Cerebellar Vermis: Essential for Long-Term Habituation of the Acoustic Startle Response

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The acoustic startle response in rats shows both short-term habituation, which recovers in seconds or minutes, and long-term habituation, which is effectively permanent. Lesions of the cerebellar vermis significantly attenuated long-term habituation without affecting the short-term process or altering initial response levels. In this response system the cerebellar vermis is part of an essential circuit for long-term habituation.

HABITUATION, THE WANING OF A response as a result of repeated application of a stimulus, is a near universal form of behavioral plasticity (*1*). Thorpe (*2*) considered habituation the simplest form of learning, and habituation and learning share many characteristics (*3*). Although often considered a transient process (*1*), an ever-growing body of evidence (*4–6*) shows that habituation can be a relatively permanent behavioral change, being retained for periods of days, weeks, or months. Such data enhance the applicability of habituation as a model for more complex forms of learning.

In many response systems this relatively permanent process is accompanied by an independent short-term process that lasts for seconds or minutes (*4–6*). One of the most commonly used response systems for studies of habituation, the acoustic startle response in the rat, shows these two independent processes. The neural circuits essential for the response itself are in the lower brainstem (*7*), and the mechanisms for short-term habituation are assumed to involve synaptic depression intrinsic to that neural pathway (*1*). The mechanisms for long-term habituation apparently are extrinsic to that pathway and involve an inhibitory process superimposed upon the pathway (*6*). However, we know little of the structures essential for this long-term behavioral change.

It has been argued that the cellular basis of behavioral change cannot be understood until the problem of localization is solved (*8*). Significant progress has been made in analyzing the cellular basis of habituation and associative learning in invertebrates (*9*), and, essential to this enterprise, has been the analysis of the neural wiring diagrams. In mammals the problem of localization for a basic form of associative learning, the classically conditioned eyelid response in the rabbit, may soon be solved (*8, 10–11*). The lateral cerebellum and its associated nuclei and pathways appear to be essential for acquisition and retention of this response. We now report that the cerebellar vermis is essential for long-term habituation of the acoustic startle response in rats.

In experiment 1 the cerebellar vermis was partially removed by aspiration in ten 120-day-old, experimentally naïve male albino rats. Seven rats served as unoperated controls. Behavioral testing began a minimum of 2 weeks after surgery. The acoustic startle response was measured after 96-dB, 1-second bursts of white noise superimposed on a 72-dB background of white noise (*12*). Animals first received three 3-minute adaptation sessions in the startle chamber on two consecutive days. On each of the next 6 days rats were given three 3-minute sessions separated by 1 hour. The startle stimulus was presented once in each session immediately following the first minute of the session. After these sessions short-term habituation was tested with 18 trials at 20-second interstimulus intervals on each of three consecutive days. Five days later long-term habituation was assessed again in the original three-

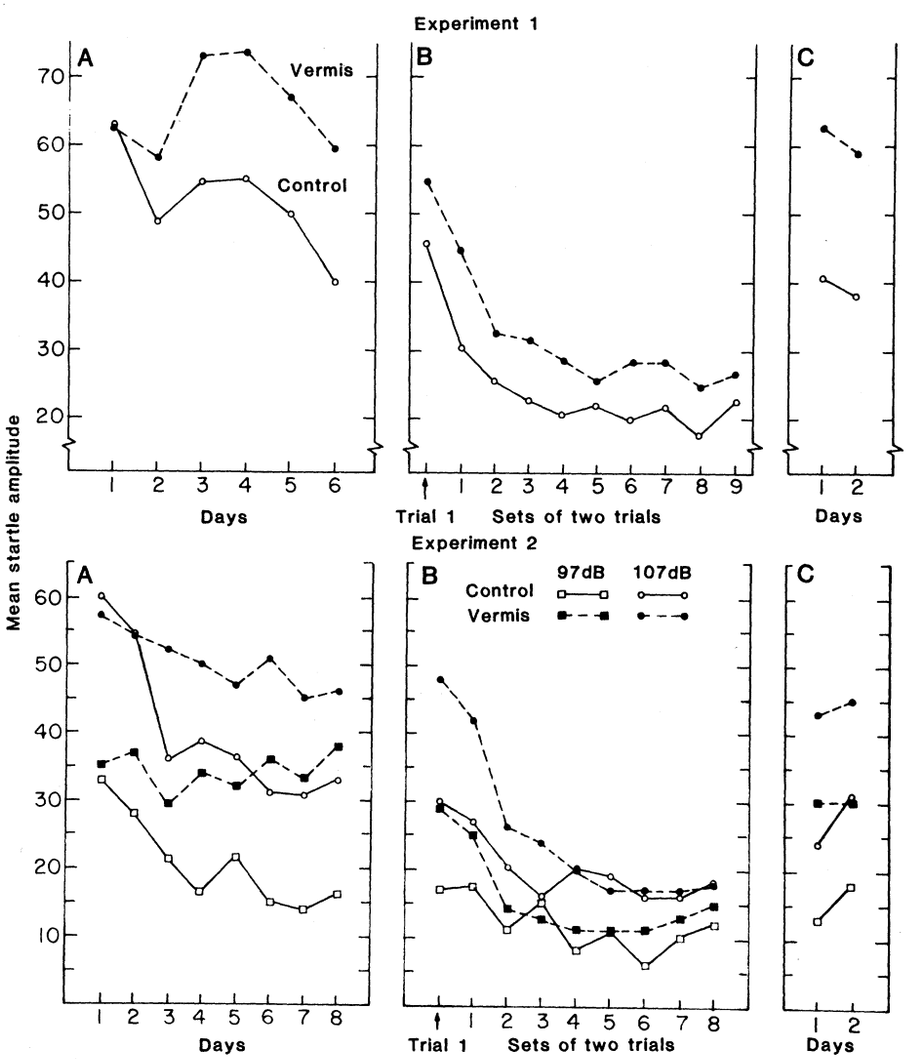

Fig. 1. The results of experiment 1 are shown across the upper panel and experiment 2 across the lower panel. For each experiment the results are shown serially from the initial long-term habituation tests (A) through the short-term tests (B) to the retests for long-term habituation (C). In (A) and (C) startle amplitude is shown as the mean for the first trial of each test and retest day. For short-term habituation (B) the first trial of the session is shown separately followed by sets of two trials for the 18 trials in experiment 1 and the 16 trials in experiment 2. In (B) the data represent three nonsignificantly different test days for experiment 1 and one short-term test session in experiment 2. The data for experiment 2 are shown for the two intensities of the startle stimulus (107 and 97 dB).

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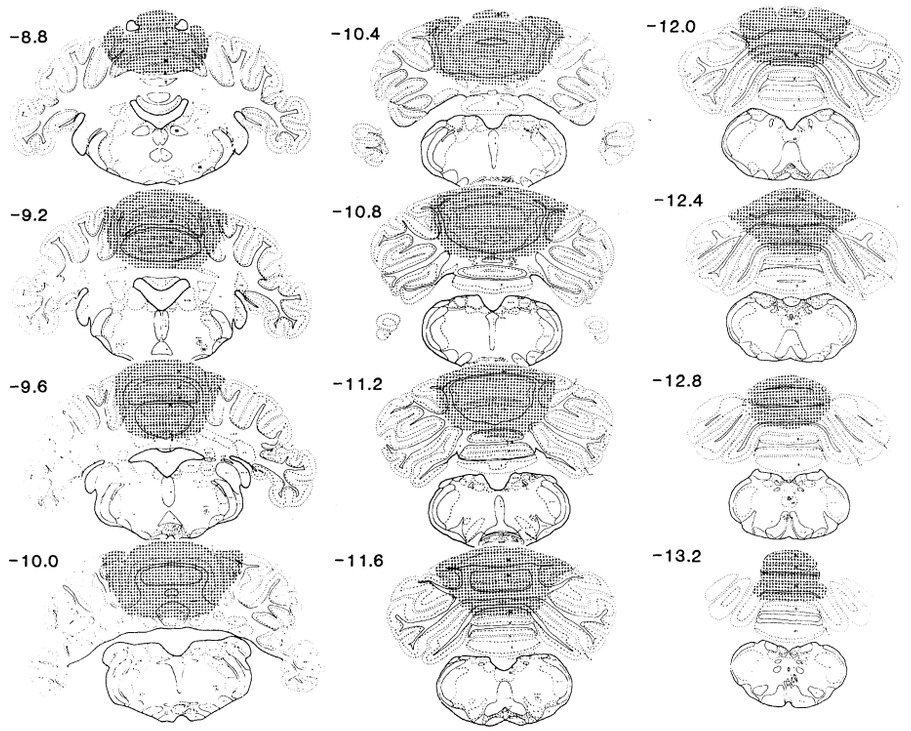

Fig. 2. Shaded areas show the extent of a representative vermal lesion on frontal sections. The reference numbers are millimeters posterior to bregma (*20*).

session-per-day, one-trial-per-session procedure on 2 days.

The first trials of each day most clearly reflected long-term habituation and therefore were analyzed. Repeated measures analysis of variance over the first six test days (Fig. 1, experiment 1, A) showed a significant difference between the groups [$F(1, 15) = 6.21$, $P < 0.05$]. Although the interaction was not significant, the two groups were not significantly different on day 1 [$t < 1$] but were on day 6 [$t(15) = 2.51$, $P < 0.05$]. The clearest difference between the groups in long-term habituation was seen between day 1 and the mean of the two retest days (Fig. 1, experiment 1, C). Analysis of these two data points yielded a significant groups by trials interaction [$F(1, 15) = 8.0$, $P < 0.05$]. The controls showed a significant decrease [$F(1, 6) = 20.82$, $P < 0.01$], while the animals with vermal lesions did not [$F < 1$]. From day 1 to retest the vermal-lesioned animals showed a 0.7 percent increase in mean responsiveness while the controls showed a 36 percent decrease. In contrast to the long-term effects, there was no apparent difference between the groups in short-term habituation (Fig. 1, experiment 1, B). This parameter decreased significantly over trials in both groups [$F(17, 255) = 10.5$, $P < 0.01$], and the interaction did not approach significance.

The results of experiment 1 could have been contaminated by ceiling effects whereby the lesioned animals were initially more responsive than controls, yet the measuring device was insensitive to these higher response levels. To assess this possibility and to replicate experiment 1, we tested independent groups of vermal-lesioned animals and sham-operated controls at two intensities of the startle stimulus, 97 and 107 dB (Fig. 1, experiment 2). There were 11 rats in each of the four groups. In addition, to assess any nonspecific effects of the lesions, general behavior was recorded in one of eight categories every 5 seconds for the first minute in each adaptation and test session. The mutually exclusive behavioral categories were locomotion, turning, head moving, sniffing, grooming, rearing, freezing, and other. The only other differences from experiment 1 were minor procedural changes.

In experiment 2 we replicated the major points of experiment 1 with the added statistical power of increased group size (Fig. 1, experiment 2). Analysis of variance over the eight initial test days (Fig. 1, experiment 2, A) yielded a significant difference between vermal-lesioned and control groups [$F(1, 40) = 11.36$, $P < 0.01$], between stimulus intensities [$F(1, 40) = 25.03$, $P < 0.01$], and across trials [$F(7, 280) = 7.01$, $P < 0.01$]. Most important, the appropriate lesion and control groups were not significantly different on trial 1 [all t values were less than 1], and the lesion by trials interaction was significant [$F(7, 280) = 2.96$, $P < 0.01$]. The controls decreased significantly across trials [$F(7, 140) = 10.49$, $P < 0.01$], while the vermal-lesioned groups did not [$F < 1$]. Comparison of day 1 with the mean of the two retests days (Fig. 1, experiment 2, C) also yielded a significant lesion by trials interaction [$F(1, 40) = 9.55$, $P < 0.01$], but both the controls [$F(1, 20) = 42.43$, $P < 0.01$] and the vermal-lesioned groups [$F(1, 20) = 5.42$, $P < 0.05$] showed significantly decreased responsiveness from day 1 to retest. The vermal-lesioned animals' mean responsiveness decreased 5.2 percent while the controls decreased 48.1 percent. None of the lesion by intensity interactions approached significance, ruling out any possible contamination from ceiling effects. All groups showed statistically significant short-term habituation (Fig. 1, experiment 2, B), and the lesion by trial interaction was not significant ($P > 0.05$).

The general behavior record taken during adaptation and test trials showed no systematic differences between vermal-lesioned and control groups. In both experiments vermal-lesioned animals showed slight motor impairments for a few days after surgery, but these had disappeared completely by the time of testing.

Lesions were reconstructed from stained 40-μm frozen sections (Fig. 2). The damage was restricted to the cerebellar vermis with only slight invasion of the hemispheres. Anterior to the primary fissure the dorsal portion of the central lobule and the ventral and dorsal portions of the culmen were almost completely destroyed. Posterior to the fissure, the declive, tuber vermis, and pyramid were completely destroyed. The uvula was only slightly damaged. The dentate and interpositus nuclei were intact, but as much as one quarter of the fastigial nucleus was invaded dorsally.

Lesions to the cerebellar vermis abolished or greatly attenuated long-term habituation of the acoustic startle response while leaving short-term habituation unaffected. The vermal lesions did not alter initial response levels. Just as the effects of brain damage on associative learning cannot be interpreted if the unconditioned response is disrupted, the effects of a lesion on habituation cannot be unambiguously interpreted if the lesion alters initial response levels. In our experiments the essential neural pathway for the response was intact as was the short-term habituation mechanism which is presumed to be intrinsic to that pathway. The vermal lesions removed an independent mechanism which apparently mediates long-term habituation by superimposing a tonic or phasic suppression upon the startle circuit.

Lesions to the mesencephalic reticular formation attenuate long-term habituation of the acoustic startle response (*6, 13*), but to a much lesser extent than did our lesions to the cerebellar vermis. In addition, the decerebrate rat with an intact cerebellum shows no long-term but significant short-term habituation in this response system (*14*). If we assume that these various lesions interfere with the same pathway, a useful working assumption, we can conclude that structures rostral to the cerebellum are involved and that the vermis is an area of convergence or focus, but we do not know whether the vermis is on the ascending or descending limb of the pathway. There are numerous pathways into and out of the cerebellum that could mediate these effects. There are rich auditory projections to the vermis (*15, 16*), which were removed by our vermal aspirations. The vermis projects widely into the reticular formation and throughout the brainstem and receives rich projections from more rostral brain areas (*17*). Whatever the rostral limb of this pathway may be, it is not the classical auditory pathway (*18*). It is interesting to note that the cerebellar vermis, in contrast to the cerebellar hemispheres and related structures, is not directly involved in conditioning of the rabbit eyelid response to an acoustic stimulus (*10*).

Our data contribute to the evidence that the cerebellum is involved in subtle and complex ways in many behavioral processes (*19*). Snider and Stowell (*16*) noted no sensory defects following cerebellar damage in spite of its rich auditory, visual, and tactile projection areas. They wondered "... whether loss of the cerebellar representations of these three exteroceptive systems does not produce objective and subjective effects which are so subtle that they have escaped present methods of study." We believe long-term habituation may be one of those subtle behavioral effects.

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12. A similar startle apparatus has been described previously (*5*). The 20 by 12 by 14 cm steel-rod and Plexiglas chamber was sandwiched between compression springs attached to a rigid superstructure. Vertical displacement of the chamber induced a voltage in the attached transducer which was digitized, rectified, and integrated by a microcomputer system into 200-msec epochs. Startle amplitude was taken as the difference between the computer output for the 200-msec epochs before and after stimulus onset.
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Calcium Antagonist Receptors in Cardiomyopathic Hamster: Selective Increases in Heart, Muscle, Brain

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The Syrian cardiomyopathic hamster has a hereditary disease in which a progressive myocardial necrosis mimics human forms of cardiac hypertrophy. Lesions are associated with calcium overload and can be prevented with the calcium antagonist verapamil. Numbers of receptor binding sites for calcium antagonists in heart, brain, skeletal muscle, and smooth muscle were markedly increased in cardiomyopathic hamsters. The uptake of calcium-45 into brain synaptosomes was also increased in cardiomyopathic hamsters. The increase in calcium antagonist receptors and related voltage-sensitive calcium channels may be involved in the pathogenesis of this cardiomyopathy.

THE SYRIAN CARDIOMYOPATHIC (CM) hamster (BIO 14.6, Bio Research, Cambridge, Massachusetts) is an inbred strain with a hereditary abnormality in skeletal and cardiac muscle involving ventricular and atrial hypertrophy with subsequent development of congestive heart failure (*1*). This strain has been used as a model for certain disturbances, such as hypertrophic obstructive cardiomyopathy and Freidrich's ataxia, the latter involving brain pathology as well as cardiac hypertrophy (*2*). Calcium overload of myocytes has been implicated in the etiology of the cardiac abnormalities in these hamsters. The calcium concentration in cardiac myocytes of CM hamsters is elevated (*3*), and calcium antagonist drugs such as verapamil are the most effective agents in relieving cardiac dysfunction (*4*). Calcium entry into myocytes and neurons (*5*) occurs through voltage-sensitive calcium channels (VSCC), which are blocked by calcium antagonist drugs of several classes including nitrendipine, verapamil, and diltiazem. Specific receptors for these drugs can be labeled with radioligand binding techniques (*5, 6*) and voltage-dependent calcium entry measured by $^{45}Ca^{2+}$ flux determinations. We now report a selective increase in numbers of calcium antagonist receptors in heart, brain, skeletal muscle, and smooth muscle of CM hamsters. In addition, synaptosomal preparations from CM hamster brain show increased calcium uptake, suggesting a link to VSCC.

[^{3}H]Nitrendipine labels the dihydropyridine class of calcium antagonist receptors (*5*). Both heart and brain exhibit 50 to 100 percent increased [^{3}H]nitrendipine binding in 30-day-old CM hamsters compared with age- and sex-matched random-bred controls (Fig. 1). Scatchard analysis indicates that the augmentation is in the number of binding sites (B_{max}) with virtually no change in affinity (K_D). [^{3}H]Desmethoxyverapamil labels high- and low-affinity forms of a second type of calcium antagonist receptor, which is allosterically linked to the dihydropyridine site (*6*). Saturation analysis reveals more than a twofold increase in numbers of both the high- and low-affinity [^{3}H]desmethoxyverapamil sites in brain. Binding affinity is decreased, but to a lesser extent. Because of

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