

dew and that significantly inhibits the growth of cucumber roots.

Although the production of growth inhibitors has been demonstrated for a variety of plants, we believe this to be the first demonstration that a volatile inhibitor may be effective in the field. Its suggested deposition in dew would constitute a novel mechanism of ecological interaction.

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2. Detailed analysis in preparation.
3. Supported by contract GB-149, National Science Foundation.

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### Nucleus of the Trapezoid Body:

#### Dual Afferent Innervation

**Abstract.** *Lesions of the anterior ventral cochlear nucleus caused degeneration of synaptic endings in the anterior half of the nucleus of the trapezoid body. Lesions of the posterior ventral cochlear nucleus caused similar degeneration in the posterior half of the nucleus of the trapezoid body. Thus, the cochlea projects twice upon the nucleus of the trapezoid body.*

In a series of experiments now being conducted, we wish to determine the projections of the cochlear nuclei upon the components of the superior olivary complex. This work is concerned with two general problem areas. First, we are interested in understanding the ascending connections of the cochlear nucleus, as these shed light upon the way in which the nucleus may be morphologically subdivided. We are also interested in supplying an anatomical basis for the interpretation of physiological studies of the lower auditory system. In this experiment we were concerned with the projection of the cochlear nuclei upon the (medial) nucleus of the trapezoid body.

The nucleus of the trapezoid body is extremely well developed in a number of animals including the bat (*Myotis*), hamster, marmoset, mole (*Scalopus aquaticus*), mouse, rabbit, and the rat. It is clearly present in the cat, dog,

giant panda, gorilla, and in man. In the rat, the animal used in this experiment, the nucleus forms the largest component of the superior olivary complex. It has been shown that cells located somewhere in the cochlear nucleus give rise to large fibers which terminate in large synaptic endings (the chalice of Held) upon the cells of the contralateral nucleus of the trapezoid body (1). In this experiment, we determined the dual loci of these cells and their mode of termination.

Basically, the method consisted of estimating (by counting) degeneration of the chalice of Held in the nucleus of the trapezoid body of animals in which lesions had been induced in the cochlear nuclei. Lesions were induced in the cochlear nuclei of nine albino rats, and in the cochlea of one rat for control purposes. After 7 to 14 days, the brain of each animal was embedded in paraffin, sectioned at 16  $\mu$ , and impregnated according to the protargol method of Bodian (2). All chalice of Held were counted in each section, and the results were plotted graphically to show the number of chalice at each level in the nucleus of the trapezoid body from the anterior to the posterior end.

The results of the experiment are summarized in Table 1, from which the following points can be made.

The anterior ventral cochlear nucleus sends fibers to the anterior half of the contralateral nucleus of the trapezoid body (rats P25, P26, and P28). As an example, the graph of the chalice count of rat P25 is shown in Fig. 1. In this animal the anterior ventral cochlear nucleus of the left side was extensively destroyed, especially at its anterior end. Other parts of the

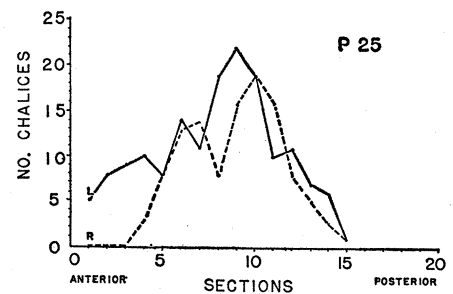


Fig. 1. Rat P25. Count of synaptic endings (chalice of Held) in the nuclei of the trapezoid body (transverse section). The number of chalice is given on the vertical axis and the ordinal position of each section (from the anterior end) on the horizontal axis. The anterior ventral cochlear nucleus of left side was damaged (L, left; R, right).

cochlear nucleus of the left side were intact, as was the cochlear nucleus on the right side. This damage is reflected in complete degeneration of the chalice of Held in the anterior portion of the right (contralateral) nucleus of the trapezoid body and the normal number of chalice on the left side (Fig. 1). The graph also shows that the number of chalice in the posterior part of the nucleus of the trapezoid body of both sides was approximately the same.

The posterior ventral cochlear nucleus sends fibers to the posterior half of the contralateral nucleus of the trapezoid body (rats P18, P24, and P54). As an example, the graph of the chalice count of rat P54 is given in Fig. 2. In this animal, the posterior ventral cochlear nucleus of the left side was completely destroyed and the anterior ventral cochlear nucleus was virtually intact. On the right side the cochlear nucleus was intact. Figure 2 shows that there was extensive degener-

Table 1. Summary of experimental lesions and synaptic degeneration in the nucleus of the trapezoid body. Abbreviations: Ant., anterior; AVC, anterior part of ventral cochlear nucleus; DCN, dorsal cochlear nucleus; Deg, degeneration of chalice of Held; ICP, inferior cerebellar peduncle; NTB, nucleus of the trapezoid body; Post., posterior; ST, spinal tract of trigeminal nerve. Damage: 0, less than 10 percent of structure damaged; 1, between 10 percent and 50 percent; 2, between 50 percent and 90 percent; and 3, more than 90 percent damage. L, left, R, right side.

Animal	Lesion			Degeneration of Held chalice	
	AVC	PVC	Other	Ant. part NTB	Post. part NTB
P16	0	0	DCN slight	Normal	Normal
P18	0	L, 1; R, 2	—	Normal	L, deg; R, deg.
P23	0	0	ICP slight	Normal	Normal
P24	0	L, 0; R, 2	—	Normal	L, deg.; R, normal
P25	L, 2; R, 0	0	—	L, normal; R, deg.	Normal
P26	L, 1; R, 0	0	—	L, normal; R, deg.	Normal
P27	0	0	ST	Normal	Normal
P28	L, 2; R, 0	0	—	L, normal; R, deg.	Normal
P54	0	L, 3; R, 0	DCN slight	Normal	L, normal; R, deg.
MH12	0	0	Cochlea	Normal	Normal

ation of the chalices of Held in the posterior part of the right (contralateral) nucleus of the trapezoid body.

In the control animal, MH12, and in rats P16, P23, and P27, the lesions of the cochlea, or lesions which failed

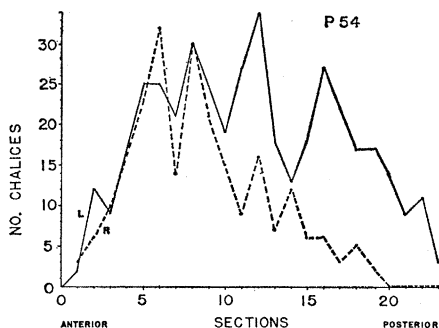


Fig. 2. Rat P54. Count of synaptic endings (chalices of Held) in the nuclei of the trapezoid body (transverse section). The posterior ventral cochlear nucleus was completely destroyed on the left side; the anterior ventral cochlear nucleus was intact on the same side (L, left; R, right).

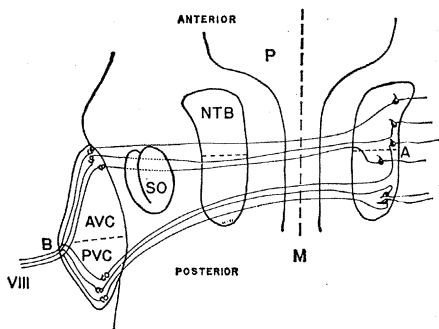


Fig. 3. Rat P23. Count of synaptic endings (chalices of Held) in the nuclei of the trapezoid body (transverse section). This animal sustained no damage to auditory structures and constitutes a control for the data Figs. 1 and 2. (L, left; R, right).

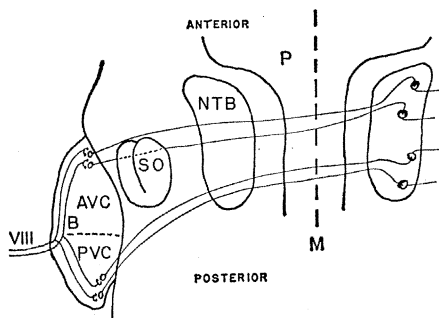


Fig. 4. Schematic representation (in horizontal section) of the connections of the nucleus of the trapezoid body. A, line dividing nucleus into anterior and posterior parts; AVC, anterior ventral cochlear nucleus; B, bifurcation of fibers of acoustic nerve; M, midline; NTB, nucleus of the trapezoid body; P, pyramidal tract; PVC, posterior ventral cochlear nucleus; SO, superior olive; VIII, acoustic nerve.

to damage the cochlear nuclei, were without effect upon the chalices of Held in the nuclei of the trapezoid body. The chalice count of rat P23 is shown in Fig. 3, as an example.

These results show that the nucleus of the trapezoid body receives afferent fibers from two sources. The anterior half of the nucleus receives fibers from the contralateral anterior ventral cochlear nucleus. These are large fibers which travel in the anterior half of the trapezoid body. The posterior half of the nucleus receives fibers from the posterior ventral cochlear nucleus. These are also large fibers which travel in the posterior half of the trapezoid body. Our data also suggest that the two projection fields overlap to some extent in the middle region of the nucleus. The anatomical arrangement is shown in Fig. 4.

The discovery of dual innervation led us to reexamine the nucleus of the trapezoid body to see if it has the appearance of two nuclei. The nucleus appears quite homogeneous, however, when examined in transverse, sagittal, and horizontal sections.

The implications of our data for understanding the projection of the cochlea upon the nucleus of the trapezoid body can be arrived at by considering the connections of the auditory system. The fibers of the acoustic branch of the stato-acoustic nerve bifurcate upon entry into the medulla into an ascending and a descending branch. The ascending branch terminates in the anterior ventral cochlear nucleus and the descending branch terminates in the posterior ventral cochlear nucleus (3). Thus, the cochlea projects upon both the anterior and posterior ventral cochlear nuclei. We have shown that the anterior and posterior ventral cochlear nuclei both project to the nucleus of the trapezoid body, hence the cochlea is represented twice in this nucleus. One representation is in the anterior half of the nucleus and the second is in the posterior half (Fig. 4).

Electrophysiological studies of the nucleus of the trapezoid body have not indicated any clear topographical representation of frequency in the nucleus (4). Our data clearly indicate that a single representation of frequency is not to be expected.

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## Identity of Tarichatoxin and Tetrodotoxin

**Abstract.** *Tarichatoxin* ( $C_{10}H_{17}N_3O_8$ ), a potent neurotoxin recently isolated in pure form from the eggs of the California newt, *Taricha torosa*, has been found to be identical to tetrodotoxin from the ovaries of *Sphoeroides rubripes*, the Japanese Fugu or puffer fish. As yet this substance has been detected only in a single family of fish, Tetraodontidae, and in a single family of amphibia, Salamandridae.

A remarkable similarity has been noted already between the chemical (1) and physiological (2) properties of tarichatoxin isolated from the eggs of the California newt *Taricha torosa* and tetrodotoxin (3), which was first isolated in crystalline form in 1950 by Yokoo and Morosawa (4) from the ovaries of a species of Japanese puffer fish or globe fish, *Sphoeroides rubripes*. We have now carried out a direct comparison of these two substances in their crystalline form and find them to be indistinguishable. Furthermore, we find no difference in the properties of two crystalline acetates which have been prepared both from tarichatoxin (5) and tetrodotoxin.

In addition to the close similarity in pharmacological properties already noted (2), we find that a massive dose (crude toxin equivalent to as much as 1000  $\mu$ g of pure material per kilogram of body weight) of either tarichatoxin or tetrodotoxin injected intraperitoneally into *Taricha* fails to paralyze or kill these animals. On the other hand, mice, frogs (*Rana pipiens*), gold fish, and tiger salamanders (*Ambystoma*) are killed by far smaller doses of the two toxins. The LD<sub>50</sub> in mice is about 10  $\mu$ g/kg for both toxins with intraperitoneal administration. Tarichatoxin blocked the action potential of desheathed frog nerves in a few minutes when applied in concentrations of 1 to 10  $\mu$ g/lit. When sci-