pression of this defect may share some features with the expression of the retinal sensitivity defect, our results cannot establish the site of gene action for the sensitivity defect. The fact that the ERG responses are relatively normal while ganglion cell sensitivity is reduced implies that the retinal location where sensitivity is affected lies between the photoreceptors and the ganglion cells. However, this affected location may not be the site of gene action because restoration of sensitivity is possible in the isolated retina. Finally, our findings in pearl (reduced sensitivity in the dark-adapted condition, normal a-wave response, normal concentration of visual pigment, and absence of photoreceptor degeneration) suggest that this single gene mutation (*19*) may provide a mutant model for some forms of human stationary night blindness (*20*).

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17. Mice were tested in a six-choice swimming maze. The maze was white. A piece of black sheet metal was placed against the back wall of one of the six arms of the maze. Only this "dark" compartment contained an escape ramp. The position of the black sheet and escape ramp was randomly selected for each trial. Mice were conditioned to swim to the dark compartment containing the escape ramp. After conditioning (90 to 100 percent correct responses for each of ten consecutive trials) the luminance of the maze was lowered and the mice were tested at each of many lower luminances.
18. Difference spectra were measured for isolated retinas bathed in $NaHCO_3$-buffered Ringer containing 5 m*M* NH_2OH HCl. Wild-type (N = 3): mean change in absorption (± standard error), 0.12 ± 0.3, λ_{max}, 509; pearl (N = 4): mean change in absorption, 0.16 ± 0.3, λ_{max}, 503.
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Temporal Selectivity in the Central Auditory System of the Leopard Frog

Abstract. *Amplitude modulation is a predominant temporal feature in many vocal signals. The leopard frog,* Rana pipiens, *has a class of neurons in the central auditory system that respond selectively to particular rates of amplitude modulation; these neurons can be characterized by a temporal tuning curve. Such selectivity is absent in the peripheral auditory system. This type of transformation may be fundamental in processing temporal information in the vertebrate sensory nervous system.*

The temporal structure (*1*) of species-specific vocal signals plays an important role in animal communication (*2*). The question of how the vertebrate auditory nervous system processes this information is therefore of interest. Although a great deal is now known about neural processing in the frequency domain, relatively little is known of the neural mechanisms responsible for processing information in the time domain (*3*).

A common feature in many bioacoustic signals is a periodic change in amplitude—amplitude modulation (AM)—, which is encoded in the periodicity of group discharge of single auditory nerve fibers (*4*). This raises the question, Is the neural basis of temporal processing simply the relay of this peripheral code along the central auditory pathways? We have electrophysiologically studied the peripheral and central auditory systems of leopard frogs (*Rana pipiens*). We selected anurans since temporal features, particularly AM, play a significant role in their recognition of species-specific vocal signals (*5*). We found that a novel transformation of the peripheral temporal code occurs in the leopard frog's central auditory system. This transformation gives rise to specialized neurons in the midbrain, which respond selectively to characteristic rates of AM (*6*). Each neuron's selectivity can be represented by a temporal tuning curve with a distinct "best rate" of AM.

To analyze the temporal selectivity of single neurons in the auditory nerve and in the central auditory system, we utilized sinusoidally amplitude-modulated white noise as a stimulus. The rate of AM could be varied while the spectral content of the stimulus remained constant (*7*). The eighth nerve and the optic

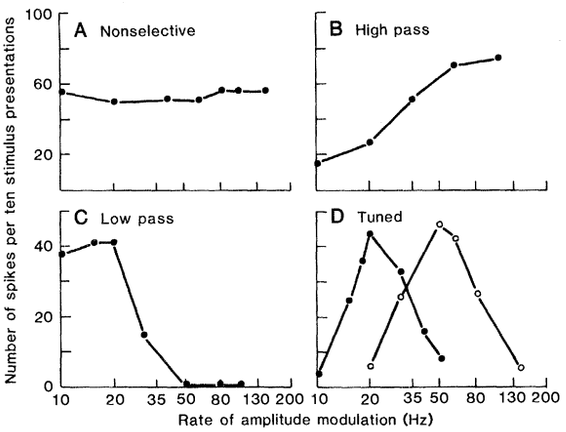

Fig. 1. Response versus rate of amplitude modulation (AM) curves from five units in the torus semicircularis. In each case the sound pressure level was held constant at 10 dB above each unit's threshold. (A) In a nonselective unit, response is independent of the rate of AM. (B) In a high-pass unit, response increases as the rate of AM in increased. (C) In a low-pass unit, response is greatest at rates of AM below 20 Hz. (D) Response versus rate of AM for two tuned units; one unit is tuned to 20 Hz and the other to 50 Hz. In all of these measurements, the depth of modulation was held constant at 100 percent.

tectum were exposed while the frogs were under urethane anesthesia (immersion in 2.5 percent urethane). The exposed areas were covered with mineral oil and the animals were allowed to recover. Two days later, they were reanesthetized and *d*-tubocurarine chloride (6 μg per gram of body weight) was injected intramuscularly to minimize any extraneous movements during the subsequent recording session.

Sinusoidally amplitude-modulated white noise was generated by multiplying white noise (Grason-Stadler 455A) with the sum of a sinusoidal voltage and d-c component (Hewlett-Packard audio oscillator 3300A) through a multiplier circuit (Analog Devices AD534LD). The AM signal was gated (using Grason-Stadler 1216A timers and 1287B electronic switches) into a 300-msec burst with 50-msec rise and fall times and was repeated once every second. The stimulus intensity could be varied by a set of audio attenuators (Hewlett-Packard 350D). The depth of modulation was controlled by varying the amplitude of the sinusoidal voltage to the multiplier circuit. Pure tones were generated by passing the output of an audio oscillator through the gating and timing system. Stimuli were delivered monaurally through a closed acoustic system (*8*). Single auditory fibers in the eighth nerve were recorded with micropipettes (20 to 40 megohms) filled with 3*M* KCl. In recording from single auditory units in the torus semicircularis contralateral to the ear being stimulated, we used micropipettes of lower impedance (3 to 20 meghoms) filled with either 3*M* KCl or 3 percent alcian blue (*9*). The recording sites in the torus semicircularis were verified by iontophoresis of alcian blue from the recording pipette (with a positive current applied for approximately 5 minutes). Action potentials were amplified, displayed on an oscilloscope (Tektronix 564B), broadcast over a loudspeaker, counted on-line with a gated electronic counter (Hewlett-Packard 5326B), and stored on a tape recorder (TEAC A2340) for off-line analysis on a computer (Digital Equipment MINC-11).

When a single unit was isolated, its best excitatory frequency to pure tones was first determined. Next, while the depth of modulation was held at 100 percent, the rate of AM was varied from 10 to 200 Hz, and thresholds in that range were measured (*10*). With the lowest threshold used as a reference, the root mean square (r.m.s.) sound pressure level was increased by 10 to 20 dB and held constant. The number of action potentials in response to ten stimulus presentations was computed at various rates of AM in the range of 10 to 200 Hz. On the basis of this iso-intensity curve, a unit was considered temporally selective only if the number of spikes at the least-preferred rates was at most 50 percent of that recorded at the most-preferred rate.

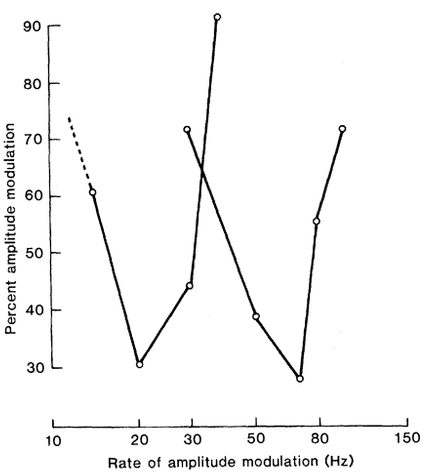

Fig. 2. Temporal tuning curves of two auditory units in the torus semicircularis. One unit is tuned to 20-Hz AM, and the other to 70-Hz AM. In all of these measurements the stimulus intensity was held constant while the depth of modulation was varied to maintain a threshold criterion. The dashed line below 14 Hz indicates that a threshold criterion number of spikes was not reached. At 100 percent AM, the signal envelope sinusoidally varies from full amplitude to zero amplitude. At lower percentages of amplitude modulation, the amplitude of the modulation envelope decreases. Zero percent AM corresponds to unmodulated white noise.

According to this criterion for temporal selectivity, all of the 114 auditory nerve fibers recorded from were nonselective in their response to amplitude-modulated noise. While the temporal periodicity of their clusters of spikes changed with modulation rate, the average number of spikes did not exhibit a significant peak indicative of a preferred rate. For approximately 33 percent of the 193 midbrain auditory neurons sampled, the response magnitude was independent of the rate of AM (Fig. 1A). Thus, like fibers of the eighth nerve, these units were not temporally selective. A smaller proportion of the auditory units in the torus (10 percent) could be classified as temporal high-pass units (Fig. 1B). Their distinguishing feature is that their activity increased monotonically as the rate of AM was increased. These neurons also responded poorly to bursts of unmodulated white noise; a phasic response usually occurred only at the beginning of a noise burst. Conversely, temporal low-pass units (16 percent) responded well to slow rates of AM but poorly at intermediate or high values (Fig. 1C). In addition, temporal low-pass neurons responded poorly to unmodulated white noise. Temporal band-suppression units (8 percent), like low-pass units, responded strongly to slow rates of AM and weakly to intermediate rates. Band-suppression neurons could be distinguished from low-pass units in that their activity increased at the higher rates of AM (> 100 Hz), and they always responded well to unmodulated white noise.

The most interesting of the temporally selective units are those tuned to particular rates of AM (*11*). These neurons (approximately 32 percent) responded maximally over a narrow range of modulation rates, whereas lower or higher rates were less effective (Fig. 1D). The temporal selectivities within this last class of units could be characterized by iso-response temporal tuning curves (Fig. 2). These temporal tuning curves were constructed in the following manner. The r.m.s. sound pressure level was held at the value used in producing the 100 percent modulation depth response function, and at each rate of AM, the

depth of modulation was increased until a threshold criterion was reached (*12*). At the best rate of AM, threshold was reached at the smallest depth of modulation; at lower or higher rates, the depth of modulation had to be increased in order to maintain a threshold level of response (*13*). In some cases, units sharply tuned to a particular rate of AM were entirely unresponsive to pure tones. (Rise and fall times were varied from 10 to 50 msec.)

Since the r.m.s. sound pressure level and spectral content of amplitude-modulated white noise remained constant as the rate of modulation was varied, the differential selectivity of auditory units in the torus is a function only of the temporal information in the stimulus (the rate at which the amplitude is modulated over time). This selectivity was not related in any direct way to a unit's best excitatory frequency to tones. Our finding that some AM tuned units failed to respond to pure tones supports the notion that the response of higher order auditory neurons to complex stimuli often cannot be predicted on the basis of their responses to pure tones alone (*14*).

Our results indicate that a transformation occurs in the manner in which the rate of AM is coded in the anuran's peripheral and central nervous systems. Unlike auditory nerve fibers, many central auditory neurons respond selectively to particular rates of AM. Further studies should reveal at what level of the auditory system this selectivity first develops. Similar transformations may underlie the encoding of other forms of temporal information in acoustic signals and in other sensory systems.

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8. The stimulus was delivered through an earphone (Permoflux PDR-10) housed in a T-shaped brass coupler. A condenser microphone (Bruel and Kjaer 4134) was inserted in one end of the T. A tapered rubber fitting, having a tip diameter slightly larger than the outer margin of the tympanic ring, was located on the other end of the coupler. The end of this fitting was placed close to but not touching the tympanum, and then sealed around the tympanic ring with silicone grease. In this manner a closed acoustic system (± 5 dB over the range 50 to 4000 Hz) was achieved without any direct mechanical connection to the eardrum. All distortion products were at least 46 dB below the level of the primary tone.
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11. A midbrain auditory unit was classified as being tuned to a particular rate of AM only if the following criteria were met: (i) The response at the preferred rate of AM had to be at least twice as great as that to very low or very high rates of AM. (ii) The temporal selectivity of the unit could not be altered by high-pass filtering the AM signal at 200 Hz.
12. Our criterion for threshold of each unit (in spikes per second) was based on its firing rate to modulated white noise compared with that to unmodulated white noise:

$$\text{Threshold} = \frac{R_m - R_u}{4} + R_u$$

where R_m represents the response to white noise 100 percent amplitude modulated at the best rate of AM and R_u represents the response to unmodulated white noise.
13. The temporal selectivity of these units was invariant over at least a 30-dB range in sound pressure level.
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Free-Running Activity Rhythms in the Rat: Entrainment by Melatonin

Abstract. *The pineal gland hormone melatonin may play a role in synchronization of rat circadian rhythms. Free-running activity rhythms of the rat were entrained by a daily melatonin injection, with entrainment occurring when the onset of activity coincided with the time of daily injections. When injections were stopped, activity rhythms became free-running again. Thus in pharmacological experiments, the time of day of melatonin administration is crucial.*

For more than a decade, it has been suggested that the rodent's pineal gland is involved in entrainment of daily activity rhythms to abrupt shifts in the light-dark cycle. Pinealectomized young rats, hamsters, and feral mice entrain faster to reversals in the lighting schedule than rats subjected to a sham operation, whereas pinealectomized old rats are slower to entrain than controls (*1*). A complete inversion of lighting (180° shift) is not necessarily the optimum strategy for testing the rate of entrainment. When an 8-hour advance of the dark period is applied, the entrainment rate of adult rats, pinealectomized when young, is greatly enhanced (*2*). Because of methodological problems in many studies, alternative interpretations of findings have been suggested (*3*). We found that entrainment is much faster for pinealectomized adult rats than for controls after a 5-hour advance of the dark period (*4*). The mechanism by which the pineal facilitates entrainment is not known.

In our laboratory the effect on free-running activity rhythms of substances that are produced by or act upon the pineal have been investigated. Daily injections of arginine vasotocin, melanocyte-stimulating hormone, and the β-adrenergic blocker, pindolol, did not entrain free-running activity rhythms. Some interference with the expression of the free-running rhythm was evident in the rats treated with arginine vasotocin. However, of particular interest was the finding that in three out of four rats the free-running activity rhythm was entrained by daily melatonin injections (*5*). We now report that daily administration of melatonin can entrain free-running circadian activity rhythms in the rat. This demonstration of entrainment of mammalian circadian rhythms by chemical means may provide a methodology for examining the process by which neural signals triggered by light are transduced into chemical messages that synchronize internal biological rhythms.

Twenty Long-Evans male rats (280 to 340 g) were housed individually in a five-