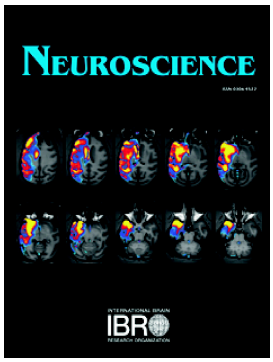

Serotonin 5-HT1A Receptor-Mediated Reduction of Excitatory Synaptic Transmission in Layers Ii/Iii of The PARASUBICULUM

Francis Carter, C. Andrew Chapman

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SEROTONIN 5-HT$_{1A}$ RECEPTOR-MEDIATED REDUCTION OF EXCITATORY SYNAPTIC TRANSMISSION IN LAYERS II/III OF THE PARASUBICULUM.

Francis Carter and C. Andrew Chapman

Center for Studies in Behavioral Neurobiology,
Department of Psychology, Concordia University,
Montréal, Québec, Canada H4B 1R6

Running Title: Serotonergic reduction of EPSPs in the parasubiculum.

Corresponding Author:
C. Andrew Chapman
Center for Studies in Behavioral Neurobiology
Department of Psychology
Concordia University
7141 Sherbrooke Street W., Rm. SP-244
Montréal, Québec, Canada H4B 1R6
Tel: (514) 848-2424 x2220
Fax: (514) 848-2817
E-mail: andrew.chapman@concordia.ca

LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine, or serotonin
ACSF	artificial cerebrospinal fluid
fEPSPs	field excitatory postsynaptic potentials
GABA	gamma aminobutyric acid
N-K	Newman-Keuls test
NMDA	N-methyl-D-aspartate

ABSTRACT

Serotonin (5-HT) has important effects on cognitive function within the hippocampal region where it modulates membrane potential and excitatory and inhibitory synaptic transmission. Here, we investigated how 5-HT modulates excitatory synaptic strength in layers II/III of the parasubiculum in rat brain slices. Bath-application of 1 or 10 µM 5-HT resulted in a strong, dose-dependent, and reversible reduction in the amplitude of field excitatory postsynaptic potentials (fEPSPs) recorded in the parasubiculum. The 5-HT reuptake blocker citalopram (10 µM) also reduced fEPSP amplitudes, indicating that 5-HT released within the slice inhibits synaptic transmission. The reduction of fEPSPs induced by 5-HT was blocked by the 5-HT_{1A} receptor blocker NAN-190 (10 µM), but not by the 5-HT_7 receptor blocker SB-269970 (10 µM). Moreover, the 5-HT_{1A} agonist 8-OH-DPAT induced a reduction of fEPSP amplitude similar to that induced by 5-HT. The reduction was prevented by the 5-HT_{1A} receptor blocker NAN-190. The reduction in fEPSPs induced by either 5-HT or by 8-OH-DPAT was accompanied by an increase in paired-pulse ratio, suggesting that it is due mainly to reduced glutamate release. Our data suggest that the effects of serotonin on cognitive function may depend in part upon a 5-HT_{1A}-mediated reduction of excitatory synaptic transmission in the parasubiculum. This may also affect synaptic processing in the entorhinal cortex, which receives the major output projection of the parasubiculum.

Key words: Parasubiculum, EPSP, rat, serotonin, 5-HT_{1A} receptor.

INTRODUCTION

The parasubiculum receives inputs from the hippocampal CA1 region, subiculum, basolateral amygdala, and anterior thalamus, and the single major output projection of the parasubiculum is to layer II of the medial and lateral entorhinal cortex, where it contacts neurons originating in all cell layers (Köhler, 1985, van Groen and Wyss, 1990, Shibata, 1993; Caballero-Bleda and Witter, 1994; Canto et al., 2012). The superficial layers of the entorhinal cortex receive inputs from sensory and associational cortices, and provide most of the cortical sensory input to the dentate gyrus and hippocampus (Witter et al., 1989; Burwell, 2000). The parasubiculum is thought to contribute to cognitive functions including spatial memory and navigational processes in large part through its inputs to the entorhinal cortex (Taube, 1995; Boccara et al., 2010; Tang et al., 2016). Stimulation of parasubicular inputs to the entorhinal cortex can heterosynaptically enhance entorhinal cortex responses to inputs from the olfactory cortex, consistent with a role for the parasubiculum in modulating entorhinal processing of sensory information (Caruana and Chapman, 2004; Sparks and Chapman, 2016). The parasubiculum also generates 4-12 Hz theta-frequency electroencephalographic activity that is coordinated with theta activity in the hippocampus and entorhinal cortex (Buzsaki, 2002; Glasgow and Chapman, 2007, 2008), and theta activity may enhance parasubicular activation of the entorhinal cortex (Chrobak and Buzsaki, 1994; Sparks and Chapman, 2013). Modulation of excitatory synaptic transmission within the parasubiculum may therefore affect not only local synaptic integration, but also how layer II entorhinal neurons respond to sensory inputs and impact hippocampal function.

Serotonin (5-hydroxytryptamine, 5-HT) is a major modulatory transmitter with diffuse forebrain projections originating in median raphe nuclei, that is linked to variations in the sleep wake cycle, mood and psychiatric disorders (Urbain et al., 2006; Puig and Gulledge, 2011). Serotonergic effects on cognitive functions mediated by the hippocampal region may arise through effects of 5-HT on cholinergic and dopaminergic systems (Fink and Göthert, 2007; Ogren et al., 2008; Seyedabadi et al., 2014). 5-HT, by disrupting rhythmic activity in the medial septum may interfere with learning by disrupting septal cholinergic and non-cholinergic projections to the hippocampal region (Vertes and Kocsis, 1997; Crooks et al., 2012). 5-HT is likely to have similar effects on cholinergic theta activity within the parasubiculum (Glasgow and Chapman, 2007). In addition, the local effects of 5-HT on neuronal excitability, which vary markedly within the entorhinal cortex versus hippocampus (Schmitz et al, 1998a; Lei, 2012) may

affect the strength of synaptic transmission within the parasubiculum and modulate the output of the parasubiculum to the entorhinal cortex.

The parasubiculum contains both serotonin immunopositive terminals and receptors that show the greatest density in superficial layers (Köhler, 1984; Köhler et al., 1981, 1986; Bjarkam et al., 2005), but the local effects of serotonin on excitatory synaptic responses in the parasubiculum have not been investigated. The activation of 5-HT_{1A} receptors hyperpolarizes membrane potential via the activation of a potassium conductance in prefrontal (Andrade, 2011; see also Behr et al., 1997) and entorhinal cortex (Grünschlag et al., 1997; Schmitz et al., 1998b; Ma et al., 2007; Deng et al. 2007). Excitatory synaptic transmission in the entorhinal cortex is decreased by 5-HT, and a postsynaptic effect of 5-HT on excitatory synaptic transmission has been suggested by observations of reduced responses to iontophoretically applied glutamate (Sizer et al., 1992), and the stability of paired-pulse ratio in combination with reduced cellular input resistance (Grunschlag et al., 1997). Other evidence indicates that activation of 5-HT_{1A} receptors inhibits excitatory transmission in layers II/III of the entorhinal cortex presynaptically, with a reduced frequency of miniature excitatory postsynaptic currents, and a facilitation of paired-pulse ratio (Schmitz et al., 1995a, 1998c, 1999) and similar results have been observed in the anterior cingulate cortex (Tian et al., 2017). In the hippocampus, in contrast, 5-HT reduces excitatory synaptic responses only at very high concentrations (Schmitz et al., 1995b), and its predominant effect is a reduction in inhibitory synaptic transmission via the inhibition of GABAergic interneurons (Oleskevich and Lacaille, 1992; Schmitz et al., 1995c; Schmitz et al., 1998a).

In the present study, we recorded field excitatory postsynaptic potentials (fEPSPs) in layer II/III of the parasubiculum in vitro evoked by stimulation of afferents in layer I to determine the effects of bath application of 5-HT on the strength of excitatory synaptic transmission. The potent and selective 5-HT reuptake blocker citalopram was used to determine effects of release of endogenous 5-HT (Hyttel, 1982; Owens et al., 2001), and paired-pulse tests were used to assess if reductions in fEPSP amplitudes were due to a reduction in presynaptic release of neurotransmitter or due to postsynaptic mechanisms. The dependence on 5-HT_{1A} receptors was assessed by blocking the effects of either 5-HT or the 5-HT_{1A} agonist 8-OH-DPAT with the 5-HT_{1A} antagonist NAN-190. Because 8-OH-DPAT can also activate 5-HT_7 receptors, the role of 5-HT_7 receptors was assessed by applying 5-HT in the presence of the selective 5-HT_7 blocker SB-269970.

EXPERIMENTAL PROCEDURES

In vitro slice preparation

Experimental methods were conducted in accordance with the guidelines of the Canadian Council on Animal Care. Acute brain slices were taken from 26 four to eight week-old rats that were anesthetized with isoflurane. Brains were placed in 4 °C ACSF containing, in mM, 250 sucrose, 2 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 26 $NaHCO_3$, 0.5 $CaCl_2$ and 10 dextrose, saturated with 95% O_2 and 5% CO_2. Horizontal slices for field potential recordings (400 µm-thick) were cut with a vibratome (Leica, VT1200; Concord, ON, Canada), and then placed in normal ACSF (124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose) for 30 min at 32 °C. Slices were then kept at room temperature for at least 1 hr prior to recordings.

Slices were transferred to a nylon net in a temperature-regulated gas-fluid interface chamber (Fine Science Tools, North Vancouver, BC, Canada), and slices were visualized using a dissecting microscope (Leica, MS5). The upper surfaces of slices were exposed to a humidified 95% O_2 and 5% CO_2 atmosphere, and oxygenated ACSF flowed through the recording chamber at a rate of 1.5 - 2.0 ml/min at 22-24 ºC. Effects of serotonin on synaptic transmission have been demonstrated previously in the hippocampus at both at room temperature and at higher recording temperatures (e.g.s, Costa et al., 2012; Schmitz et al., 1999).

Stimulation and recording

Field potential recording electrodes were made from borosilicate glass (1.0 mm OD) using a pipette puller (Sutter Instruments, P-97, Novato, CA, USA; 3-6 MΩ). Electrodes were filled with ACSF and placed at the border of layer II/III, 50-180 µM below the surface of the slice (see inset in Figure $1A_2$). Recordings were obtained using an Axoclamp 2B amplifier and HS-2Ax0.1LU headstage (DC-3 kHz, Molecular Devices, Sunnyvale, CA, USA), and digitized (20 kHz; Molecular Devices, Digidata 1322A) using the pClamp 8.2 software package (Molecular Devices).

Bipolar stimulating electrodes were made from two tungsten electrodes (1 MΩ; FHC Inc., Bowdoin, ME, USA) and electrode tips were placed in layer I of the parasubiculum, parallel to the cortical surface, approximately 0.3-0.4 mm anterior to the recording electrode. Cathodal monophasic square-wave constant current pulses (0.1 ms in duration) were delivered using a stimulus generator (WPI, Model A300; Sarasota, FL, USA) and a stimulus isolation unit (Model A360). Stimulation intensities were adjusted to evoke fEPSPs with an amplitude of ~65-75% of the maximal response (typically < 100 µA).

Pharmacology

Evoked field potential responses were monitored once every 30 sec. To assess the effects of serotonin (5-hydroxytryptamine, 5-HT) on synaptic responses, after a 10 min period of stable baseline recordings in normal ACSF, 1 or 10 µM 5-HT was bath-applied for 15 min, followed by a 45 min washout period in normal ACSF. All drugs were obtained from Tocris Bioscience (Oakville, ON, Canada), and were stored frozen as concentrated stock solutions. Reduced release of transmitter can result in increased paired-pulse ratio due to an increase in the pool of readily releasable transmitter available during the response to the second stimulation pulse, and changes in paired-pulse depression during application of 5-HT were therefore examined to assess if the reduction of fEPSPs was due to changes in transmitter release or to post-synaptic factors. Paired-pulse tests, in which 10 samples were recorded using a 30 ms interpulse interval (Glasgow et al., 2012), were recorded during the start of the baseline period, the end of the 5-HT application period, and at the end of the washout period.

To determine if endogenous 5-HT can induce a reduction of fEPSPs similar to that observed during bath application of 5-HT, recordings were obtained before, during and after application of the serotonin reuptake inhibitor citalopram hydrobromide (10 µM). The role of 5-HT_{1A} and 5-HT_7 receptors in the 5-HT-induced reduction of fEPSPs was assessed by first applying either the 5-HT_{1A} receptor antagonist NAN-190 hydrobromide (10 µM), or the 5-HT_7 receptor antagonist SB-269970 hydrochloride (10 µM; Thomas et al., 2002) for a period of 15 min, followed by co-application of 5-HT (1 µM) for 15 min. Recordings in the presence of the receptor antagonist were continued for a further 30 min.

To further test the role of 5-HT_{1A} receptors, following a 10 min baseline period in normal ACSF, the 5-HT_{1A} receptor agonist 8-OH-DPAT hydrobromide (10 µM) was applied for 15 min, prior to a 45 min washout period. Paired-pulse responses were also obtained during these tests as they were during 5-HT application. Then, to determine if the effects of 8-OH-DPAT are mediated by 5-HT_{1A} receptors, the 5-HT_{1A} antagonist NAN-190 was applied for 15 min prior to co-application of 8-OH-DPAT for a period of 15 min.

Data analysis

Peak amplitudes of fEPSPs and the fiber volley were measured relative to the baseline prior to the stimulation pulses using pClamp 8.2 software (Molecular Devices), and SigmaPlot 11.0 (Systat Software Inc.) was used for statistical analysis and preparation of figures. The fiber volley was not analyzed in 3 slices in which it was not distinguishable from the stimulus artefact.

Data for each slice were expressed as a percentage of the average amplitude of baseline responses, and mean values for groups of slices were expressed as the mean ± one SEM. Paired-pulse ratio was determined by expressing the amplitude of the fEPSP response to the second pulse in each pair as a percentage of the amplitude of the response to the first pulse. The average amplitudes obtained during the last five min of the baseline period, and last two min of both the drug and washout periods were assessed using separate repeated measures analyses of variance, to determine if drug application resulted in statistically significant changes in fEPSP amplitude. Significant effects were investigated using Student Newman-Keuls tests to assess the significance of drug-induced changes in fEPSPs and their reversibility during washout. Assumptions of normality and equal variance were met for all statistical tests.

RESULTS

Bath application of 5-HT for 15 min resulted in a concentration-dependent, reversible reduction of excitatory synaptic responses in the parasubiculum (Figure $1A_{1,2}$). The amplitude of evoked field EPSPs recorded in layers II/III was rapidly reduced by 24 ±5.6 % during 15 min bath-application of 1 µM 5-HT (-0.27 ±0.02 vs. -0.36 ±0.02 mV; n = 8 slices; $F_{2,14}$= 21.21, $p < 0.001$; Newman-Keuls (N-K) $p < 0.01$ for baseline vs. 5-HT) and responses recovered from this reduction after approximately 15 min of washout in normal ACSF (N-K $p < 0.071$ for wash vs. baseline). Bath application of 10 µM 5-HT resulted in a stronger reduction in EPSP amplitudes of 39.6 ±4.0 % (-0.23 ±0.01 vs. -0.38 ±0.02 mV; n = 8; $F_{2,14}$= 19.99, $p < 0.001$; N-K $p < 0.001$ for 5-HT vs. baseline) and this effect was reversed after about 15 min of washout in normal ACSF (N-K $p = 0.214$ for wash vs. baseline). Changing 5-HT concentration from 1 to 10 µM led to a significant increase in reduction of synaptic transmission in layers II/III of the parasubiculum (t_{14}= 2.24, $p < 0.05$).

Serotonin may modulate synaptic responses through both pre- and postsynaptic mechanisms (Grünschlag et al., 1997; Schmitz et al., 1999). The amplitude of the fiber volley (Figure $1A_3$) reflects sodium entry in activated fibers during the compound action potential, and was not significantly affected during application of 1 or 10 µM 5-HT (1 µM -0.23 ±0.03 vs. -0.23 ±0.03 mV; n = 8; $F_{2,14}$= 0.76, $p = 0.48$; 10 µM -0.31 ±0.02 vs. -0.30 ±0.02 mV; n = 7; $F_{2,12}$= 2.3, $p = 0.13$). The reduction in the EPSP is therefore not associated with reduced action potentials in presynaptic fibers. The volley was increased non-significantly after washout of 10 µM 5-HT (-0.34 ±0.03 vs. -0.30 ±0.02 mV), however, and this may have contributed to the non-

significant increase in mean EPSP amplitudes at the end of the recording period in this group of slices.

In the CA1 region, the fiber volley is not sensitive to 5-HT-induced reductions in calcium influx into terminals that results in reduced transmitter release and depression of fEPSPs (Ropert, 1988; Schmitz et al., 199b). Paired-pulse tests were therefore used to determine if the reduction of fEPSPs was likely due to a presynaptic reduction in glutamate release (Glasgow et al., 2012). Responses evoked during baseline recordings in normal ACSF displayed moderate paired-pulse depression of the responses to the second pulse. Application of 1 or 10 µM 5-HT resulted in a reduction of the amplitude of the response to the first pulses, but also resulted in a concentration-dependent increase in paired-pulse ratios that reversed during washout in normal ACSF (Figure 1B). Bath application of 1 µM 5-HT increased paired pulse ratio from 74.8 ±4.6 to 95.7 ±7.4, ($F_{2,14}$= 9.82, $p < 0.01$; N-K $p < 0.01$), and 10 µM 5-HT increased paired pulse ratio from 79.1 ±7.5 to 120.2 ±7.9 ($F_{2,14}$= 57.63, $p < 0.001$; N-K $p < 0.001$). Note that mean paired-pulse depression observed during baseline recordings was changed to paired-pulse facilitation during application of 10 µM 5-HT, and this effect was also observed in the slice from which sample traces were obtained for 1 µM 5-HT (Figure 1B). The increase in paired-pulse ratio was significantly greater for 10 µM 5-HT versus 1 µM 5-HT (t_{14}= 2.26, $p < 0.05$), and ratios returned to baseline values during washout in both groups of slices. Because a reduction in transmitter release in response to the first stimulation pulse can increase the pool of readily releasable transmitter during the second stimulation pulse, the larger increases in paired-pulse ratio induced by 10 µM 5-HT are consistent with a stronger reduction of presynaptic glutamate release induced by 5-HT.

To determine if the reduction of fEPSP amplitudes induced by bath application of 5-HT could be mimicked by enhancing effects of 5-HT released from neurons, recordings were conducted during application of the selective 5-HT reuptake inhibitor citalopram (Figure. 2). The amplitude of synaptic responses was reduced by 12.3 ±4.7 % during 15 min bath-application of citalopram (10 µM; -0.40 ±0.05 vs. -0.45 ±0.04 mV; n = 9). The repeated measures analysis of variance did not reach significance due to variability in recovery of responses during the wash period ($F_{2,16}$= 1.76, $p = 0.205$), but pairwise comparison reflected a significant reduction in responses recorded during application of citalopram versus the baseline period ($t_8 = 2.63$, $p < 0.05$). Note that the slower onset of effects of citalopram versus bath application of 5-HT is similar to results obtained by Schmitz et al. (1999) with the re-uptake inhibitor fenfluramine in

the entorhinal cortex, likely due to a gradual accumulation of 5-HT during block of re-uptake. The amplitude of the fiber volley was not significantly altered by citalopram, but there was a trend towards an increase in volley amplitude during the washout period (-0.32 ±0.04 during baseline vs. -0.32 ±0.05 mV in citalopram, and -0.35 ±0.05 at wash; n = 7; $F_{2,12}$= 3.37, p = 0.07).

The contributions of 5-HT_{1A} receptors, or 5-HT_7 receptors which can be activated by the 5-HT_{1A} agonist 8-OH-DPAT (Costa et al., 2012), were assessed by application of 1 µM 5-HT during constant bath application of receptor blockers. The 5-HT_7 receptor antagonist SB-269970 (10 µM) had no significant effect on fEPSP amplitudes, and also did not prevent the reduction of fEPSPs induced by 5-HT (Figure 3A). Field EPSP amplitudes were reduced by 26.4 ±4.5 % after 15 min application of 5-HT (-0.26 ±0.03 vs. -0.36 ±0.03 mV; n = 5, $F_{2,8}$= 17.17, p < 0.001; N-K p < 0.01 for 5-HT vs. baseline), and returned to baseline during washout (N-K p =0.46 for wash vs. baseline). The amplitude of the fiber volley was not significantly altered by 5-HT in the presence of SB-269970 (-0.15 ±0.03 vs. -0.15 ±0.03 mV; n = 5; $F_{2,8}$= 1.49, p = 0.282). In contrast, the 5-HT_{1A} receptor antagonist NAN-190 (10 µM) also had no effect on fEPSP amplitudes when applied alone, but blocked the reduction of fEPSP amplitude induced by 5-HT (Figure. 3B). Field EPSP amplitudes did not change significantly after application of 5-HT (-0.32 ±0.04 vs. -0.32 ±0.03 mV; n = 5, $F_{2,8}$= 0.261, p = 0.78; N-K p = 0.71) and remained stable during the washout period (N-K p = 0.81), suggesting that 5-HT_{1A} receptors mediate the 5-HT-induced reduction of fEPSPs in the parasubiculum.

The 5-HT_{1A} receptor agonist 8-OH-DPAT was used to assess whether more selective activation of the 5-HT_{1A} receptors is sufficient to cause the reduction of synaptic responses. Application of 8-OH-DPAT (10 µM) led to a reduction of fEPSP amplitudes similar to that induced by 5-HT (Figure. 4A). Responses were reduced by 17.7 ±7.4 % (-0.31 ±0.03 vs. -0.38 ±0.03 mV; n = 7, $F_{2,12}$= 7.25, p < 0.01; N-K p < 0.05), and returned towards baseline values during the washout period (N-K p = 0.21). The amplitude of the fiber volley was not significantly altered by 8-OH-DPAT (-0.11 ±0.07 vs. -0.12 ±0.08 mV; n = 7; $F_{2,12}$= 0.33, p = 0.724). The initial increase in mean fEPSP amplitude during application of DPAT is likely not due to drug application, because drug effects in our recording system require several minutes for drug concentrations to increase within the recording chamber. Similar to results obtained for 5-HT application, paired pulse ratios were enhanced during application of 8-OH-DPAT (from 66.1 ±4.6 at baseline to 88.4 ±11.7 in 8-OH-DPAT, and 60.9 ±4.6 at washout; n = 4, $F_{2,6}$= 9.64, p

<0.05; data not shown), consistent with a presynaptic mechanism for the reduction of fEPSPs mediated by activation of 5-HT_{1A} receptors.

To confirm that effects of 8-OH-DPAT were mediated by 5-HT_{1A} receptors, 8-OH-DPAT was applied during constant bath application of the 5-HT_{1A} receptor antagonist NAN-190 (10 µM; Figure 4B). Application of NAN-190 alone was associated with an increase in mean fEPSP amplitude, but this was not a reliable effect (see Figure $3B_2$). Field EPSP amplitudes were reduced nonsignificantly by 3.7 ±3.3 % during addition of 8-OH-DPAT ($n = 6$, $F_{2,10} = 0.84$, $p = 0.46$) and also remained stable during the washout period (N-K $p = 0.85$). This reinforces the conclusion that the reduction of fEPSPs induced by 5-HT in the parasubiculum is mediated by 5-HT_{1A} receptors.

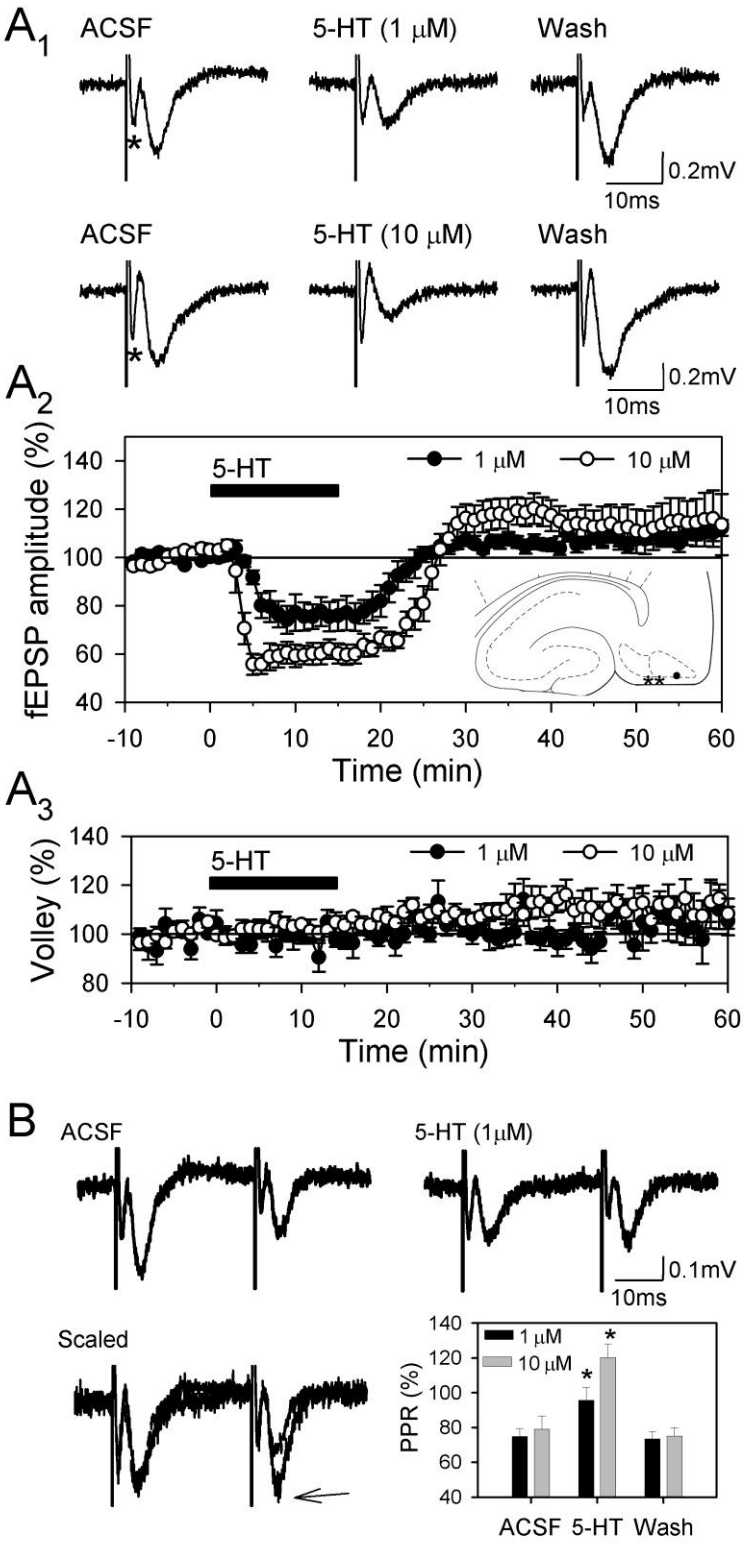

Figure 1. Bath-application of serotonin (5-HT) results in a reversible reduction the amplitude of evoked field excitatory postsynaptic potentials (fEPSPs) in layers II/III of the parasubiculum in vitro. **A**. The amplitudes of fEPSPs evoked by stimulation of layer I were reduced in a concentration-dependent manner by addition of 1 or 10 µM 5-HT to ACSF for 15 min. The responses recovered during washout. Averaged recordings of fEPSPs, using five consecutive responses obtained at the end of each recording period, are shown for representative slices tested with either 1 or 10 µM 5-HT (A_1; asterisks indicate the fiber volley). Mean amplitudes of the fEPSP (A_2) and fiber volley (A_3) are shown for each group of slices. The inset in A_2 indicates positions of the bipolar stimulation electrode (**) and recording electrode (●) on a horizontal section adapted from the atlas of Paxinos and Watson (1998). **B**. The serotonergic reduction of fEPSPs was associated with reduced paired-pulse depression (30 ms interpulse interval), suggesting that the inhibition of fEPSPs results from a reduction in transmitter release. Representative averaged traces obtained during the baseline period in ACSF and in the presence of 1 µM 5-HT are shown (top). Traces are superimposed and scaled to the amplitude of the first response in baseline recordings (bottom, left) to reflect the increase in paired-pulse ratio during application of 5-HT (arrow). The histogram displays average paired pulse ratios among slices receiving 1 or 10 µM 5-HT (asterisks indicate $p < 0.01$ and < 0.001 with respect to baseline for 1 and 10 µM respectively).

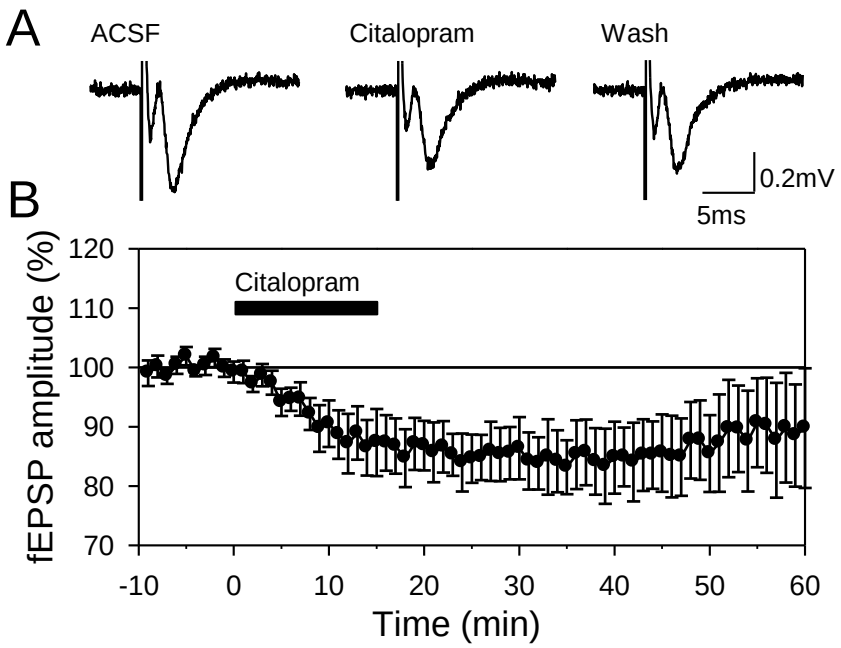

Figure 2. Bath-application of the selective 5-HT reuptake inhibitor citalopram reduces the amplitude of field EPSPs evoked in layers II/III of the parasubiculum. **A**. Representative averaged fEPSPs recorded at the end of each recording period are shown. **B.** The mean amplitude of fEPSPs among the group of slices was reduced during 15 min bath-application of 10 µM citalopram.

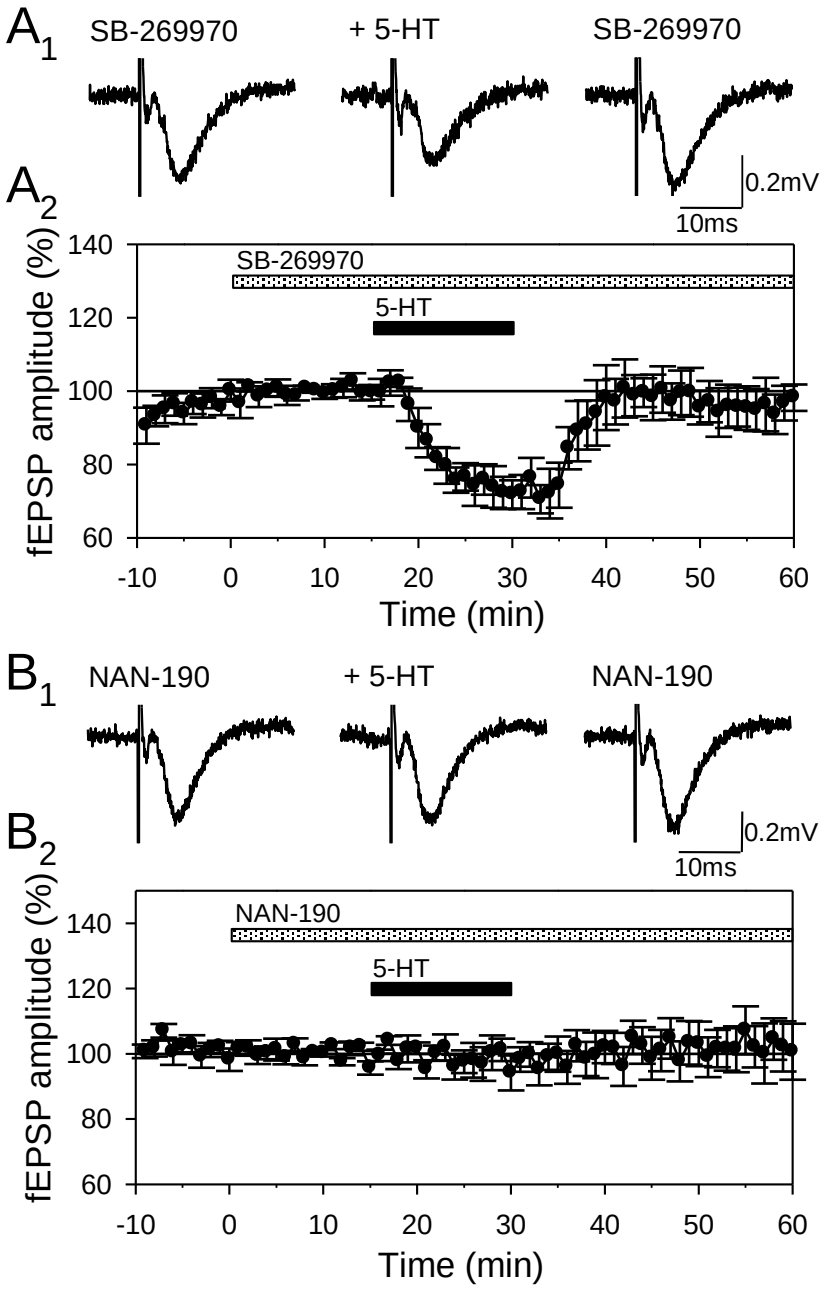

Figure 3. The serotonergic reduction of synaptic transmission is dependent on activation of 5-HT_{1A} receptors but not 5-HT_7 receptors. **A**. The 5-HT_7 receptor antagonist SB-269970 did not significantly affect baseline field EPSP amplitudes, and did not prevent the reduction in fEPSP amplitudes induced by 1 µM 5-HT. Averaged field EPSP recordings (A_1) and mean amplitudes for the group of slices (A_2) are shown. **B**. The 5-HT_{1A} receptor antagonist NAN-190 blocked the reduction in representative averaged field EPSPs (B_1) and mean field EPSP amplitude (B_2) induced by 1 µM 5-HT, indicating that 5-HT acts through 5-HT_{1A} receptors.

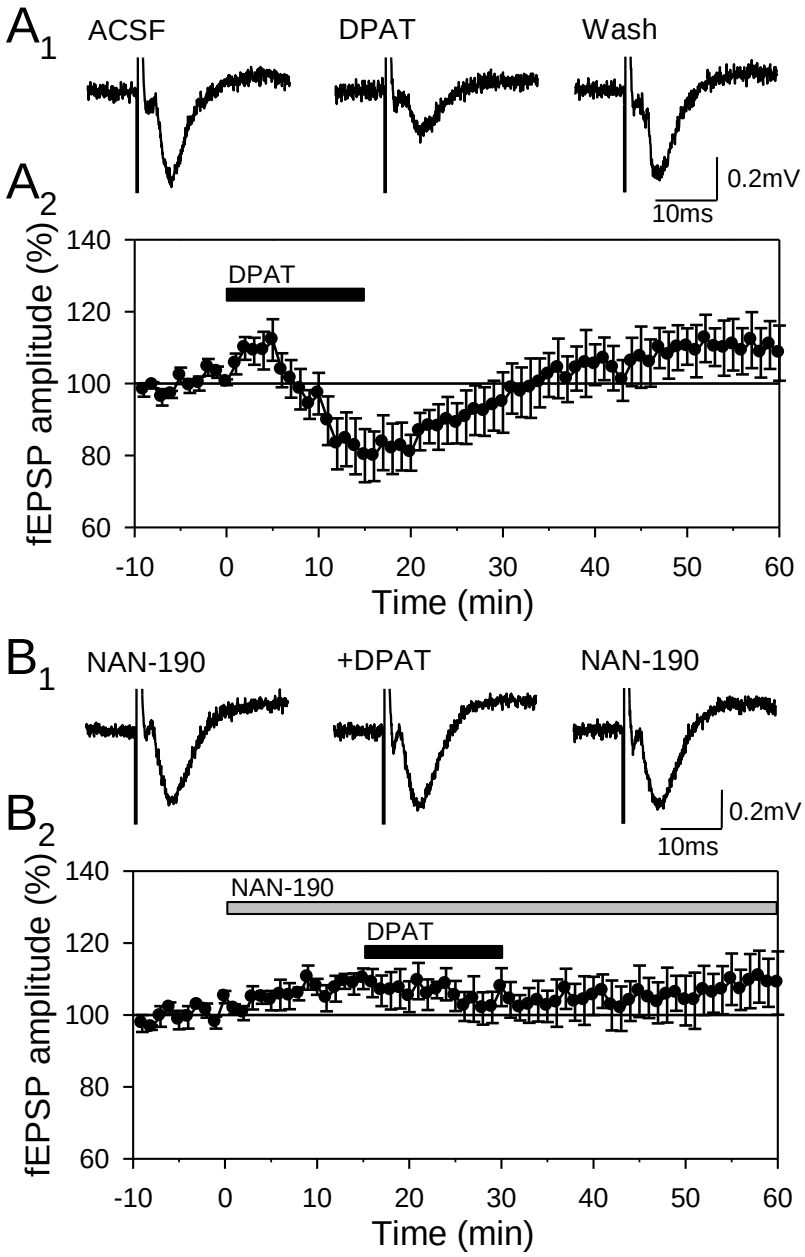

Figure 4. The 5-HT_{1A} receptor agonist 8-OH-DPAT results in a reduction in the amplitude of synaptic responses, and this effect is blocked in the presence of the 5-HT_{1A} receptor antagonist NAN-190. **A.** Application of 10 µM 8-OH-DPAT resulted in a reversible reduction of synaptic responses, reflected in averaged field EPSP recordings from a representative slice (A_1) and the mean amplitudes of field EPSPs in the group of slices tested (A_2). **B.** Constant bath application of the 5-HT_{1A} antagonist NAN-190 prevented effects of 8-OH-DPAT on averaged field EPSP recordings (B_1) mean amplitude of field EPSPs (B_2).

DISCUSSION

We have found here that application of serotonin (5-HT) results in a strong, concentration-dependent reduction in the amplitude of excitatory synaptic responses in layer II/III of the parasubiculum evoked by stimulation of layer I in acute rat brain slices in vitro. Similar to other findings in entorhinal cortex (Schmitz et al., 1998c; 1999), the reduction of fEPSPs was associated with an increase in paired-pulse ratio, suggesting that 5-HT reduces fEPSPs through a presynaptic reduction in glutamate release. The reduction in synaptic responses was mimicked by the potent and selective 5-HT reuptake blocker citalopram (Hyttel, 1982; Owens et al., 2001); this is most likely due to an enhancement of the effects of 5-HT release within the parasubiculum (Köhler, 1984; Köhler et al., 1981, 1986; Bjarkam et al., 2005) although the relative contribution of synaptic an extrasynaptic serotonin, and release from nearby regions in the slice, is not clear. Results also indicate that the reduction of fEPSPs induced by 5-HT is mediated by 5-HT_{1A} receptors; the reduction of fEPSPs was mimicked by the 5-HT_{1A} receptor agonist 8-OH-DPAT, and the inhibitory effects induced by either 5-HT or 8-OH-DPAT were blocked by the 5-HT_{1A} receptor blocker NAN-190.

The 5-HT_{1A} agonist 8-OH-DPAT can also activate 5-HT_7 receptors (Costa et al., 2012), but we found here that the reduction of fEPSP amplitude induced by 8-OH-DPAT was blocked by NAN-190. Further, the 5-HT_7 receptor blocker SB-276690 did not affect the reduction of fEPSPs induced by 5-HT. 5-HT_7 receptors, which can affect hippocampal synaptic responses (Andreetta et al., 2016; Costa et al., 2012), therefore do not contribute the 5-HT receptor-mediated reduction of fEPSPs in the parasubiculum.

The depression of fEPSP amplitudes observed here is likely to be primarily due to a presynaptic mechanism resulting in reduced glutamate release. Paired-pulse depression was reduced during the reduction of fEPSPs induced by either 5-HT or by the 5-HT_{1A} agonist 8-OH-DPAT, consistent with reduced release of transmitter in response to the first stimulation pulse that provides a larger remaining pool of readily releasable transmitter during the response to the second stimulation pulse. This is consistent with findings in layers II/III of entorhinal cortex where inhibition of evoked synaptic responses by 5-HT_{1A} receptors is accompanied by an increase in paired-pulse ratio (Schmitz et al., 1995a, 1999). Findings in the entorhinal cortex also show that the frequency distribution, but not the amplitude, of miniature excitatory postsynaptic currents is reduced by 5-HT_{1A} receptor activation (Schmitz et al., 1998c), and that 5-HT does not affect the amplitude of glutamate-evoked postsynaptic currents in isolated patches

(Schmitz et al., 1998c). At corticostriatal synapses, 5-HT also reduces EPSCs through a presynaptic mechanism, but the reduction is mediated by 5-HT_{1B} receptors, and is a long-lasting effect rather than the transient reduction observed here (Mathur et al., 2011). A reduction in action potentials in presynaptic fibers does not do not appear to contribute to the present results, and the stability of the fiber volley observed here during application of 5-HT is consistent with findings in the CA1 region where 5-HT reduces fEPSPs and calcium entry into presynaptic terminals without affecting the fiber volley (Schmitz et al., 1995b).

Although a presynaptic reduction in transmitter release likely mediates most of the reduction of fEPSPs observed here, postsynaptic factors might contribute to some degree. Activation of 5-HT_{1A} receptors in lateral entorhinal cortex increases a potassium conductance and hyperpolarizes neurons, and the associated reduction in cellular input resistance mediates a reduction in EPSP amplitude (Grunschlag et al., 1997). Parallel effects are induced by serotonin in subicular neurons (Behr et al., 1997), and are also possible in the parasubiculum. A postsynaptic effect on glutamate receptors is also possible, as 5-HT has been shown to reduce depolarizations induced by glutamate in entorhinal layer II/III neurons (Sizer et al., 1992), and 5-HT_{1A} receptor activation can inhibit NMDA-mediated synaptic currents in prefrontal neurons (Yuen et al., 2005). In the anterior cingulate cortex, the reduction of EPSCs induced by 5-HT is associated with increased paired pulse ratio and reduced frequency of miniature EPSCs; these effects are blocked by NAN-190, suggesting 5-HT_{1A} receptors mediate the reduction in transmitter release (Tian et al., 2017). However, the authors found that 5-HT induces a residual depression in the presence of NAN-190, and that blocking postsynaptic G-proteins also reduces the depression of EPSCs induced by 5-HT, suggesting that postsynaptic 5-HT receptors also contribute.

Changes in inhibitory synaptic transmission, which is stronger in superficial versus deep layers of the parasubiculum (Funahashi and Stewart, 1998), is unlikely to have contributed. Activation of 5-HT_{1A} receptors reduces polysynaptically evoked IPSCs in entorhinal neurons (Schmitz et al., 1998b), and also reduces inhibitory synaptic transmission in the hippocampus (Oleskevich and Lacaille, 1992; Schmitz et al., 1998a; Fink and Gothert, 2007), but reduced inhibition cannot contribute to the reduction of fEPSPs observed here. *Increases* in inhibition, however, have also been observed in the entorhinal cortex; 5-HT can increase firing frequency in inhibitory neurons (Lei, 2012), and increase the frequency of spontaneous IPSC without affecting miniature IPSCs (Deng and Lei, 2008). Both of these effects appear to be due to

depolarization of inhibitory cells induced by a 5-HT_{2A}-mediated inhibition of a potassium conductance (Deng and Lei, 2008), but this is unlikely to have contributed to the reduction of fEPSPs observed here because the reduction was completely blocked by the 5-HT_{1A} blocker NAN-190. Similarly, excitation of inhibitory cells in prefrontal cortex induced by stimulation of the raphe nuclei is mediated by 5-HT_3 receptors rather than by 5HT1A receptors (Puig et al., 2004). However, possible effects of 5-HT on the excitability of GABAergic neurons and IPSCs in the parasubiculum are yet to be determined.

The parasubiculum receives inputs from the hippocampus and subiculum in addition to subcortical inputs from thalamus and amygdala, and the contribution of modulatory effects of 5-HT in the parasubiculum on cognitive function are likely to depend both on reduced synaptic integration within the parasubiculum, and changes in its output to the entorhinal cortex (Witter et al., 1989; Tang et al., 2016). A 5-HT-induced reduction of EPSPs is likely to reduce firing in parasubicular inputs to layer II of the entorhinal cortex, and might also inhibit epileptiform activity as 5-HT does in the entorhinal cortex (Lei, 2012). It has been proposed that reduced excitatory transmission in the entorhinal cortex induced by 5-HT may result in reduced feedforward inhibition and enhanced excitability within the dentate gyrus (Schmitz et al., 1998a). Synaptic inhibition in the entorhinal cortex might be similarly reduced by reduced excitatory transmission in the parasubiculum. In the prefrontal cortex, serotonin has complex actions, and has both excitatory effects on prefrontal network activity through 5-HT_2 receptors, and a depressive effect on firing of pyramidal neurons via 5-HT_{1A} receptors (Puig and Gulledge, 2011). In the parasubiculum, 5-HT may be associated with a generalized reduction of excitatory transmission that may enhance the relative strength of more active excitatory inputs. It is also possible that periods of reduced 5-HT activity associated with rapid eye movement sleep (Urbain et al., 2006) may result in a generalized increase in excitatory transmission that may promote mechanisms of memory consolidation. A further assessment using intracellular recordings will be required to determine the effects of 5-HT on membrane potential and firing activity of parasubicular neurons.

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Highlights for "*Serotonin 5-HT1A receptor-mediated reduction of excitatory synaptic transmission in layers II/III of the parasubiculum*." by F. Carter and C.A. Chapman.

1) Serotonin reduces the amplitude of evoked field excitatory postsynaptic potentials in the parasubiculum in vitro.

2) The reduction of excitatory synaptic transmission induced by serotonin is concentration-dependent and reversible.

3) The reduction is mimicked by the 5-HT_{1A} receptor agonist 8-OH-DPAT, and is blocked by the 5-HT_{1A} receptor blocker NAN-190.

4) The serotonin reuptake blocker citalopram also reduces the amplitude of excitatory synaptic responses.

5) Serotonin and 8-OH-DPAT increase paired-pulse ratio, consistent with a reduction in glutamate release.