Citalopram reduces glutamatergic synaptic transmission in the auditory cortex via activation of 5-HT_{1A} receptors

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Serotonin modulates cognitive processes and is related to various psychiatric disorders, including major depression. Administration of citalopram reduces the amplitude of auditory evoked potentials in depressed people and animal models, suggesting that 5-HT has an inhibitory role. Here, we characterize the modulation of excitatory post-synaptic currents by application of either 5-HT or agonists of 5-HT_{1A} and 5-HT_2 receptors, or by endogenous 5-HT evoked by citalopram on pyramidal neurons from layer II/III of rat auditory cortex. We found that application of 5-HT concentration-dependently reduces excitatory post-synaptic currents amplitude without changing the paired-pulse ratio, suggesting a post-synaptic modulation. We observed that selective agonists of 5-HT_{1A} and 5-HT_2 receptors [8-OH-DPAT (10 μM) and DOI (10 μM), respectively] mimic the effect of 5-HT on the excitatory post-synaptic currents. Effect of 5-HT was entirely blocked by co-application of the antagonists NAN-190 (1 μM) and ritanserin (200 nM). Similarly, citalopram application (1 μM) reduced the amplitude of the evoked excitatory post-synaptic currents. Reduction in the magnitude of the excitatory post-synaptic currents by endogenous 5-HT was interpolated in the dose-response curve elicited by exogenous 5-HT, yielding that citalopram raised the extracellular 5-HT concentration to 823 nM. Effect of citalopram was blocked by the previous application of NAN-190 but not ritanserin, indicating that citalopram reduces glutamatergic synaptic transmission via 5-HT_{1A} receptors in layer II/III of the auditory cortex. These results suggest that the local activity of 5-HT contributes to decrease in the basal excitability of the auditory cortex for enhancing the detection of external relevant acoustic signals. *NeuroReport* 30: 1316–1322 Copyright © 2019 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

Serotonin (5-HT) is a major neurotransmitter in the central nervous system, related to different psychiatric disorders like depression. A selective serotonin reuptake inhibitor (SSRI) citalopram has been indicated as the first option for the treatment of major depression due to its better efficacy/side effects ratio than other antidepressant drugs [1]. Theoretically, the influence of citalopram is determined by the relationship between the locus of 5-HT release, the activity, and location of the reuptake transporters and the localization of 5-HT receptor subtypes [2]. Thus, physiological studies may be helpful to characterize brain circuits relevant to the therapeutic action of antidepressant drugs and to improve their response [2].

Depression has been generally associated with impairment of sensory-motor function. On this point, it has been noted that 5-HT modulates the information processing of the sensory areas of the brain [3] and the information processing of the auditory receptive fields [4]. Specifically, the administration of citalopram reduces the amplitude of the auditory evoked potentials measured both in depressed people [5] and in rodents models of depression [6]. In order to understand this relationship, the first step would be to determine how 5-HT modulates synaptic transmission in the auditory cortex [7,8]. However, the specific mechanisms by which 5-HT modulates glutamatergic synaptic transmission in the auditory cortex are still unknown.

Morphological studies have shown the localization of 5-HT_{1A} [9] and 5-HT_{2A} receptors in the auditory cortex [10]. Also, a recent post-mortem study in depressed humans showed that the expression of the 5-HT_{1A} receptors in the auditory cortex was higher than in healthy controls, whereas the expression of the 5-HT_2 receptors was the opposite. Hence, altered expression of the 5-HT receptors in the auditory cortex might be associated with major depression [11]. Previous evidence in layer II/III of

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the juvenile rat auditory cortex showed that activation of 5-HT_{1A} and 5-HT_2 receptors reduce GABAergic synaptic transmission through pre- and post-synaptic mechanisms, respectively [7]. Notably, the SSRI citalopram, which increases the levels of 5-HT in the synaptic cleft, may activate both 5-HT_{1A} and 5-HT_2 receptors. We hypothesized that 5-HT and citalopram would reduce glutamatergic transmission via activation of 5-HT_{1A} and 5-HT_2 receptors in layer II/III of the auditory cortex. Here we characterized the effect of exogenous and endogenous 5-HT on glutamatergic synaptic transmission in layer II/III of auditory cortex. To explore the role of endogenous 5-HT in glutamatergic synaptic transmission in the auditory cortex, the potent and selective 5-HT reuptake blocker citalopram was used. To investigate the participation of 5-HT_{1A} receptors in the inhibitory effects of the 5-HT on glutamatergic synaptic currents, the selective agonist 8-OH-DPAT and the specific blocker of 5-HT_{1A} receptors, NAN-190 were applied. The role of 5-HT_2 receptors was assessed by application of the selective 5-$HT_{2A/C}$ receptor agonist (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride, (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride (DOI), and the selective antagonist ritanserin. Finally, glutamatergic synaptic currents were recorded in the presence of the $GABA_A$ receptor blocker picrotoxin and, to ensure that the observed synaptic currents were due to a-Amino-3-hydroxy-5methylisoxazole-4-propionic acid (AMPA) receptors, in some experiments 6,7-Dinitroquinoxaline-2,3(1H,4H)-dione (DNQX) was used.

Methods

Sixty male Wistar rats 25–30 days old were used in the present study. The animals were housed in a 12:12 hours light/dark cycle with unrestricted access to water and food. All the methods were in accordance with the Ethics Committee of the Autonomous University of Yucatan, CIR-B 2012-0018 and CEI-14-2018, and were according to the guidelines of the National Institute of Health for animal research. Slice preparation of coronal auditory cortex was performed as described previously [12]. Rats were anesthetized with isoflurane (99.9%; Baxter, Round Lake, Illinois, USA) until they lost all their reflexes, and afterwards the brain was extracted. Auditory cortex coronal slices (270 µm thick) were obtained with a vibratome (VT1000; Leica, Biosystems Inc. Illinois, USA) in a cold solution (4°C) containing (in mM): 126 NaCl, 3.5 KCl, 10 Glucose, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 1.5 $CaCl_2$, and 1.5 $MgCl_2$, pH 7.4 and saturated with 95% O_2 and 5% CO_2. Slices were incubated in artificial cerebro-spinal fluid (ACSF) at 32°C for 1 hour before being placed in the recording chamber. All drugs used in this work were purchased from Sigma (México). Stock solutions of DOI and ritanserin were diluted in methanol; NAN-190 and 8-OH-DPAT were diluted in DMSO, and appropriate dilutions were made with ACSF to achieve the desired concentrations in the bath fluid. All drugs were prepared the same day of the experiment and were bath applied in ACSF solution.

Electrophysiology

Pyramidal neurons were visually selected using a BX51WI microscope (Olympus, Tokyo, Japan) with an infrared camera system (DAGE-MTI, Michigan City, Indiana, USA). Post-synaptic glutamatergic currents (EPSCs) were recorded in a whole-cell configuration, in voltage-clamp mode, with a holding potential of –60 mV through 3–5 MΩ borosilicate electrodes in the bath (P-97 Sutter Instruments) filled with an intracellular solution (in mM): 125 K^+-gluconate, 15 KCl, 0.5 EGTA, 1 lidocaine N-ethyl bromide (QX314), 1 $MgCl_2$, 10 HEPES, 4 glutathione, 3 $ATPMg_2$, 0.3 $GTPNa_2$ and 20 phosphocreatine, with a pH of 7.2 and an osmolarity of 275 ± 5 mOsm. The intracellular solution also contained picrotoxin (1 mM) to block $GABA_A$ receptors [13,14]. EPSCs were evoked by two identical electric stimuli (paired-pulse, 0.1 ms, <50 µA). The intensity of the stimuli was adjusted to obtain pairs of EPSC separated by 50 ms, and the stimuli were applied every 10 seconds with an isolation unit (A365; World Precision Instruments, Sarasota, Florida, USA), through a monopolar glass stimulation electrode filled with ACSF and placed at approximately 100–200 µm from the recording electrode. A voltage step of 2 mV was applied at the beginning of each episode to monitor access resistance (Ra: <20 MΩ) and input resistance (Ri: >100 MΩ). Cells were excluded from the analysis only if these parameters changed ≥20% during the experiment. Signals were filtered at 2 kHz and, digitized to 10 kHz. All the experiments were analyzed off-line using pCLAMP10.5 software (Molecular Devices) and Sigma Plot V10 (Systat, software Inc., California, USA).

Data analysis

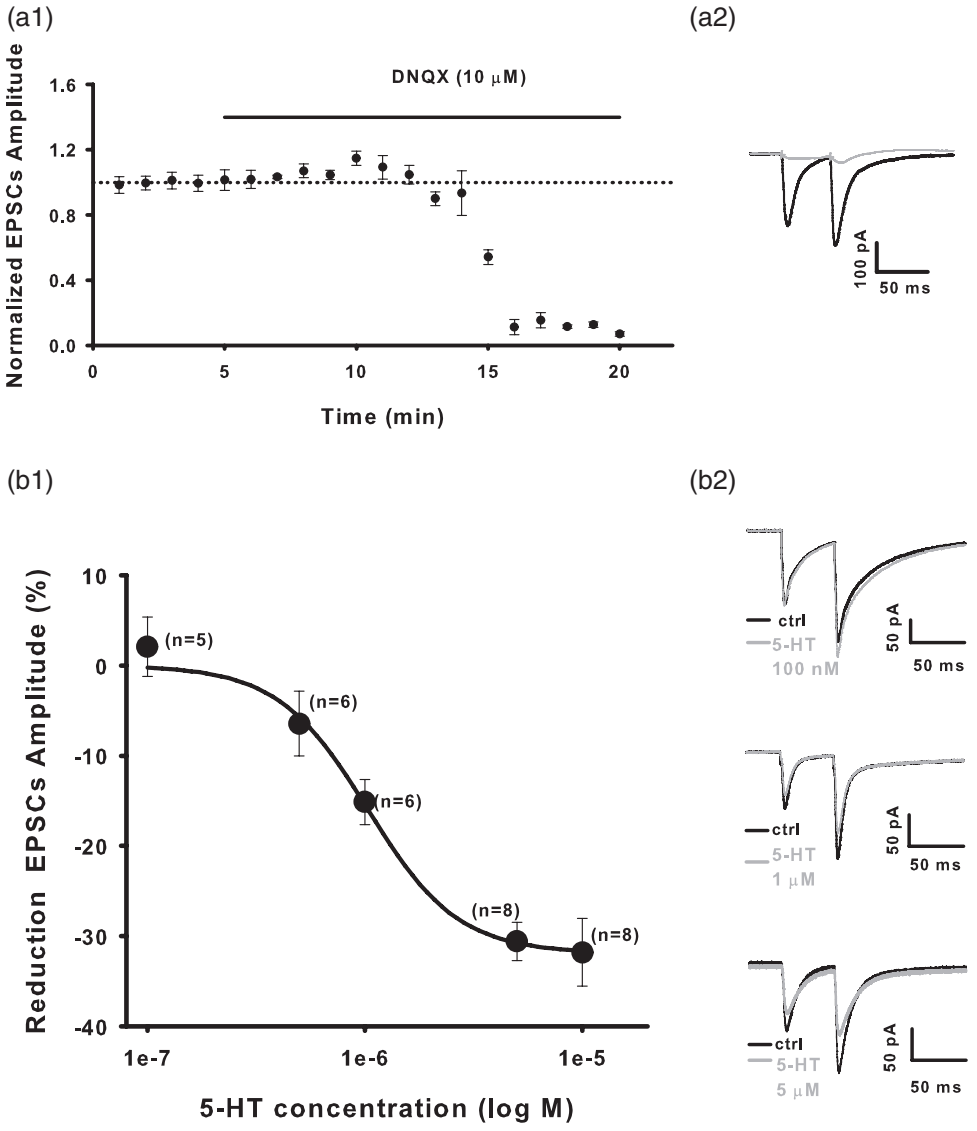
A statistically stable period (baseline condition) was defined as a time interval (10 minutes) in which the EPSCs amplitudes did not vary, according to analysis of variance (ANOVA) repeated measures. After recording an initial baseline for 10 minutes, 5-HT and the drugs were applied for 20 minutes until reaching a stable condition. The effect of citalopram took longer to stabilize and was applied for 40 minutes. The changes in glutamatergic synaptic currents were calculated as the average of the 60 EPSCs current amplitudes measured during the last 10 minutes of drug application, and subsequently were normalized by dividing them by the average control conditions (baseline). All data were expressed as mean ± SE. The paired-pulse ratio (PPR) was calculated by dividing the mean amplitude of the second EPSCs why the mean amplitude of the first EPSCs, both in basal conditions and during the last 10 minutes of drug exposure. Statistical analysis was performed using Sigma Plot v.10. The average of EPSCs amplitude in control condition was compared with the last 10 minutes of drug application by using paired Student's *t*-test. Differences between treatments were assessed by one-way ANOVA, followed by post-hoc Bonferroni test. Differences between means were considered significant only if $P < 0.05$. *Indicate P

< 0.05, **indicate $P < 0.01$. The concentration-response curve of 5-HT was fitted using the Hill equation: I Imax {1/[1 (EC_{50}/[ligand])nH]}, where Imax is the maximum response, EC_{50} is the concentration of ligand producing a half-maximal response and nH the Hill coefficient.

Results

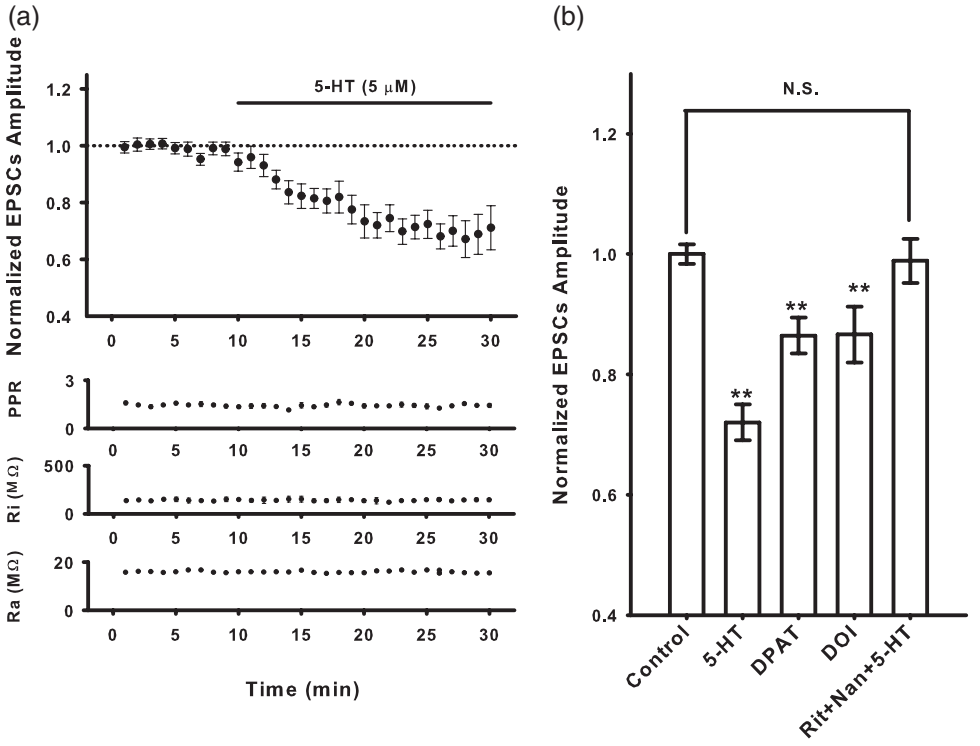
$GABA_A$R was blocked in the recorded neurons by adding 1 mM of picrotoxin to the intracellular solution [12,13]. Figure 1a shows that the EPSCs amplitudes were almost completely blocked by the AMPA/kainite antagonist, DNQX (10 μM). Under these conditions, bath application of 5-HT for 20 minutes dose-dependently reduced the amplitude of EPSCs recorded in pyramidal neurons of layer II/III from the auditory cortex. The reduction of glutamatergic synaptic currents by 5-HT reached statistical significance after 1 μM concentration, with an EC_{50} of 1.03 ± 0.24 μM, achieving its maximal response at 5 μM (Fig. 1b1). No effect was observed for bath application of 5-HT 0.1 μM (n = 5, $P = 0.69$, paired *t*-test), and for 0.5 μM of 5-HT (n = 6, $P = 0.14$, paired *t*-test). Representative traces of the EPSCs recorded before (black) and during 5-HT application (gray) in three different concentrations are shown in Fig. 1b2.

Fig. 1



5-HT dose-dependently depresses excitatory post-synaptic currents in pyramidal neurons of layer II/III of rat auditory cortex. (a1) Time course of DNQX (10 μM) inhibitory effect on the EPSC amplitude in auditory pyramidal neurons. (a2) Representative traces of two superimposed EPSCs before and after DNQX application. (b1) Best fit of the dose-dependent inhibitory effect of 5-HT on the EPSC amplitude. (b2) Representative traces of superimposed EPSCs, before and after the indicated 5-HT concentrations. *Indicate $P < 0.05$, **indicate $P < 0.01$, with post-hoc Bonferroni test after one-way ANOVA. ANOVA, analysis of variance; DNQX, 6,7-Dinitroquinoxaline-2,3(1H,4H)-dione; EPSC, excitatory post-synaptic current.

Fig. 2



5-HT_{1A} and 5-HT_2 receptors mediate the inhibitory effect of 5-HT on EPSCs in pyramidal neurons from layer II/III of rat auditory cortex. (a) Time-course of 5-HT (5 μM) inhibition of EPSC amplitude. No changes in PPR, input resistance (Rin), or access resistance (Ra) were detected. (b) Bar plot showing that the inhibitory effect of 5-HT on the normalized EPSC amplitudes is blocked by the concurrent application of a 5-HT_{1A} antagonist (NAN-190, NAN) and a 5-HT_2 antagonist (Ritanserin, RIT), and is mimicked by 8-OH-DPAT (DPAT) a selective 5-HT_{1A} agonist, and DOI, a selective 5-HT_2 agonist. DOI, (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride, (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride; EPSC, excitatory post-synaptic current; PPR, paired-pulse ratio.

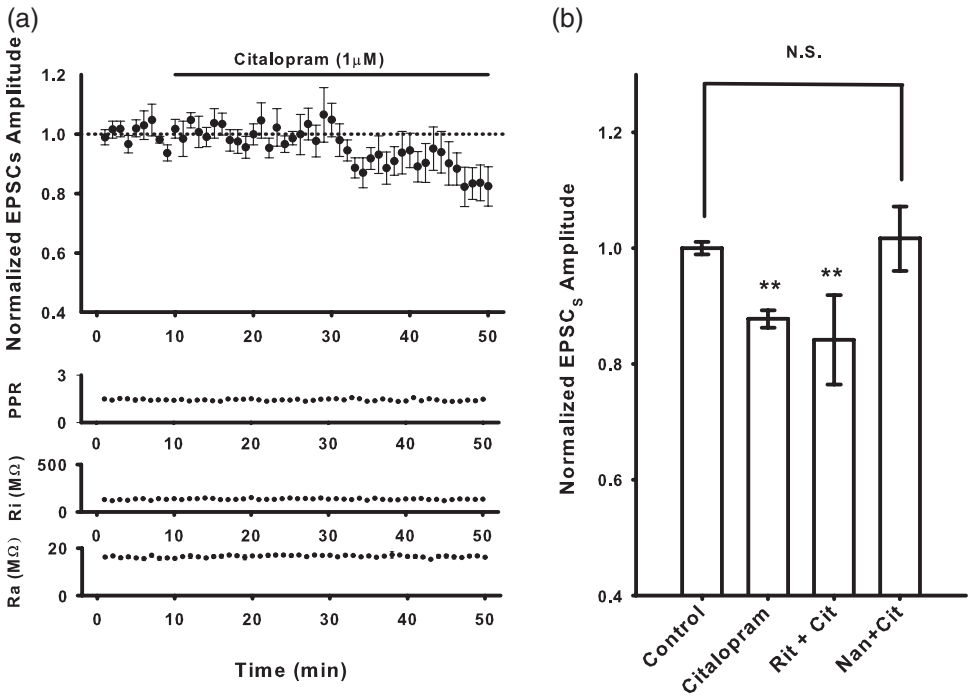
Figure 2a shows the time course of the inhibition of EPSCs amplitude produced by 5-HT at the concentration of 5 μM. On average, the EPSCs amplitude was reduced by 5-HT to 72 ± 4% of baseline (n = 8, $P < 0.01$, paired *t*-test).

5-HT may modulate synaptic transmission in a pre- or post-synaptic way [7]. Hence, PPR was measured to determine whether EPSCs modulation by 5-HT occurred at the pre-or post-synaptic locus. None of the 5-HT concentrations tested produced a significant change in PPR of the EPSCs: 1.54 ± 0.05 in control vs. 1.6 ± 0.06 in 5 μM 5-HT (n = 8, $P = 0.883$, paired *t*-test; Fig. 2a), suggesting that the effect of 5-HT occurred in the post-synaptic cell.

On the other hand, the reduction of the EPSCs amplitude elicited by 5-HT was mimicked by bath application of the 5-HT_{1A} or 5-HT_{2A} receptor agonists, 8-OH-DPAT (10 μM) and DOI (10 μM), respectively. As shown in Fig. 2b, bath application of the 5-HT_{1A} agonist 8-OH-DPAT reduced the amplitude of EPSCs to 86.45 ± 3% of control values (n = 10, $P = 0.001$, paired *t*-test), without changing the PPR: 1.61 ± 0.08 in control condition vs. 1.52 ± 0.14 in the presence of 8-OH-DPAT (n = 10, $P = 0.242$, paired *t*-test). Similarly, bath application of the 5-$HT_{2A/C}$ agonist DOI reduced the amplitude of EPSCs to 86.63 ± 4% of baseline (n = 10, $P = 0.001$, paired *t*-test), also without changing the PPR: 1.41 ± 0.03 in control vs. 1.43 ± 0.04 in presence of DOI (n = 10, $P = 0.623$, paired *t*-test). Next, the effect of 5-HT was tested in the presence of the 5-HT_{1A} and 5-HT_2 receptor antagonists, NAN-190 (1 μM) and ritanserin (200 nM), respectively. Under these conditions, bath application of 5-HT was unable to reduce the EPSCs amplitude, 100 ± 2% in the control condition vs. 98.8 ± 3.6% (n = 10, $P = 0.537$, paired *t*-test; Fig. 2b). The inserts in Fig. 2a shows that changes produced by 5-HT on the EPSCs amplitude were not due to changes in input resistance (Rin) or of access resistance (Ra).

To determine whether the reduction of the glutamatergic synaptic transmission in auditory cortex induced by bath application of 5-HT could be imitated by endogenous 5-HT, EPSCs were recorded during 40 minutes in presence of the SSRI citalopram. Figure 3a shows that citalopram (1 μM) reduced the EPSCs amplitude to 87.8 ± 1.5% of baseline (n = 6, $P = 0.001$, paired *t*-test), again without

Fig. 3



5-HT_{1A}, but not 5-HT_2 receptors, mediate the inhibitory effect of citalopram on EPSCs amplitude recorded in pyramidal neurons from layer II/III of rat auditory cortex. (a) Time-course of the inhibitory effect of citalopram (1 μM) on EPSC amplitude. No changes in PPR, Rin, or Ra were detected. (b) Bar plot showing that the reduction of EPSCs amplitudes caused by citalopram (1 μM) was completely blocked by the 5-HT_{1A} antagonist NAN-190 (1 μM), but not the 5-HT_2 antagonist ritanserin (200 nM). *Indicate $P < 0.05$, **indicate $P < 0.01$, with post-hoc Bonferroni test after one-way ANOVA. ANOVA, analysis of variance; EPSC, excitatory post-synaptic current; PPR, paired-pulse ratio.

effects on the PPR: 1.35 ± 0.03 in control vs. 1.32 ± 0.04 in presence of citalopram (n = 6, $P = 0.3$, paired *t*-test) and in the Rin and Ra (Fig. 3a). The inhibitory effect of citalopram on the EPSCs amplitude was completely blocked by the prior application of the 5-HT_{1A} antagonist NAN-190 (1 μM) (n = 6, $P = 0.424$, paired *t*-test; Fig. 3b), but not by the 5-HT_2 antagonist ritanserin (200 nM). In the presence of ritanserin, citalopram reduced the EPSCs amplitude to 84.7 ± 6% of baseline (n = 6, $P = 0.001$, paired *t*-test; Fig. 3b), without changes in the PPR: 1.48 ± 0.1 in control vs. 1.44 ± 0.09 in citalopram plus ritanserin (n = 6, $P = 0.773$, paired *t*-test). In order to estimate the concentration of endogenous 5-HT that reduces the amplitude of EPSPs in the presence of citalopram, we interpolated its effect in the dose-response curve obtained by bath application of 5-HT, revealing that this SSRI raised the extracellular concentration of 5-HT up to 823 ± 53 nM.

Discussion

The results of this study show the modulation of glutamatergic synaptic transmission by exogenous and endogenous 5-HT in layer II/III of the auditory cortex from juvenile rats. We found that bath application of 5-HT caused a concentration-dependent reduction of the evoked EPCSs in layer II/III of the auditory cortex, which is similar to its previously reported inhibitory modulation of GABAergic synaptic transmission (1.1 μM) in the same cortical area [7], but opposite to that observed in layer II/III of entorhinal cortex, where 5-HT reduced the firing frequency (EC_{50} of 0.48 μM) of pyramidal neurons through 5-HT_1 receptors activation [15].

In this study, bath application of 8-OH-DPAT and DOI, which are selective agonists for 5-HT_{1A} and 5-HT_2 receptors, respectively, mimicked the reduction of the EPSCs amplitude by 5-HT, and their effects were prevented by the simultaneous presence of their respective antagonists, NAN-190 and ritanserin, indicating that the reduction of EPSCs amplitude was mediated by both 5-HT_{1A} and 5-HT_2 receptors. Consistent with these results, the activation of 5HT_{1A} receptors also reduced the firing frequency of pyramidal neurons in layer II/III of the auditory cortex [16]. Similar results were found in the prefrontal cortex where 5-HT_{1A} receptors reduce AMPA synaptic currents recorded in pyramidal neurons, an effect produced through the inhibition of the adenylyl cyclase/protein kinase A pathway [17], opening the possibility that the same signaling pathway may be activated by 5-HT_{1A} receptors to modulate the excitatory input in pyramidal neurons of layers II/III of auditory cortex [17].

Also, Timmermann *et al.* [18] reported that the administration of 5-HT_2 receptor agonist, lysergic acid diethylamide, reduced the intrinsic connectivity in auditory cortex [18].

Here we showed that bath application of the selective 5-HT_2 agonist, DOI, consistently reduced EPSCs amplitude in the auditory cortex, which differs to that reported in other cortical regions where the activation of 5-HT_2 receptors enhances the excitability of neurons, manifested by an increase in the frequency and amplitude of the EPSCs [19,20]. These results strongly suggest that 5-HT_2 receptors have a different function in the auditory cortex compared to other cortical areas. In this context, we previously reported that in the auditory cortex the adrenergic α1 receptors activate a Gq-PLC pathway to reduce glutamatergic synaptic transmission [21], opening the possibility that the 5-HT_2 receptors activate the same signaling pathway to reduce the EPSCs in layers II/III of auditory cortex.

The reduction of glutamatergic synaptic transmission was also partially reproduced by citalopram, whose maximal inhibitory effect on EPSCs amplitude was equivalent to that produced by an estimated increase in the extracellular concentration of 5-HT of about 823 nM. Importantly, the inhibitory effect of endogenous 5-HT (unmasked by citalopram) on glutamatergic synaptic transmission was mediated by 5-HT_{1A} receptors, since the reduction of EPSCs amplitude by citalopram was entirely blocked by NAN-190, but not the 5-HT_2 receptor antagonist, ritanserin. Altogether, these results indicate that citalopram acts on serotonergic synapses that release 5-HT only in nanomolar concentrations that are sufficient to activate 5-HT_{1A} but not 5-HT_2 receptors expressed in the pyramidal neurons of layers II/III of the rat auditory cortex. These results can be explained because 5-HT_2 receptors require micromolar concentrations in order to be activated, as shown with the inhibition of the EPSCs amplitude by exogenous application of 5-HT (5 μM) that only was blocked by a joint application of 5-HT_{1A} and 5-HT_2 receptor antagonists, indicating that both receptors (5-HT_{1A} and 5-HT_2) contribute to the global inhibitory effect of 5-HT on the excitatory synaptic transmission in layer II/III of auditory cortex.

On the other hand, both effects of 5-HT (endogenous or exogenous) on the glutamatergic synaptic transmission were located at the post-synaptic neurons, because the paired-pulse ratio remained unchanged, suggesting that the reduction of EPSCs amplitudes was primarily due to the modulation of AMPA/kainate receptors on the post-synaptic neuron. These results are similar with findings reported in layer II/III of parasubiculum, where bath application of 5-HT or citalopram also reduced the amplitude of the evoked fEPSP via 5-HT_{1A} receptors activation, although in this region the inhibition was accompanied by increases in the PPR, suggesting that presynaptic 5-HT_{1A} receptors reduced glutamate release [22].

The results presented here suggest that a function of serotonergic input to pyramidal neurons of layer II/III of the auditory cortex is to inhibit glutamatergic synaptic transmission via activation of 5-HT_{1A} and 5-HT_2 receptors. However, as the results show, since 5-HT can act through both 5-HT_{1A} and 5-HT_2 receptors, this modulation may depend on the affinity by the 5-HT. For example, a low concentration of 5-HT (endogenous 5-HT) would only activate 5-HT_{1A} receptors, whereas a high concentration (exogenous 5-HT) would activate both 5-HT_{1A} and 5-HT_2 receptors, respectively. Of peculiar relevance for the present study is the observation that citalopram, which likely increases the concentration of endogenous 5-HT in the synaptic cleft, reduces the amplitude of evoked auditory potentials in healthy and depressed humans [6], opening the intriguing possibility that this effect could be mediated by activation of post-synaptic 5-HT_{1A} receptors.

From the above findings, it could be speculated that citalopram, through increasing the extracellular concentration of 5-HT, could decrease the continuous local synaptic input from other pyramidal neurons of layer II/III in auditory cortex (synaptic noise), whereas at the same time, would favor and give greater relevance to the glutamatergic synaptic inputs with auditory sensory information from the medial geniculate nucleus of the thalamus thus enhancing the signal-to-noise ratio from the sensory auditory system [4,23].

Nevertheless, the increase in the concentration of the endogenous 5-HT could be limited by the activation of the 5-HT_{1A} auto-receptors present at the serotonergic fiber which would inhibit serotonergic discharge of action potentials and produce a decrease of the 5-HT release [24]. In our study, the possible participation of 5-HT_{1A} auto-receptors was not proven, since to date, there are no specific antagonists that differentiate between 5-HT_{1A} auto and hetero-receptors. In any case, the differential inhibition of glutamatergic transmission exerted by endogenous 5-HT through activation of 5-HT_{1A} but not 5-HT_2 receptors of pyramidal neurons of the auditory cortex described here may have critical functional implications, like the reduction of the unspecific intrinsic synapses, while enhancing specific relevant auditory signals coming from the auditory thalamic-cortical pathway [22,25].

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V.C.-R., M.C.-B., and D.A.-M. performed experiments. V.C.-R., E.A.P.-P., and H.H. performed data analysis and statistics. J.L.G.-A., J.C.P., and M.A. gave intellectual contributions and reviewed the manuscript. H.S. developed the ideas of the project and the experimental plan. J.C.P. and H.S. wrote the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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