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Echo-Ranging Neurons in the Inferior Colliculus of Bats

Abstract. *Bats measure the distance to an object in terms of the time lag between their outgoing orientation sounds and the returning echo. For measurement of the time lag, the latency of response of a neuron to a stimulus must be nearly constant regardless of the stimulus amplitude and envelope. Otherwise, a large error would be introduced into the measurement. Bats have neurons that are specialized for echo ranging.*

For echolocation, some insectivorous bats emit tone pulses which are variously modulated in frequency and amplitude (*1*). Echoes coming back from objects at different places overlap and show complex envelopes and structures which are quite different from those of the outgoing sounds. The bats analyze these complex echoes from different aspects in order to echolocate. One of the fundamental aspects is echo ranging. A basic clue for measurement of the distance to an object is given by the time lag between the outgoing orientation sound and the returning echo (*2*). This time lag is presumably coded in the difference in time between the response of a neuron to the outgoing sound and that to the echo.

The latency of response of a neuron to a stimulus generally changes as a function of stimulus amplitude and rise time. If the amplitude of a stimulus such as an outgoing sound is very large, and quickly reaches the threshold of a neuron, the latency of response of the neuron will be short. If the amplitude of a stimulus such as an echo is just above the threshold of the neuron, or if it slowly increases up to the threshold, the latency of response will be long. When this occurs, a large error will be introduced into distance measurement. For echo ranging, there must therefore be neurons which show relatively constant latency regardless of stimulus amplitude and rise time. If such neurons discharge multiple impulses in response to a single stimulus, there will be no way to distinguish between one strong echo and a few weak echoes. There must therefore be neurons with constant latency which do not fire repetitively. Furthermore, the neurons must be spontaneously inactive. Any neurons which satisfy the above requirements may be considered to be specialized for the measurement of distance, and may be called echo-ranging neurons.

Neurons in the cochlear nucleus of bats fire repetitively in repsonse to a tone burst, and some of them are spontaneously active (*3*). Therefore, echo-

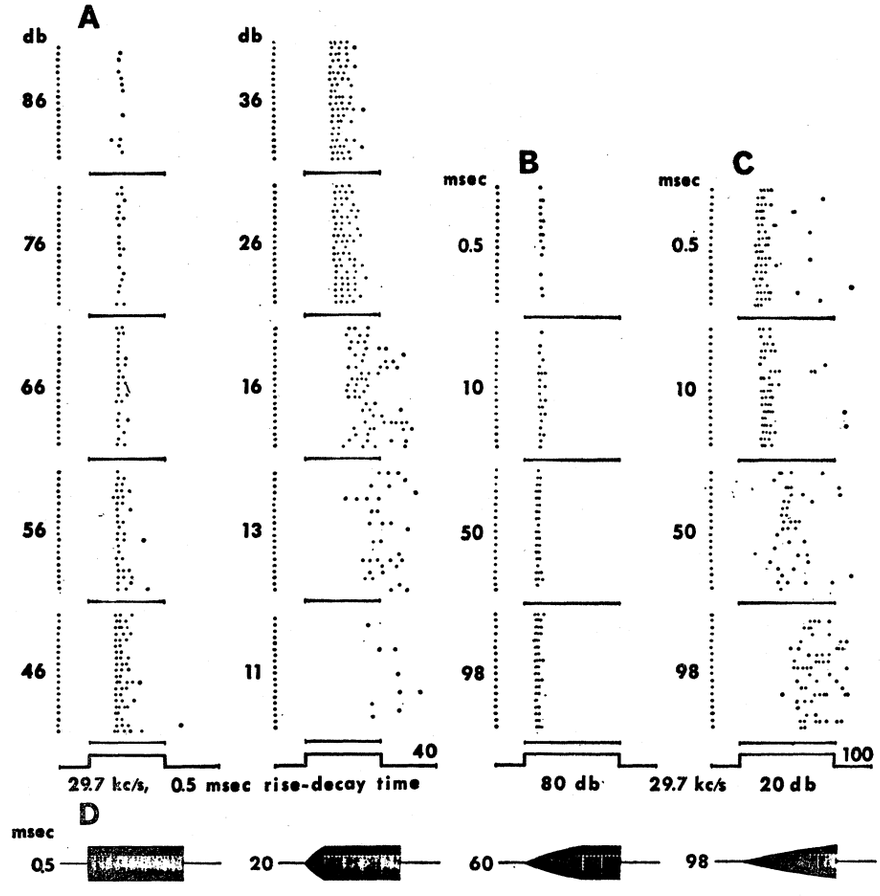

Fig. 1. Responses of an inferior collicular neuron which was not specialized for echo ranging. Responses to repeated presentations of the same tone pulse are shown by a dotted pattern in which one dot corresponds to the peak of one action potential. The sweep of a cathode-ray oscilloscope was displaced vertically before each stimulus. The dots to the left mark the start of the sweep. The sound simulus is represented by a horizontal bar and square wave at the bottom of each dotted pattern. (A) The amplitude of a 29.7-khz tone burst with a 0.5-msec rise-decay time and a 40-msec duration was attenuated from 86 db to 11 db. (B and C) The rise time of a 29.7-khz tone burst with a 100-msec duration was changed, as indicated by the number to the left of each dotted pattern. The decay time was always 0.5 msec. The peak amplitude of the tone burst was (B) 80 db and (C) 20 db. Note the changes in the latency of response and number of impulses. (D) Four samples of the 100-msec tone bursts used are shown.

ranging neurons have not been observed in this nucleus.

The inferior colliculus of bats receives impulses from the lateral lemniscus which synchronize remarkably with shortly spaced tone pulses (*4, 5*). Severe bilateral ablation of the inferior colliculus causes failure in echolocation, in spite of an unusually frequent emission of orientation sounds (*5*). Unlike the cochlear nuclear neurons, most neurons in the inferior colliculus show no spontaneous discharges and fire only a few impulses at the onset of a sound stimulus (*3*). Therefore, if echo-ranging neurons exist, they are to be found in the inferior colliculus. The superior olivary complex of bats is another area which should be investigated in this context. This complex has not yet been studied electrophysiologically.

In the present experiments, the little brown bat (*Myotis lucifugus*) and the Indiana bat (*Myotis sodalis*), anesthetized with pentobarbital sodium, were mounted on a small platform in a sound-proofed room in which the temperature was maintained at about 35°C. A small hole was made in the skull covering the inferior colliculus, and a glass micropipette electrode filled with 3*M* KCl was inserted into the colliculus through the hole while sound stimuli were delivered at a rate of two per second from a loudspeaker located 68 cm in front of the head. When the action potential of a single neuron was recorded with conventional electronic instruments, its tuning curve (or excitatory area) was measured with a 40-msec tone burst that had a 0.5-msec rise-decay time (*6*). The best frequency of the neuron was also determined. A 100-msec tone burst at the best frequency was then delivered. The amplitude increased linearly with time at the onset. The rise time was changed from abrupt to 98 msec, but the decay time was kept at 0.5 msec (Fig. 1D). A study was made of the effect of the changes in the rise time and peak amplitude of the stimulus on the response of the neuron. The peak amplitude of the stimulus was expressed in sound pressure level (decibels referred to 0.0002 dyne/cm^2 root-mean-square).

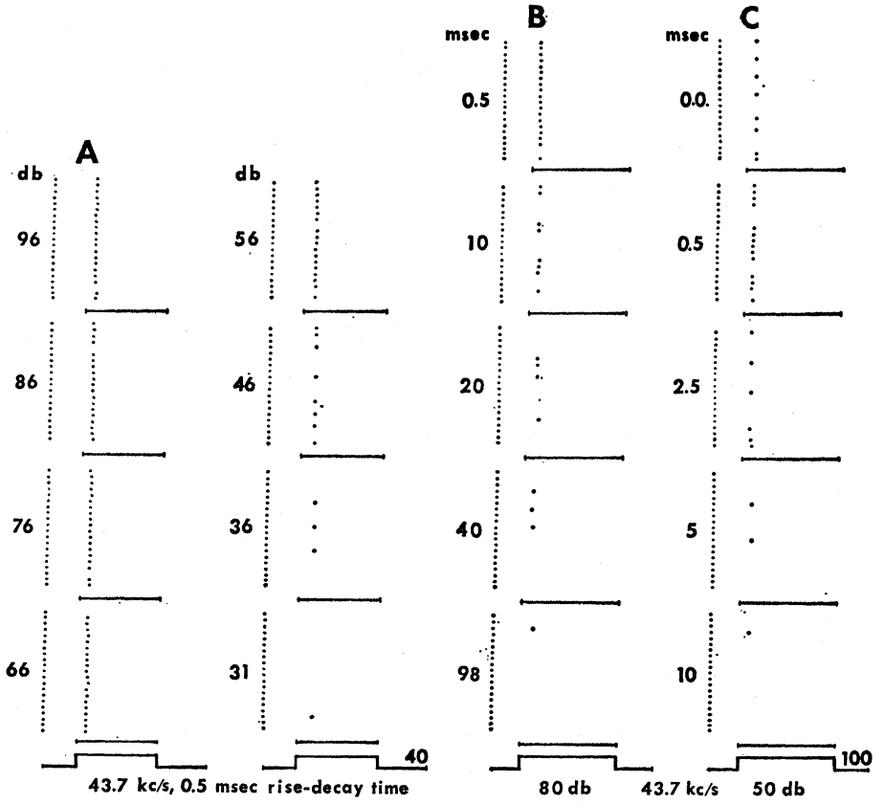

Fig. 2. Responses of an echo-ranging neuron in the inferior colliculus. (A) The amplitude of a 43.7-khz tone burst with a 0.5-msec rise-decay time and a 40-msec duration is attenuated from 96 db to 31 db. (B and C) The rise time of a 43.7-khz tone burst with a 100-msec duration was changed, as indicated by the number to the left of each dotted pattern. The decay time was always 0.5 msec. The peak amplitude of the tone burst was (B) 80 db and (C) 50 db. Note that the number of impulses is always one or less than one, and the latency of the response does not fluctuate more than ± 1.0 msec, even if the probability of response decreased down to 0.5.

Responses of 57 inferior collicular neurons to the tone bursts were studied. Most of the neurons studied discharged only a few impulses during the rising phase of the stimulus amplitude, but did not fire during the falling phase. In terms of latency of responses to tone bursts with different amplitudes and rise times, there were at least two types of phasic on-responding neurons.

In about 32 percent of the phasic neurons studied, the latency of response changed more than 60 msec as a function of stimulus amplitude and rise time (Fig. 1). The number of impulses per stimulus was either monotonically or nonmonotonically related to stimulus amplitude. When the impulse-count function was nonmonotonic, the latency of reponse sometimes changed nonmontonically. In Fig. 1A, the latency is 15.7 ± 1.51 msec at 86 db, 13.4 ± 0.67 msec at 46 db, and 26.3 ± 5.23 msec at 16 db, 5 db above the threshold. Since the rate of change in amplitude is a function of both rise time and peak amplitude, tone bursts with different peak amplitudes have different rates of change in amplitude for the same rise time. In Fig. 1, B and C, the rise time of a tone burst is changed, but the peak amplitude is kept at 80 and 20 db, respectively. Large changes occur in the number of impulses and in the latency of response with rise time. The threshold of response of such a neuron did not increase more than 10 db with a change in rise time from 0.5 to 98 msec (Fig. 3*c*). This type of neuron was not at all specialized for echo ranging.

In about 34 percent of phasic neurons, the latency of response was nearly constant, regardless of the stimulus amplitude (Fig. 2A). The change in latency with rise time was also very limited (Fig. 2, B and C). The number of impulses per stimulus changed monotonically, or slightly nonmonotonically, with the stimulus amplitude. When the rise time increased, the number of impulses per stimulus decreased, and sometimes no impulses at all were discharged for a sound with a rise time of longer than 60 msec, even at the maximum amplitude available (Fig. 2B). When the rise time was less than 0.2 msec, an acoustic transient appeared which could be heard by a human at 5 cm from the loudspeaker.

Therefore, there was a possibility that the good responses to tone bursts with a short rise time were due to the acoustic transient. The threshold of the response was, however, not lower for a tone burst with an abrupt rise time than for one with a rise time of 0.5 msec. Furthermore, the neuron showed a very high threshold for a click sound produced by a 0.5-msec square pulse. The responses to rapidly rising tone bursts were therefore not due to the acoustic transient.

When the rise time was lengthened, the threshold of the response of such a neuron increased. The difference in threshold between responses to a 100-msec tone burst with a 0.5-msec rise time and that with a 98-msec rise time was less than 10 db in 32 percent of the total phasic neurons studied, 10 to 30 db in 34 percent, and more than 30 db in 34 percent. The threshold measured with a 4-msec tone burst having a 0.5-msec rise-decay time was the same as that measured with a 100-msec tone burst having the same rise-decay time. Therefore, the increase in threshold that occurred with a slow rise was not due to the decrease in the total energy of the tone burst, caused by lengthening the rise time, but was due to the slow rise time itself.

The minimum slope of the stimulus envelope necessary for excitation of the neuron was obtained by plotting the threshold of the response against the rise time (Fig. 3). In Fig. 3, curves *a* and *b* make contact at 4 to 5 msec with the straight dashed lines, which indicate the minimum slope. This means that the neuron can respond only to a tone burst with an amplitude which reaches the threshold within 4 to 5 msec, and that the "critical latency shift" due to the rise time is 4 to 5 msec. The smallest critical latency shift obtained was 1 msec. Since only a tone burst which quickly increased in amplitude could excite the neuron, the change in latency that accompanied an increase in rise time was very limited in these neurons. These phasic on-responding neurons may be called "latency-constant units."

The latency-constant neurons usually did not show spontaneous discharges during an observation period of more than 10 minutes. They were, however, not necessarily echo-ranging units, because some of them discharged more than one impulse per stimulus. Only less than 20 percent of the neurons studied appeared to be echo-ranging

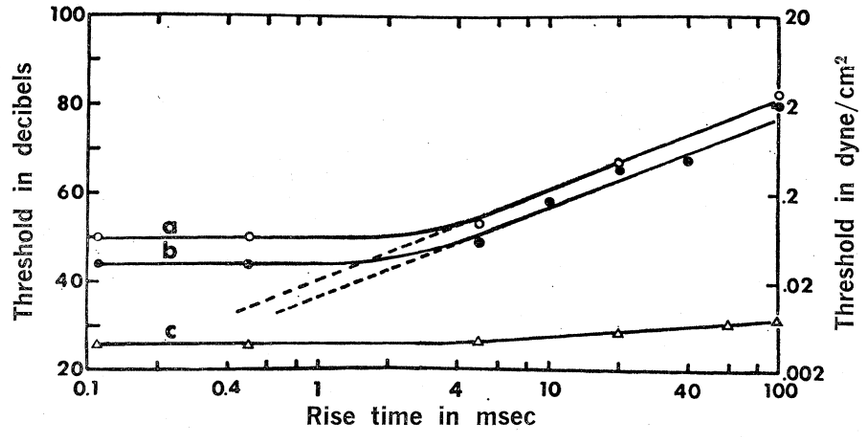

Fig. 3. Change in the threshold of response of three neurons (*a*, *b*, and *c*) with the rise time of a tone burst set at the best frequency. The duration and the decay time of the tone burst were 100 and 0.5 msec, respectively. The frequency of the tone burst was 70 khz for *a*, 32 khz for *b*, and 50 khz for *c*. The dashed lines indicate the minimum slopes of the stimuli which excited neurons *a* and *b*.

neurons. The responses of one of them are described in detail below.

The neuron in Fig. 2 had a best frequency of 43.7 khz and a threshold of 31 db for a 43.7-khz, 40-msec tone burst with a 0.5-msec rise-decay time. The *Q* value was 11 (*7*). The number of impulses was always one or less than one per stimulus, regardless of stimulus frequency, amplitude, and rise time. This extremely phasic response of the echo-ranging neuron can be explained by forward inhibition (*8*) or accommodation. At 96 db, the latency of the response was 5.7 ± 0.27 msec. Even if the amplitude was attenuated down to 56 db (one one-hundredth), the latency was 6.2 ± 0.20 msec. Both the latency of response and the number of impulses per stimulus changed only a little, so the neuron can hardly code stimulus amplitude in this range. If an outgoing sound of 96 db at the oval window and a returning echo of 56 db are assumed, the error in distance measurement for this neuron, due to amplitude difference, would be 8.6 cm on the average. Further attenuation from 56 db reduced the number of impulses per stimulus and lengthened the latency of response. The latency at 36 db (5 db above the threshold) was 9.0 ± 1.1 msec (Fig. 2A). The error, 8.6 cm, is large compared with the minimum discriminable difference in distance, which is shown by bats trained for tests of distance discrimination (*2*). A neuron which shows a much smaller change in latency than does the above example may be found in the future. In Fig. 2, B and C, the peak amplitude of a 100-msec tone burst was kept at 80 and 50 db, respectively, and the rise time was changed. The threshold of the response increased and the number of impulses per stimulus decreased with a lengthening of rise time, but the latency stayed nearly constant. When the number of impulses per stimulus decreased down to about 0.5, the latency was still 7.6 ± 1.0 msec at 80 db, and 7.8 ± 0.87 msec at 50 db. The shift in critical latency of this neuron was not very short—10 msec.

The inferior colliculus of bats receives impulses which are remarkably synchronized with the outgoing sound and with echoes coming back shortly after it (*4*, *5*). The collicular neurons are generally differentiated for processing echoes returning from different distances because they show various types of recovery cycles (*9*). This also appears to be true in the echo-ranging neurons. The echo-ranging neurons are particularly specialized for processing information about distance. The time lag between their responses to an outgoing sound and its returning echo is an accurate clue for echo ranging.

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6. The response of an auditory neuron to a stimulus can be either an augmentation or a

suppression of background activity, if any exists, so that the area above the tuning curve may be called the excitatory or inhibitory response area. Here, the excitatory response area is simply called the excitatory area.
7. The Q value is the best frequency divided by the band width of an excitatory area at 10 db above the minimum threshold.
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Coagulation Inhibitor Elicited by Thrombin

Abstract. *A protein fraction present in bovine prothrombin complex, or in prothrombin-free preparations obtained from serum, is converted by thrombin into an anticoagulant that strongly interferes with the intrinsic pathway of blood coagulation and with thrombin formation, initiated also by the extrinsic mechanism. If one assumes that a similar phenomenon takes place in vivo, the association between thrombosis and hemorrhage may be better understood.*

Thrombin, the basic procoagulant and the unique clotting factor that accounts for fibrin formation, when infused or continuously released into circulation brings about a reaction in which bleeding rather than thrombosis is a predominant symptom (*1*, *2*). The marked hemorrhagic tendency is often manifested by a prolongation of clotting time to the limit of uncoagulability, for which the accepted knowledge of hemostasis does not provide a sufficient explanation. The decrease of platelets and coagulation factors is not great enough to delay the clot formation that extensively, and the presence of heparin in blood was never convincingly demonstrated. Fibrinolysis with formation of breakdown products does not occur regularly and, at least in some experimental thrombinemias, was ruled out as a cause of impaired clotting (*2*). The present study, although made in vitro, may clarify certain mechanisms underlying abnormalities in coagulation under circumstances in which thrombin is present in the blood.

An earlier observation (*3*) indicated that a coagulation inhibitor can be isolated by chromatography from preparations of bovine prothrombin complex treated with a small amount of thrombin or converted into thrombin in strong salt solutions. Since in both cases prothrombin undergoes functional and structural alterations (*4*, *5*), the anticoagulant that became evident at this stage was considered to be a prothrombin derivative that inhibits prothrombin conversion on a competitive basis. I have now obtained evidence that a protein fraction normally present in bovine blood, and independent of prothrombin, is converted by thrombin into a clot-retarding factor of great potency. The precursor (proinhibitor) absorbs together with the vitamin K–dependent coagulation factors from plasma and serum and is therefore present in preparations of prothrombin complex.

For study, preparations were obtained according to the method described previously (*5*), with the use of barium carbonate or barium sulfate for the initial absorption of proteins. The starting material was either oxalated bovine plasma or normal bovine serum. From the chromatograms shown in Fig. 1, it is evident that inhibitor activity was elicited by thrombin in regular prothrombin preparations absorbed from plasma, as well as in prothrombin-free preparations obtained in a similar manner from serum. Thrombin as a procoagulant was eluted early from the column, and then a spectacular clot-retarding activity became evident. It is not represented by a distinct protein peak in chromatograms; therefore, it may be assumed that the corresponding protein fraction was rather small. I was also able to separate first the proinhibitor from nonactivated preparations as a fraction trailing the prothrombin peak (fraction 2) and subsequently activate it with thrombin. The prothrombin portion from the same ion exchange procedure (fraction 1) generated no anticoagulant activity upon contact with thrombin. Nonactivated serum preparations contained a weak and preformed clot-retarding activity, probably elicited by thrombin during blood clotting. Additional contact with thrombin brought about a severalfold potentiation of inhibitor. Prolongation of partial thromboplastin time in bovine plasma with inhibitor-containing fractions was often over 20 minutes. In many assays only a partial clot appeared first, and a solid one formed several minutes later. In those samples, the prothrombin consumption was greatly impaired. One hour after coagulation the two-stage determination indicated 75 to 100 units of residual prothrombin per milliliter (less than 10 units in the control).

Table 1. Influence of inhibitor on coagulation tests in bovine plasma. TPLN, thromboplastin.

Reagents used in the test	Clotting time (sec) of bovine plasma (0.1 ml)	
	Plus saline (0.1 ml)	Plus inhibitor* (0.1 ml)
Calcium	74	190
Partial thromboplastin†	58	720
Partial TPLN + kaolin	51	380
TPLN (bovine brain)†	18	28
Stypven†	24	27
Stypven + phospholipid†	13	48
Factor Xa†	31	40
Factor Xa + phospholipid†	17	83
Thrombin (purified)	15	15

* Fraction from chromatography of serum product activated with thrombin. † Calcium also present.

Table 2. Bovine inhibitor in coagulation of plasmas from different species.

Species	Partial thromboplastin time (sec) of species plasma (0.1 ml)	
	Plus saline (0.1 ml)	Plus inhibitor* (0.1 ml)
Cow	57	> 600
Man	61	50
Dog	21	42
Horse	85	61
Sheep	50	> 600
Goat	48	420

*Fraction from activated proinhibitor.

The new anticoagulant also interferes with other coagulation tests in bovine plasma (see Table 1). In all the assays, only the thrombin time remained undisturbed. Of interest is the almost paradoxical fact that the clotting mechanism was much more inhibited in the presence of lipids than in their absence. There was also a striking inhibition of the thromboplastin generation test composed of bovine reagents. Certain fractions of activated proinhibitor (Fig. 1), when used as a diluent for one of the four components, gave less than 1 percent of the original activity in the test. Human plasma was used as a substrate to eliminate a possibility of a secondary inhibition, since, as presented in Table 2, the same inhibitor that renders bovine plasma almost unclottable has little or