

RECORDINGS of unit activity showing 4–6 ms latency responses to a click stimulus provided evidence that the dentate nucleus could function as a short-latency auditory relay. On the basis of these findings, plus fiber fillings from injections of phaseolus leucoagglutinin into the dentate, a new auditory pathway between dorsal and ventral cochlear nuclei, dentate nucleus, and rostral thalamus is proposed. The pathway could provide direct, short-latency transmissions to the motor cortex that bypass the classical auditory receptive cortex.

**Key words:** Hearing, Acoustic information transmission, Primary sensory pathways, Auditory function, Cerebellum, Thalamus, Motor cortex, Cochlear nuclei

## The dentate nucleus is a short-latency relay of a primary auditory transmission pathway

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### Introduction

The dentate nucleus has not previously been recognized to be part of any primary ascending auditory system, but a significant role of cerebellum and related brainstem nuclei such as the dentate and interpositus has been demonstrated in the production of long-latency eyeblink and nictitating membrane conditioning to auditory conditioned stimuli. One earlier study<sup>1</sup> found that neurons of the dentate nucleus had onset latencies of response of >40 ms to auditory stimuli used for conditioning, and another<sup>2</sup> found responses of <20 ms, but neither disclosed responses sufficiently rapid (4–8 ms onset) to serve a primary auditory transmission pathway.

To test the hypothesis that such responses might be found, we made recordings of activity from single units of the dentate nuclei of conscious cats during presentations of click stimuli of 70 db intensity. The animals were trained to perform a conditioned blink response (CR) to the click as a conditioned stimulus (CS). After finding responses of 4–6 ms onset, injections of phaseolus leucoagglutinin (PHA-L) were made in the dentate nucleus to determine if fibers consistent with an ascending auditory pathway could be identified.

### Materials and Methods

Conditioning of short-latency (20 ms onset) blink CRs was produced by forward pairing of click as CS with glabella tap and hypothalamic electrical stimulation (570 plus 10 ms interstimulus intervals; 10 s intertrial interval; see<sup>3</sup> for complete details). Five adult cats were studied weighing between 2.5 and 3.0 kg.

Extracellular and intracellular recordings of unit activity (see<sup>4</sup> for criteria) were measured with a Dagan 8100-1, high impedance amplifier and stored on a Vetter FM tape recorder (Model D) at DC to 5000 Hz (<1% falloff). Spike occurrences were detected with a threshold discriminator (Frederick Haer and Co.). Data were collected at 1 ms intervals and analyzed in histograms of 2 ms bin width. Loci of recordings were confirmed by serial sections of tissue showing electrode tracks and marking of intracellularly recorded units with biocytin<sup>5</sup> (Fig. 1A–C). Electrodes were pulled from 1.5–2.0 mm (o.d.) theta tubing. When filled with 2% biocytin (Sigma) in 2.5 M KCl and connected on both sides with Ag/AgCl wire, the resistances of the electrodes ranged from 40–10 MΩ. The animals were surgically prepared under Na pentobarbital anesthesia (35 mg kg<sup>-1</sup>, i.p.) for later recording from the dentate nucleus using a stereotaxic guide tube.<sup>6</sup> The methods were described in detail previously.<sup>7,8</sup> Penicillin G (150 000 units, i.m.) and benzathine penicillin G (150 000 units, i.m.) were given on the day of surgery, three days later during the recovery period, and at one week intervals thereafter, as needed. During the training/conscious recording sessions, the head of the animal was fixed to a stabilizing frame and the body placed in a loose cloth sleeve.<sup>7</sup> The behavior of the animal was continuously observed to evaluate its comfort, and the study was discontinued if the animal gave any signs of discomfort such as vocalization and hyperactivity. At the conclusion of the studies the animals received lethal doses of Na pentobarbital, and serial sections of perfused brain tissue were examined.<sup>8</sup> The procedures met APS, USPHS, Society of Neuroscience, and University of California guidelines.

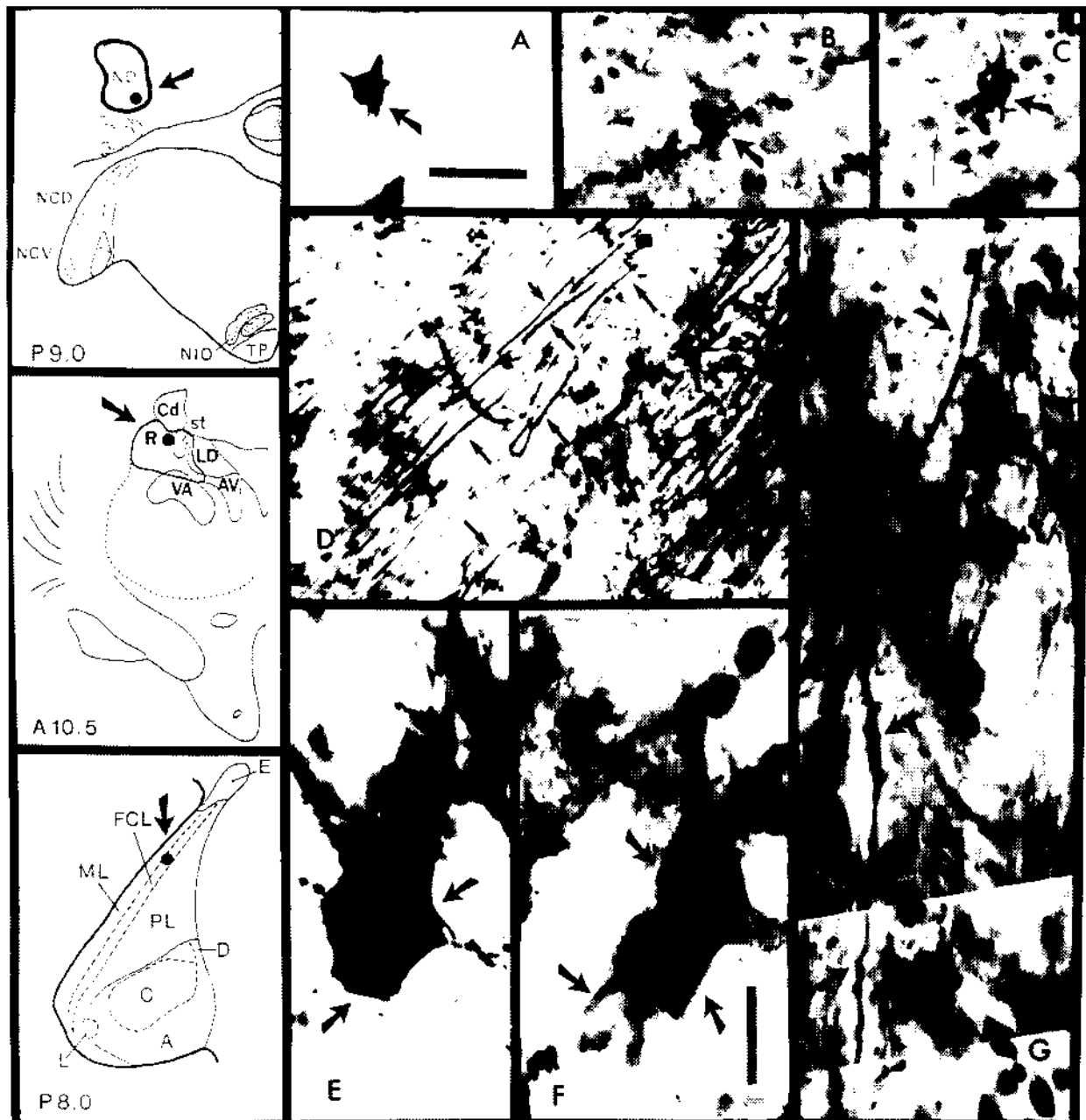


FIG. 1. Biocytin markings of intracellularly recorded neurons of dentate nucleus (A–C). Scale (25  $\mu\text{m}$ ) in A applies to A–C. Location of cells (dot) in dentate nucleus is shown at upper left (arrow). Extracellular injection of PHA-L (0.1  $\mu\text{l}$  plus 5 nA hyperpolarizing current for 1 min) in dentate nucleus resulted in fiber fillings (D) and retrograde soma fillings (E–F) in dorsal cochlear nucleus (lower left, arrow), and fiber fillings (G) without soma fillings in rostral thalamic auditory area (middle left, arrow) described earlier.<sup>20</sup> Scale in F (25  $\mu\text{m}$ ) applies to E–G (and for D = 125  $\mu\text{m}$ ). Abbreviations: nuclei-dentate, ND; dorsal cochlear, NCD; ventral cochlear, NCV; inferior olivary, NIO; caudate, Cd; reticularis, R; lateralis dorsalis, LD; ventralis anterior, VA; anterior ventral, AV. Pyramidal tract, TP; stria terminalis, st; granular region of cochlear nucleus, E; fusiform cell layer, FCL; molecular layer, ML; polymorphic layer, PL; and dorsal (D), central (C), lateral (L) and anterior (A) part of the ventral cochlear nucleus. Numbers are anterior (A) and posterior (P) stereotaxic coordinates.

Physical parameters of the click have been described previously (see<sup>9</sup> for amplified earphone recordings of the stimuli). The clicks were of 70 dB intensity (measured at the ears of the animals with General Radio Company dB meter type 1565-A at the standard SPL level of 20  $\mu\text{N m}^{-2}$ ), and were generated by a rectangular pulse of 1 ms duration, delivered to a loudspeaker placed 0.5–0.75 m in front of the animal. During testing, clicks were presented every 10 s.

## Results

Peristimulus time histograms of spike occurrences were made with reference to times of click onset (uncorrected for 1 ms air conduction delay between sound source and ears of animal) for each cell that was studied. Averages were then made of the activity of all units in the periods immediately preceding and following each click presentation. Histograms of the mean post-CS activity of a total of 263 units studied

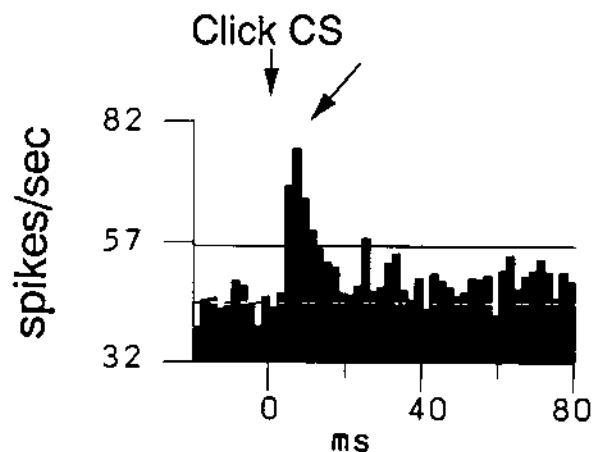


FIG. 2. Histogram averages of the activity of 263 units from cats conditioned to blink to the click CS. The 4–6 ms onset of unit responses to the click CS is clearly seen. The click stimulus was delivered at time 0. Dashed line shows mean baseline level of spike discharge. Solid horizontal line is 3 standard deviations above mean of baseline spike activity.

showed an increase in activity much greater than 3 standard deviations above the baseline with onset 4–6 ms after the click (Fig. 2). The increase in discharge was brief, returning to baseline 20 ms after click presentation just prior to the onset of the conditioned blink response.

Of the 263 units of the dentate nucleus from which recordings were obtained, 33 units responded to the CS at 4–8 ms latency. The auditory responsive units were defined on the basis of increased activity in any 4 ms period of activity during the 160 ms period following click presentation ( $\geq 2$  discharges above the peak of twenty 4 ms periods of baseline activity).<sup>7</sup> These results indicate that a significant number (13%) of units of the dentate nucleus lie along a short-latency auditory transmission pathway.

Injections of an anterograde pathway tracing agent, PHA-L (2.5%, Vector labs), were made into the dentate nucleus of three cats (for details of methods, see<sup>10</sup>). Fibers were traced (Fig. 1G) between dentate nuclei and another newly discovered auditory region, the rostral thalamus, that was shown to respond with increased activity at latencies of 6–8 ms to these same auditory stimuli.<sup>7</sup> Since conspicuous markings of cell bodies were not observed in this region, we assume that the fillings were anterograde. Nuclei in this region project to the rostral complex.<sup>10,11</sup> Fibers were also traced between dentate and dorsal and ventral cochlear nuclei (Fig. 1D–F). Since conspicuous fillings of cell bodies were observed in these nuclei, we assume that the fillings were retrograde. (Previously described fiber connections<sup>12,13</sup> with vestibular nuclei, posteriorly, and VL thalamic nuclei, anteriorly, were also found.)

## Discussion

Ablation of classical auditory receptive regions of the cortex that receive collicular-geniculo-cortical projections does not prevent acquisition of short-

latency Pavlovian conditioned blink responses to click CSs or some other types of auditory discriminations, and long-latency conditioned blinking and conditioned nictitating membrane movements to auditory CSs can develop in decorticate preparations.<sup>14–16</sup> Development of short-latency blink CRs performed 20 ms after delivery of a click CS depends, however, on a functionally intact rostral cortex.<sup>14,17,18</sup> If 25% KCl is applied to the rostral cortex to produce spreading depression in conditioned animals, the short-latency blink CR is abolished reversibly, returning later after recovery, whereas the unconditioned blink response to glabella tap is maintained throughout.<sup>17</sup> This occurs in rabbits as well as cats.<sup>18</sup> Bilateral ablation of the rostral cortex prevents acquisition of short-latency conditioned blinking.<sup>14</sup> The impairment persists despite extensive training for a period of three months after surgery.

If the motor cortex is essential for development of the short-latency blink CR, and if the classical auditory cortex and surrounding areas are not needed for development of the short-latency blink CR to an auditory CS, then there should be another short-latency auditory transmission pathway to motor cortex apart from the classical inferior colliculus — medial geniculate pathway. This possibility is further supported by findings<sup>19</sup> which have shown that layer V pyramidal cells of the rostral (pericruciate) cortex can respond to click and hiss stimuli at latencies as short as those of primary auditory cortex.

We propose that a primary ascending auditory transmission pathway exists between dorsal and ventral cochlear nuclei, dentate nucleus, rostral thalamus, and motor cortex to support the 8–12 ms unit activation of the cortical units by click stimuli similar to those used in the present study.<sup>19</sup>

The primary ascending auditory pathways of the brain that are presently recognized can be broadly characterized into three systems on the basis of differences in afferent and efferent connections, neuronal cell types, and response properties. The systems have been labelled lemniscal line (also cochleotopic), lemniscal adjunct (also non-cochleotopic) and non-specific. All begin with spiral ganglion projections to neurons in the dorsal and ventral cochlear nuclei. The higher levels of the lemniscal line auditory system have been defined as consisting of the central nucleus of the inferior colliculus, the ventral division of the medial geniculate, and primary auditory cortex.<sup>20,21</sup> The lemniscal adjunct system has been defined as consisting of the external and pericentral nucleus of the inferior colliculus, the thalamic posterior nuclear group, including the medial division of the medial geniculate and areas surrounding the primary auditory cortex.<sup>22,23</sup> A third group of nuclei has been characterized as nonspecific.<sup>24</sup> At the thalamic level these include the midline and intralaminar nuclei. Earlier recordings from the latter regions did not

disclose responses to auditory stimuli of latencies <14 ms.<sup>25</sup> The dentate nucleus has not previously been recognized as part of any of these primary ascending auditory pathways.

## Conclusion

We conclude that the dentate nucleus can function as a short-latency relay of a primary ascending auditory pathway by virtue of the 4–6 ms onset latency of its response to auditory stimuli. Further anatomical and electrophysiological evidence suggests that the primary auditory pathway, of which the dentate is a part, runs between dorsal and ventral cochlear nuclei, dentate nucleus, rostral thalamus and motor cortex.

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ACKNOWLEDGEMENTS. Supported by NS25510. J. Landeira Fernandez was supported by a Brazilian Conselho Nacional de Pesquisa fellowship.

Received 20 May 1991;  
accepted 23 May 1991