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Research paper

Audiograms, gap detection thresholds, and frequency difference limens in cannabinoid receptor 1 knockout mice

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ABSTRACT

The cannabinoid receptor 1 (CB1R) is found at several stages in the auditory pathway, but its role in hearing is unknown. Hearing abilities were measured in CB1R knockout mice and compared to those of wild-type mice. Operant conditioning and the psychophysical Method of Constant Stimuli were used to measure audiograms, gap detection thresholds, and frequency difference limens in trained mice using the same methods and stimuli as in previous experiments. CB1R knockout mice showed deficits at frequencies above 8 kHz in their audiograms relative to wild-type mice. CB1R knockouts showed enhancements for detecting gaps in low-pass noisebursts relative to wild-type mice, but were similar for other noise conditions. Finally, the two groups of mice did not differ in their frequency discrimination abilities as measured by the frequency difference limens task. These experiments suggest that the CB1R is involved in auditory processing and lay the groundwork for future physiological experiments.

1. Introduction

The endocannabinoid signaling pathway plays an important role in regulating neuronal activity. The primary receptor for endocannabinoids in the nervous system is the CB1 receptor (CB1R), which has widespread expression throughout the brain (Mechoulam and Parker, 2013; Ohno-Shosaku et al., 2012; Pettit et al., 1998), presumably reflecting its importance in many aspects of neuronal processing. Behavioral evidence has been found for its role in various systems, including motor, memory, anxiety, and appetite (reviewed in Chaperon and Thiebot, 1999). At the cellular level, CB1Rs are responsible for depolarization-induced suppression of inhibition and excitation (DSI and DSE, Kreitzer and Regehr, 2001; Wilson and Nicoll, 2001), and also play a role in long-term depression (LTD, Safo and Regehr, 2005; Sjostrom et al., 2003; Tzounopoulos et al., 2007). However, it is not clear how such cellular effects contribute to behavior.

One approach to linking behavior with cellular function is to investigate how CB1Rs influence perception. CB1Rs are expressed at several stages in the auditory pathway, including the medial nucleus of the trapezoid body (MNTB, Kushmerick et al., 2004), the ventral cochlear nucleus (VCN, Zheng et al., 2007), and the dorsal cochlear nucleus (DCN, Zhao et al., 2009). Studies in zebra finches (*Taeniopygia guttata*) have revealed the importance of CB1R expression for the production of stereotyped birdsong through synaptic plasticity (Soderstrom and Tian, 2004) and have shown high levels of CB1R expression in brain regions involved in song learning and vocal production (Soderstrom and Johnson, 2000). CB1Rs have been implicated in tinnitus (Zheng et al., 2007, 2015). However, the function of CB1Rs in hearing is largely unknown.

To address this, we compared the hearing abilities of CB1R knockout (KO) mice to mice without any known hearing impairment (wild-type CBA/CaJ mice). Three experiments were performed with these KO mice: audiograms, gap detection thresholds, and frequency difference limens (FDLs). We first measured audiograms, which are a measure of general hearing ability across different frequencies. We used the same methods and stimuli as Radziwon et al. (2009) to obtain comparable audiograms for the CB1R KO mice, and we found that the KO mice had poorer hearing at mid-range and high frequencies than the wild-type CBA/CaJ mice. We next measured gap detection thresholds to assess the

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sensitivity of the auditory system for temporal features of sounds. Radziwon et al. (2009) obtained gap detection thresholds for wild-type CBA/CaJ mice using the same methods used here. We found that the KO mice were *more* sensitive, i.e., lower thresholds, to small gaps than wild-type mice. Finally, we measured FDLs, which are a finer test of frequency sensitivity than detection thresholds, using the same methods and stimuli as Radziwon and Dent (2014) used in wild-type CBA/CaJ mice. We found no differences between the two populations, suggesting the detection impairments at mid-frequencies do not extend to discriminating between those same frequencies. These experiments provide an important starting point for understanding how CB1Rs contribute to hearing and also provide direction for future cellular studies.

2. Methods

2.1. Subjects

Ten adult, CB1R KO mice (*Mus musculus*) were used as subjects in these experiments. The knockouts were originally a gift from Andreas Zimmer, in a C57/B6 background. These mice were back-crossed into the CBA/CaJ line for at least ten generations before establishing a breeding line of homozygous KOs, which have been maintained for several generations. Six subjects (all M) were used to obtain audiograms. Four subjects (2 M, 2 F) were used to obtain thresholds for gap detection, and three of those gap detection subjects (2 M, 1 F) also completed the frequency difference limens experiment. Mice began training at approximately two months of age and the experiments lasted approximately 12 months. The mice were individually housed and kept on a reverse day/night cycle (lights off at 6 am and on at 6 pm). The mice were tested during the dark portion of their cycle. All of the mice were water restricted and maintained at 85% of their free-drinking weight during the course of the experiment. Food was available ad libitum, except during testing sessions. All procedures were approved by the University at Buffalo, SUNY's Institutional Animal Care and Use Committee.

2.2. Apparatus

The mice were tested in a wire cage (23 × 39 × 15.5 cm, see Fig. 1) located in a sound attenuated chamber (53.5 × 54.5 × 57 cm) lined with 4 cm thick Sonex sound attenuating foam (Illbruck, Inc., Minneapolis, MN). The chamber contained an overhead web camera (Logitech QuickCam Pro, Model 4000) and a small 25 W white-light bulb to monitor the animal during test sessions. Sounds were played from an electrostatic speaker (Tucker–Davis Technologies (TDT), Gainesville, FL, Model ES1). The cage also contained two nose-poke holes surrounded by infrared sensors (Med Associates Model ENV-254), and a response dipper (Med Associates Model ENV-302M-UP).

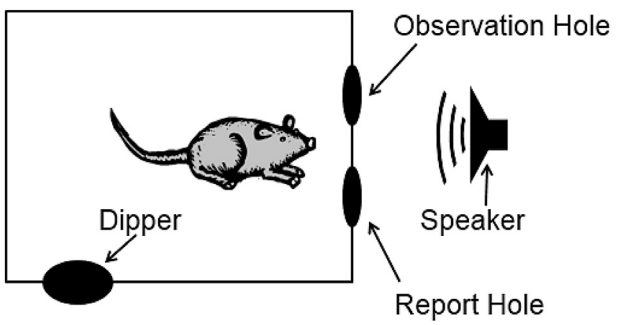

Fig. 1. Schematic of experimental setup. Mice began a trial by nose poking to the observation hole. When they detected or discriminated between stimuli presented from the speaker, they poked to the report hole. If correct, the mice received Ensure® from the dipper.

The experiments were conducted with Dell Optiplex 580 computers running Matlab R2007b software, which operated Tucker–Davis Technologies (TDT, Gainesville, FL) modules and RPvds software. Stimuli were sent through an RX6 processor, an RP2 signal processor, a PA5 attenuator, an ED1 speaker driver, and then finally to the speaker. Inputs to and outputs from the testing cages were controlled by the RX6 processor. The response dipper and infrared sensors were powered by Elenco Precision (Models XP-603 and XP-650 respectively) power supplies. A Tektronix (model TDS 2014) oscilloscope was attached to the PA5 attenuator to ensure that the stimuli were being sent to the speaker.

2.3. Stimuli

The stimuli were created and edited in Adobe Audition. Sound pressure levels for frequencies up to 12 kHz were calibrated using a sound level meter (Larson Davis system 824) with a condenser microphone (PRM902) placed at the position where the animal's head would be during testing. Sound pressure levels for higher frequencies were calibrated using an ultrasound recording system (Avisoft, Model USG 116-200) and Raven Pro (v 2.3, Cornell University) software, with the microphone placed at the same location. Calibrations were conducted weekly, and did not vary by more than 2 dB at any frequency tested throughout the course of the experiments.

Audiogram stimuli were the same as those used in Radziwon et al. (2009). Mice were tested on eight pure tones (1, 2, 4, 8, 12, 16, 24, and 42 kHz) with a 2 s duration and cosine rise/fall times of 20 ms. In the gap detection task, the repeated background was a noise burst with an 800 ms duration, and cosine rise/fall times of 100 ms. Stimuli were broadband or band passed, and only one stimulus type was presented per session. The four band-limited stimuli that were used consisted of (1) noise high passed at 18 kHz, or noise low passed at (2) 18 kHz, (3) 12 kHz, or (4) 8 kHz. The broadband stimuli contained frequencies up to 100 kHz. All stimuli were presented at a sensation level (SL) of 30 dB (calculated by measuring detection thresholds for the broadband noise stimuli) and roved by ±3 dB from presentation to presentation to eliminate any possible level cue for the mice. For each condition, target stimuli with gaps were created from the background stimuli. That is, for the target stimuli, silence was inserted into the center of the stimulus while the rest of the stimulus remained identical to the background stimulus. All stimuli remained 800 ms in duration, and the gaps ranged from 1 to 100 ms, depending on the animal's performance.

In the FDL experiment, stimuli were 500 ms pure tones with cosine rise/fall times of 20 ms. The four background reference frequencies tested were 12, 16, 24, and 42 kHz. The tones were presented at 10 dB SL (calculated from the audiogram experiment above) and roved by ±3 dB from presentation to presentation to eliminate any possible level cue for the mice. The target tones were higher in frequency than the reference stimulus, with step sizes varying by subject and frequency (usually 2–5% of the baseline frequency), although this was adjusted dependent upon each mouse's performance.

2.4. Audiogram procedures

In the audiogram experiment, the mice were trained using a go/no-go operant conditioning procedure to respond to pure tones in a detection task. The mouse began a trial by nose poking through an observation nose-poke hole two times, which initiated a variable