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Habituation and Sensitization of Startle Reflexes Elicited Electrically from the Brainstem

Abstract. *Repetitive elicitation of startle-like responses by electrical stimulation of the cochlear nucleus led to sensitization followed by habituation. In contrast, repetitive elicitation of startle-like responses by electrical stimulation of the reticular formation led only to sensitization. Since these different locations represent different points along the acoustic startle circuit, the data suggest that sensitization may be related to the motor side of reflex arcs, whereas habituation may be related to the sensory side.*

Repetitive elicitation of reflex behavior is thought to involve both decremental (habituation) and incremental (sensitization) processes that interact to determine response strength (*1*). In invertebrates, the cellular mechanisms underlying habituation and sensitization are independent (*2*). In more complex vertebrate systems, the two processes are assumed to be mediated by different neuronal systems, although only a few direct demonstrations support this assumption (*3*). Habituation results from stimulus repetition; sensitization can be caused by either stimulus repetition or exposure to stimuli other than those used to elicit the reflex. The acoustic startle reflex in the rat shows habituation to a repeated acoustic stimulus but sensitization to high levels of background white noise or a repeated visual stimulus (*4*). Recently we have attempted to delineate the neural circuit that mediates acoustic startle and have found that startle-like responses can be elicited by electrical stimulation at various points along this circuit (*5*). If habituation and sensitization involve different neural processes, they might be separated by eliciting startle electrically from different parts of the acoustic startle circuit. We now report that startle elicited by electrical stimulation of the cochlear nucleus (CN) becomes sensitized and then habituated, whereas that elicited by stimulation of the reticulo-spinal tract becomes sensitized but not habituated.

Male albino rats had bilateral monopolar electrodes implanted in either the CN, which forms the first central synapse in the acoustic startle circuit, or in the nucleus reticularis pontis caudalis (RPC), whose cell bodies form the reticulo-spinal tract that mediates the motor side of startle (*6*). One week later, the rats were placed in cages in which startle reactions were recorded (*7*), and 1 minute later were given single stimuli (1 msec, 25 to 100 μA to each electrode) bilaterally in either the CN ($N = 8$) or the RPC ($N = 8$) every 30 seconds (*8*). A total of 60 stimuli were applied over the 30-minute test session. Throughout testing, a constant level of 80-dB background noise was maintained. In addition, control rats ($N = 8$) were tested for acoustic startle elicited by 60 50-msec, 110-dB tones spaced 30 seconds apart.

Figure 1A shows that startle elicited acoustically or electrically through the CN showed an initial increase in amplitude followed by a gradual decrease toward the end of the session [for acoustic stimuli $F(14, 98) = 2.68$, $P < .01$; for CN stimulation $F(14, 98) = 2.71$, $P < .01$]. In contrast, startle elicited through the RPC increased across the session and did not subsequently decline [$F(14, 98) = 4.24$, $P < .001$].

Under these conditions, startle behaved differently when electrically elicited through the CN or the RPC. The results with acoustic and CN stimulation are consistent with prior reports (*4*), in which it was theorized that repetitive presentation of the eliciting stimulus produced habituation, whereas concomitant exposure to background noise produced sensitization. The net decrease in startle amplitude across the session was thought to result because the decremental effects of habituation overcame the incremental effects of sensitization. The progressive increase in startle amplitude with repetitive RPC stimulation in our study could have resulted from (i) repetitive stimulation of the RPC itself producing sensitization but no habituation, leading to a net increase in startle across the session, and (ii) exposure to background noise producing sensitization which led to a progressive increase across the session, since RPC stimulation did not produce habituation.

Previous experiments (*4*) have shown that the amplitude of the startle reflex elicited by a single tone increased as the duration of prior exposure to background noise was increased. This facilitatory effect was greatest after 30 minutes of prior exposure to background noise. When startle-eliciting tones were delivered repetitively following a 30-minute exposure to background noise, startle showed a pronounced and relatively smooth decline in amplitude within the test session. That is, once sensitization to background noise had reached a maximum (after 30 minutes), a relatively pure

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measure of habituation to the tones could be measured. If the response increments shown in Fig. 1A resulted primarily from continuous exposure to background noise, 30 minutes of exposure to noise itself should be sufficient to enhance response amplitude to a high level in all three groups. Moreover, if habituation occurs when the reflex is activated by tones or electrical stimuli to the CN but not to the RPC, one would expect a response decrement during repetitive tones or CN stimulation, but none during RPC stimulation.

To test this, we implanted rats with electrodes in the CN ($N = 8$) or RPC ($N = 8$). Eight more rats were set aside for subsequent acoustic testing. One week later each rat was placed into the startle test cage and 1 minute later given two electrical stimulations or two tones separated by 30 seconds to establish a presensitized baseline. All rats were then exposed to 80-dB noise for 30 minutes followed by 60 electrical or acoustic stimuli, one stimulus every 30 seconds. Noise was maintained at 80-dB throughout testing.

All three groups had responded similarly at the beginning of the session (Fig. 1B). After 30 minutes of exposure to background noise, all three groups showed a marked and essentially equivalent increase in amplitude when startle elicitation finally began [$F(1, 23) = 14.81$, $P < .001$]. This result indicates that exposure to noise by itself was sufficient to produce sensitization with no need for repetitive reflex elicitation. Thereafter, both the acoustic and CN groups showed a decrease in startle amplitude [F_{tones} (14, 98) = 3.45, $P < .001$; $F_{CN} = (14, 98) = 3.72$, $P < .001$]. In contrast, the RPC rats showed no decline over the comparable test period, leading to a significant interaction of group with trial block [$F(28, 196) = 2.32$, $P < .001$]. In fact, the RPC curve was essentially flat over the period of reflex excitation, as one would predict if sensitization to noise had reached its maximum and was not followed by habituation.

The finding that noise increased startle after stimulation of the RPC or CN suggests that sensitization to noise may involve changes in later parts of the acoustic startle circuit. Thus, sensitization could occur in the RPC or in the spinal cord. On the other hand, habituation under these conditions seemed to occur only in earlier parts of the circuit, since the response decreased during repetitive stimulation through the CN but not through the RPC (*9*). Moreover, the habituation process in the earlier parts of the circuit must be effective, since it can overcome a progressive sensitization in later parts of the circuit. These data represent, therefore, the first instance (to our knowledge) in which different anatomical loci within a neural circuit in the vertebrate have been implicated in the processes of habituation and sensitization. Moreover, they support the view that the two phenomena are independent and have different underlying neural processes.

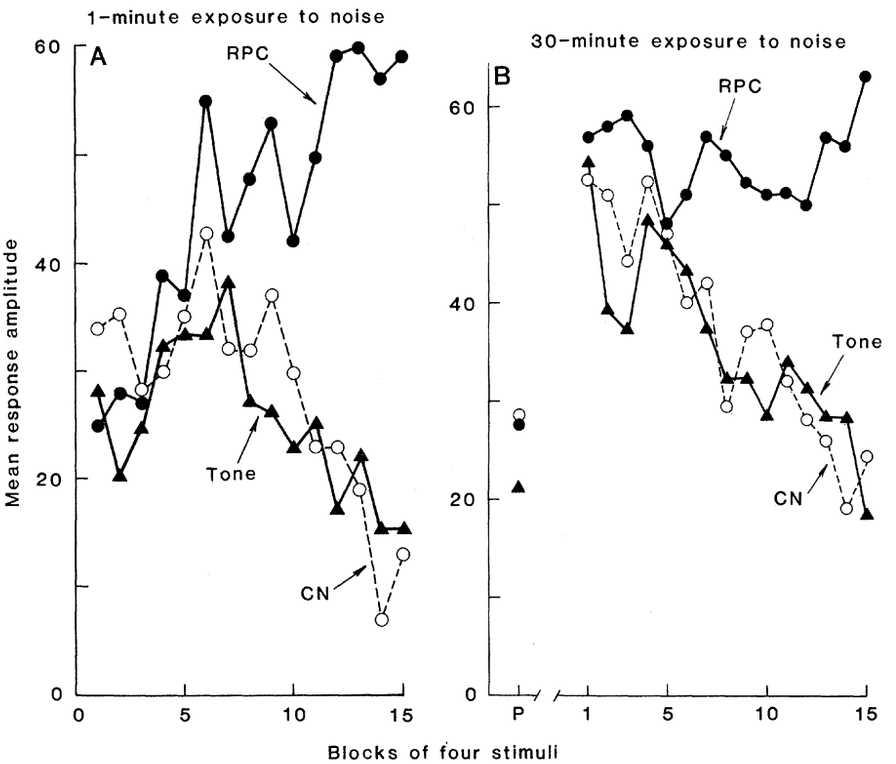

Fig. 1. (A) Responses over successive blocks of four stimuli when startle was elicited acoustically (triangles) or electrically from the cochlear nucleus (*CN*) (open circles) or the nucleus reticularis pontis caudalis (*RPC*) (closed circles). (B) Responses over two initial stimuli and then over successive blocks of four stimuli that began 30 minutes later when startle was elicited acoustically (triangles) or electrically from the cochlear nucleus (*CN*) (open circles) or the nucleus reticularis pontis caudalis (*RPC*) (closed circles).

More generally, the data suggest that sensitization might be prominent in the circuitry involved in the motor side of reflexes, whereas habituation may occur only when longer sensory-motor pathways are activated. The generality of this idea is greatly strengthened by the results of Sanes and Ison (*10*) using the corneal reflex in humans. In this case repetitive presentation of the eliciting stimulus led to sensitization of the early component of the electromyographic response (EMG) mediated by a short, presumably disynaptic pathway. Repetitive presentation of the same stimulus, however, led to habituation of a later component of the EMG measured in the same muscles, mediated by a longer, polysynaptic pathway. Our startle data and those obtained from humans suggesting that sensitization may be especially important in modulating motor aspects of reflex behavior in multisynaptic pathways have implications for theories of habituation and sensitization.

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6. Electrodes were made from 00 insect pins (diameter, 0.25 mm) insulated to within 0.5 mm of the tip. After being sterilized, they were implanted bilaterally in rats lightly anesthetized with chloral hydrate (400 mg per kilogram of body weight). For CN rats, the coordinates were 2.5

mm posterior to lambda, 3.4 mm lateral to the midline and 8.5 mm beneath the top of the skull. For RPC rats they were 2.0 mm posterior, 1.0 mm lateral, and 9.2 mm deep. The electrodes were then cemented in place on the skull and attached to a 22-gauge wire to which "bubble clips" or jeweler's "pin clutches" could be attached. Another 22-guage wire was attached to a skull screw to serve as an indifferent electrode. After being tested, the rats were killed and perfused with 10 percent Formalin. The brains were removed and electrode placements in the CN or RPC were verified by examining serial histological sections stained with cresyl violet.

7. The apparatus to measure startle has been described [G. T. Weiss and M. Davis, *Pharmacol. Biochem. Behav.* **4**, 713 (1976)]. Briefly, an 8 by 15 by 15 cm Plexiglas and wire mesh cage suspended between compression springs within a steel frame was used. Cage movement resulted in displacement of an accelerometer where the resultant voltage was proportional to the velocity of displacement. Startle amplitude was defined as the maximum accelerometer voltage that occurred during the first 200 msec after the startle stimulus was delivered and was measured with a specially designed sample and hold circuit. The stabilimeter was housed in a lighted, ventilated, sound-attenuated chamber, 50 cm from a high-frequency speaker. The acoustic startle stimulus was a 50-msec, 110-dB burst of white noise having a rise-decay time of 0.1 msec. Background white noise was 80 dB. Sound-level measurements were made within the cages with a sound-level meter (General Radio 1551-C) (A scale).
8. Electrical stimuli were monophasic, negative-going single pulses 1 msec long delivered through a mercury slip ring from a constant-current stimulator. One or two days before the main experiment, rats were tested with a few bilateral shocks to determine the intensity required to produce a startle of about 20 to 40 units. These intensities, which ranged from about 25 to 100 μA to each electrode, were then used in the main experiment. Subsequent histology showed that higher currents were generally required to elicit startle when electrode tips were somewhat off target. However, placements on target occasionally required higher currents, perhaps because of local tissue damage. The electromyographic topologies of the responses to acoustic stimuli were similar to those to electrical stimulation of the CN or RPC (*5*).
9. If rapid rates of stimulation through the RPC are used (for example, 1 stimulus per second), responses will decline. Hence a separation of habituation and sensitization may depend on the exact interstimulus intervals used.
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A New Role for Temperature in Insect Dormancy: Cold Maintains Diapause in Temperate Zone Diptera

Abstract. *In early autumn, high temperatures terminate diapause in the alfalfa blotch leafminer* Agromyza frontella*; low temperatures maintain diapause. These responses subserve a thermally malleable dormancy and allow flexibility in the annual number of generations. The view that favorable conditions cannot reverse the course of diapause are contradicted by the data on* A. frontella. *A better understanding of the diverse seasonal adaptations that insects have evolved may add precision to life history studies and phenological models in insect pest management.*

Diapause, a physiological state of developmental and reproductive suppression, is a prime synchronizer of insect seasonal cycles. It is regulated by token stimuli (primarily photoperiod and temperature) that allow the insect to anticipate approaching seasonal changes (*1–4*). The specific action of natural photoperiod and temperature has been characterized for very few natural populations, particularly as they undergo overwintering (*3, 5, 6*). This omission limits the interpretation of how life histories evolve, and it impedes the construction of accurate phenological models in insect pest management. For example, it is generally considered that once fully initiated, diapause is not reversible, even under favorable conditions, until after certain physiological changes have occurred. In many species of insects from the temperate region these changes take place at low temperatures (compare vernalization) (*1, 3, 7, 8*). Despite evidence to the contrary (*3*), researchers and writers of general texts (*9, 10*) frequently assume that low temperatures are required to accelerate diapause termination. Our results with the alfalfa blotch leafminer *Agromyza frontella* (Rondani) (Diptera: Agromyzidae) contradict the common finding that the course of diapause is irreversible under environmental conditions that favor growth and development. The results also show that low temperature can delay, rather than hasten, the completion of diapause in the temperate zone.

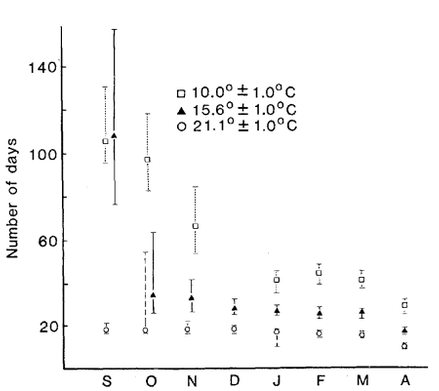

Fig. 1. Median time to emergence from September through April after transfer of overwintering *A. frontella* puparia from outside locations into three thermal conditions in a light-dark photoperiod of 16 hours of light and 8 of darkness (LD 16:8). Variation indicates emergence of individuals at 10th and 90th percentiles. Sample sizes 22 to 78; mean ± 1 standard deviation = 43 ± 11. See (*16, 17*) for details.

Agromyza frontella is a European species that was introduced into North America in the 1960's. Since then, it has become widespread in the northeastern United States and in Canada (*11, 12*), where it has caused significant damage to alfalfa. Females oviposit into the mesophyll of alfalfa leaflets, and the three larval instars mine and feed between the epidermal layers. Mature larvae exit from the mines and drop to the soil for pupation. In eastern North America, *A. frontella* produces three complete, and in some areas, a fourth or fifth generation per year (*11–14*). Overwintering is accomplished by partially developed pupae within puparia, at a soil depth of approximately 2 cm (*13*).

To investigate the overwintering of a natural population, specifically to establish the influence of naturally occurring temperatures on diapause maintenance and termination, we tested the thermal responses of a field population throughout dormancy (*15*). Our data (Table 1 and Fig. 1) (*16*) show that both photoperiod and temperature have roles in regulating diapause; however, temperature dominates. At 21°C, emergence of *A. frontella* individuals occurs within 18 to 19 days during all months from September through March; this means diapause is reversed at that temperature. During September and October, development at 10°C proceeds at approximately one-sixth the rate of that at 21°C; in November the rate of development at 10°C increases to about one-fourth of that at 21°C. Subsequently, in January, February, March, and April, development at 10°C stabilizes at approximately one-third the rate at 21°C—the level characteristic of postdiapause (*17*) and nondiapause (*18*) development.

Diapause in *A. frontella*, as in other insects (*19*), is a dynamic state, and the thermal responses of the insects change throughout its course. During September, diapause development is very sensitive to diapause-maintaining temperatures; both 10°C and 15.6°C retard the rate of diapause development. During October and November, thermal maintenance of diapause decreases; for example, by the end of October 15.6°C no longer decelerates diapause development, and by the end of November the

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