Neuroscience 208 (2012) 49–57

CELL'S INTRINSIC BIOPHYSICAL PROPERTIES PLAY A ROLE IN THE SYSTEMATIC DECREASE IN TIME-LOCKING ABILITY OF CENTRAL AUDITORY NEURONS

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Abstract—Studies in the vertebrates have shown that the time-locking ability of central auditory neurons decreases progressively along the ascending auditory pathway. This decrease is presumably attributed to a progressive reduction in the fidelity of synaptic transmission and an increase in the influence of synaptic inhibition along the cascade. The extent to which neurons' intrinsic biophysical properties contribute to the change in time-locking ability is unclear. We carried out whole-cell patch clamp recordings from the auditory thalamus of leopard frogs and compared their biophysical properties and time-locking abilities (determined by cell's responses to depolarizing pulse trains applied intracellularly) with those of lower auditory brainstem neurons. We found that frog thalamic neurons were homogeneous, exhibiting uniformly sustained, regular firing patterns, but not having low-threshold transient Ca^{2+} current which mammal thalamic neurons generally possess. Furthermore, intrinsic biophysical properties of the thalamic neurons are such that the time-locking ability of these neurons was very poor. The homogeneity of thalamic auditory neurons is in contrast to the heterogeneity of lower auditory brainstem neurons, with different phenotypes exhibiting different time-locking abilities and with sustained-regular phenotype consistently showing the worst time-locking ability among all biophysical phenotypes. Auditory nuclei along the ascending auditory pathway showed a progressive increase in the population of sustained-regular phenotype—this corresponded to a systematic decrease in the overall time-locking ability, with neurons in the dorsal medullary nucleus showing the best, and thalamic neurons exhibiting the poorest time-locking ability, whereas neurons in the torus semicircularis displayed intermediate time-locking ability. These results suggest that the biophysical characteristics of single neurons also likely play a role in the change in temporal coding ability along the ascending auditory pathway. Published by Elsevier Ltd on behalf of IBRO.

Key words: frog, thalamus, brainstem, dendritic morphology, temporal coding, low-threshold Ca^{2+} current.

The temporal pattern of sounds is important for sound discrimination in humans (e.g. for speech perception) as well as in various vertebrates and invertebrates (Langner, 1992; Shannon et al., 1995). For animals that produce species-specific vocal signals, the temporal cue is often critical for species recognition. This is best exemplified by call discrimination for two species of gray tree frogs, whose advertisement calls have similar spectral characteristics but show distinct temporal properties. Specifically, the rate of amplitude modulation (AM) and the duration and rise/fall times of individual syllables differ between these species (Gerhardt, 2001).

In anurans, the coding of AM stimuli is transformed from a periodicity code in the auditory periphery to a rate code in the auditory midbrain and thalamus, where neurons function as temporal filters and respond selectively to different AM rates (Feng et al., 1990; Hall, 1994; Rose and Gooler, 2006). The transformation of the coding scheme is partly attributed to the progressive decrease in time-locking ability of single neurons, from a maximum of 250 Hz at the auditory nerve and dorsal medullary nucleus (DMN, a homolog of the avian and mammalian cochlear nucleus) to $<$100 Hz at the torus semicircularis (TS, a homolog of the inferior colliculus) (Rose and Capranica, 1985; Feng et al., 1990; Simmons et al., 2000). Responses of neurons in the auditory thalamus to repetitive acoustic stimuli show rapid adaptation, with little or no time locking (Feng et al.1990). Studies in mammals have similarly revealed the progressive decrease in time-locking ability along the central auditory pathway (Fitzpatrick et al., 1999; Llano and Feng, 1999; Joris et al., 2004). The change in time-locking ability is generally assumed to be because of a decrease in the fidelity of synaptic transmission with increasing numbers of synapses in the cascade, and to an increase in the strength of synaptic inhibition at successive stages of sound processing (Rouiller et al., 1979; Rhode and Greenberg, 1992; Feng and Lin, 1994; Edwards et al., 2007).

There is growing evidence that cell's intrinsic properties contribute to various auditory response properties, including cell's time-locking ability and frequency preference (Llinas, 1988; Oertel, 1999; Hutcheon and Yarom, 2000; Yang et al., 2009). For example, the intrinsic membrane properties of neurons (e.g. low input resistance and short membrane time constant) as well as the compositions of ion channels are vitally important for the cell's

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Abbreviations: AM, amplitude modulation; CPP, 3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; DMN, dorsal medullary nucleus; I_{kl}, low-threshold potassium channel; I_{kir}, inwardly rectifying potassium channel; I_T, low-threshold transient Ca^{2+}; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione; pps, pulses per second; TS, torus semicircularis; TTX, Tetrodotoxin.

0306-4522/12 $36.00 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2012.01.043

ability to transmit signals rapidly and precisely (Manis and Marx, 1991; Sivaramakrishnan and Oliver, 2001; Soares et al., 2002; Trussell, 1999). To determine whether or not the neurons' intrinsic biophysical properties play a role in the systematic change in time-locking ability of central auditory neurons, we performed *in vitro* whole-cell patch recordings from neurons in the auditory thalamus of northern leopard frogs, *Rana pipiens pipiens*. We determined their membrane biophysical properties and their entrainment ability in response to depolarizing pulse trains at different repetition rates; these properties were compared with those of lower brainstem neurons (Yang and Feng, 2007; Yang et al., 2009). Anatomically, the auditory thalamus, which is at the top of the cascade in the frog central auditory system, comprises two divisions: the posterior and the central thalamic nuclei. They receive differential innervations from the TS and perform frequency analysis and temporal analysis, respectively (Hall and Feng, 1987; Feng and Lin, 1991). We found that, whereas lower auditory brainstem neurons are heterogeneous, thalamic neurons are homogeneous exhibiting a uniform biophysical phenotype (i.e. giving sustained-regular discharge pattern when membrane was depolarized) that shows the longest membrane time constant and the poorest entrainment ability among all biophysical phenotypes. However, these neurons' dendritic morphology was diverse. These data indicate that the previously described decrease in temporal precision shown *in vivo* may, in part, be attributed to the progressive increase in the population of sustained-regular phenotype with their particular intrinsic biophysical properties.

EXPERIMENTAL PROCEDURES

Brain slice preparation and electrophysiological recordings

The protocols were reviewed and approved by the University of Illinois Institutional Animal Use and Care Committee. Experimental procedures focused on minimizing the number of animals used and their suffering. To prepare frog brain slices, young adult northern leopard frogs (*Rana pipiens pipiens*) weighing 10–25 g (6–9 months old) were anesthetized in 0.2% tricaine methanesulfonate (MS-222, Sigma, St. Louis, MO, USA) and then decapitated. All experiments were done in April to August. Once the brain was extracted, it was immediately placed in an appropriate oxygenated (95% O_2/5% CO_2) Ringer solution (in mM) with a pH of 7.6: 104 NaCl, 4 KCl, 1.4 $MgCl_2{\cdot}6H_2O$, 10 D-glucose, 25 $NaHCO_3$, and 2.4 $CaCl_2{\cdot}2H_2O$. After removal of the dura mater, the tissue was embedded in a 3.2% solution of low-melting point agarose (Sigma, St. Louis, MO, USA) dissolved in Ringer solution. After hardening and cooling the agarose block in a freezer (−12 °C for 5–7 min), the block was trimmed to obtain desired slice orientation. The brainstem and forebrain were sectioned along the transverse plane into 300 μm slices using a vibratome (Ted Pella [Redding, CA, USA], model 1000 plus). Brain slices were immersed in oxygenated Ringer solution in a tissue chamber for <1 h at room temperature before the electrophysiological recording; the chamber was continuously perfused with Ringer solution at a rate of 2 ml/min.

In this study, we also made a simple comparison of the temporal firing patterns of auditory thalamic neurons in frogs and rats. To prepare rat brain slices, Sprague–Dawley rats (postnatal age: 10–16 days) were deeply anesthetized with sodium pentobarbital (55 mg/kg), the brains were quickly removed and placed into chilled (4 °C), oxygenated (5% CO_2 and 95% O_2) slicing medium containing (in mM) the following: 2.5 KCl, 1.25 NaH_2PO_4, 10.0 $MgSO_4$, 0.5 $CaCl_2$, 26.0 $NaHCO_3$, 11.0 glucose, and 234.0 sucrose. The thalamic brain slices were obtained from coronal slices. Slices were transferred to a holding chamber containing oxygenated physiological saline that contained (in mM) the following: 126.0 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $MgCl_2$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose.

A fixed-stage microscope (Olympus BX50WI) equipped with differential interference contrast optics and a 63× water-immersion objective is used to visualize individual neurons. Patch electrodes were fabricated from 1.5-mm-diameter borosilicate glass micropipettes and had impedance of 3–6 MΩ when backfilled with the following solution (in mM) having a pH of 7.5–7.6 and osmolarity of 260–270 mOsm: 117 K-gluconate, 13 KCl, 1.0 $MgCl_2{\cdot}6H_2O$, 0.07 $CaCl_2{\cdot}2H_2O$, 0.1 EGTA, 10 HEPES, 3 ATP-Mg, 0.3 GTP-Na, and 0.3% biocytin. The initial access resistance typically ranged from 10 to 13 MΩ and remained stable with a series resistance of 9–14 MΩ during the recording session. Neurons showing >15% change in the series resistance were discarded. All recordings were performed at room temperature (~22.5 °C) using Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA). Data were collected and analyzed by means of pCLAMP software (Molecular Devices).

Recording loci

Patch clamp recordings were made from two primary nuclei of the auditory thalamus: the posterior (P) and the central (C) nuclei (Neary and Northcutt, 1983; Hall and Feng, 1987). From each frog, we could obtain only two–three brain slices (300 μm) that covered the rostrocaudal extent of the posterior and central thalamic nuclei (Fig. 1). Both these nuclei are situated within the dorsal thalamus, and consisted of similarly sized neurons arranged in vertical laminae; the lateral nucleus, neighboring with posterior and central portions in rostral thalamus, can be distinguished by the lack of lamination and its lower cell density. The boundary between the posterior and central nuclei is characterized by a distinct reduction in cell density. To determine the recording locus, we took a digital photo of the location of the electrode tip after a neuron's biophysical properties were fully characterized. The locations were substantiated by biocytin staining of recorded neurons. For TS neurons, whole cell-patch clamp recordings were made from all three auditory nuclei of the TS: the principal nucleus (Tp), the laminar nucleus (Tl), and the magnocelluar nucleus (Tmc) (Yang et al., 2009).

Biocytin labeling

To identify the locations of the recorded neurons and the cells' morphological characteristics, the cells were filled with biocytin while they were held in whole-cell patch mode. After the conclusion of patch-clamp recording, we placed the slices in 4% paraformaldehyde overnight. Slices were then reacted with avidin-biotin-peroxidase complex (ABC Elite, Vector laboratories, Burlingame, CA, USA), mounted, and coverslipped with permount (Fisher Scientific, Pittsburgh, PA, USA). Labeled neurons were examined under a light microscope and drawn with the aid of a camera lucida drawing attachment.

Basic membrane properties, firing pattern, and entrainment ability

Basic membrane properties and biophysical phenotypes of auditory neurons were determined using the protocols established in an earlier study (Yang and Feng, 2007). The membrane time constant and the input resistance were calculated from the voltage change evoked by small hyperpolarizing current. To determine a cell's biophysical phenotype, we observed its temporal discharge