Neurons Sensitive to Interaural Temporal Disparities in the Medial Part of the Ventral Nucleus of the Lateral Lemniscus

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Batra, Ranjan and Douglas C. Fitzpatrick. Neurons sensitive to interaural temporal disparities in the medial part of the ventral nucleus of the lateral lemniscus. J. Neurophysiol. 78: 511 ± 515, 1997. The ventral nucleus of the lateral lemniscus (VNLL) is implicated in processing monaural sounds, because its neurons receive input chiefly from the contralateral cochlear nucleus. However, we demonstrate here that a region of the VNLL contains a distinct population of neurons that process binaural sounds and are sensitive to interaural temporal disparities (ITDs). Responses of single neurons were recorded from unanesthetized rabbits by using metal electrodes or micropipettes loaded with dextran tagged with either biotin or a fluorescent label. Reconstructions of recording sites based on a few marks indicated that ITD-sensitive neurons were located in a medial region of VNLL that has a low density of neurons or in the adjacent reticular formation. In one animal the locations of five ITD-sensitive neurons were marked directly by injection of dextran with different tags. All of these neurons lay in the medial region of the VNLL. The ITD-sensitive neurons of the VNLL had characteristic responses. Most neurons responded only at the onset of contralaterally or binaurally presented tones; many did not respond to ipsilateral stimulation alone and did not follow dynamic changes in the ITD. The presence of ITD-sensitive neurons in the VNLL that responded only at the onset of tones suggests that this center plays a role in the localization of transient sounds.

INTRODUCTION

The ventral nucleus of the lateral lemniscus (VNLL) is a major station in the auditory pathway that provides input to all parts of the inferior colliculus (Whitney and Henkel 1984). Although little is known about the responses of neurons in the VNLL, it is believed to process chiefly monaural information because most of its input comes from the contralateral cochlear nucleus (Glendenning et al. 1981). Electrophysiological studies in the cat (Aitkin et al. 1970) and in the bat Eptesicus fuscus (Covey and Casseday 1991) support this view. However, there is some anatomical evidence that the medial portion of the VNLL is specialized for binaural processing (Glendenning et al. 1981; Henkel and Spangler 1983), and one electrophysiological study (Guinan et al. 1972a,b) reported a substantial fraction of binaural neurons in the cat. In the present study we demonstrate that there is a distinct population of neurons in the medial part of VNLL that is sensitive to interaural temporal disparities (ITDs), supporting the view that the VNLL contains binaural as well as monaural neurons.

METHODS

Recording procedure and acoustic stimulation

Three female Dutch-belted rabbits (~2 kg) with clean external ears were used in these experiments. Preparatory surgery and procedures for recording from the unanesthetized animals were similar to those previously described (Kuwada et al. 1987). Briefly, each rabbit was surgically prepared for recording in two steps. During both steps the rabbit was anesthetized with a mixture of ketamine (Ketaset) and xylazine (Rompun; 35 and 5 mg/kg im, respectively). In the first step, the dorsal surface of the skull was surgically exposed by using aseptic techniques and a short rod was mounted on it. After the surgery, the animal was still anesthetized, custom ear molds were made using Audalin (Esschem, Epsom, PA). After this step, one to two weeks were spent acclimating the rabbit to body and head restraints and to the ear molds. The second step was to drill a small hole (2–4 mm) in the skull above the VNLL to permit passage of the electrode. A topical antibiotic was then applied to the exposed dura and the hole capped with elastomer. The rabbit was allowed several days to recover before recording sessions were initiated.

Calibrated acoustic stimuli were delivered via tubes inserted through the ear molds. The stimuli were tone bursts, which could be interaurally delayed, or “binaural-beat” stimuli (Kuwada et al. 1979), all with linear rise and fall times of 4 ms. The binaural-beat stimulus consisted of tones delivered to the two ears that differed in frequency by 1 Hz, with the frequency to the contralateral ear usually higher. This stimulus produced a 1-Hz cyclic variation in the ongoing ITD.

Recordings from single neurons were made with glass-coated Pt-W electrodes or with micropipettes (~15 μm tip diam). Recording sessions on each animal were conducted over a few months and lasted 2–3 h/day.

Marking of recording sites and histology

In two animals the recording sites were reconstructed from electrolytic lesions (10 μA·20 s) or injections of labeled dextran. Dextran was injected iontophoretically (2.5 μA·1.25–5 min) or by pressure using micropipettes. The micropipettes were loaded with phosphate-buffered saline containing 10% dextran tagged with one of a variety of fluorescent labels or with biotin (Molecular Probes, Eugene OR, stock Nos. D1817, D1820, D1956, D1976, D3312, and D7153). In the third animal, the locations of most ITD-sensitive units were marked directly by iontophoretic injection of dextran at the recording site.

Upon conclusion of recordings, the animal was deeply anesthetized and perfused with a washout followed by a fixative containing 4% paraformaldehyde and 0.2% glutaraldehyde, according to the procedures of Hill and Oliver (1993) for rats. The brains were blocked in the plane of the electrode tracks and placed in 30% sucrose for several days. They were frozen-sectioned (50 μm) and...
alternate sections were mounted for visualization of the fluorescent markers (e.g., in Fluormount G, Southern Biotechnology, Birmingham, AL). The remaining sections were processed with an avidin-biotin-horseradish peroxidase complex (ABC Kit, Vectorstain) to visualize injected biotinylated dextran (for detailed procedures see Hill and Oliver 1993). Electrolytic lesions were visualized in ABC-treated sections counterstained with neutral red or thionin. Locations of the marked sites were reconstructed by using a computer-aided tracing system (Neurolucida, Microbrightfield, Colchester, VT).

RESULTS

The VNLL of the rabbit consists of neurons intercalated in the fibers of the lateral lemniscus (Fig. 1A). Its lateral part (Fig. 1A, l) can be seen in Nissl-stained sections (Fig. 1B) to consist of small (\(\sim 15 \, \mu m\)), densely packed neurons. Medially (Fig. 1A, m), there is a mixed population of small and large (\(\sim 35 \, \mu m\)) neurons that are scattered through the body of the fiber tract (Fig. 1B, small arrows).

In the experiments reported here we focus on a subpopulation of 21 neurons that were sensitive to ITDs of low-frequency tones (\(< 2 \, kHz\)). Of these, 17 had best frequencies of \(< 2 \, kHz\) and the remainder had best frequencies between 2 and 5.5 kHz. The locations of 16 of these neurons were reconstructed on the basis of lesions or injections made at the end of several weeks of recording from each animal (Fig. 1A, ○). Most of these neurons were localized to the medial portion of VNLL or to the adjacent reticular formation. To more accurately determine the location of ITD-sensitive neurons, recording sites were marked directly in one animal. Two sites where ITD-sensitive neurons were studied are shown in Fig. 1B. Both sites are within the medial part of VNLL. The site at the bottom left (large arrow) was marked by an injection of biotinylated dextran, whereas the site at the top right (arrowhead) was marked by an injection of dextran that was both biotinylated and conjugated with tetramethylrhodamine. The rhodamine can be seen in the neighboring section, which was viewed by using fluorescence microscopy (Fig. 1C). Locations of these two neurons, as well as three others, are shown in Fig. 1A.

**FIG. 1.** Location of neurons sensitive to interaural temporal disparities (ITDs). A: reconstruction of recording sites based on a few lesions or injections made at the end of recordings in each animal (○) or marked directly with injections of labeled dextran (×). All directly marked sites were within the medial portion of ventral nucleus of the lateral lemniscus (VNLL, m). Dashed box indicates area shown in B and C. B: photomicrograph of a section through the right VNLL showing 1 recording site marked with biotinylated dextran (large arrow) and another marked with dextran conjugated to both biotin and rhodamine (arrowhead). Small arrows, small and large neurons in the medial portion of VNLL. Plane of section is that of the electrode tracks and is between transverse and horizontal (more dorsal is more anterior). C: same region in a neighboring section viewed through a rhodamine filter under fluorescence. This image was taken with a high-resolution grey-scale video camera and then false-colored. Site injected with doubly labeled dextrans (arrowhead) is visible, but the other site labeled in B is not. A 3rd labeled site is visible at the lower right. This site was injected with rhodamine-green conjugated dextran and could be distinguished from rhodamine fluorescence by use of a green filter. Neuron studied at this site was not sensitive to ITDs. l, lateral portion of VNLL containing a high density of small neurons; m, medial portion of VNLL containing a low density of small and large neurons; MCP, middle cerebellar peduncle; MTB, medial nucleus of the trapezoid body; Pyr, pyramids; Tz, trapezoid body; V, root of trigeminal nerve.
SENSITIVITY TO ITDS IN THE VNLL

Fig. 2. Responses of a neuron in the VNLL sensitive to ITDs. A: response to contralaterally presented tones. B: response to binaurally presented tones at the optimal ITD (−200 μs). Bar below histograms represents the duration of the stimulus. A and B top panels: dot rasters for 30 repetitions of each stimulus. Bottom panels: peristimulus-time (PST) histograms based on 150 repetitions. These responses are both of the onset type. C: response as a function of ITD for several frequencies (0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 kHz). Response at each frequency varies cyclically with ITD and neuron responds maximally at about the same ITD at all frequencies. All stimuli were 75-ms long, repeated every 200 ms. Frequency for the PSTs was 1 kHz. Contra- lateral and ipsilateral intensities, 64/64 dB SPL. Best frequency of neuron, 1.2 kHz.

(*) All five neurons were within the low density, medial portion of VNLL.

The ITD-sensitive neurons in the vicinity of the VNLL had characteristic responses. The responses of two representative neurons are illustrated in Figs. 2 and 3. The neuron of Fig. 2 was located at one of the sites in Fig. 1B (large arrow) marked by an injection. It produced an onset response to contralateral stimulation (Fig. 2A), no response to ipsilateral stimulation (not shown), and an onset response to binaural stimulation at the most favorable ITD (Fig. 2B). When the neuron was stimulated binaurally and the ipsilateral tone burst was progressively advanced or delayed relative to the contralateral tone burst, the response varied cyclically with a period equal to that of the tone (Fig. 2C). This feature indicates that the neuron was sensitive to the interaural phase difference at the onset of the tone. At some ITDs the response was totally suppressed. At all frequencies at which the neuron was ITD-sensitive, it discharged maximally at about the same ITD. Although this neuron was sensitive to static changes in ITDs, it did not follow the dynamic changes produced by binaural-beat stimuli.

The neuron of Fig. 3 was similar in that it produced chiefly

Fig. 3. Responses of a 2nd neuron in the VNLL sensitive to ITDs. A: response to contralaterally presented tones. B: response to ipsilaterally presented tones. C: response to binaurally presented tones at the optimal ITD (−200 μs). All responses are of the onset type. Dot rasters illustrate responses to 30 repetitions of each stimulus in A and B and 10 repetitions in C. PST histograms are based on 75 repetitions in A and B and 10 repetitions in C. D: response to a binaural-beat stimulus. This neuron followed dynamic changes in the ITD. E: response as a function of ITD for several frequencies (0.4, 0.6, 0.8, 1.0, and 1.2 kHz) as calculated from the response to the binaural-beat stimulus (Kuwada et al. 1987; Yin and Kuwada 1983). Tone bursts were 75 ms long, repeated every 200 ms. Frequencies in A, B, and D were 800 Hz; in C, 1 kHz. Intensities, ~55/50 dB SPL. Best frequency of neuron, 800 Hz.
an onset response to contralateral and binaural stimulation (Fig. 3, A and C) and discharged maximally at about the same ITD at each frequency tested (Fig. 3E). Unlike the neuron of Fig. 2, it also produced an onset response to ipsilateral stimulation (Fig. 3B) and did follow dynamic changes in ITD produced by a binaural-beat stimulus (Fig. 3D).

The neuron of Fig. 2 is typical of most of the neurons studied, whereas that of Fig. 3 is among the more extreme in terms of the strength of the response to ipsilateral stimulation and the strength and quality of the response to dynamically changing ITDs. Responses to ipsilateral stimuli occurred in about one-half of the neurons tested (6/14), were always onset, and were often less than the responses to contralateral stimuli. About one-third of the neurons responded (although sometimes rather poorly) to dynamic changes in ITD.

**DISCUSSION**

We have identified a distinct population of neurons in the vicinity of the VNLL that are sensitive to ITDs, particularly the ITDs at the onset of sounds. It is likely that these neurons are located in the medial portion of VNLL, because all five marked locations were in this region. Some other neurons were localized to the reticular formation medially or to the lateral part of VNLL, so we cannot exclude the possibility that some ITD-sensitive neurons lie in these regions. However, the localization of these other neurons was indirect and therefore subject to error. A medial location within the VNLL for ITD-sensitive neurons is consistent with evidence that this region receives input from the medial and lateral superior olives (Glendenning et al. 1981; Henkel and Spangler 1983) and also with evidence that low frequencies are represented there (Merchán and Berbel 1996).

A medial “paralemniscal zone” that contains neurons sensitive to binaural stimulation was described in the bat *E. fuscus* (Covey 1993). However, this region differs from the medial VNLL of the rabbit both cytoarchitecturally and physiologically. The paralemniscal zone of the bat has neurons densely packed in columns and is located more dorsally, abutting chiefly the intermediate and dorsal nuclei. Most paralemniscal neurons in the bat give a sustained response to contralaterally presented tones and are inhibited by ipsilateral stimulation. In contrast, the medial VNLL of the rabbit consisted of scattered cells; neurons in this region responded transiently to tones, and ipsilateral stimulation could either excite or inhibit depending on the ITD.

The medial VNLL is part of a region termed the “lateral tegmentum” by Glendenning et al. (1981), because they believed it to be part of a diffuse secondary auditory pathway ascending the brainstem described by Morest (1965). However, only the posterior (ventral) part of the medial VNLL appears to contribute to such a system (Henkel 1983). We consider the medial VNLL better viewed as a specialized region of the VNLL because it actually lies within the confines of the lateral lemniscus.

As far as we know no previous study has shown a population of ITD-sensitive neurons in or near the VNLL. However, in a study of the superior olivary complex (SOC), Spitzer and Semple (1995) encountered two neurons with similar properties at the margin between the VNLL and the SOC.

The responses of neurons in the VNLL differed substantially from those of neurons in the SOC. The neurons in the VNLL responded to monaural and binaural tones chiefly at stimulus onset, and most did not follow dynamic changes in the ITD produced by a binaural-beat stimulus. In contrast, the neurons in the SOC typically have a more sustained response to monaural and binaural stimulation, and readily follow dynamic changes in ITD (Batra et al. 1997a; Moushegian et al. 1964; Spitzer and Semple 1995; Yin and Chan 1990). It is therefore unlikely that our recordings were from the ascending axons of neurons in the superior olivary complex.

The ITD sensitivity in the VNLL probably does not arise directly from the bilateral convergence of phase-locked inputs. Although phase-locking is difficult to assess in neurons that produce only onset responses, we did measure phase-locking in the few neurons that were driven well by binaural-beat stimuli. We found that it was too weak (i.e., synchronization coefficients were too low) to account for the strength of ITD sensitivity in these neurons, if they were the site where bilateral convergence occurred. [A discussion of the relationship between phase-locking and the strength of sensitivity to ITDs is given in Batra et al. (1997b).] Thus ITD sensitivity of these neurons is most likely transmitted from the SOC and phase-locking degraded in the process of transmission. The differences between responses in the SOC and VNLL indicate that there is either a substantial transformation in the VNLL or that the ITD-sensitivity of these neurons originates in a population of neurons in the SOC with atypical responses.

Onset responses in the VNLL were observed previously (Adams 1997; Aitkin et al. 1970; Covey and Casseday 1991; Guinan et al. 1972a,b). Covey and Casseday (1991) suggested that the VNLL may be specialized for processing acoustic transients. We have demonstrated that neurons with onset responses in VNLL can be sensitive to ITDs and therefore to the location of the sound as well as the temporal pattern. This result suggests that the VNLL may play a role in the localization of acoustic transients.

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