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Time-dependent modulation of glutamate synapses onto 5-HT neurons by antidepressant treatment

Sean D. Geddes [a, b, c], Saleha Assadzada [a, b, c], Alexandra Sokolovski [a, e], Richard Bergeron [a, e], Samir Haj-Dahmane [f], Jean-Claude Béïque [a, b, c, d, *]

[a] Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, K1H 8M5, Canada
[b] Neuroscience Graduate Program, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada
[c] Canadian Partnership for Stroke Recovery, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada
[d] Centre for Neural Dynamics, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada
[e] Ottawa Hospital Research Institute, Ottawa, Ontario, K1Y 4E9, Canada
[f] Research Institute on Addictions, University at Buffalo, State University of New York, Buffalo, NY 14203, USA

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ABSTRACT

Antidepressants, including the selective serotonin reuptake inhibitors (SSRIs), are thought to exert their clinical effects by enhancing serotonin (5-HT) transmission. However, animal studies show that the full magnitude of this enhancement is reached only following prolonged treatments with SSRIs, consistent with the well-described therapeutic delay of this class of medications. Thus, the clinical efficacy of SSRIs most likely does not emerge from their acute pharmacological actions, but rather indirectly from cellular alterations that develop over the course of a sustained treatment. Here, we show that sustained administration of the SSRI citalopram leads to a homeostatic-like increase in the strength of excitatory glutamate synapses onto 5-HT neurons of the dorsal raphe nucleus that was apparent following one week of treatment. A shorter treatment with citalopram rather induced a paradoxical decrease in the strength of these synapses, which manifested itself by both pre- and postsynaptic mechanisms. As such, these results show that an SSRI treatment induced a concerted and time-dependent modulation of the synaptic drive of 5-HT neurons, which are known to be critically involved in mood regulation. This regulation, and its time course, provide a mechanistic framework that may be relevant not only for explaining the therapeutic delay of antidepressants, but also for the perplexing increases in suicide risks reportedly occurring early in the course of antidepressant treatments.

1. Introduction

Major depression is one of the most prevalent and life-threatening forms of mental illness and is associated with significant disabilities and mortality (Chopra et al., 2011). Although modern antidepressants, including the widely prescribed Selective Serotonin Reuptake Inhibitors (SSRIs), provide much welcomed therapeutic benefits, they are unfortunately plagued by a lack of efficacy in a high proportion of patients and by a delay in their clinical actions (Slattery et al., 2004). These distressing limitations resolutely advocates for the need to gain a clearer understanding of the neural substrates of the clinical effects of this class of drugs.

The mere presence of a therapeutic delay suggests that the clinical efficacy of antidepressants stems from the development of a neuroadaptive cellular mechanism rather than from their acute pharmacological actions. In part reinforced by the clinical findings that the serotonin (5-HT) system appears to be required for the antidepressant effect (Delgado et al., 1990; Neumeister, 2003; Young et al., 1985), the behavior of 5-HT neurotransmission during the time course of administration of several types of antidepressants has been under scrutiny for close to 40 years (*e.g.*, (De Montigny and Aghajanian, 1978). Whereas the acute administration of SSRIs rapidly blocks 5-HT transporters and increases net 5-HT output in target regions (Bel and Artigas, 1993; Artigas, 1993), the magnitude of this increase is constrained by activation of inhibitory somatodendritic 5-HT_{1A} autoreceptors that suppresses

* Corresponding author. University of Ottawa, Faculty of Medicine, Office: 3501N, 451 Smyth Road, Ottawa, ON K1H 8M5, Canada. Tel.: +1 613 562 5800 4968; fax: +1 613 562 5434.
E-mail address: jbeique@uottawa.ca (J.-C. Béïque).

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the firing activity of 5-HT neurons. Remarkably, the firing activity of these neurons recovers over the time course of sustained SSRI treatments, likely as a result of the desensitization of these 5-HT_{1A} autoreceptors (De Montigny and Blier, 1984; Blier and De Montigny, 1994; Albert and Lemonde, 2004). Because the time course of these cellular events broadly matches the delayed therapeutic efficacy of SSRIs in humans, it may explain the delay in the clinical efficacy of these drugs.

Unfortunately, despite some progress, capitalizing on this knowledge for therapeutic benefits has been difficult, likely because of the challenge in pharmacologically discriminating between pre- and postsynaptic 5-HT_{1A} receptors (Blier et al., 1998; Segrave and Nathan, 2005). Moreover, the framework elaborated from these studies has mainly conceptualized 5-HT neurons as semi-autonomous units, largely ignoring the dynamic synaptic network in which these cells are embedded. Indeed, the raphe receives strong afferent projections from a remarkably vast amount of cortical and subcortical regions of the brain, forming an extensive network that synapses onto both 5-HT neurons and local GABAergic neurons (Weissbourd et al., 2014; Pollak Dorocic et al., 2014; Pan and Williams, 1989; Haj-Dahmane and Shen, 2005; Li and Bayliss, 1998). Yet, the basic characteristics of these glutamatergic synapses have been relatively little investigated, let alone how they are altered by chronic antidepressant treatments.

Here, using *ex vivo* whole-cell electrophysiological recordings, we found, unexpectedly, that the strength of glutamate synapses onto 5-HT neurons was markedly reduced early in the time course of a treatment with an SSRI. Remarkably, a homeostatic-like synaptic plasticity mechanism emerged later during the treatment such that, by about one week of treatment, synaptic strength was not only no longer suppressed, but was rather potentiated. Because 5-HT neurons play a key role in mood regulation, this delayed, homeostatic-like, increase in synaptic drive onto these neurons by SSRIs may contribute to their clinical effectiveness.

2. Materials and methods

2.1. Animals

Sprague Dawley rats (50–90 g; Charles River, St. Constant, Quebec, Canada) were received at least 7 days prior to implantation of mini-pumps for drug delivery and housed two to three per cage. They were kept on a 12:12h light/dark cycle, with access to food and water *ad libitum*. All experiments and procedures were performed in accordance with approved procedures and guidelines set forth by the University of Ottawa Animal Care and Veterinary Services.

2.2. In vivo treatments

Citalopram (Toronto Research Chemicals; North York, Canada) was delivered to animals via subcutaneously implanted osmotic minipumps (Alzet; Cupertino, California) at a dose of 20 mg/kg/day. Animals (26–29 days old) were anesthetized with isofluorane at the time of minipump implantation. Citalopram was dissolved in 45% w/v 2-Hydroxypropyl-β-cyclodextrin (2-HβC) to enhance drug solubility in water. Citalopram or 45% w/v 2-HβC (as vehicle) was administered for 2 or 7 days before the electrophysiological experiments.

2.3. Slice preparation

Brainstem slices containing the dorsal raphe nucleus were prepared from 28 to 37-day old Sprague Dawley rats following 2 or 7 days of treatments with citalopram or vehicle. Rats were anesthetized by inhalation of isofluorane (Baxter Corporation, Canada) and sacrificed by decapitation. The brain was rapidly removed and placed in ice-cold choline chloride-based cutting solution of the following composition (in mM): 119 choline-Cl, 2.5 KCl, 1 $CaCl_2$, 4.3 $MgSO_4$-$7H_2O$, 1 NaH_2PO_4, 1.30 sodium L-ascorbate, 26.20 $NaHCO_3$, and 11 glucose, and equilibrated with 95% O_2, 5% CO_2. Two or three coronal slices (300 μm thick) containing the DRN were sectioned from a block of brainstem tissue in ice-cold choline chloride-based cutting solution using a Vibratome slicer Series 1000 Plus (Shepreth, England) or a Leica VT1000s vibrating blade microtome (Nussloch, Germany). Slices were then transferred into a recovery chamber containing standard Ringer's solution of the following composition (in mM): 119 NaCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$-$7H_2O$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, and 11 glucose, at a temperature of 37°C, continuously bubbled with a mixture of 95% O_2, 5% CO_2. Slices were then allowed to recover for 1h in the recovery chamber and equilibrate to a temperature of approximately 25°C until recordings were performed.

Hippocampal neuron recordings were performed from *ex vivo* organotypic slices cultures as previously described (Soares et al., 2013). In brief, individual hippocampi were removed from sprague dawley rats between the ages of 6–8 days. Coronal slices from hippocampi were gathered using a MX-TS Tissue Slicer (Siskiyou). As previously described, individual hippocampal slices were placed in membrane inserts and kept in six-well plates at 34°C in 95% O2 and 5% CO2 in culture media (Soares et al., 2013). Whole-cell recordings were performed following 8–10 DIV.

2.4. Whole-cell electrophysiology

DRN neurons were visualized using an upright microscope (Examiner D1; Zeiss, Oberkochen, Germany) equipped with Dodt-contrast or differential-interference contrast (DIC) (40×/0.75NA objective). 5-HT neurons were identified by morphological and biophysical characteristics using previously established criteria (Haj-Dahmane, 2001; Aghajanian and Vandermaelen, 1982; Calizo et al., 2011). Using the aqueduct as a landmark, we surmise that our recordings were primarily from the rostral half of the DRN. Whole-cell recordings were carried out using an Axon Multiclamp 700B amplifier, sampled at 10 kHz, digitized with an Axon Digidata 1440A digitizer and filtered at 2 kHz. Whole-cell recordings were performed using borosilicate glass patch electrodes (3–6 MΩ; World Precision Instruments, Florida or Sutter, California) pulled on a Narishige PC-10 pipette puller (Narishige, Japan). All experiments were performed at room temperature in Ringer containing (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1.0 NaH_2PO_4, 11 glucose, and 26.2 $NaHCO_3$ (or low Mg^{2+} Ringer containing 0.1 $MgSO_4$, 3.0 $CaCl_2$) saturated with 95% O_2 and 5% CO_2 (pH = 7.3; 295–310 mOsm/L). Excitatory post-synaptic currents (EPSCs) were recorded using an intracellular solution of either of the following compositions (in mM): (1) 115 cesium methane-sulfonate, 5 tetraethylammonium-Cl, 10 sodium phosphocreatine, 20 HEPES, 2.8 NaCl, 5 QX-314, 0.4 EGTA, 3 ATP(Mg^{2+}), and 0.5 GTP, pH 7.25 (adjusted with CsOH; osmolarity, 280–290 mOsmol/L; or (2) 77 cesium methane-sulfonate, 10 tetracesium BAPTA, 5 tetraethylammonium-Cl, 3 calcium-chloride, 10 sodium phosphocreatine, 20 HEPES, 5 QX-314, 4 ATP(Mg^{2+}), and 0.5 GTP, pH 7.25 (adjusted with CsOH; osmolarity, 280–290 mOsmol/L. In some recordings, we bath applied the non-selective 5-HT_{1A} receptor agonist 5-Carboxamidotryptamine (5-CT) to elicit a 5-HT_{1A}-medated outward current (or hyperpolarization). These recordings were carried out using an internal solution of the following composition (in mM): 115 potassium gluconate, 20 KCl, 10 sodium phosphocreatine, 10 HEPES, 4 ATP(Mg^{2+}), and 0.5 GTP, pH 7.25 (adjusted with KOH; osmolarity, 280–290 mOsmol/L). For post-hoc immunohistochemical identification of 5-HT neurons, Alexa-594 hydrazide (Na-salt; 30 μM; Invitrogen) was included in the recording pipette. For all voltage clamp recordings, access resistance was continuously monitored by applying a 125 ms, 2 mV hyperpolarizing pulse, 245 ms prior to stimulation. Recordings were discarded when the access resistance changed by >30%. Liquid junction potential was not compensated for.

Excitatory post-synaptic currents were elicited using patch electrodes filled with standard Ringer solution and placed 50–100 μm dorsolateral to the recorded neurons. EPSCs were evoked with a single square pulse (100 μs) delivered at either 0.067 or 0.1 Hz. Unless stated otherwise, all recordings were performed in the presence of the $GABA_A$ receptor antagonist bicuculline (20 μM) or picrotoxin (100 μM).

For determining NMDAR/AMPAR ratios (N/A ratios) of evoked EPSCs (eEPSCs), mixed NMDAR and AMPAR eEPSCs were recorded while voltage clamping the cell at +40 mV. NBQX (20 μM) was then bath applied in order to isolate the NMDA receptor-mediated component. The AMPAR component was computed off-line by subtracting the NMDAR component from the mixed current traces.

Paired-pulse ratios (PPRs) were determined by eliciting eEPSCs with an inter-stimulus interval (ISI) of 50 ms while holding the cell at −70 mV. These paired pulses were elicited at a stimulation frequency of 0.1 Hz. Miniature EPSCs (mEPSCs) were acquired at −70 mV in the presence of Tetrodotoxin (TTX, 1 μM). Current–voltage relationships (I–V curves) of evoked AMPAR-mediated EPSCs were performed as previously described (Soares et al., 2013) with 0.1 mM spermine added to the internal solution and with D,L-APV (100 μM) in the extracellular solution.

The sensitivity of AMPAR-mediated currents to NASPM (3 μM) was established by obtaining a 4-min baseline of AMPAR-EPSC and bath applying NASPM for 25 min. Similar to NASPM applications, the sensitivity of NMDAR-mediated currents to ifenprodil was established by obtaining a 4-min baseline NMDAR-mediated EPSC at +40 mV in the presence of NBQX (20 μM) and bath applying ifenprodil (3 μM) for 25 min. NMDAR sensitivity to ifenprodil was calculated based on the 4-min baseline and the last 4 min from the 29 min recording. The decay of NMDAR-mediated eEPSCs was determined by a weighted tau value calculated using a bi-exponential fit as previously described (Beique et al., 2006).

2.5. Immunohistochemistry

Brain slices used for immunohistochemistry were prepared from 28 to 37 day old rats with 1X phosphate buffered saline (PBS) used as the solvent for all solutions. Rats were anaesthetized through an intraperitoneal injection of 100 mg/kg pentobarbital. They were then intracardially perfused with 10 U/mL ice-cold heparin, followed by 4% PFA. Post fixation in 4% PFA was carried out for 2 h prior to sequential