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Research Paper

Serotonin modulates response properties of neurons in the dorsal cochlear nucleus of the mouse

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ABSTRACT

The neurochemical serotonin (5-hydroxytryptamine, 5-HT) is involved in a variety of behavioral functions including arousal, reward, and attention, and has a role in several complex disorders of the brain. In the auditory system, 5-HT fibers innervate a number of subcortical nuclei, yet the modulatory role of 5-HT in nearly all of these areas remains poorly understood. In this study, we examined spiking activity of neurons in the dorsal cochlear nucleus (DCN) following iontophoretic application of 5-HT. The DCN is an early site in the auditory pathway that receives dense 5-HT fiber input from the raphe nuclei and has been implicated in the generation of auditory disorders marked by neuronal hyperexcitability. Recordings from the DCN in awake mice demonstrated that iontophoretic application of 5-HT had heterogeneous effects on spiking rate, spike timing, and evoked spiking threshold. We found that 56% of neurons exhibited increases in spiking rate during 5-HT delivery, while 22% had decreases in rate and the remaining neurons had no change. These changes were similar for spontaneous and evoked spiking and were typically accompanied by changes in spike timing. Spiking increases were associated with lower first spike latencies and jitter, while decreases in spiking generally had opposing effects on spike timing. Cases in which 5-HT application resulted in increased spiking also exhibited lower thresholds compared to the control condition, while cases of decreased spiking had no threshold change. We also found that the 5-HT_2 receptor subtype likely has a role in mediating increased excitability. Our results demonstrate that 5-HT can modulate activity in the DCN of awake animals and that it primarily acts to increase neuronal excitability, in contrast to other auditory regions where it largely has a suppressive role. Modulation of DCN function by 5-HT has implications for auditory processing in both normal hearing and disordered states.

1. Introduction

The serotonergic system contributes to a variety of important behavioral functions including arousal, stress response, and attention (Jacobs and Fornal, 1999; Kähkönen and Ahveninen, 2002). In addition, this system provides widespread input to numerous central auditory regions that allow serotonin (5-hydroxytryptamine, 5-HT) to modulate sound-evoked properties including response magnitude (Ebert and Ostwald, 1992), spike timing (Hurley and Pollak, 2005b), and frequency tuning (Hurley and Pollak, 2001). In addition to its role in normal auditory function, the serotonergic system has been implicated in a variety of behavioral disorders, some of which have a substantial auditory component (Lucki, 1998). For instance, there is evidence that disturbance of the serotonergic system contributes to the development of tinnitus, a condition marked by the perception of phantom sounds, typically following acoustic trauma (Simpson and Davies, 2000). Although the underlying mechanisms that lead to tinnitus are not entirely clear, trauma-induced neuronal hyperactivity, which is first observed in the central auditory system in the dorsal cochlear nucleus (DCN), may be a leading cause (Marriage and Barnes, 1995; Kaltenbach, 2007).

The DCN is a direct target of the auditory nerve and it receives additional inputs from interneurons that convey auditory and somatosensory information (Cant and Benson, 2003; Shore, 2005) and from 5-HT neurons of the raphe nuclei (Klepper and Herbert, 1991; Thompson and Thompson, 2001). Several functional roles for the DCN have been proposed that reflect its multimodal integration including sound localization in the vertical plane (Nelken

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and Young, 1996; Oertel and Young, 2004), suppression of self-generated sounds (Shore, 2005; Shore and Zhou, 2006), and sound identification (Young and Davis, 2002; Roberts and Portfors, 2015). The involvement of the DCN in the suppression of self-generated sounds may act to enhance the relative salience of sounds from external sources. Due to its roles in arousal and attention, the serotonergic system is positioned to impact the activity of DCN neurons by modulating responsiveness to incoming sounds that may have behavioral importance. The serotonergic system may also play a key role in hyperactivity, as local stimulation of 5-HT fibers in the DCN can induce increased spiking of post-synaptic neurons (Tang and Trussell, 2015), which resembles hyperexcitability reported in animal models of tinnitus (Brozoski et al., 2002; Kaltenbach, 2007). The output of the DCN is primarily transmitted by fusiform cells that target the inferior colliculus (IC). The IC is a prominent midbrain nucleus that subserves a multitude of auditory functions. Like the DCN, is heavily innervated by 5-HT fibers (Thompson et al., 1994; Hurley et al., 2002) and thus, may play an important role in auditory disorders marked by neuronal hyperexcitability (Jastreboff and Sasaki, 1986; Bauer et al., 2008). In this study, we examined whether exogenous application of 5-HT affects basic auditory response properties of DCN neurons in awake mice. We found that although 5-HT modulated responses in a heterogeneous manner, the predominant effect was an increase in neuronal excitability. We also found that the 5-HT_{2A} receptor is likely mediating serotonin effects. These findings demonstrate that neuromodulation mediated by 5-HT can shape neural responses to sound at low levels of the ascending central auditory system.

2. Materials and methods

2.1. Animals

Single unit responses were recorded in 19 awake CBA/CaJ mice aged three to six months (7 female, 12 male). Animals were housed with same-sex littermates on a reversed 12-h light/dark schedule and had free access to food and water. All animal care and use procedures were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Washington State University, an AAALAC-accredited institution.

2.2. Surgical procedures

Surgical procedures were similar to previous studies (Roberts and Portfors, 2015) and have been described in detail elsewhere (Muniak et al., 2012). Briefly, a subcutaneous injection of ketoprofen was given before surgery. Anesthesia was maintained with isoflurane (0.5–2.5%) inhalation while the mouse was in a stereotaxic frame. A lightweight metal headpost was attached to the skull using dental cement. To gain access to the DCN, a craniotomy approximately one square millimeter was performed, centered 5.9 mm caudal to Bregma and 2.30 mm lateral of the midline (Paxinos and Franklin, 2004). The craniotomy was covered with bone wax and the exposed tissue was treated with lidocaine (3%), as well as a triple antibiotic ointment that contained neomycin, polymyxin B, and bacitracin. The animal was returned to its home cage for a recovery period lasting at least one day. The bone wax overlying the craniotomy was removed prior to each recording session and reapplied at the conclusion of the session.

2.3. Acoustic stimulation

Stimulus generation and data acquisition were controlled by custom-written software (SSHF, Amy Boyle, Washington State University Vancouver). Acoustic stimuli were output through a 16-bit digital-to-analog converter (500,000 samples/s; National Instruments), sent to a programmable attenuator (PA-5, Tucker Davis Technologies), and routed to a ribbon tweeter (LCY K100, Ying Tai Corporation) placed 10 cm from the ear ipsilateral to the recording site. The speaker output was calibrated before each recording session over a range of 3–120 kHz using a ¼ inch calibrated microphone (model 4135, Brüel & Kjær) positioned at the location normally occupied by the animal's ear. The gradual roll-off of intensity measured at high frequencies was corrected online so that the sound pressure level for each frequency at a given intensity varied by less than 2 dB SPL. Distortion components of tonal stimuli were buried in the noise floor at least 50 dB below the signal level as determined by analyzing fast Fourier transforms of the digitized microphone signals.

2.4. Recording and drug application procedures

Experiments were conducted in a sound-attenuating chamber. The mouse was given a mild sedative (acepromazine, 2 mg/kg) at the beginning of each session that aided in placement of the animal in a foam body restraint. This small dose of acepromazine has no effects on basic auditory response properties (Felix et al., 2012). The headpost was secured to a stereotaxic device to render the head immobile and maintain a consistent position during recordings. The animal was monitored frequently and the experiment was terminated if the animal appeared distressed. Experimental sessions lasted no more than four hours, and each animal was used in one to three recording sessions separated by at least one day.

Recordings and application of pharmacologic agents were conducted using piggy-back electrodes (Havey and Caspary, 1980) made by gluing a single-barrel recording pipette onto a three-barreled glass pipette (10–15 μm total tip diameter), such that the tip of the recording pipette extended 10–15 μm beyond the tip of the multibarrel pipette. The recording pipette (1–2 μm tip diameter) was filled with 1 M NaCl solution, which yielded a pipette impedance of 15–30 MΩ. Two of the drug delivery barrels were filled with one of the following agents dissolved in water (pH 4.5): 30 mM of serotonin creatinine sulfate (5-HT; Sigma), 30 mM of the 5-HT_{2A} receptor agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI; Tocris), or 10 mM of the 5-HT_{2A} receptor antagonist Ketanserin (KS; Tocris). The remaining barrel was filled with 1 M NaCl and was used as a sum channel to balance the currents of the drug barrels.

Electrodes were advanced in the brain using a hydraulic micropositioner (David Kopf Instruments) and neural activity was amplified (Multiclamp700B, Axon Instruments), bandpass filtered (300–6000 Hz; Krohn-Hite), and digitized with a 16-bit analog-to-digital converter (50,000 samples/s; National Instruments). Individual raw waveforms were viewed online, recorded, then stored for offline analysis. Single-unit responses were isolated by advancing the electrode slowly through the DCN (located at a depth approximately 2.5–3.5 mm from the surface of the brain) while presenting a broadband noise search stimulus (70 dB SPL). Upon isolation of a unit, the characteristic frequency (CF) was determined, defined as the frequency that required the lowest sound level to elicit an evoked response. Thresholds were determined audiovisually for each neuron prior to data collection and were defined as a clear response of at least 0.5 spikes per stimulus above the rate of spontaneous activity. Spontaneous and evoked activity were determined by windowing the recording window online into separate epochs before and during the stimulus presentation and measuring the spiking rates for each time period. We targeted fusiform cells whose responses consisted of simple spikes, moderate levels of spontaneous activity, and V-shaped frequency tuning

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