.** For callosal terminals, PPR was also increased in response to wash-in of CP 93129 (300 nM), and returned to baseline levels following wash-out. **D.** There was no effect on PPR when CP 93129 application was preceded by application of SB 216641 (10 μM). **E.** Summary graph showing the drug-induced change in PPR of EPSCs evoked by optogenetic stimulation of callosal projections (relative to the pre-drug period). The PPR increase following CP 93129 (CP) wash-in was blocked following pre-application of SB 216641 (SB). **F.** Example of pyramidal cell responses to pairs of light flashes (IFI: 50 ms) activating vHPC input in response to serotonin (5-HT) and CP 93129. **G.** For vHPC input, 5-HT (3 μM) elicited a non-significant increase in the PPR (IFI: 50ms). **H.** For vHPC input, CP 93129 reversibly increased PPR. **I.** Pre-application of SB 216641 blocked the effect of CP 93129 on the PPR of responses to vHPC input. **J.** Summary plot for vHPC terminals: the CP 93129-induced increase in PPR was blocked by pre-application of SB 216641. **K.** Example traces showing asynchronous unitary EPSCs (uEPSC) induced by photostimulation of callosal terminals in ACSF in which Ca^{2+} has been replaced with 2 mM Sr^{2+}. **L.** There was no difference in

callosally induced uEPSC amplitude following wash-in of 5-HT. **M.** For uEPSCs elicited by callosal terminal stimulation, the cumulative probability plot of inter-event intervals is shifted to the right following serotonin application. The summary plots show that the frequency of uEPSCs is lower in 5-HT. **N.** vHPC stimulation-induced uEPSCs did not show a change in amplitude following 5-HT application. **O.** The frequency of uEPSCs evoked by vHPC terminal stimulation is reduced following 5-HT wash-in as shown in the cumulative probability and summary plot. Data are represented as mean ± SEM. *$p < 0.05$, **$p < 0.01$; PPR: n = 6/8/5 for callosal terminals; n = 5/6/8 for vHPC terminals. uEPSC: n = 11 for callosal terminals; n = 12 for vHPC.

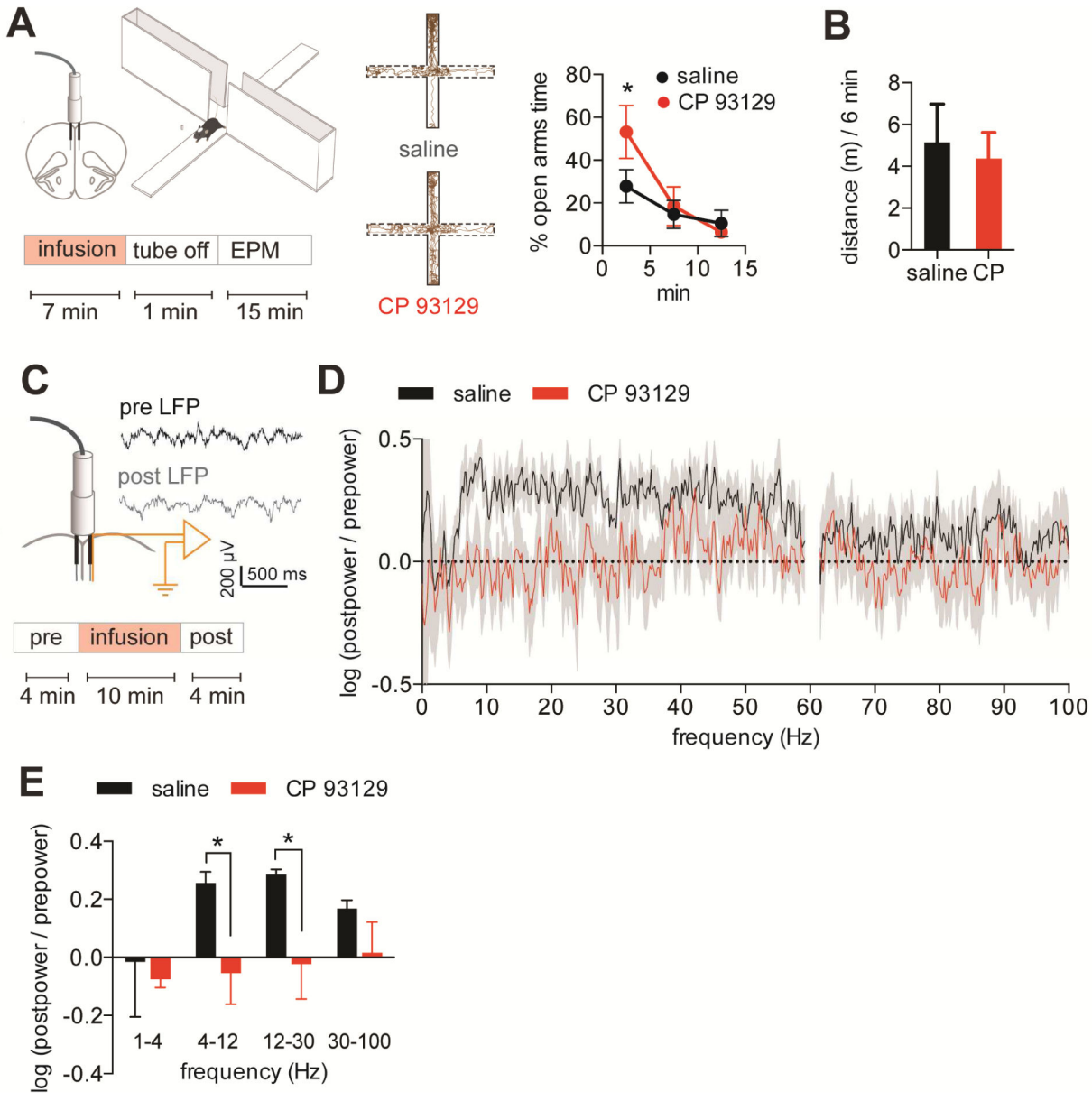

FIGURE 4. Intra-mPFC infusion of a 5-HT1B receptor agonist reduces innate anxiety and mPFC theta power

A. Left: experimental set-up. Freely moving mice were infused with saline +/− drug through a guide cannula implanted bilaterally in the mPFC. Following infusion, the animal was placed in an elevated plus maze (EPM) for 15 min. Right: infusion of the 5-HT1B receptor agonist, CP93129 (4.3 ng in 0.5 µL), increased time spent in the open arms of the EPM compared to saline infused mice. **B.** There was no effect of CP 93129 infusion in distance moved in the first 6 min of the open field test. **C.** A tungsten electrode was attached to a bilateral guide cannula and implanted within the mPFC. Local field potentials (LFP) were recorded continuously during infusion and power spectra for 4-min pre- and post-infusion periods were compared. Example traces showing recordings before and after infusions of saline are shown. **D.** The logarithm of the ratio between the pre- and post-drug power spectra

for saline (black) and CP 93129 (red) infusion are shown. **E.** The summary plot shows the mean log ratio power for saline and CP 93129 infusions in different frequency bands. Theta (4–12 Hz) and beta (12–30 Hz) are selectively reduced following CP 93129 infusion. Data are represented as mean ± SEM. *$p < 0.05$. EPM: n = 4 for saline and n = 5 for CP 93129; LFP: n = 11 for saline and n = 8 for CP 93129.