Neuropharmacology 126 (2017) 224–232

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

# Serotonin gating of cortical and thalamic glutamate inputs onto principal neurons of the basolateral amygdala

Ji-Dong Guo*, Brendan M. O'Flaherty, Donald G. Rainnie

*Division of Behavioral Neuroscience & Psychiatric Disorders, Yerkes National Primate Research Center, Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA, USA*

ARTICLE INFO

*Article history:*
Received 5 July 2017
Received in revised form
31 August 2017
Accepted 8 September 2017
Available online 9 September 2017

*Keywords:*
Serotonin receptor
Glutamatergic transmission
Basolateral amygdala

ABSTRACT

The basolateral amygdala (BLA) is a key site for crossmodal association of sensory stimuli and an important relay in the neural circuitry of emotion. Indeed, the BLA receives substantial glutamatergic inputs from multiple brain regions including the prefrontal cortex and thalamic nuclei. Modulation of glutamatergic transmission in the BLA regulates stress- and anxiety-related behaviors. Serotonin (5-HT) also plays an important role in regulating stress-related behavior through activation of both pre- and postsynaptic 5-HT receptors. Multiple 5-HT receptors are expressed in the BLA, where 5-HT has been reported to modulate glutamatergic transmission. However, the 5-HT receptor subtype mediating this effect is not yet clear. The aim of this study was to use patch-clamp recordings from BLA neurons in an *ex vivo* slice preparation to examine 1) the effect of 5-HT on extrinsic sensory inputs, and 2) to determine if any pathway specificity exists in 5-HT regulation of glutamatergic transmission. Two independent input pathways into the BLA were stimulated: the external capsule to mimic cortical input, and the internal capsule to mimic thalamic input. Bath application of 5-HT reversibly reduced the amplitude of evoked excitatory postsynaptic currents (eEPSCs) induced by stimulation of both pathways. The decrease was associated with an increase in the paired-pulse ratio and coefficient of variation of eEPSC amplitude, suggesting 5-HT acts presynaptically. Moreover, the effect of 5-HT in both pathways was mimicked by the selective 5-$HT_{1B}$ receptor agonist CP93129, but not by the 5-$HT_{1A}$ receptor agonist 8-OH DPAT. Similarly the effect of exogenous 5-HT was blocked by the 5-$HT_{1B}$ receptor antagonist GR55562, but not affected by the 5-$HT_{1A}$ receptor antagonist WAY 100635 or the 5-$HT_2$ receptor antagonists pirenperone and MDL 100907. Together these data suggest 5-HT gates cortical and thalamic glutamatergic inputs into the BLA by activating presynaptic 5-$HT_{1B}$ receptors.



## 1. Introduction

The basolateral amygdala (BLA) is a key relay structure in emotional circuitry. The BLA receives substantial glutamatergic input from both the somatosensory cortex through the external capsule, and from the thalamic nuclei through the internal capsule. The BLA in turn projects to multiple downstream targets critically involved in the regulation of stress and anxiety-like behavior, including the central nucleus of amygdala (CeA) and the bed nucleus of the stria terminalis (BNST) (Walker et al., 2003). Significantly, abnormal hyperactivity of the BLA has been implicated in the etiology of several emotional disorders including depression and anxiety. Consistent with this observation, repeated stress increases the activity of BLA neurons *in vivo* (Zhang and Rosenkranz, 2012; Padival et al., 2013). Furthermore, activation or inhibition of neural activity in the BLA respectively enhances or reduces anxiety-like behavior (Davis, 2002; Tye et al., 2011). Additionally, stressful stimuli increase glutamate release in the BLA (Reznikov et al., 2007), and microinjection of glutamate antagonists into the BLA abolishes the expression of conditioned fear (Kim et al., 1993; Lee et al., 2001). Hence, glutamatergic transmission is essential for normal BLA function.

Importantly, glutamatergic input into the BLA can be regulated by neurotransmitters and/or neuromodulators such as serotonin (5-HT) (Bocchio et al., 2016). Indeed, dysregulation of 5-HT transmission is thought to play a major role in the etiology of emotional disorders (Lowry et al., 2005; Asan et al., 2013). For example, the BLA hyperactivity seen in major depressive disorder is normalized

* Corresponding author.
*E-mail address:* jguo4@emory.edu (J.-D. Guo).

http://dx.doi.org/10.1016/j.neuropharm.2017.09.013
0028-3908/

after successful pharmacotherapy using selective serotonin reuptake inhibitors (SSRIs). The BLA is heavily innervated by 5-HT terminals originating from the dorsal raphe nucleus (Abrams et al., 2004; Muller et al., 2007), and multiple 5-HT receptor subtypes are expressed by both BLA principal neurons and interneurons (Asan et al., 2013). Indeed, local activation of 5-$HT_{2A}$ or 5-$HT_{2C}$ receptors induces anxiety-like behavior in rodents (Campbell and Merchant, 2003; de Mello Cruz et al., 2005, Cornelio and Nunes-de-Souza, 2007; Christianson et al., 2010), an effect thought to be mediated by activating postsynaptic 5-HT receptors. Far fewer studies have examined 5-HT receptor-mediated modulation of glutamatergic transmission in the BLA. Previous studies reported that activating presynaptic 5-HT receptors inhibits glutamatergic transmission in the BLA (Cheng et al., 1998; Rainnie, 1999; Yamamoto et al., 2012). However, the receptor subtype(s) mediating this response have not been clearly established. In this study we used *in vitro* patch clamp recording from BLA slices to 1) determine the identity of the presynaptic 5-HT receptor(s) modulating BLA glutamatergic transmission, and 2) determine if presynaptic 5-HT differentially regulated cortical and thalamic glutamatergic inputs into the BLA.

## 2. Methods

### 2.1. Animals

Adolescent male Sprague-Dawley rats (35–49 days old, Charles River, Raleigh, NC) were used throughout this study. Animals were group-housed with 4–5 rats per cage and had access to food and water *ad libitum*. Efforts were taken to minimize both animal suffering and the number of animals used in experiments. Animal care and all procedures used in this study were performed in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Emory University.

### 2.2. Slice preparation

Coronal slices (350 μm) containing the BLA were obtained as previously reported (Li et al., 2011). Briefly, under deep anesthesia (isofluorane, Henry Schein Inc, Melville, NY, USA), the brains of anesthetized rats were rapidly dissected and immersed in a cold (4 °C) oxygenated artificial cerebrospinal fluid (ACSF) "cutting solution", of the following composition (in mM): NaCl (130), $NaHCO_3$ (30), KCl (3.50), $KH_2PO_4$ (1.10), $MgCl_2$ (6.0), $CaCl_2$ (1.0), glucose (10), ascorbate (0.4), thiourea (0.8), sodium pyruvate (2.0), and kynurenic acid (2.0). BLA slices were cut using a Leica VTS-1000 vibratome (Leica Microsystems Inc., Bannockburn, IL, USA). After sectioning, slices were maintained at 32 °C in "cutting solution" oxygenated with a mixture of 95% oxygen and 5% carbon dioxide for 1 h prior to recording. Slices were then transferred to a holding chamber containing "regular" ACSF maintained at room temperature, with the following composition (in mM): NaCl (130), NaHCO3 (30), KCl (3.50), KH2PO4 (1.10), MgCl2 (1.30), CaCl2 (2.50), and glucose (10), ascorbate (0.4), thiourea (0.8), and sodium pyruvate (2.0).

### 2.3. Patch clamp recording

For whole-cell patch clamp recording, slices were continuously perfused by gravity-fed oxygenated "regular" ACSF heated to 32 °C (2–3 ml per min) in a Warner Series 20 submersion-type slice chamber (0.5 ml volume; Warner Instruments, Hamden, CT). Slices were viewed using differential interference contrast (DIC) optics and infrared (IR) illumination with an IR sensitive CCD camera (Orca ER, Hamamatsu, Tokyo, Japan) mounted on a Leica DMF6000 microscope (Leica Microsystems Inc., Bannockburn, IL). Patch pipettes were fabricated from borosilicate glass (resistance 4–6 MΩ) and filled with a recording solution of the following composition (in mM): K-Gluconate (130), KCl (2), HEPES (10), $MgCl_2$ (3), phosphocreatine (5), K-ATP (2), and NaGTP (0.2). The patch solution was adjusted to pH 7.3 with KOH and had an osmolarity of 280–290 mOsm. Whole-cell recordings were made with a Multiclamp 700B amplifier (Molecular Devices Corporation, Sunnyvale, CA) using pClamp 10.4 software and an Axon Digidata 1550 A-D interface (Molecular Devices Corporation). BLA principal neurons were identified visually by their pyramidal shape and confirmed physiologically by their membrane properties. Whole-cell access resistances measured in voltage clamp were in the range 5–20 MΩ and were routinely monitored throughout each experiment; a change of <15% was deemed acceptable.

### 2.4. Evoked EPSCs

Postsynaptic currents onto BLA neurons were evoked by stimulating the external capsule (cortical input) or the internal capsule (thalamic input) with a concentric bipolar stimulation electrode (FHC, Bowdoinham, ME) as previously reported (Li et al., 2011). To isolate evoked excitatory postsynaptic currents (eEPSCs), the $GABA_A$ receptor antagonist picrotoxin (100 μM) was included in the patch solution to block inhibitory postsynaptic currents (IPSCs). Furthermore, the membrane potential was held at −65 mV, which is close to chloride equilibrium potential, to minimize contamination by residual $GABA_A$ receptor-mediated evoked IPSCs. The selective $GABA_B$ receptor antagonist CGP 53432 (1 μM) was also bath applied to block the slow component of evoked IPSCs. Two stimulation paradigms were used in this study to induce eEPSCs: 1) one train of five single square wave pulses (150 μs, 0.2 Hz) delivered every 2 min, and 2) consecutive single pulse stimulations (150 μs, 0.1 Hz) were delivered throughout the experiment. Baseline amplitude of eEPSCs was adjusted to half maximal stimulation response. For analysis, all eEPSCs values were normalized to the baseline amplitude and expressed as the percentage of baseline.

To examine the potential involvement of presynaptic 5-HT receptors in the attenuation of glutamate release, we employed a paired-pulse paradigm in conjunction with an analysis of the coefficient of variation (CV) of eEPSC amplitude, as previously reported (Guo et al., 2012). Alterations in the paired-pulse ratio (PPR) are thought to represent changes in release probability in the presynaptic terminal (Hess and Ludin, 1987; Manabe et al., 1993). A change of CV is associated with either a change of release probability or the number of release sites (Choi and Lovinger, 1997). For the paired-pulse paradigm, two electrical stimuli were delivered with an inter-stimulus-interval of 50 ms. The PPR was calculated as the mean peak amplitude of the second eEPSC (P2) divided by the first eEPSC (P1). CV was calculated as δ/μ, where δ is the stand deviation of the peak eEPSC amplitude and μ is the mean eEPSC amplitude. Here, we used 10 eEPSCs immediately before drug application and 10 eEPSCs during the maximal drug effect to calculate CV in the baseline and drug application respectively. The selection of 10 sweeps was adequate for analysis based on the low variation of eEPSCs amplitude and ensured that they were captured at the time of the maximum drug response. Including more sweeps would increase the chance that some sweeps may not have reached maximal 5-HT effect and if included in the analysis would erroneously increase CV value.

### 2.5. Drug application

The following drugs were obtained from 1) Sigma-Aldrich (St.