

cavity relative to the body cavity; this constitutes a limiting factor to lophophore size which may relate to the early loop phase characteristic of this particular species.

5) Shell outline, degree of convexity, cardinal margin, and characters of the beak [differentiated as "bouchardiform" by Thomson in 1915 (2)] all combine to produce an animal with a shape apparently well adapted to live in close association with constantly shifting sediments of a coarse sandy type.

Some terebratulid genera show great morphological conformity with *M. cumingi*; for example, *Australiarcula* (Cretaceous). Some differ in adult loop patterns and in the degree of secondary thickening (while retaining its differential character) but show the characteristic beak and muscle scar patterns; for example, the Tertiary genera *Magadinella* (Australia), *Rhizothyris* (New Zealand), and *Tanakura* (Japan). Others are functionally similar while displaying differences in some morphological characters; for example, *Bouchardia* (Recent, Brazil) possesses a cardinal process of very different shape but situ-

ated in the same position relative to the dorsal insertions of the adjustor muscles. It is conceivable that other functional replicas may be found in genera such as the Cretaceous terebratulids *Trigonosemus*, *Sympthyris*, and *Terebratrostrata*; in *Onychotretra* (Orthida, Silurian); and in *Cardiarina* (Rhynchonellida, Carboniferous), all of which possess open, transapical foramina allied with a prominent or elongate beak. Now that the assumption has proved incorrect that an open foramen of adequate size implies permanent attachment to a substrate, studies of these and other genera may give hints of the existence of other types of brachiopod progression.

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Far-Field Acoustic Response: Origins in the Cat

Abstract. *Short-latency evoked potentials recorded from the vertex of adult cats in response to click stimulation (the far-field acoustic response) were analyzed in a series of lesion experiments to determine the origins of each component. The resultant data indicate that the primary generator of potential 1 is the acoustic nerve; of potential 2, the cochlear nucleus; of potential 3, neurons of the superior olivary complex activated by projections crossing the midline; of potential 4, neurons of the ventral nucleus of the lateral lemniscus and preolivary region activated equally by crossed and uncrossed projections; and of potential 5, neurons of the inferior colliculus activated primarily by crossed projections.*

In human subjects, a series of evoked potentials with submicrovolt amplitudes can be recorded from disk electrodes placed on the scalp vertex and earlobe within 10 msec of click stimulation (1). This sequence of waves, which are separated by intervals of approximately 1 msec, has been tentatively correlated with activation of the brainstem auditory nuclei and thus has been termed the "far-field" acoustic response (1). Such a measure of brainstem auditory function could provide a sensitive and objective neurological measure that would be particularly useful among populations unable to respond appropriately in conventional audiological testing, such as in cases of severe mental retardation, as well as among patients with central hearing problems or with acute head trauma for which the locus of pathology is unknown. In the cat, similar recording procedures result in a series of evoked potentials at latencies that are slightly shorter than those recorded in the human but also show successive delays of approxi-

mately 1 msec (2, 3). These evoked potential components emerge separately in the kitten during the first 2 weeks after birth and progressively increase in amplitude and decrease in latency until an adult profile is attained at 1 to 2 months of age (4). The decrease in latency of one far-field response component has similarly been correlated with development in human infants (5). Thus, the far-field response could also represent a powerful tool for observing functional maturation within the brainstem auditory pathway. However, neither the clinical nor experimental potential of the far-field acoustic response can be fully exploited until an anatomical identification has been made of the generator loci primarily responsible for each of the short-latency waves.

The latencies of the far-field acoustic response components in the cat have been correlated with the latencies of auditory responses recorded from brainstem areas by Jewett (2). In this study, the far-field peak 1, with a latency similar to the N_1 response

at the round window, was interpreted as a reflection of acoustic nerve discharge. Far-field peak 2 was correlated with the latency of the cochlear nucleus evoked potential; peak 3, with the latency of potentials recorded in the region of the superior olivary complex; peak 4, with the latency of potentials recorded on either side and within the inferior colliculus; and peak 5, with the latency of potentials recorded within and rostral to the inferior colliculus. In order to extend these data and more clearly identify the primary origins of each far-field potential component, the present series of lesion experiments was performed on ten adult cats.

Prior to surgery, the cat was anesthetized with sodium pentobarbital (35 mg per kilogram of body weight) and placed in a stereotaxic frame with hollow ear bars. The skull was exposed and a stainless steel screw was secured at the vertex for the far-field recordings. A second reference lead was attached to the pinna of one ear. Click stimuli of 0.1-msec duration and 10-kHz frequency were generated by a Wavetek 112 audiooscillator and delivered through B & K 4144 condenser microphones attached to the end of each ear bar. At the other end of the ear bar, click intensity was measured at 70 db sound pressure level (referenced to 0.0002 dyne/cm²), or approximately 50 db human sensation level, by lengthening the click duration to 400 msec to allow for the slow response time of the calibrator (General Radio sound intensity meter 1565A). Clicks were presented at 10 per second and the resultant response was led from the recording electrodes to a Grass P511 amplifier (30 to 3 kHz band pass, 100,000 gain). The amplified response was fed into an averager (Enhancetron 1024) and the average of 600 successive responses was plotted with an x-y plotter. A series of control recordings was routinely made with binaural and monaural clicks before any lesion was made. After each lesion, the same series of recordings was repeated and at least 1 hour was allowed for stabilization of the recordings before a subsequent lesion was made. Precollicular decerebration was carried out by first aspirating the occipital lobes and then sectioning the brainstem rostral to the inferior colliculi with a blunt spatula. Hemostasis was facilitated in all procedures by Gelfoam and Oxycel. In another type of preparation, the inferior colliculi were aspirated or undercut bilaterally under visual control. In some preparations a sagittal midline section of the brainstem from the level of the colliculus to that of the cochlear nucleus was carried out with a blunt spatula held in a stereotaxic carrier to interrupt all crossed projections. A coronal brainstem section or hemisection

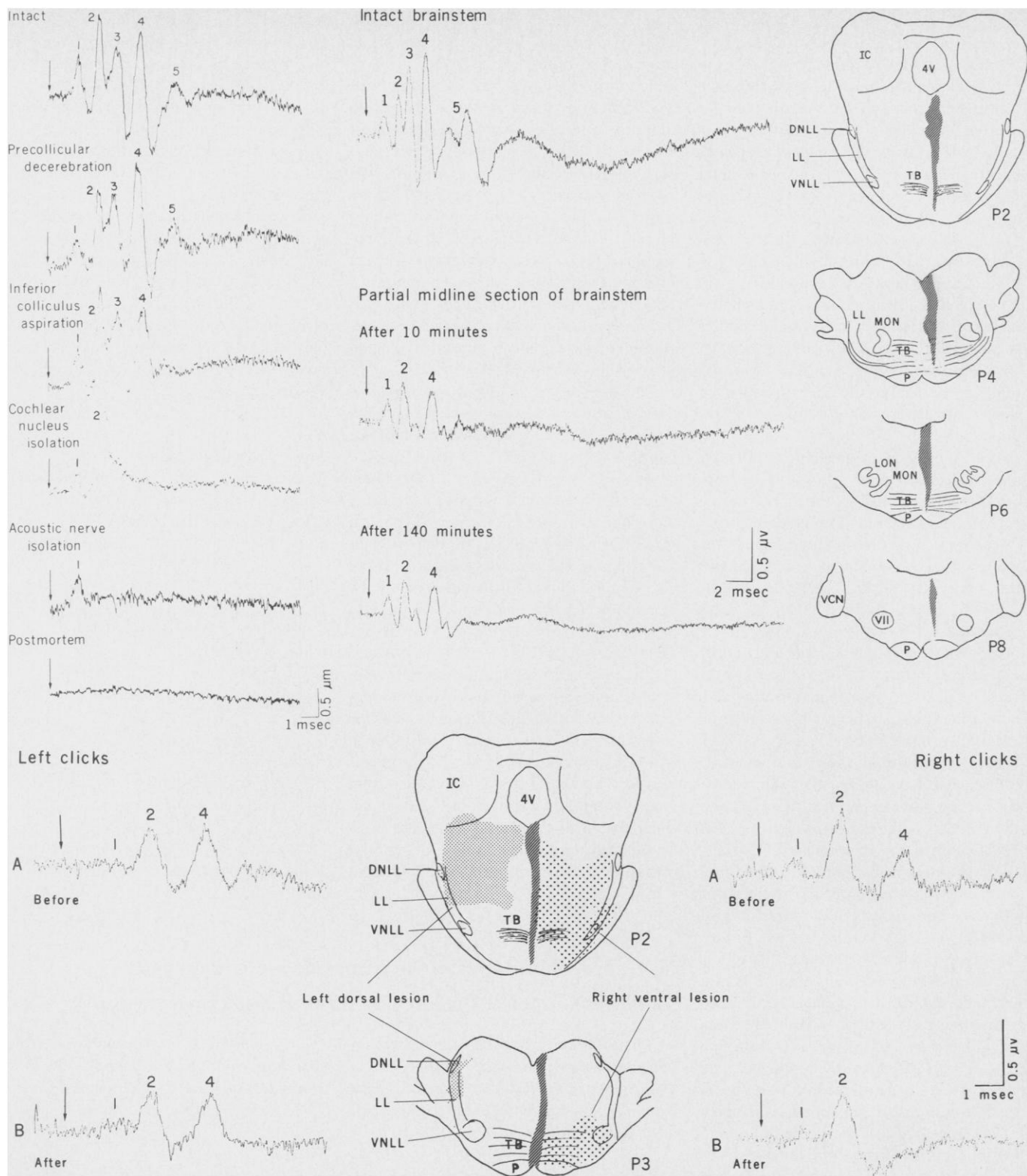


Fig. 1 (top left). Far-field potentials after various lesions. The potential sequence recorded from the intact cat in response to binaural click presentations was unaltered following precollicular decerebration. Subsequent bilateral aspiration of the inferior colliculi eliminated potential 5. Following surgical isolation of the cochlear nuclei from the brainstem, potentials 3 and 4 disappeared. Isolation of acoustic nerve from the cochlear nucleus resulted in the loss of potential 2. No potential remained in the postmortem recording (bottom trace). In this and subsequent figures, positivity is above the baseline. Fig. 2 (top right). Changes in the far-field potential sequence recorded from a decerebrate cat following sagittal midline section of the brainstem. In this split-brainstem preparation a small amount of the ventral trapezoid body remained intact at the P4 level, as indicated in the lesion reconstruction diagrams. Potential 3 showed some slight recovery by 140 minutes after the lesion; in complete sections, potential 3 was abolished with no recovery. Potentials 4 and 5 showed marked reductions whereas potentials 1 and 2 remained at control levels; *IC*, inferior colliculus; *4V*, fourth ventricle; *DNLL*, dorsal nucleus of lateral lemniscus; *LL*, lateral lemniscus; *VNLL*, ventral nucleus of lateral lemniscus; *MON*, medial olivary nucleus; *P*, pyramidal tract; *TB* trapezoid body; *LON*, lateral olivary nucleus; *VCN*, ventral cochlear nucleus; *VII*, facial nucleus. Fig. 3 (bottom). Typical far-field potential sequence recorded from a decerebrate cat following sagittal midline section of the brainstem (A). Left clicks stimulate the left split-brainstem and induce far-field potentials 1, 2, and 4, which remain unchanged after a lesion of the left dorsal nucleus and tract of the lateral lemniscus. Right clicks independently stimulate the right split-brainstem and induce far-field potentials 1, 2, and 4; following a lesion of the ventral nucleus of the lateral lemniscus and preolivary region, potential 4 disappears; abbreviations are as defined in Fig. 2 legend.

at the P3 stereotaxic level was similarly carried out in order to separate the lateral lemniscus complex from the superior olivary complex (6). In some cases, the cochlear nucleus was exposed by aspirating the overlying cerebellum and was then surgically isolated from the adjacent brainstem under visual control. The acoustic nerve was similarly isolated from the cochlear nucleus by carefully sectioning the nerve at the internal acoustic meatus. Each of these lesions was made with subsequent successful recordings in at least two animals. The animals were killed and were immediately perfused with 10 percent formalin. Frozen sections were cut at 80 μ m and stained with thionine. Lesion reconstructions were made by projecting the relevant brain sections onto appropriate brain atlas diagrams (6).

In Fig. 1, the first trace indicates the typical sequence of far-field evoked response components elicited in the intact cat by binaural click stimuli. The average latency of each potential and its range were determined from recordings in seven cats, with the following values obtained: peak 1, 1.4 ± 0.2 msec; peak 2, 2.6 ± 0.3 msec; peak 3, 3.5 ± 0.4 msec; peak 4, 4.8 ± 0.5 msec; and peak 5, 6.7 ± 0.7 msec. Following decerebration, all potentials continued to be elicited at approximately the same amplitude and latency, which indicated that the primary loci of generation were caudal to the level of midbrain transection. Subsequent aspiration of the inferior colliculi or, in other experiments, isolation of the colliculi from the brainstem by bilateral undercutting eliminated potential 5 (Fig. 1). After stable recordings had been obtained from the acollicular preparation, the cochlear nucleus was surgically isolated from the adjacent brainstem. Following this procedure, potentials 3 and 4 disappeared, while potentials 1 and 2 continued to be elicited by the click stimuli at approximately control amplitude and latency. When the acoustic nerve was subsequently isolated from the cochlear nucleus, potential 2 disappeared while potential 1 continued to be elicited. This potential disappeared following a lethal dose of pentobarbital. These data indicated that potential 5 required the integrity of the inferior colliculus, while potentials 2 and 1 required the integrity of the cochlear nucleus and the acoustic nerve, respectively.

To assess the contribution of crossed projections, particularly to potentials 3 and 4, the split brainstem preparation was next studied. With binaural click stimulation, the typical sequence of evoked potential components was induced in the decerebrate cat with intact brainstem (Fig. 2). After sagittal midline section of the brainstem, there was little change in ampli-

tude or latency of potentials 1 and 2. In contrast, potential 3 was largely abolished in the split brainstem preparation illustrated, even though a ventral fragment of the trapezoid body remained intact; in other preparations, with complete interruption of the trapezoid body, potential 3 was completely abolished. Potential 4 was reduced approximately 50 percent after the sagittal brainstem section and potential 5 largely disappeared. Hence, far-field potentials 1 and 2 were independent of fibers crossing the midline, whereas potentials 3 and 5 were largely dependent upon such crossed projections and potential 4 was approximately equally dependent upon crossed and uncrossed projections.

In order to analyze further the origins of potentials 3 and 4, sections were made between the lateral lemniscus and superior olivary complex before or after sagittal midline section. As noted above, far-field potentials 1, 2, and 4 remained following the sagittal brainstem section when click stimuli were delivered to the left or right ear. Subsequent transverse section of the left hemibrainstem, which destroyed the dorsal nucleus and interrupted the tract of the lateral lemniscus, produced no change in the potentials (Fig. 3, left clicks, A and B). In contrast, a section through the right hemibrainstem, which destroyed the ventral nucleus of the lateral lemniscus and the adjacent preolivary complex, resulted in the loss of potential 4 (Fig. 3, right clicks, A and B). When transverse sections were made bilaterally through the ventral nuclear region of the lateral lemniscus prior to the sagittal midline section, potential 4 was almost completely abolished, whereas

potential 3 showed only a slight reduction in amplitude. Thus, potential 4 required the integrity of the ventral nucleus of the lateral lemniscus and the adjacent preolivary complex and was dependent to almost an equal extent upon the crossed and uncrossed projections to this area. Potential 3, in contrast, was dependent upon a more caudally situated structure which received primarily crossed inputs, which suggests the medial superior olivary nucleus as the primary generator of this potential.

These data indicate the primary origin of each of the far-field acoustic response components in the cat. Such identification may enhance the usefulness of this measure in both basic and clinical neurophysiological studies.

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Tetrahedral Intermediate in a Specific α -Chymotrypsin Inhibitor Complex Detected by Laser Raman Spectroscopy

Abstract. *Laser Raman spectroscopy indicates that inhibition of α -chymotrypsin by 2-phenylethaneboronic acid occurs in two steps: (i) the formation of a loosely bound complex, in which the boron remains in a trigonal configuration; and (ii) reaction of the boron with a functional group in the catalytic site of the enzyme, resulting in a reversible, stable tetrahedral adduct.*

The design of a number of specific enzyme inhibitors has been based on the hypothesis that reactive intermediates, or transition states, of compounds whose reactions are being catalyzed bind considerably better to enzymes than do the compounds themselves (1-3). Among these compounds is 2-phenylethaneboronic acid, a powerful inhibitor of α -chymotrypsin, prepared by Koehler and Lienhard (4). These authors suggest that a tetrahedral adduct between the γ -oxygen of a serine residue in the catalytic site of the enzyme

and the inhibitor is responsible for the strong binding, and they consider this intermediate analogous to the transition states of chymotrypsin-catalyzed reactions. However, a number of different boronic acid derivatives have been used as enzyme inhibitors (5-6a), and several mechanisms have been considered (4, 6, 6a) to account for their properties.

In view of the importance of boronic acids as specific enzyme inhibitors and their possible use in studies of mechanisms, we have investigated by means of laser Ra-