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Research paper

Comparison of auditory responses in the medial geniculate and pontine gray of the big brown bat, *Eptesicus fuscus*

Kimberly Miller, Ellen Covey*

Department of Psychology, University of Washington, Seattle, Box 351525, WA 98195, USA

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ABSTRACT

The inferior colliculus has been well studied for its role of transmitting information from the brainstem to the thalamocortical system. However, it is also the source of a major pathway to the cerebellum, *via* the pontine gray (PG). We compared auditory responses from single neurons in the medial geniculate body (MGB) and PG of the awake big brown bat. MGB neurons were selective for a variety of stimulus types whereas PG neurons only responded to pure tones or simple FM sweeps. Best frequencies (BF) in MGB ranged from 8 kHz to > 80 kHz. BFs of PG neurons were all above 20 kHz with a high proportion above 60 kHz. The mean response latency was 19 ms for MGB neurons and 11 ms for PG neurons. MGB and PG contained neurons with a variety of discharge patterns but the most striking difference was the proportion of neurons with responses that lasted longer than the stimulus duration (MGB 13%, PG 58%). Both nuclei contained duration-sensitive neurons; the majority of those in MGB were band pass whereas in the PG they were long pass. Over half of the neurons in both nuclei were binaural. Differences between these nuclei are consistent with the idea that the thalamocortical pathway performs integration over time for cognitive analysis, thereby increasing selectivity and lengthening latency, while the colliculo-pontine pathway, which is more concerned with sensory-motor control, provides rapid input and a lasting trace of an auditory event.

1. Introduction

One important function of sound, including communication signals, is to direct attention and motor behavior toward stimuli that are of potential interest to the organism. The inferior colliculus (IC) is the first level at which there are integrative processes that could perform something akin to cognitive processing (Bergan and Knudsen, 2009; Malmierca et al., 2009; Metzger et al., 2006; Perez-Gonzalez et al., 2005) and distribute this information to pathways that access different functional systems.

It is a well-known fact that one of the principal targets of output from the IC is the medial geniculate body (MGB), which in turn projects to the auditory cortex (AC). The MGB of echolocating bats is structurally similar to that of other mammals, and large relative to total brain size. It has been extensively studied for its cytoarchitecture, basic response properties, and input and output pathways (Llano and Feng, 1999; Olsen and Suga, 1991a,b; Razak et al., 2007; Wenstrup et al., 1994; Wenstrup, 1999; Winer and Wenstrup, 1994; Winer et al., 2005). In bats, the MGB neurons that pass information from the IC to the auditory cortex have been shown to be highly selective for duration, binaural properties, and sound frequency (Shen et al., 1997). Both MGB and AC retain some order of tonotopic organization, but orthogonal to this there may be other topographic organizations such as binaural properties (Wenstrup et al., 1994).

The auditory ponto-cerebellar pathway is less well known than the thalamocortical pathway. Projections from the IC to the pontine gray (PG) have been shown to be robust in the bat (Wenstrup et al., 1994; Frisina et al., 1989; Schweizer, 1981; Schuller et al., 1991), but limited in the guinea pig (Thompson, 2006), and cat (Andersen et al., 1980; Hashikawa, 1983), and reportedly absent in the rat (Aas, 1989; Mihailoff et al., 1989). The PG projects to certain areas in the cerebellum that control flight motion and vocalization, suggesting that it plays an important role in coordinating or fine-tuning echolocation calls, perception of objects in the environment,

Abbreviations: μA, micro-ampere; μPa, micro pascal; ABL, Average binaural level; AC, Auditory cortex; BF, Best frequency; dB, Decibel; E, Excitatory; F, Facilitation; FG, Fluoro-gold; FM, Frequency modulation; FR, Fluoro-ruby; FRAs, Frequency response area; I, Inhibition; IC, Inferior colliculus; KHz, Kilohertz; MGB, Medial geniculate body (d, v, m, dorsal, ventral, medial subdivisions); ms, Millisecond; O, Not excitatory; PBS, Phosphate buffered saline; PG, Pontine gray; SFM, Sinusoidally frequency-modulated tone; SG, Supragenicular nucleus; SPL, Sound pressure level.

* Corresponding author. Tel.: +1 206 616 8348; fax: +1 206 685 3157.
E-mail address: ecovey@u.washington.edu (E. Covey).

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and flight patterns (Schuller et al., 1991; Kamada and Jen, 1990). In all of the bats that have been studied, tonotopy appears to be limited or absent in the PG (Schuller et al., 1991; Kamada et al., 1992). Wu and Jen (1995) reported that IC and PG neurons follow higher pulse repetition rates than cortical neurons, suggesting that they have a shorter recovery time. Kamada et al. (1992) found that there were more non-monotonic neurons in the PG than monotonic, suggesting that neurons in this area are most responsive to low intensity sounds. Kamada and Jen (1990) reported that cerebellar neurons were more sensitive to FM tones than pure tones, they were onset responders, and tended to focus predominantly on sound sources located near the midline of the auditory field.

The goal of our study was to compare the basic response properties of neurons in the MGB and PG of same species, using the same stimulus paradigms. Our hypothesis was that these two pathways process information differently, with the MGB performing enhancement and filtering necessary for cognitive processing, and the PG extracting information necessary for ongoing regulation of motor responses.

2. Methods

Eighteen big brown bats (*Eptesicus fuscus*) of both sexes were used in this study. To prepare a bat for electrophysiological recording, a small stainless steel post was attached to the skull. This surgical procedure has previously been described by Miller et al. (2005). On the day that the post was attached, a small opening was made in the skull overlying both the MGB and PG for insertion of the electrode. The opening was then sealed with bone wax until the day of the recording. Recording began 1–4 days after surgery. Each bat was used in 2–8 recording sessions lasting ~6 h/day. Experiments were terminated if the bat showed signs of discomfort. Between sessions, the opening was covered with Gelfoam and coated with sterile petroleum jelly. Bats were housed in individual cages in a temperature- and humidity-controlled environment and were given *ad libitum* access to food and water. All procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

2.1. Acoustical stimuli

Acoustical stimuli were synthesized in the same manner as previously described by Faure et al. (2003). Stimuli were presented to the two ears *via* two Bruel & Kjaer (B&K) type 4135 1/4-inch condenser microphones modified for use as loudspeakers with a circuit to correct for nonlinearities in the transfer function (Frederiksen, 1977). The transducer was positioned so that its diaphragm was ~1 mm in front of the external auditory meatus. The output of the loudspeaker, measured with a B&K type 4138 1/8-inch condenser microphone calibrated with a B&K Type 4220 sound level calibrator, is expressed in decibels of sound pressure level (SPL root mean square with regard to 20 μPa) equivalent to the peak amplitude of continuous tones of the same frequency (Stapells and Picton, 1981). The transfer function of the transducer was flat ±5 dB from 26 to 118 kHz (measured 1–134 kHz). All signals had rise–fall times of 0.4 ms and were presented randomly at a repetition rate of 2 or 3 pulses/s. Stimuli included pure tones, broadband noise, frequency sweeps (FM) and sinusoidally frequency-modulated tones (SFM). Crosstalk between the two ears was tested as previously described by Ehrlich et al. (1997). Attenuation at the ear opposite the sound source was determined to be 30 dB or greater.

When a single unit was isolated, we conducted routine tests to determine whether it responded best to pure tones, noise, FM, or SFM, and whether its responses were monaural or binaural. Neurons were defined as selective for a given stimulus type if they responded exclusively to that class of stimulus. If the unit responded to pure tones, the best frequency (BF) and minimum threshold were determined. If the response was exclusively to sweeps, we determined the best sweep depth, center frequency, and minimum threshold. If the response was specific to SFM, we varied modulation rates (10–300 Hz) and modulation depths (1–10 kHz). All units were tested for duration sensitivity by varying duration in two tests (1–20 ms in 1 ms increments and 10–100 ms in 10 ms increments) using stimuli 20 dB above threshold at the unit's BF or best sweep parameters. A neuron was considered duration sensitive if it responded best over a specific range of durations. To be considered duration sensitive, the spike count in response to non-optimal durations had to be less than 50% of the spike count in response to the optimal duration range. Short-pass units fired at all durations under a specific length, long pass units fired at all durations longer than a specific length, and band-pass units fired most strongly at some intermediate range of durations with decreased response on either side. If the unit responded binaurally as determined by comparing average binaural level to interaural level difference (Nakamoto et al., 2004), we determined its binaural class according to the nomenclature provided by Fuzessery et al. (1990). Units were classified as binaurally suppressed if the firing rate decreased by ≥20%, or binaurally facilitated if the firing rate increased by ≥20% of the monaural firing rate. Rate-level functions plotted spike counts as a function of sound level for stimuli at BF or best sweep/SFM parameters, and best duration. Nonmonotonic rate-level functions were defined as those in which an initial increase in spike count was followed by ≥25% decrease in spike count at higher levels.

2.2. Electrophysiology

Electrophysiological recordings were conducted in a double-walled, sound-attenuating chamber (Industrial Acoustics Co., Inc., New York, NY). Before recording, each bat was given a subcutaneous injection of a neuroleptic (19.1 mg/kg Fentanyl/Droperidol mixture, Abbott Laboratories, North Chicago, IL). Bats were then placed in a foam-lined body restraint that was suspended in a flexible sling supported by springs within a stereotaxic frame (ASI Instruments, Warren, MI) mounted atop a floating vibration table (TMC, Inc., Peabody, MA). The head post was clamped in a customized holder mounted on a stereotaxic micromanipulator (David Kopf Instruments, Tujunga, CA). A chlorided silver wire was placed under the temporal musculature to serve as a reference electrode. The bone wax that covered the opening in the skull was removed and the dura was cut for insertion of the recording electrode. Neural responses were recorded with glass micropipettes filled with one of the following solutions: 5% Fluororuby (FR, tetramethylrhodamine dextran, 10,000 MW, Invitrogen, Carlsbad, CA, in 0.9% sterile saline), 5% Fluorogold (FG, 2-hydroxy-4, 4′-diamidinostilbene; Fluorochrome Inc., Denver, CO, in 0.9% sterile saline), or a 0.9% solution of sterile saline. The tip diameters of the electrodes ranged from ~1 to 10 μm with impedances that varied from 10 MΩ to 80 MΩ. Electrodes were aimed and lowered to MGB or PG based on stereotaxic coordinates. Electrodes were advanced with a stepping hydraulic micropositioner (David Kopf Instruments model 650). Action potentials were recorded with a Neuroprobe amplifier (A-M Systems model 1600), the 10× output of which was further amplified and band-pass-filtered (Tucker Davis Technologies (TDT), Alachua, FL, PC1; filter cutoff, 700 Hz and 3 kHz), and passed through a spike discriminator (TDT SD1). Spike times were logged on a computer by feeding the output of the spike discriminator into an event timer (TDT ET1) synchronized to a timing generator (TDT TG6). Stimulus generation and on-line data visualization were controlled by custom software. Spike times were displayed as dot rasters ordered by the acoustic parameter that was randomized during testing.