that we had available for experimentation, some were taken by a hind leg, others by the flank or midsection of the belly. Toads that we pulled away from larvae as they were being drawn into the mud had only small cuts in the skin to mark the place of larval attachment.

Our laboratory tests indicate that *T. punctifer* larvae invariably kill the toads that they capture. They abandon the prey within hours, after ingesting blood and body fluids only. Tabanid larvae are generally fluid feeders (*1*), and *T. punctifer* is not unusual in that respect.

Since toads still showed some responsiveness to poking half an hour after capture by larvae, larval venom (*2, 3*), if injected at all into the toads, must be slow-acting at best. When we handled the larvae, we often felt mildly painful (and eventually itchy) skin punctures, which they visibly inflicted with their mouthparts. Quick immobilization of insect prey, presumably caused by envenomization, and painful "bites" to humans, have been reported for other tabanid larvae (*2, 3*). The single orifices at the tip of the larval mandibles (Fig. 1G) are presumed to be the openings of the venom glands (*3*).

To measure the forces that larvae exert when pulling on toads, we fastened toads that were partly submerged and dead or nearly dead (no muscular response to poking) to an electronic force transducer by means of a wire harness strapped around their front ends. The transducer was pulled vertically upward at a constant rate (0.72 cm per minute) by a motorized lift, to which the larvae offered a resistance that was registered graphically (force as a function of time) on an oscilloscope screen. Three larvae were pulled upward in this fashion until they released the toads. One oscilloscope tracing, typical for all three, is shown in Fig. 2. The larvae held onto the prey for several minutes (7.4 ± 0.7 minutes). Maximum resistance forces (12.9 ± 1.4 g) were 20 to 30 times the larval body weight (0.56 ± 0.08 g) and 10 to 16 times the toad body weight (0.97 ± 0.03 g) (*4*).

Larvae caught feeding on toads in the field were all full grown (*5*). We do not know whether younger larvae might also feed on *Scaphiopus*, but we suspect larval *T. punctifer* of all stages to subsist primarily on insects, as other tabanid larvae apparently do (*1, 6*). Our captive larvae ate crickets and were capable even of catching bombardier beetles, against whose defensive spray they were shielded by the mud (*7*). Because mass emergences of *Scaphiopus* occur only at lengthy intervals (*8*), it seems likely that

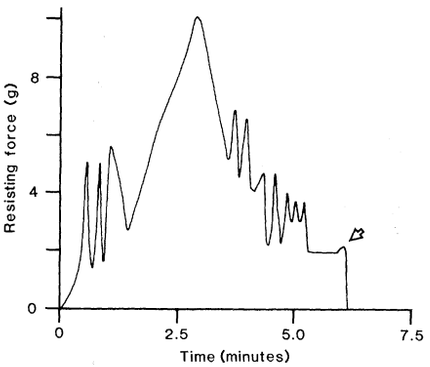

Fig. 2. Resisting force exerted by *T. punctifer* larva as the toad it was grasping was slowly pulled away by vertical lift. Oscillations were caused by sporadic body contractions of the larva. The arrow denotes point where larva released the toad.

T. punctifer feeds on this toad (as well as possibly other anurans) on an opportunistic basis only.

While we know of no previous records of tabanid larvae feeding on Amphibia, cases have been cited of insects feeding on vertebrates. A number of aquatic insects (beetles, Hemiptera, and nymphal dragonflies) have been observed to take fish, tadpoles, and frogs (*9*). Frogs, as well as small birds and a mouse, have been seen to be eaten by preying mantids (*10*). The case we report is a reversal of the usual toad-eats-fly paradigm, although in the case of *Scaphiopus* and *Tabanus* the paradigm may also prevail in its conventional form. Adult *Scaphiopus* might well on occasions have predatory access to the very *Tabanus* flies that as larvae preyed on their conspecifics.

RODGER JACKMAN
Combeinteignhead, Newton Abbot, South Devon TQ12 4RT, England
STEPHEN NOWICKI
DANIEL J. ANESHANSLEY
THOMAS EISNER
Division of Biological Sciences and *School of Electrical Engineering, Cornell University, Ithaca, New York 14853*

References and Notes

1. H. Oldroyd, *The Natural History of Flies* (Norton, New York, 1964).
2. J. O. Schmidt, *Annu. Rev. Entomol.* **27**, 337 (1982).
3. H. J. Tesky, *Mem. Entomol. Soc. Can.* **63**, 1 (1969).
4. Values are the means ± standard error of the mean.
5. The larvae went on to pupate without molting; the emergent adults were identified as *T. punctifer*, confirming the larval identification.
6. J. L. Webb and R. W. Wells, *U.S. Dept. Agr. Bull. 1218* (1924), p. 1; J. F. Burger, *Trans. Am. Entomol. Soc.* **103**, 145 (1977).
7. S. Nowicki and T. Eisner, *Psyche* **90**, 119 (1983).
8. A. N. Bragg, *Gnomes of the Night* (Univ. of Pennsylvania Press, Philadelphia, 1965).
9. A. D. Imms, *A General Text Book of Entomology*, revised by O. W. Richards and R. G. Davies (Methuen, London, ed. 9, 1957).
10. F. C. Morse, *Emu* **22**, 74 (1922); K. M. Nash, *Victorian Nat.* **79**, 11 (1962); M. G. Ridpath, *J. Aust. Entomol. Soc.* **16**, 153 (1977); D. A. Nickle, *Proc. Entomol. Soc. Wash.* **83**, 801 (1981).
11. We thank D. A. Nickle and L. Pechuman for helpful discussion, J. F. Burger and R. Ruibal for information and for identifying the larva and toad, respectively, and Maria Eisner for help with electron microscopy.

16 March 1983; revised 15 June 1983

The Development of Human Fetal Hearing

Abstract. *Blink-startle responses to vibroacoustic stimulation were monitored ultrasonically in human fetuses of known gestational age. Responses were first elicited between 24 and 25 weeks of gestational age and were present consistently after 28 weeks. Defining the developmental sequence for audition provides a foundation for diagnosing deafness and recognizing aberrant responses antenatally.*

The late third-trimester fetus has long been known to respond to sound (*1*). The end points used for testing hearing antenatally have been a change in overall fetal activity (*2*) or heart rate acceleration (*1, 3*). We have attempted to reopen investigation of fetal audition through the use of high-resolution ultrasound imaging for observing eye-blink responses [auropalpebral reflex (APR) (*4*)] to a specific vibroacoustic stimulus pattern.

Studies of evoked auditory potentials in prematurely born infants suggest that the auditory system is functional by the start of the third trimester (*5*). We studied 236 fetuses between 16 and 32 weeks of gestational age. Their mothers were women from the general population who had been referred for ultrasound studies; gestational age had been established by known date of conception, ultrasonic staging before 22 weeks of gestational age, or both. Infants (singletons) were subsequently born in good health at term. None of the mothers had taken any medication other than iron and vitamin supplements, none had consumed any alcohol or smoked within 24 hours of testing. Mothers with diabetes, hypertension, rhesus factor sensitization, premature labor, or any systemic medical process were excluded.

Each fetus was stimulated with a hand-held, battery-powered, vibroacoustic noise source (Electrolarynx, model 5B, Western Electric, 16-mm circular disk contact surface) applied firmly to the maternal abdomen directly overly-

ing a fetal ear (*6*) (Fig. 1). The stimulator has an approximate 110-dB output intensity as measured in air 2 inches from the probe surface with broad spectral peaks at about 250 and 850 Hz. We would expect from studies of sound transmission through the maternal abdominal and uterine walls (*7*) that intensity would be reduced on average some 15 dB with little change in spectral composition. Background noise intensity within the gravid uterus has been estimated in the range of 75 dB (*7*). The stimulus pattern was an initial 0.5-second duration pulse, followed after 30 seconds with a manually delivered series of ten 0.5-second pulses spaced about 1 second apart. The eyelids (Fig. 2) were observed continuously during stimulator placement and throughout the stimulus sequence with ultrasound imaging equipment having $\leq$ 2 mm lateral resolution and an imaging rate of 30 frames per second.

Spontaneous eyelid movements were observed with increasing frequency after 26 weeks of gestational age, but the APR's were unique in the forcefulness of lid clenching. The blink responses were also immediate (latency less than 0.5 second) and bilateral. Blink responses were scored triadically in the ten-burst sequence as no response, blink occurring with the first stimulus and continuing serially, and inconsistent blink or aversive head movement occurring somewhere in the ten-burst sequence but not initially and not sequentially. Responses are given in Table 1 for 227 of 236 subjects in whom testing was attempted. These three categories were separated by gestational age [$\chi^2(16) = 279.5$, $P < 0.001$].

The APR's were typically associated with contraction of the cheek and frontalis muscles. Other components of the startle reaction, such as head aversion, arm movement, and leg extension, occurred with the initial pulse. There was an increase in yawning activity after the stimulus sequence was concluded. The overall response magnitude was always greater for blink-startle responses than for the temporarily inconsistent type of reaction.

Table 1. Number of fetuses making no, inconsistent, or blink responses to vibroacoustic stimulation at different gestational ages. Gestational age groups below 24 and above 29 weeks have been combined.

Gestational age (weeks)	Auditory response			
	No response	Inconsistent	Blink	Total
12–15.9	17	0	0	17
16–19.9	26	0	0	26
20–23.9	32	0	0	32
24–24.9	14	7	0	21
25–25.9	9	14	1	24
26–26.9	5	20	3	28
27–27.9	2	12	9	23
28–28.9	0	3	17	20
29–32	0	0	36	36

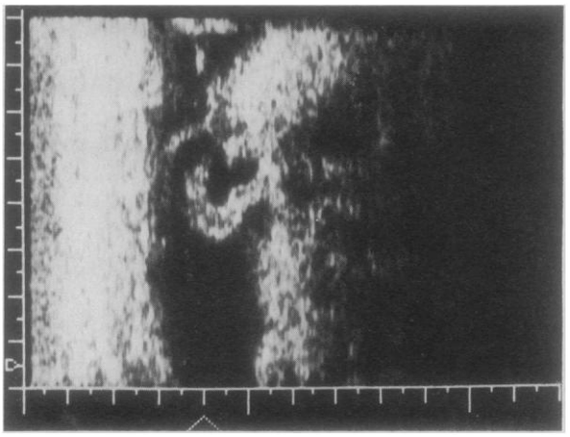

Fig. 1. Ultrasound image of the fetal external ear.

We conclude that hearing is established as a functionally interactive sensation by the start of the third trimester for the specific stimulus used and with the restriction to short latency craniofacial motor reactions. Arm or leg movements of longer latency (> 1.5 seconds delay), without associated head movement or blink, were seen in 3 of 24 additional subjects younger than 21 weeks of gestational age in whom this activity had specifically been sought. The sharp transitional occurrence of auditory startle behavior at the 25-week frontier of extrauterine viability provides an additional indicator of neuromotor maturity that may be applied in clinical practice.

An invariant APR after 28 weeks of gestational age in the normal study population suggests the potential for antenatal screening for gross deafness. In reviewing our experience with an additional 680 subjects between 28 and 36 weeks of gestational age, we encountered eight with no observable blink response, despite multiple trials. In two of these, deafness with severe sensory deficit was demonstrated audiologically after delivery. One occurred in a diabetic pregnancy (class C) and had associated microtia. Neither had other anomalies, renal insufficiency, or pertinent family history. Both had eye (*8*), diaphragm, and body movements appropriate for gestational age in each of several examinations. Two hearing failures occurred in severely compromised fetuses who also lacked spontaneous body movements. One with (nonimmune) hydrops at 30 weeks of gestational age was studied four times on 2 days preceding intrauterine demise. The other was referred at 35 weeks of gestational age and was delivered emergently 1 hour later. Severe fetal anemia (hematocrit 5) was presumed as a result of spontaneous fetomaternal hemorrhage. This infant did not survive the immediate perinatal period. The remaining four cases had multiple structural anomalies, including two cases with advanced hydrocephalus (30 and 32 weeks gestational age), trisomy 13 (36 weeks), and achondrogenesis (30 weeks). No instance of significant hearing deficit was found postnatally for any of the fetuses having a demonstrable blink response.

Hearing may be tested antenatally through the use of ultrasonic monitoring of the blink response. Our preliminary experience suggests that the absence of a blink response under the test conditions outlined indicates a serious, primary hearing impairment or significant depression of the central nervous system from developmental or exogenous causes. We recommend that clinical application follow development (and standardization) of quantitative measures of response latency.

Antenatal hearing testing with ultrasonic monitoring should also be refined

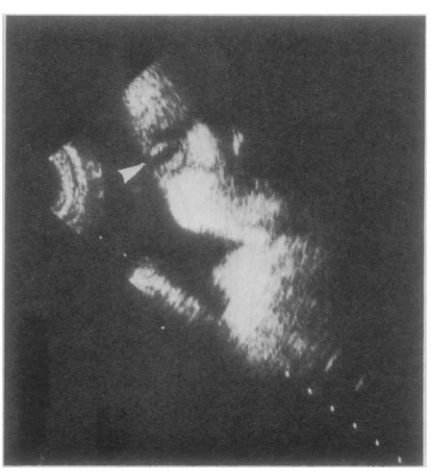

Fig. 2. Lateral coronal view of the face showing the eyelid (arrow).

by varying the test stimulus, evaluating other motor responses, or by studying response decrement to repetitive stimulation. We suggest that these studies be directed to the broad topic of the developing central nervous system and not be restricted conceptually to the issue of hearing alone.

JASON C. BIRNHOLZ
Rush Medical College, Rush-Presbyterian–St. Luke's Medical Center, Chicago, Illinois 60612
BERYL R. BENACERRAF
Diagnostic Ultrasound Associates, Boston, Massachusetts 02115

References and Notes

1. L. Sontag and R. Wallace, *Am. J. Dis. Child.* **51**, 583 (1936); A. Peiper, *Monatsschr. Kinderheilkd.* **29**, 236 (1925); H. S. Forbes and H. B. Forbes, *J. Comp. Physiol. Psychol.* **7**, 353 (1927).
2. S. Gelman, S. Wood, W. Spellacy, R. Abrams, *Am. J. Obstet. Gynecol.* **143**, 484 (1982); L. Leader, P. Baillie, B. Martin, E. Vermeulen, *Br. J. Obstet. Gynaecol.* **89**, 441 (1982).
3. E. Wedenberg, *Acta Oto-Laryngol. Suppl.* **206**, 27 (1965).
4. W. Preyer, *The Mind of the Child* (Appleton, New York, 1888); C. A. Froding, *Acta Oto-Laryngol.* **52**, 31 (1960).
5. E. Weitzman and L. Graziani, *Dev. Psychobiol.* **1**, 79 (1968); P. Despland and R. Galambos, *Pediatr. Res.* **14**, 154 (1980); A. Starr, R. Amlie, W. Martin, S. Sander, *Pediatrics* **60**, 831 (1977).
6. J. Birnholz, *Radiology* **147**, 819 (1983).
7. J. Bench, *J. Genet. Psychol.* **113**, 85 (1968).
8. J. C. Birnholz, *Science* **213**, 679 (1981).

28 March 1983; revised 7 June 1983

Extensive Dendritic Sprouting Induced by Close Axotomy of Central Neurons in the Lamprey

Abstract. *Massive dendritic sprouting was induced in identified giant reticulospinal neurons of the lamprey by axotomy close to the soma. An axonal lesion slightly farther from the cell body induced new growth from both dendrites and axon. The amount of new growth per cell was the same whether it originated from the dendrites alone or from axonal and dendritic compartments. The location of the axonal lesion therefore determines where, in the neuron, membrane is inserted to produce the new neurites. The dendritic tree of a differentiated vertebrate central neuron was shown to have sufficient plasticity to extend new growth for several millimeters beyond the normal dendritic domain.*

The characteristic form of a mature neuron results from selective growth in different regions of the cell leading to the generation of an axon and dendrites. The mechanisms underlying this selective growth can be studied by examining the location and extent of sprouting after axotomy of mature neurons. Generally, the most prominent response to axotomy is the partial or complete regeneration of the severed axon, which may be accompanied by relatively minor changes in the dendritic tree (*1–3*). However, in recent invertebrate studies, axotomy close to the soma induced profuse intraganglionic sprouting with relatively little axonal regeneration into the connectives (*4*). In the cricket, the sprouting definitely originated from the dendritic tree. We now report that "close" axotomy (< 500 μm) from the soma of identified central neurons in a primitive vertebrate, the sea lamprey, induced extensive dendritic sprouting while greatly decreasing axonal regeneration. Axotomy farther from the soma (1000 to 1400 μm) results in less dendritic and more axonal sprouting than close axotomy, with the total amount of growth induced by the two types of lesion being roughly equal. These results suggest that close axotomy causes a shunting of new growth into the dendrites and away from the axon stump without altering the total extent of regenerative sprouting.

Larval sea lampreys (*Petromyzon marinus*) 9 to 13 cm long were anesthetized by immersion in a saturated aqueous benzocaine solution. An incision was made dorsally across the skull at the level of the rostral midbrain. The top of the cartilaginous skull and the choroid plexus were then partially freed and reflected caudally to expose the hindbrain. A partial hemisection of the brain was made at one of two locations in the hindbrain with a No. 11 scalpel blade (Fig. 1A), and the choroid and skull were then replaced. No suturing was necessary. For distant axonal transection in the spinal cord, a scalpel blade was used to sever the cord with a dorsal incision. All lesions extended through the entire dorsoventral dimension of the brain or spinal cord. The animals were allowed to recover in lamprey saline (*5*) for 3 days, and were gradually returned to well water by 6 days where they were kept at 15°C until examined histologically.

Intracellular staining with Lucifer yellow (*6*) was used to study the morphology of normal and axotomized cells. Somata of the three most anterior pairs of large bulbar Müller cells (*7*) (anterior bulbar cells or ABC's; see Fig. 1) were

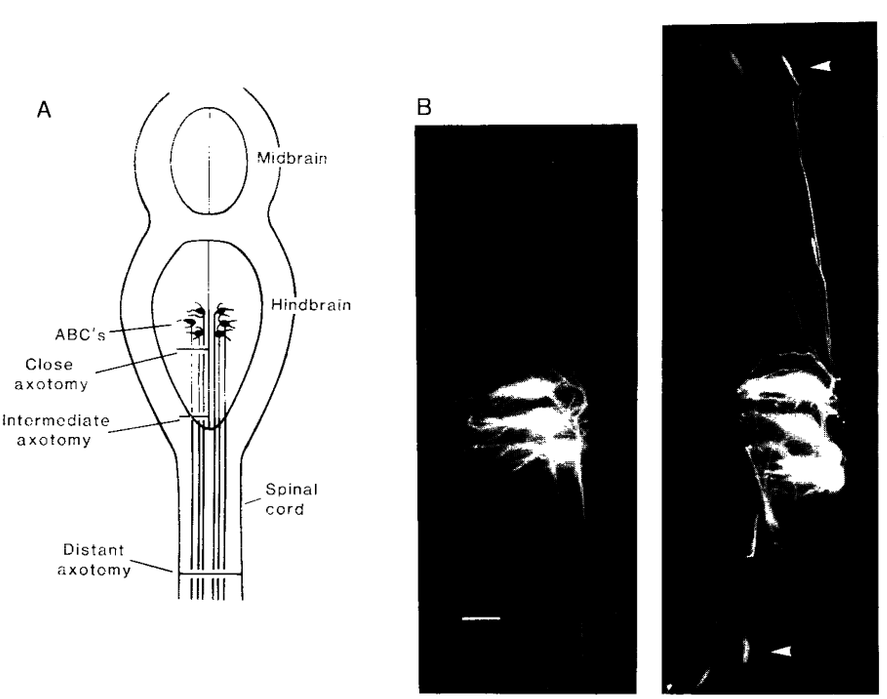

Fig. 1. (A) Diagram showing the lamprey brain in dorsal view with the cell bodies of the three most anterior pairs of bulbar Müller cells (ABC's) in the hindbrain and their axons extending caudally down the spinal cord. In various experiments, these neurons were axotomized at the three locations indicated. (B) Whole mounts of lamprey brain showing three ABC's from a normal animal (left) and from another animal 49 days after close axotomy (right). Arrows indicate sprouts with swollen tips extending from the dendrites of the axotomized cells. No axonal regeneration is apparent. Cells were stained by intracellular injection of Lucifer yellow. Scale bar, 100 μm.