Montagna and W. Sadler, Eds. (Plenum, New York, 1974).
6. C. H. Del Campo and O. J. Ginther, *Am. J. Vet. Res.* **33**, 2561 (1972).
7. The gestation period for this strain is 23 days.
8. Lure females had been ovariectomized and were brought into behavioral estrus by 10 μg of estradiol benzoate and 1.0 mg of progesterone injected intramuscularly 48 and 6 hours, respectively, before behavioral testing.
9. Since the last female on the ovarian end of the uterine horn had only one adjacent littermate, the data from these animals were excluded from the contiguity classification. However, these females were included in the analyses based on the number of males located on the caudal or ovarian side of the female. This accounts for the differences in total sample size, given in Table 1 for the three methods of classification.
10. Responding females are those exhibiting two or more mounts during the five tests for male copulatory behavior.
11. Comparisons of the average number of mounts exhibited by females in the several groups were made with Kruskal-Wallis tests. Parametric statistics could not be used because of the large number of zero scores in the FFF and 0M groups.
12. Since the lordosis quotient is a proportional measure, the analyses of variance were performed on scores transformed according to the formula $x' = 2 \arcsin\sqrt{x}$ [B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1971)].
13. Fetal body weight apparently is determined by a number of interacting variables. Although male rat fetuses tend to be heavier than their female littermates, this sex difference appears as early as day 12 of gestation [W. J. Scott and J. F. Holson, *J. Embryol. Exp. Morphol.* **40**, 249 (1977)]. Since the gonads differentiate after this time, it is unlikely that the difference is due to the action of fetal androgens. Furthermore, the female offspring of mothers injected with testosterone propionate or androstenedione during pregnancy show reductions in body weight that persist into adulthood [(*3*); I. L. Ward, *Horm. Behav.* **1**, 25 (1969); H. B. Popolow and I. L. Ward, *J. Comp. Physiol. Psychol.* **92**, 13 (1978)]. Paradoxically, prenatal exposure to antiandrogens has no effect on the birth weight of female rats (*4*). These conflicting data make it difficult to interpret the differences found in the birth weight of females classified according to their location in utero relative to male littermates. However, in agreement with our data, Clemens (*5*) reported that females derived from all-female litters had higher birth weights than females developing among male littermates.
14. J. Weisz and I. L. Ward, *Endocrinology* **106**, 306 (1980); I. L. Ward and J. Weisz, *Science* **207**, 328 (1980).
15. F. S. vom Saal and F. H. Bronson, *Science* **208**, 597 (1980).
16. R. Gandelman, F. S. vom Saal, J. M. Reinisch, *Nature (London)* **266**, 722 (1977); N. J. McDermott, R. Gandelman, J. M. Reinisch, *Physiol. Behav.* **20**, 661 (1978); F. S. vom Saal, *Horm. Behav.* **12**, 1 (1979); _____ and F. H. Bronson, *Biol. Reprod.* **19**, 842 (1978).
17. Within the combined 1M and 2M groups of the caudal male classification system, there were approximately equal numbers of females in uterine positions 2 through 6 (1 being the position closest to the cervix). These females did not show any significant morphological or behavioral differences when they were classified according to absolute uterine position. Thus, it is unlikely that a general nutritional gradient or any other variable related primarily to absolute position in the uterine horn, independent of the locations of males, had any important influence. On the other hand, secretions from the maternal ovary might have localized effects on female fetuses occupying positions close to the ovary. This possibility is not supported by the ovarian male classification data presented in Table 1. Furthermore, a comparison between females located immediately adjacent to the ovary and all other females indicated no statistically significant effect on any measure of morphology.
18. We thank Drs. Byron Ward and Benjamin Sachs for critically reading this manuscript. Supported by grant HD-04688 from the National Institute of Child Health and Human Development and by research scientist development award II 1-K2-MH00049 from the National Institute of Mental Health.
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23 October 1980; revised 25 March 1981

Reformation of Organized Connections in the Auditory System After Regeneration of the Eighth Nerve

Abstract. *Binaural cells in the superior olive normally have identical frequency sensitivities when acoustically stimulated via either ear. The precision with which central connections are reformed after auditory nerve regeneration can be determined by comparing the frequency sensitivities of the two binaural inputs to these cells. Three months after cutting the nerve and subsequent regeneration in the leopard frog, binaural cells once again have well-matched frequency sensitivities. Thus, the specificity of central connectivity that characterizes the auditory system in normal animals is restored after regeneration.*

A striking feature of the vertebrate nervous system is the specificity with which connections between neurons are made (*1*). The auditory system is exemplary in this regard. Each auditory nerve fiber is "tuned" so that at low stimulus intensities it will respond to a tone of a characteristic frequency, namely the fiber's best excitatory frequency (BEF), whereas at higher tonal levels it responds to a broader frequency band. The threshold of the fiber, plotted as a function of tonal frequency and intensity, represents its tuning curve and is consequently V shaped. Within the vertebrate central auditory system at least up to the midbrain, many neurons maintain V-shaped excitatory tuning curves (*2*), which are almost as narrowly tuned as those in the auditory nerve itself (*3–6*). This preservation of frequency selectivity suggests that nerve afferents with very different BEF's do not innervate the same central cell. Binaural cells, which receive input from both ears, provide further evidence of precise interconnections within the central auditory system. In response to tones, binaural cells, whether excited by both ears (E-E) or excited by one ear and inhibited by the other (E-I), usually exhibit similar best frequencies for both ears (*4, 6*). Thus, similarly tuned afferents from both sides of the brain must converge systematically onto a common target cell.

To gain insight into how these highly precise connections may be made, we have developed a preparation based on the regenerative properties of auditory nerve fibers in anurans (frogs and toads). The VIIIth nerve of amphibians, unlike that of mammals (*7*), is able to regenerate back into the central nervous system after its fibers have been severed (*8*). We have exploited this phenomenon to study the characteristics of tuning curves of central auditory neurons after reinnervation. It is critical to know whether the regenerating afferents return to specific cells, or whether the afferent fibers determine the innervation pattern among themselves, independently of the identity of the postsynaptic cells. This question can be approached by studying the similarity of the best frequencies from the two ears for binaural cells. Since most binaural cells in the anuran's central auditory system have similar frequency sensitivities when stimulated by either ear (*5, 6*), the tuning curve derived from the side with the intact nerve can serve as a marker to indicate the best frequency for the original innervation. If, after reinnervation, binaural cells have matching best frequencies again, it would suggest that the regenerating afferents have remade contact with their former postsynaptic cells. On the other hand, if the best frequencies of the binaural cells are largely mismatched, regenerating fibers would seem not to have returned to their original target cells within the dorsal medullary nucleus, but to have sorted out independently of the identity of the postsynaptic cell.

For our electrophysiological study, we selected the superior olivary nucleus, a second-order auditory nucleus, as our recording site. In anurans this nucleus receives its dominant excitatory input from the contralateral dorsal medullary nucleus, the presumed homolog of the mammalian cochlear nucleus, which in turn receives input from its ipsilateral VIIIth nerve (*6, 9*) (Fig. 1A). Binaural cells are reliably encountered in the anuran's superior olive (*6*). Adult leopard frogs (*Rana pipiens*) were anesthetized with MS-222, and the VIIIth nerve on one side was exposed through the roof of the mouth. With a tungsten microhook, the nerve was totally severed from the brain at the point where it penetrated the medulla. The animals were then housed in individual tanks at 20°C and allowed to recover for 3 months or longer. No attempt was made to control the acoustic environment. Ambient noise levels in the recovery tanks were generally around 72 dB sound pressure level (relative to 20 $\mu N/m^2$) and included energy from 50 Hz to 4kHz, which encompasses the frequency range of hearing of this species (*10*).

During recording, the animals were anesthetized with Nembutal (sodium

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pentobarbital, Abbott, 0.05 mg per gram of body weight), and a small opening was made in the roof of the mouth over the medulla contralateral to the regenerated nerve. Each ear was driven by a separate PDR-10 earphone so that binaural stimuli could be presented independently to the two ears. The earphones were housed in special couplers, which were sealed around the outer margin of each tympanic ring. A condenser microphone (Bruel and Kjaer 4134) within each housing permitted absolute calibration of the sound pressure levels at the two ears.

A glass-coated tungsten microelectrode, inserted into the ventral surface of the medulla directly over the superior olive, was advanced by means of a Kopf hydraulic microdrive until auditory-evoked activity was encountered; during this search phase, a broadband (50 Hz to 10 kHz, 70 to 80 dB) acoustic stimulus was presented separately to each ear or to both ears simultaneously. When a binaural unit was isolated, tones from a bank of frequency oscillators were presented to each ear to determine whether the neuron could be excited by both ears, or only one. If excited by only one ear, a tuning curve was taken for that ear and the presence of inhibition from the other ear was tested. Inhibitory binaural interactions were studied in the following way. A tone was presented to the excitatory ear at the unit's BEF at 10 dB above threshold. The intensity at which a tone to the other ear suppressed this evoked activity by 50 percent was determined over a broad range of frequencies. This procedure generated an inhibitory tuning curve and identified the unit's BIF (best inhibitory frequency) (*11*).

To provide a basis for comparison of the precision of matched sensitivity from the two ears, 22 binaural cells were first recorded in the superior olive of nine normal leopard frogs. Then, in six experimental animals with regenerated VIIIth nerves, 24 binaural cells were next isolated in their superior olivary nucleus. In both normal frogs and those with regenerated nerves, the most common type of binaural cell was the E-I type in which the contralateral ear was excitatory (via the regenerated nerve in the regenerates) and the ipsilateral ear was inhibitory (Fig. 1B). The BEF of this representative binaural unit to a contralaterally presented tone is 600 Hz. As the tone to the ipsilateral ear approached 600 Hz, the resulting inhibition became more marked. Increases in relative intensity of the inhibitory tone reduced baseline firing and broadened the inhibitory frequency range. The inhibitory tuning curve was also V shaped and exhibited a distinct BIF that remained unchanged by stimulus intensity (Fig. 1C).

When inhibitory tuning curves are constructed for binaural cells in normal animals, the BIF generally matches the BEF very well (Fig. 1D). Binaural cells appear to be well matched again 3 months after nerve transection (Fig. 1, C and D). For example, unit 4_1-1-1 in Fig. 1C has a BEF of 910 Hz and a BIF of 980 Hz. These best frequencies and the frequency ranges of the excitatory and inhibitory tuning curves are very close. For unit 18_2-3-3, the BEF and BIF are virtually identical, and the frequency ranges of the excitatory and inhibitory tuning curves overlap. Most of the binaural units recorded in both groups of animals fell on, or close to, a line of best fit for perfectly matched tuning curves from both ears (Fig. 1D).

In most cases, the best frequencies of binaural cells are similar, suggesting that regenerating fibers have returned to their former target cells. Alternatively, it would be possible for normal-appearing matched binaural cells to occur in another way: regenerating auditory fibers could initially segregate out with no regard for the previous innervation of the postsynaptic cells upon which they had terminated and, through rearrangements of the projections from the dorsal medullary nuclei onto the superior olive, binaural cells could exhibit the matching best frequencies observed in this study. One reason for favoring the hypothesis that primary afferent fibers do, in fact, return to their original sites of innervation is that the results are consistent with those obtained in studies of regeneration in the retinotectal system, in which cut optic nerve fibers return to their correct retinotopic loci in the tectum (*12*).

Our demonstration of the reformation of such organized projections in the central auditory system generates a number of questions concerning the origin of the

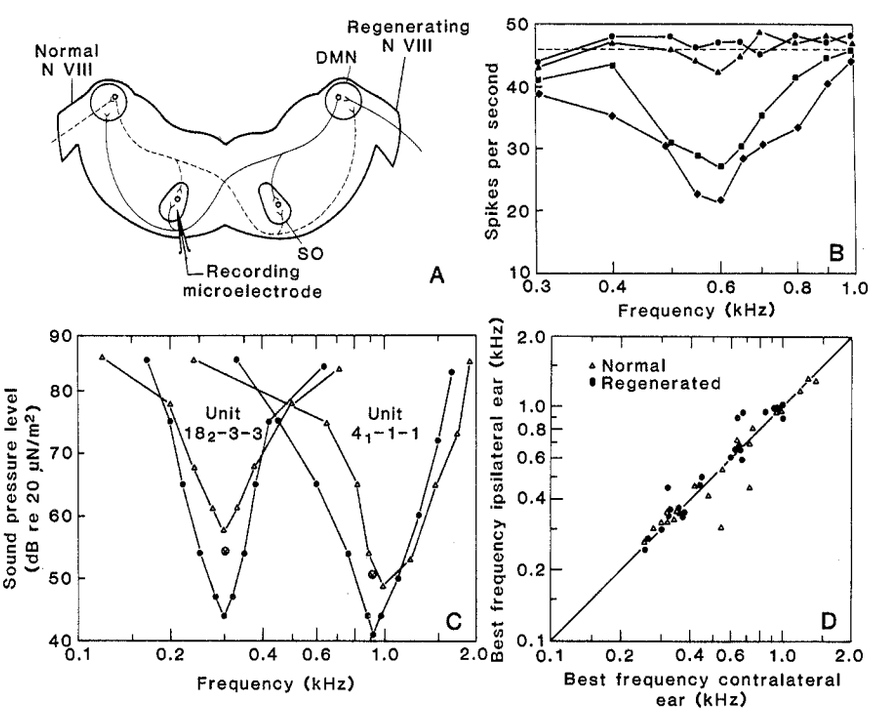

Fig. 1 (A) Schematic diagram of a cross-section of the brain at the level of the superior olive showing the auditory nuclei and pathways and the positioning of the microelectrode relative to the regenerating nerve. *DMN*, dorsal medullary nucleus; *SO*, superior olivary nucleus. (B) Inhibitory interactions for an E-I type binaural cell in the superior olive of a frog with a regenerated VIIIth nerve. This cell exhibited a BEF of 600 Hz and a threshold of 29 dB for auditory stimulation of the contralateral ear. A 600-Hz tone was presented to the contralateral ear at 39 dB, and tones of different frequencies from 300 Hz to 1 kHz were presented at various sound pressure levels (●, 44 dB; ▲, 49 dB; ■, 54 dB; ◆, 59 dB) to the ipsilateral ear. The horizontal dotted line along the top of the diagram represents the baseline firing elicited by the contralateral excitatory tone alone. At every intensity level where inhibition occurred, the maximum decrease in the spike count occurred when the frequency presented to the ipsilateral ear was also 600 Hz. As the intensity of the tone to the ipsilateral ear was increased, the inhibitory frequency range broadened. (C) Binaural tuning curves of two E-I cells in the superior olive of a frog with a regenerated VIIIth nerve. The contralateral ear (via the regenerated nerve) was excitatory (●) in both cases. ⊗, tone presented at the unit's BEF at 10 dB above threshold. △, inhibitory tuning curve. (D) Graph for E-I binaural cells depicting the agreement between the best frequencies of their binaural tuning curves. The diagonal line represents the line of best fit if the two best frequencies were perfectly matched.

final order. Is the binaural matching of best frequencies accompanied by any form of cooperative reorganization within the superior olive itself, or is it achieved solely by selective specificity of the regenerating afferents for their former target cells? If reorganization is largely a result of the specificity of a regenerating afferent for its target, do regenerating fibers synapse directly and unerringly on their former target cells, or do they locate an appropriate postsynaptic neuron only after a period of trial connections? Under our experimental conditions, the reformation of functional synapses can occur 5 to 6 weeks after the nerve is cut (*13*). Hence, the regenerated nerve had been in contact with central cells for at least 6 weeks before the data in this study were collected. But these questions could be pursued by documenting the similarity of the best frequencies for binaural cells in the superior olive early in the reinnervation process.

In other sensory systems, the study of regenerative processes has been instrumental in yielding insights into the mechanisms that guide the initial formation of connections during ontogeny (*12*). The preparation we have developed should lead to such insights in the auditory system, where, at present, there are few studies of the dynamics of formation of central connections during development (*14*).

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References and Notes

1. C. Cotman, Ed., *Neuronal Plasticity* (Raven, New York, 1978); R. D. Lund, *Development and Plasticity of the Brain* (Oxford Univ. Press, New York, 1978); M. Jacobson, *Developmental Neurobiology* (Plenum, New York, ed. 2, 1978).
2. Cells with complexly structured or very broad tuning curves are found in specific nuclei of the brainstem [dorsal cochlear nucleus, E. Young and W. Brownell, *J. Neurophysiol.* **39**, 282 (1976); dorsomedial periolivary nucleus, N. Y.-S. Kiang, K. Morest, D. Godfrey, J. Guinan, E. Kane, in *Basic Mechanisms in Hearing*, A. Møller, Ed. (Academic Press, New York, 1973)]. Nevertheless, most research reports on brainstem auditory neurons in terrestrial vertebrates strongly emphasize the common and widespread occurrence of V-shaped excitatory tuning curves [H. Scheich, *Verh. Dtsch. Zool. Ges.* **72**, 155 (1979)].
3. N. Y.-S. Kiang, *Discharge Patterns of Single Fibers in the Cat's Auditory Nerve* (Research Monogr. 35, MIT Press, Cambridge, Mass., 1965); J. Goldberg and W. Brownell, *Brain Res.* **64**, 35 (1973); G. Mousheigian and A. Rupert, *J. Neurophysiol.* **33**, 351 (1970); E. Evans, in *Handbook of Sensory Physiology*, vol. 5, part 2, *Auditory System*, W. D. Keideland W. D. Neff, Eds. (Springer-Verlag, Berlin, 1975), pp. 1–108; H. Scheich, in *Recognition of Complex Acoustic Signals*, T. Bullock, Ed. (Dahlem Konferenzen, Berlin, 1977), pp. 161–182.
4. C. Tsuchitani and J. Boudreau, *J. Acoust. Soc. Am.* **46**, 979 (1969); J. Boudreau and C. Tsuchitani, *J. Neurophysiol.* **31**, 442 (1968); J. Goldberg and P. Brown, *ibid.* **32**, 613 (1969); J. Guinan, S. Guinan, B. Norris, *Int. J. Neurosci.* **4**, 101 (1972).
5. A. Feng and R. Capranica, *J. Neurophysiol.* **39**, 871 (1976).
6. ———, *ibid.* **41**, 43 (1978).
7. H. Spoendlin, *Acta Otolaryngol.* **79**, 266 (1975); *ibid.* **81**, 228 (1976); E. Wever and W. Neff, *J. Comp. Physiol. Psychol.* **40**, 217 (1947).
8. H. Zakon and R. Capranica, *Brain Res.* **209**, 325 (1981).
9. I. Grofova and N. Corvaja, *ibid.* **42**, 189 (1972).
10. K. Mudry, M. Constantine-Paton, R. Capranica, *J. Comp. Physiol.* **114**, 1 (1977); H. Liff, *J. Acoust. Soc. Am.* **45**, 512 (1969).
11. Unpublished measurements made in this species, as well as in other species of anurans (*6*), indicate that under these recording conditions the coupling between the two ears due to mechanical cross talk is −25 to −30 dB. Binaural interactions were therefore assumed to have a neural origin only if the intensity differences between the two ears necessary to elicit such an interaction did not exceed 20 dB.
12. R. Gaze, *Q. J. Exp. Physiol.* **44**, 290 (1959); ——— and M. Jacobson, *Proc. R. Soc. London Ser. B* **157**, 420 (1963); R. Gaze and M. Keating, *Brain Res.* **21**, 183 (1970); D. Ingle, *Science* **181**, 1053 (1973); R. Sperry, *J. Neurophysiol.* **7**, 57 (1944).
13. H. Zakon, in preparation.
14. E. Rubel, in *Handbook of Sensory Physiology*, vol. 9, *Development of Sensory Systems*, M. Jacobson, Ed. (Springer-Verlag, Berlin, 1978), pp. 135–237.
15. We thank W. Wilczynski, B. Finlay, and S. Udin for their helpful comments on early versions of this manuscript. Supported by NIH grant NS 09244.

12 January 1981

Brain 5β-Reductase: A Correlate of Behavioral Sensitivity to Androgen

Abstract. *Testosterone is converted in the dove* (Streptopelia risoria) *brain to 5β-reduced metabolites that do not affect behavior. In long-term castrated birds, which are relatively insensitive to the behavioral effects of testosterone, the activity of preoptic 5β-reductase is increased. The increase, which is specific to the preoptic area, is reversed by estrogen. Inactivation of testosterone by 5β-reduction may be involved in the control of brain sensitivity to androgen.*

Hormones affect sexual behavior by direct action on the brain (*1*). Their action is influenced in two ways: either the hormone itself can change in amount and availability or the sensitivity to hormone of the tissues mediating the behavior can change. Although sensitivity to androgen is known to be influenced by the genetic constitution of the individual (*2*), exposure to hormones during early development (*3*), environmental stimuli (*4*), and hormonal condition in adulthood (*5*), the brain mechanisms involved have not been identified. In the dove *Streptopelia risoria*, male courtship depends on the action of androgen on the preoptic-anterior hypothalamus (AHPOA) of the brain (*6*). The effectiveness of implants of testosterone propionate (TP) in the AHPOA declines with time after the male is castrated, suggesting that sensitivity to androgen changes according to hormonal status and that target cells in the AHPOA participate in the change.

Metabolism to active hormones is an important step in the action of androgen on target cells (*7*). In addition to converting testosterone to 17β-estradiol and 5α-dihydrotestosterone (5α-DHT), both of which have behavioral effects, the AHPOA of the male dove is also active in producing 5β-androstanes, 5β-dihydrotestosterone (5β-DHT), and the two corresponding diols (3α- and 3β-, 17β-diols) which have no behavioral effects (*8*) and appear to have no androgenic action in other species (*9*). The evidence (*8*) suggests strongly that the 5β-reduction pathway is part of a steroid inactivation mechanism that could influence the concentration of active androgen in the AHPOA.

Recently, we found that formation of 5β-DHT is increased in the preoptic area (POA) of long-term castrated doves compared to intact males (*8*). This is consistent with the view that testosterone inactivation increases with decline in behavioral sensitivity to androgen. Measurement of total 5β-reduction is required to test this hypothesis. Because there may have been individual differences in the effects of interaction with the female, 5β-reduction of testosterone was very variable in males tested for courtship. Therefore, we have examined the possibility that decreased behavioral sensitivity to androgen is related to increased 5β-reduction of testosterone by measuring the male vocal behavior, perch calling, which is highly sensitive to androgen and is displayed independently of the female (*10*). We report here that testosterone inactivation by 5β-reduction is markedly increased in the POA of long-term castrated doves which are relatively unresponsive to the behavioral action of testosterone. To our knowledge, this is the first report of a change in brain metabolism of a sex hormone, which can be related to both hormonal condition and the behavioral effectiveness of the hormone.

We measured total 5β-reduction (*11*) in intact, sexually active males, two groups of short-term (30-day) castrates, and three groups of long-term (200-day) castrates ($N = 6$ in each group). The short-term castrates were treated with

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