ceptors may have evolved into internal sensory organs encased in cartilage or bone.

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Initiation of Behavior by Single Neurons: The Role of Behavioral Context

Abstract. *Flying crickets avoid sources of ultrasound, possibly echolocating bats, by making rapid steering movements that turn them away from the stimulus. Electrical stimulation of a single, identified sensory interneuron (Int-1) elicits avoidance steering; depressing its response to ultrasound abolishes avoidance steering. Int-1 is necessary and sufficient for this behavior but only while the cricket is in flight. Thus, the sufficiency of Int-1 for eliciting this behavior is contingent on behavioral context.*

The extent to which discrete and identifiable behavioral acts are controlled by single interneurons has led to the conceptualization of key neural elements in the control of behavior, ranging from feature detectors (*1*) to command neurons (*2*). Although it is clear that certain behavioral acts can be attributed to activity in particular neurons, the role of initiating behavior has usually been assigned to premotor interneurons (*2, 3*). We now report an escape behavior in a cricket that is elicited by an identified sensory interneuron, but only when specific sensorimotor conditions are met. Moreover, activity of the neuron is necessary for production of the behavior. Thus, the accepted tests of a neuron's direct role in behavior—necessity and sufficiency (*2*)—can yield different outcomes depending on the behavioral state of the animal. We found that under specific, behaviorally relevant conditions, a sensory interneuron can take on some of the characteristics associated with decision-making (*2, 3*).

Tethered, flying crickets respond to acoustic ultrasound stimulation with short-latency movements of their appendages, head, and abdomen, which are directed away from the source; this avoidance response is reminiscent of that of moths (*4*) and is probably a bat avoidance behavior (*5–8*). We have developed an experimental preparation for intracellular recording from a minimally dissected cricket (*Teleogryllus oceanicus*) without interfering with flight behavior ["flight" was defined as visible movement of the wing stubs or rhythmic movement of the thorax and assumption of the characteristic posture of flight (*9*)] (Fig. 1A). A dye-filled microelectrode was inserted into the axon or a dendrite of a single, identified interneuron, Int-1, to permit intracellular recording of its response to ultrasound, intracellular current injection to alter its membrane potential, and iontophoretic injection of Lucifer yellow (*10*) to allow identification of Int-1 by its unique morphology (*6, 7, 11, 12*). Avoidance steering during flight was monitored from electromyograms (EMG's) recorded in the dorsal longitudinal muscles (DLM's) of the abdomen (*13*). Thus, we were able to alter the excitability of Int-1 in an animal during flight and directly observe the effects of such alteration on the behavioral response to ultrasound.

The neuromotor correlates of steering are shown in Fig. 1A. Ultrasound from the right speaker excited the right Int-1, and after a short latency (30 to 70 msec) the left DLM's of the second abdominal segment fired while the right DLM's were silent. Avoidance steering is characterized by the activation of the DLM's contralateral to the side of acoustic stimulation (*14*), which in free flight would cause the animal to turn away from the speaker. Steering never occurred unless the animal was in flight, regardless of how strongly Int-1 was stimulated by ultrasound. Int-1 responded to ultrasound with a high-frequency burst of action potentials (up to 500 spikes per over 30 to 100 msec); its response was graded with respect to sound level from 40 to 95 dB [sound pressure level (SPL)] (Fig. 1B) (*6, 7, 15*). Only when Int-1 discharged above about 220 spikes per second (*16*) were the contralateral DLM's activated.

To test whether activity in Int-1 alone is sufficient to elicit a steering response, we excited Int-1 after "anode break" (*17*). In Fig. 2A, Int-1 discharged at a high rate, 400 spikes per second (a typical response to an 85-dB, 30-kHz tone pulse), which turned on the contralateral DLM's; the ipsilateral DLM's remained inactive. Both Int-1 and the DLM's discharged for a prolonged period until Int-1 was again hyperpolarized (not shown). The latency between the first Int-1 spike and the start of the DLM response (49 ± 16.1 msec, $\bar{X}$ ± standard error of the mean) was comparable to that elicited by ultrasound in this animal (51 ± 10.3 msec) [$t(18) = -0.05$, $P > 0.10$]. Furthermore, above a rate of 220 spikes per second in Int-1, the DLM response was positively correlated with spike rate

Table 1. Activity in Int-1 and avoidance behavior. Values are given as means ± standard errors of the mean.

Measure	*N*	Int-1 membrane potential		Mean difference	*t**	*P*
		Normal	Hyperpolarized			
Int-1 spike rate (spike per second)	9	325 ± 16.8	167 ± 13.5	157 ± 9.20	17.1	<0.0005
Relative DLM response (arbitrary units)	9	1.0 ± 0.30	0.03 ± 0.05†	1.00 ± 0.16	6.44	<0.0005

*One tailed *t*-test for paired samples. †Not significantly different from zero [$t(9) = 1.33$, $P > 0.10$].

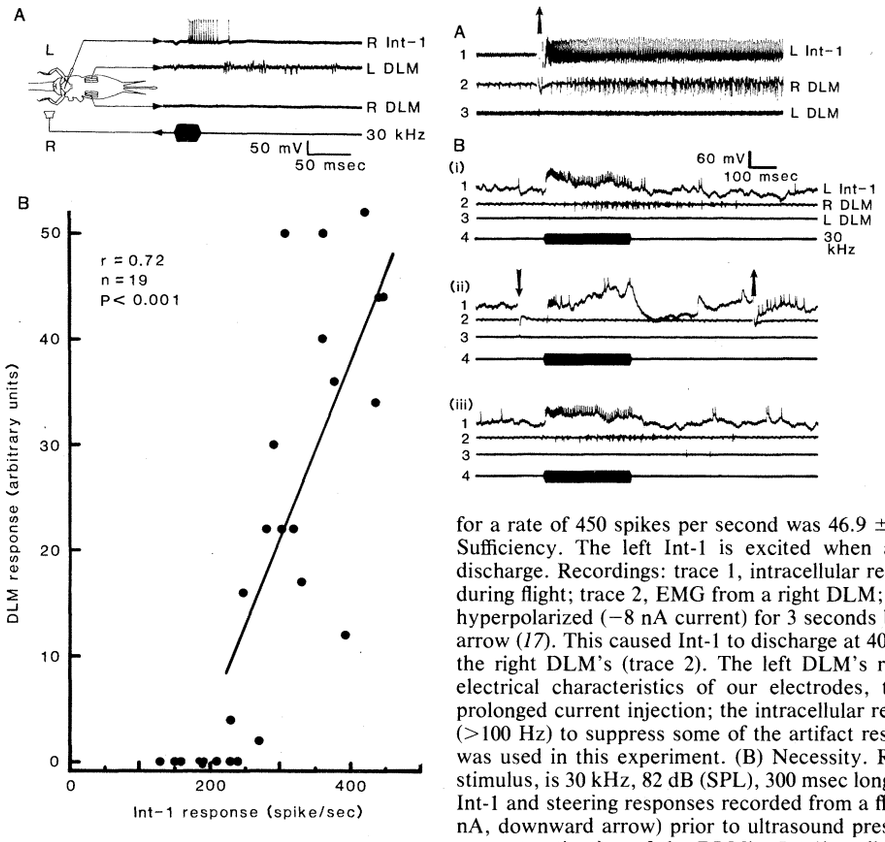

Fig. 1 (left). (A) The experimental preparation. The cricket was positioned ventral side up. Simultaneous recordings were made during flight from Int-1 (*21*) and both the left (L) and right (R) abdominal DLM's (*13*). A 30-kHz, 85-dB, 30-msec sound pulse was presented from a loudspeaker 90° to the animal's long axis. In this example, the microelectrode crosses the midline to record from the axon of the right Int-1; the sound pulse is from the right. The Int-1 record is shown above that of the contralateral DLM's. Ordinal calibration applies only to the intracellular records. (B) Relation between the discharge rate of Int-1 and steering in flying crickets (*22*). The line $y = 0.174x - 31.4$ was calculated from the linear regression of the DLM response for Int-1 spike rates greater than 220 spikes per second. The slope was 0.174 ± 0.10 (±95 percent confidence interval); the predicted DLM response for an Int-1 spike rate of 250 spikes per second was 12.1 ± 4.41 and that for a rate of 450 spikes per second was 46.9 ± 5.69 (± standard error). Fig. 2 (right). (A) Sufficiency. The left Int-1 is excited when anode break (*17*) causes the right DLM's to discharge. Recordings: trace 1, intracellular recording from a dendritic region of the left Int-1 during flight; trace 2, EMG from a right DLM; trace 3, EMG from a left DLM. Int-1 had been hyperpolarized (−8 nA current) for 3 seconds before the current was turned off at the upward arrow (*17*). This caused Int-1 to discharge at 400 spikes per second initially (*21*), which excited the right DLM's (trace 2). The left DLM's remained silent (trace 3). Because of the poor electrical characteristics of our electrodes, the bridge would not stay in balance during prolonged current injection; the intracellular recording of Int-1 (trace 1) was high-pass filtered (>100 Hz) to suppress some of the artifact resulting from anode break. No acoustic stimulus was used in this experiment. (B) Necessity. Recordings are as in (A). Trace 4, the acoustic stimulus, is 30 kHz, 82 dB (SPL), 300 msec long, played to the left ear (*22*). (i) Control: Normal Int-1 and steering responses recorded from a flying cricket (*9*). (ii) Hyperpolarizing Int-1 (−15 nA, downward arrow) prior to ultrasound presentation depressed its discharge sufficiently to prevent activation of the DLM's. Int-1's spike rate was reduced from about 330 spikes per second to less than 170 spikes per second. Hyperpolarizing current was terminated at the upward arrow (*18*). (iii) Control trial following hyperpolarization in (ii): both the left Int-1 and the right DLM regained responsiveness to ultrasound. Approximately 200 to 300 msec elapsed between (i) and (ii) and between (ii) and (iii). Voltage calibration refers only to the Int-1 records in (B).

($r = 0.91$, $P < 0.01$, $n = 8$). In an intact flying animal, such activation of the contralateral DLM's would be sufficient to produce a strong steering response, similar to that produced in response to a pulse of ultrasound (30 kHz, 80 to 85 dB). As long as anode break caused Int-1 to discharge above about 220 spikes per second, the contralateral DLM's were activated; if the rate was too low, the DLM's did not respond. Finally, if the animal was not flying, the DLM's did not respond, even if Int-1 discharged at rates in excess of 500 spikes per second. Hence, only under the behaviorally relevant conditions of flight was the firing of Int-1 sufficient to initiate steering.

To test whether Int-1 is necessary for ultrasound avoidance steering during flight, we stimulated Int-1 with a hyperpolarizing current, which moved its membrane potential farther away from spike threshold and so lowered its excitability to the synaptic currents generated by an ultrasound stimulus. When this was done just before acoustic stimulation [Fig. 2B(ii)], the number of action potentials produced by ultrasound was reduced, coinciding with the complete failure of the abdominal steering muscles to respond to ultrasound [compare with Fig. 2B(i)]. When the hyperpolarizing current was turned off, Int-1's membrane potential rebounded to resting level (*18*). The effects of hyperpolarizing Int-1 were completely reversible [Fig. 2B(iii)]: both Int-1 and the DLM's recovered their responsiveness to ultrasound as soon as the current ceased (Table 1). It was not necessary to suppress activity in Int-1 completely to eliminate steering, but only to lower the spike rate below about 220 spikes per second. We conclude that activity in Int-1, above a critical threshold level, is necessary to initiate ultrasound avoidance steering.

Examples of single-neuron control of behavior, in which the criteria of necessity and sufficiency can be applied in "naturally" behaving preparations are rare (*2*). Perhaps our most interesting finding is that Int-1, a primary auditory interneuron (*6*), is sufficient for avoidance steering only in a "flying" animal (the proper behavioral context) and only if it discharges above a relatively high, but behaviorally relevant, spike rate. Int-1 does not make direct connections with the motoneurons responsible for abdominal steering; rather, it receives innervation from auditory receptors in the first thoracic ganglion and sends its axon to the brain (*19*). Because of Int-1's position in the sensory-to-motor hierarchy, its influence on the behavior is unique; Int-1 functions as a feature-sensitive unit (a "bat-detector") that sends a graded signal to the flight steering system (*6, 7*). The ability of Int-1 to affect steering, however, is contingent on the behavioral state of the animal; steering is gated by the action of the flight oscillator. Similar gating of sensory information by a flight oscillator has been reported in locusts (*20*).

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12. Int-1 was initially identified physiologically with subsequent verification by intracellular staining with Lucifer yellow (*10*). The "necessity" and "sufficiency" results were repeated in four and five animals respectively.
13. The abdominal DLM's are selectively activated during abdominal steering movements in crickets (*14*). The avoidance steering behavior involves the wings, all the appendages, and major body segments, as well as the abdomen (*5, 7*). Conventional EMG recordings, using differential a-c amplification, were made from the left and right DLM's of the second abdominal segment. The spike-coded DLM response was electronically integrated after full-wave rectification. Initiation of the behavior was quantified from the integrated EMG response, measured over the duration of the 300-msec ultrasound pulse. The values used in Fig. 1B and Table 1 are expressed in arbitrary units.
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15. Root-mean-square SPL's, expressed in decibels referred to 20 μPa, were measured with a 1/4-inch Bruel & Kjaer 4135 microphone and 2203 meter. The sound field was uniform to ±1 dB within 3 cm of the cricket.
16. In other experiments the critical spike rate in Int-1 ranged from 180 to 250 spikes per second.
17. We were unable to pass sufficient depolarizing current through our electrodes to excite the cell above the threshold spike level required for the behavior. However, we could cause Int-1 to discharge on release of negative current (anode break). The strength and duration of the discharge depended on the amount and duration of current. High discharge rates lasting as long as 3 seconds were typical after several seconds of stimulation. The mechanism of such dramatic anode break excitation is not clear, but it is a characteristic of several cricket auditory interneurons (*7*). To allow comparisons with acoustic stimulation, only the first 300 msec of the integrated EMG response was used in these sufficiency tests (*13*).
18. Because of the short period of current stimulation, the rebound excitation after anode break in Fig. 2B(ii) produced only a small discharge compared with that shown in Fig. 2A (*17*); this modest discharge was insufficient to cause steering.
19. Crickets have one bilaterally symmetrical pair of Int-1's. The dendrites ramify ipsilaterally (with respect to the side of origin of the major auditory innervation) in the auditory neuropile of the prothoracic ganglion. The axon projects through the ipsilateral neck connective, terminating in the ipsilateral subesophageal ganglion and ipsilateral regions of the brain (*6*).
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21. Int-1 was recorded intracellularly with a d-c electrometer (and conventional bridge circuitry) through the use of 100- to 200-megohm Lucifer yellow electrodes. The spike rate in Int-1 was defined as the number of action potentials during the first 100 msec of auditory response divided by 0.10 seconds.
22. Int-1 responds to ultrasound over a wide range of pulse durations between 0.5 msec and >1 second. For convenience in the experiments illustrated in Figs. 1B and 2B and Table 1, we used a 300-msec pulse of ultrasound, which produced responses in Int-1 and the DLM's similar to those produced by a batlike train of short pulses (*6, 7*).
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Smooth Curve of Evolutionary Rate: A Psychological and Mathematical Artifact

Gingerich (*1*) has shown that many of our orthodox comparisons for evolutionary rates between different groups of organisms are wrongly stated as a result of measurement over different temporal scales. Thus, he demonstrates that vertebrates may not evolve more rapidly than invertebrates, while Pleistocene mammals may not exceed their earlier Tertiary forebears in rate of change.

Nevertheless, Gingerich's quantitative apparatus for illustrating this phenomenon is an artifact of human psychology, and his chosen mode of mathematical plotting is not a property of the empirical world. He bases his conclusions on the continuous and straight array of data, with slope of −1.0, for a plot of the natural logarithm of the evolutionary rate (in darwins) as the ordinate and the natural logarithm of the measurement interval (in millions of years) on the abscissa.

The formula for rate in darwins (*2*) is the ratio of final to initial state, or ln x_2 − ln x_1 (where x is the morphological character being measured at times t_2 and t_1) divided by time in millions of years. (Comparison of rates in darwins is valid regardless of the time interval if the organisms' rate of change is exponential. If rates of change are not exponential, then comparisons are valid only if the same time interval is used for both organisms.) Gingerich then regresses this ratio against time in millions of years. In other words, Gingerich had plotted time (on the abscissa) against its own reciprocal ($1/t$) on the ordinate, and his negative slope arises necessarily from this artificial redundancy. Hallam (*3*) has commented on this problem, while Bonner (*4*) avoided just such an artifact by considering rate in darwins with respect to time as a univariate problem (*5*).

The plotting of a variable against its reciprocal is not devoid of empirical information if the numerator of the ratio is free to vary. The slope must be negative (unless the numerator varies directly with and at least as strongly as its denominator), but it may take a variety of forms with interesting implications. However, in Gingerich's case, as he notes with some puzzlement (*1*, p. 160), the numerator has an average value of 1.2 at all values of the abscissa. In such a situation, the slope must be −1.0 because the plot really does reduce to k/t as a function of t, where k is a constant.

But why should the numerator, a measure of absolute evolutionary change in a lineage, be constant? Surely this invariance cannot be a property of the world. Amounts of evolutionary change smaller than ln x_2 − ln x_1 = 1.2 must exist unless all changes are *per saltum* to this degree. Likewise, larger amounts of change must occur if mammals have their ultimate ancestors in prokaryotes and all organisms are related by evolutionary descent. The average value of 1.2 at all scales must be a property of human perception, not of the world. That is, we rarely notice smaller degrees of change, while larger amounts are accompanied by so much uncertainty about actual ancestors and descendants that we do not identify lineages with confidence and do not make the evolutionary links. Gingerich says as much when he states: "Organisms differing by a factor of much more (or less) than 1.2 are so different (or so similar) that they are rarely compared in calculating rates" (*1*, p. 160). Nonetheless, whatever the reason for a constant average numerator, the simple fact of its invariance renders the smooth and straight form of Gingerich's plot a mathematical necessity, not a source of empirical information.

Incommensurability of categories represents another problem with Gingerich's analysis. The very fast rates (up to 60,000 darwins) for laboratory selection experiments and the very low rates of longest intervals (less than 1 darwin) are not random samples of their scales and cannot, therefore, be directly compared. As Schindel argues (*6*), the fastest rates are for populations when they change, and do not include the millions of natural populations that are not evolving and would display rates in darwins of flat zero. The slowest rates do represent a potential average for all lineages (since all change to some degree over such long intervals), but they are so low primarily because they average long periods of stasis with shorter intervals of change at higher rates. Even Gingerich, a leading gradualist in the so-called gradualist-punctuationalist debate, says as much: "The longer the interval, the more stasis and evolutionary reversal are likely to be averaged in the result" (*1*, p. 160).

This analysis does not deny the value of Gingerich's main practical point: proper comparison of rates must either