raised on PA-deficient *Taraxacum* contained only traces (or none) of this aldehyde (less than 5 ng per male) (*14*). The size of the coremata, which ranged from very small to large, also depended on the access of the larvae to PA-containing plants. We added the monocrotaline to the *Taraxacum* diet of final-instar larvae and we found hydroxydanaidal in the coremata, and the scent organs were much larger than those of control males fed on *Taraxacum* only.

We used standard electrophysiological methods (electroantennograms) (*15*) to test the olfactory effectiveness of the coremata of males that as larvae had been fed diets supplemented with monocrotaline. While very small coremata elicited electrical responses indistinguishable from those of control stimuli (air only), properly developed coremata elicited good responses in the antennae of both sexes and both species; the electroantennogram amplitudes indicate a correlation between organ size and stimulatory power (Fig. 4).

The information available so far demonstrates an insect-plant relationship without parallel. Both the biosynthesis of an odorophore and the maximal development of the organ that disseminates and secretes that odorophore require access to a specific secondary plant metabolite.

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3. Excised coremata were preserved in ampules containing CS_2. Extracts were studied by gas chromatography–mass spectroscopy (GC-MS) and by high-performance liquid chromatography (HPLC). The GC-MS identification of hydroxydanaidal in coremata extracts was accomplished by direct comparison with an authentic sample (Finnigan 3000 mass spectrometer). Samples were injected onto a silane-treated glass column (0.2 by 91 cm) packed with 3 percent OV-1 on 80/100 mesh Supelcoport operated isothermally at 117°C. Prominent ions in the electron impact spectra of authentic and natural samples were observed at mass-to-charge ratios of 151 (M+), 134, 123, 122, 95, 94, 79, 61, and 51. Gas chromatographic analyses were conducted with a silane-treated glass column (0.2 by 244 cm) packed with 3 percent OV-17 on 100/120 mesh Gas Chrom Q operated isothermally at 150°C with flame ionization detection. As little as 10 ng per animal could be detected. The quantity of aldehyde in each injection was determined either by electronic integration or by measurement of peak heights. Some samples were also analyzed by HPLC on a μBondepak C_{18} column (0.4 by 30 cm) eluted with a mixture of water and acetonitrile (50:50) with detection by ultraviolet absorbance at 280 nm (0.01 absorbance units full scale). As little as 0.5 ng per injection (2.5 ng per animal) was detected by this method.
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14. We have not yet established whether these small amounts are due to contamination (in the course of excising the organs or from some other source) or if small amounts of PA's are transferred to the larvae by the eggs and subsequently utilized for hydroxydanaidal synthesis; larvae of *Nyctemera* ingest PA's from *Senecio*, which appear in the adult moths and even in their eggs (approximately 1 μg per egg) [M. Benn, J. DeGrave, C. Gnanasundersam, R. Hutchins, *Experientia* **35**, 731 (1979)].
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Testosterone Uptake in the Brainstem of a Sound-Producing Fish

Abstract. *Three nuclear areas in the medulla were implicated in the control of sound production in the oyster toadfish* Opsanus tau. *The sonic motor nucleus was labeled by retrograde transport of horseradish peroxidase injected into swimbladder sonic muscles, and an adjacent ventrolateral and a more anterior periventricular nucleus of the medulla were revealed by autoradiography with ^3H-labeled testosterone. These androgen uptake sites occur in brainstem areas corresponding to areas predicted to contain the neural centers controlling the duration and fundamental frequency of the toadfish mating call.*

Neurons that concentrate steroids have been found in the forebrain, brainstem, and spinal cord of a number of vertebrates (*1, 2*). The functions of these neurons are largely unknown, although in frogs, birds, and mammals the androgen concentrating motor nuclei of the final common pathway to the sonic muscles are involved in sound production (*3*).

The oyster toadfish (*Opsanus tau*) produces an agonistic grunt and a courtship boatwhistle. Although most fish sounds, including the grunt of the toadfish, are of short duration and pulselike, the boatwhistle is unusual in that it is of relatively long duration and tonal (*4*). Behaviorally relevant aspects of the boatwhistle are its fundamental frequency, duration, and repetition rate (*5*). In view of the close association of sound production and reproductive behavior in toadfish, we examined the question of whether the neurons that innervate the sonic muscles concentrate labeled testosterone.

Steroid target neurons in homologous brain regions of fish, amphibians, reptiles, birds, and mammals show certain similarities (*1–3*). However, in four teleosts, no brainstem steroid-binding neurons were described; these teleosts were the paradise fish (*Macropodus opercularis*) (*6*), goldfish (*Carassius auratus*) (*7*), platyfish (*Xiphophorus maculatus*) (*8*), and green sunfish (*Lepomis cyanellus*) (*2, 9*). The absence of steroid target neurons in these species has not been explained. Of these species only the sunfish is known to produce sound (*10*), although it is typically thought of as visually oriented (*11*). Brain stimulation in the sunfish has evoked nest building, color change, aggression, courtship, and sperm release (*9*), but not sound production (*12*). However, the correlation of steroid uptake with song in birds and mating calls in frogs suggests that brainstem target cells might well be present in a vocal teleost such as the toadfish.

Pattern generators controlling the duration of the boatwhistle call have been localized grossly within the anterior medulla, whereas those controlling fundamental frequency have been found in the

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posterior medulla (*13*). The duration and fundamental frequency of the boatwhistle, and hence the output of these pattern generators, vary seasonally (*14*). Assuming that these changes depend on variations in male sex hormones, we predicted that [^{3}H]testosterone injected into toadfish would label at least two brainstem nuclei and that two of these nuclei would be likely candidates for neural centers controlling the fundamental frequency and duration of the boatwhistle call.

The toadfish's sound-producing organ, the swimbladder, is bounded on each side by an intrinsic sonic muscle band (*15*). To identify motoneurons projecting to these muscles we made several injections of 50 μl of 10 percent horseradish peroxidase (HRP; Sigma type VI) into either the left or right member of this muscle pair in two toadfish. After 10 to 13 days the fish were anesthetized and perfused through the heart with saline and mixed fixative (1 percent paraformaldehyde and 1.25 percent glutaraldehyde in 0.1*M* phosphate buffer). We then prepared 50-μm frozen sections of the medulla and anterior spinal cord and examined them for retrograde labeling using the tetramethylbenzidine HRP protocol (*16*). The label was traced through the occipital nerve into large motoneurons in the ipsilateral half of the sonic motor nucleus (Fig. 1, A and B), a large midline structure just below the central canal and above the medial longitudinal fasciculus (*17, 18*).

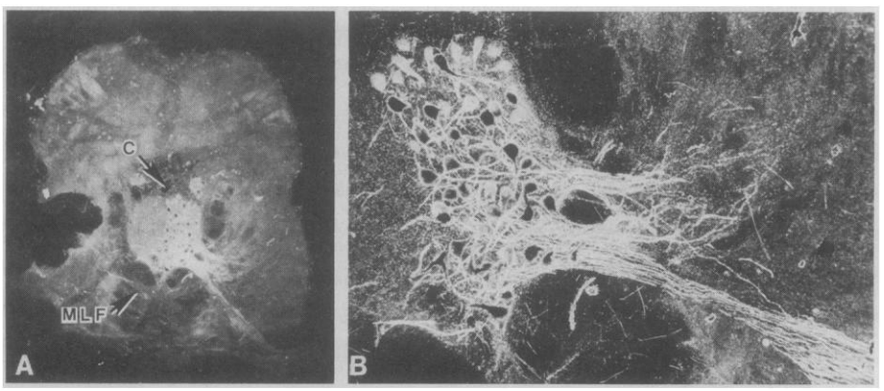

Fig. 1. (A) Low power and (B) high power dark-field micrographs of the anterior spinal cord of the oyster toadfish illustrating the labeled ipsilateral sonic motor nerve and nucleus after injection of HRP into the sonic (swimbladder) muscle. The symmetrical contralateral half of the nucleus is visible but unlabeled. The indentation in (A) was made to mark the contralateral side of the cord during sectioning. Abbreviations: *C*, central canal; *MLF*, medial longitudinal fasciculus.

In another series of experiments, castrated fish were given intramuscular injections of 200 μCi of [^{3}H]testosterone. Two hours later the fish were killed and their brains were removed and frozen in liquid propane. The brains were serially sectioned and processed for steroid autoradiography (*19*). After 90 to 245 days, sections were developed and stained with methyl green pyronine. We classified the cells as labeled or unlabeled according to criteria established by the Poisson distribution (*20*).

We found no steroid-binding cells in the sonic motor nucleus, but did find them in two other sites in the medulla. Since connectivity patterns and functions of these nuclei have not been established, we are naming them tentatively for convenience. A posterior site, nucleus ventrolateralis medullae, is ventrolateral to the sonic motor nucleus above the floor of the posterior medulla and anterior spinal cord (Fig. 2, C and D). A more anterior site, nucleus periventricularis medullae, is present in the dorsal medulla at cerebellar levels, just lateral to the fourth ventricle (Fig. 2B). The labeling pattern for these nuclei was similar in both sexes. Occasional labeled cells were also scattered in the medulla and basal optic tectum (Fig. 2, A to D) and in the torus semicircularis (Fig. 2A), a midbrain structure implicated in hearing, lateral line sensation, and sound production (*17*).

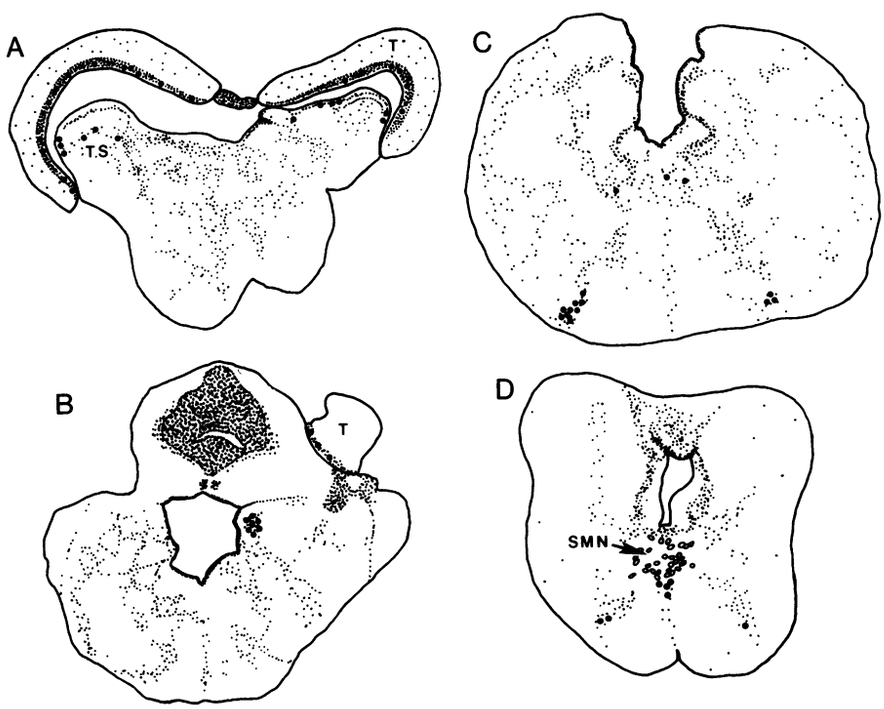

Fig. 2. Representative frontal sections of the toadfish brainstem showing all neurons (small dots) and cells (large dots) concentrating [^{3}H]testosterone. (A) Section through midbrain showing diffuse labeling of the optic tectum and torus semicircularis. Labeling occurred in superficial and deeper cells scattered throughout the medial-lateral extent of the torus. (B) Section through anterior medulla (beneath cerebellum) demonstrating periventricular nucleus. Tectal cells are also labeled. (C) Section through caudal medulla showing ventrolateral nucleus. (D) Section through anterior spinal cord (slightly rostral to Fig. 1) showing caudal portion of ventrolateral nucleus. Medial cells labeled in (C) and (D) are small and appear to be glial. Abbreviations: *SMN*, sonic motor nucleus; *T*, optic tectum; *TS*, torus semicircularis.

Although it is possible that androgen-binding sites have simply been overlooked in other fishes, investigators of the paradise fish specifically examined the brainstem and spinal cord with negative results (*6*). The absence of androgen target neurons in sunfish is not particularly surprising even if one does not accept our assertion that they are "weak" sound producers (*10–12*). Sound production has evolved independently in numerous teleost taxa, and thus it would not be surprising if some teleosts capable of sound production did not possess ste-

roid target neurons. Steroid-binding neurons would be most likely in those species, like the toadfish, that produce a seasonal, sexually dimorphic mating call with functions similar to those of bird song (*4*, *14*).

Except for the sexually dimorphic nuclei of rats involved in female lordosis (*21*), neurons innervating perineal muscles in males (*22*), and the nuclei associated with sound production in various vertebrates (*3*), the behavioral functions of steroid target neurons are largely unknown. Steroids are usually considered to have two types of actions on the central nervous system (*21*), an organizational effect (that is, sexual differentiation of the brain and determination of the structure and complexity of neural circuits) and an activating or inhibiting effect on preexisting neural circuits (by affecting neuronal electrical activity and synaptic transmission). Activation of behavior also parallels the ethologists' notion of the effect of steroids on the fixed action pattern. The two types of actions do not, however, include the possibility that steroids can modulate quantitative aspects of a behavior pattern. For instance, the pitch of the contact call of the Japanese quail varies directly with androgen concentration (*23*), and quantitative seasonal changes in the toadfish boatwhistle are only partially related to temperature (*14*). Identification of two sets of target neurons in the toadfish medulla, at the approximate levels predicted for pattern generators by Demski (*13*), makes control and modulation of call duration and fundamental frequency an attractive hypothesis for the function of these nuclei. It also extends the correlation between sound production and neuronal steroid uptake to a member of the largest vertebrate class, the fishes.

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In vivo Mapping of Local Cerebral Blood Flow by Xenon-Enhanced Computed Tomography

Abstract. *A noninvasive technique has been developed to measure and display local cerebral blood flow (LCBF) in vivo. In this procedure, nonradioactive xenon gas is inhaled and the temporal changes in radiographic enhancement produced by the inhalation are measured by sequential computerized tomography. The time-dependent xenon concentrations in various anatomical units in the brain are used to derive both the local partition coefficient and the LCBF. Functional mapping of blood flow with excellent anatomical specificity has been obtained in the baboon brain. The response of LCBF to stimuli such as changes in carbon dioxide concentrations as well as the variability in LCBF in normal and diseased tissue can be easily demonstrated. This method is applicable to the study of human physiology and pathologic blood flow alterations.*

The importance of techniques that can be used to measure cerebral blood flow as an index of cerebral function has long been recognized. A number of techniques have been developed in an attempt to find an in vivo methodology to map local or regional cerebral metabolic rate or cerebral blood flow, or both, for normal or abnormal brain function (*1*, *2*). In most methods currently in use, one externally monitors the transit or clearance of inhaled or injected radiotracers (*2–4*). Although these techniques have proved useful, they generally yield only gross estimates of cerebral function within relatively large tissue volumes. Recent advancements in both single-photon and positron-annihilation emission computed tomography permit improved anatomical resolution (*5*, *6*). These techniques still suffer from inherent limitations of spatial resolution and require specialized imaging devices (*6*, *7*). The introduction of rapid, sequential transmission computed tomography (CT) provided a method of monitoring changing tracer concentrations over time with improved anatomical specificity (*8*). Although iodinated contrast media have been used to demonstrate qualitative flow patterns, methods developed to make quantitative measurements of local cerebral blood flow have had only limited success. In addition, clinically available contrast media do not cross the blood-brain barrier; therefore, tissue perfusion in the brain cannot be evaluated (*9*).

We have been developing techniques over the past several years in which local cerebral blood flow (LCBF) in extremely small tissue volumes in vivo can be de-

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