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11. After all the viral stocks had been prepared, an aliquot of each was thawed and titrated by the end-point dilution method (*5*). All stocks were tritrated in parallel on PBLs derived from a single donor. The median number of tissue culture infectious doses ($TCID_{50}$) of HIV-1 per milliliter was taken as the inverse of the highest dilution that on average resulted in infection of the PBL cultures. To confirm reproducibility, we titrated the virus stocks a second time with a different PBL donor; comparable results were observed.
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Stress-Induced Facilitation of Classical Conditioning

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Stress has been shown to impair subsequent learning. To determine whether stress would impair classical conditioning, rats were exposed to inescapable, low-intensity tail shock and subsequently classically conditioned under freely moving conditions with a brief periorbital shock unconditioned stimulus and a white noise conditioned stimulus. Unexpectedly stressed rats exhibited significantly more conditioned eyeblink responses and the magnitude of their individual responses was also enhanced. These results stand in contrast to the learning deficits typically observed and suggest that stress can enhance the acquisition of discrete conditioned responses.

Excessive environmental stimulation is capable of imposing far-ranging consequences on behavior, specifically on the ability to process and synthesize new information. In animals, exposure to inescapable shock typically impairs learning and is referred to as "learned helplessness" (*1*). This phenomenon affects a wide range of learning paradigms but is especially prevalent during instrumental learning (*2*). In contrast, we report here an increase in the acquisition of associative classical conditioning after exposure to inescapable shock.

Male Fischer 344 rats (n = 24) were implanted with two wires around the upper eyelid to record electromyographic (EMG) activity from obicularis oculi and two wires around the lower lid to deliver a periorbital shock. After at least 4 days of recovery, rats were adapted to a conditioning chamber for 1 hour. Half of the rats were then placed in a restraining tube in a different chamber in a different room and exposed to 90 1-s shocks (1 mA, 60 Hz) to the tail, one per min for 90 min, and the other half were returned to their home cages. Twenty-four hours later, stress was reinstated with five 1-s shocks (1 mA), one per minute. After 30 min, the animals were transferred to the conditioning chamber for training. This procedure was repeated each day for 4 days. Unstressed control rats (n = 8) were taken directly from their home cage for training. Four rats were trained per day, two stressed and two unstressed.

On the first day of training, rats were observed for orienting or startle responses to the white noise stimulus, and shock thresholds needed to elicit a blink were obtained. Training consisted of pairing a 350-ms white noise conditioning stimulus (CS) (85 dB, 5 ms rise and fall time) with a coterminating 100-ms shock to the periorbital muscles (2 mA, 60 Hz, ac). Each daily training session consisted of ten blocks of ten trials. Each block consisted of a noise-alone trial, four paired trials, a shock-alone trial, and four additional paired trials. Intertrial intervals were randomized between 20 and 40 s (mean, 30 s). Two groups (stressed, n = 4; unstressed, n = 4) were exposed to the same number of stimuli as in paired training, except that stimuli were explicitly unpaired and presented between 10 and 20 s (mean, 15 s) (*3*).

Immediately after the last training session, rats were killed and trunk blood was collected for radioimmunoassay of serum corticosterone. Trunk blood was also obtained from a group of rats (n = 4) exposed to the same amount of restraint, tail shock, and time in the conditioning chamber (without stimuli) and from a group of naïve controls (n = 4) taken directly from their home cage.

Compared to unstressed controls, rats

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restrained and exposed to inescapable shock before training exhibited a significant increase in the percentage of trials with conditioned responses (CRs) [that is, responses that occurred before the unconditioned stimulus (US) began]. This increase persisted throughout each of the 4 days of training [analysis of variance (ANOVA) with repeated measures and planned comparisons; $P < 0.0001$] (Fig. 1) and was evident and significant on the initial day of training: Stressed rats exhibited a mean ± SEM of 20 ± 5% CRs, whereas the unstressed controls exhibited 1 ± 1% CR. On the fourth and final day of training, stressed rats exhibited 60 ± 6% CRs, whereas unstressed rats exhibited 24 ± 10% CRs. The stressed rats showed an increase in learning from day 1 to day 2 ($P < 0.0001$) but not for days 2, 3, and 4. The unstressed rats also showed an increase in learning between days 1 and 2 ($P < 0.005$) but not for days 2, 3, and 4 (4). The lack of a further increase in learning after day 2 indicates that both groups had reached asymptotic performance by day 2. Nevertheless, the rate of acquisition was significantly increased for stressed rats compared to unstressed controls between days 1 and 2 ($P < 0.0001$).

These stress-induced effects were specific to learning because animals exposed to unpaired stimuli did not exhibit CRs. On day 4, stressed-unpaired rats showed 9 ± 2% CRs, and unstressed-unpaired rats showed 12 ± 4% CRs. During the 4 days of training, paired rats exhibited conditioned responding and unpaired rats did not ($P < 0.001$). On days 1 and 2 of training, no differences occurred between unstressed-unpaired and unstressed-paired rats, but a significant difference arose on days 3 and 4: Unstressed-paired rats exhibited significantly more CRs than the unstressed-unpaired rats. Moreover, stressed-paired rats exhibited significantly more CRs than stressed-unpaired rats ($P < 0.005$), indicating that the effect of stress on learning was not attributable to sensitization or alpha conditioning. We were able to rule out the effects of stress on performance because stress did not have a significant effect on the threshold needed to elicit a blink ($P = 0.11$) and threshold did not correlate with the percentage of CRs; correlation between current threshold and the percentage of CRs for all stressed-paired rats was 0.07 ($P = 0.16$) and for unstressed-paired rats was 0.04 ($P = 0.28$).

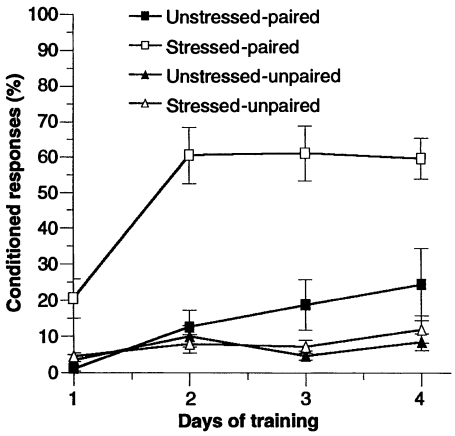

Fig. 1. Mean (± SEM) percentage of CRs from stressed rats exposed to paired ($n = 8$) and unpaired ($n = 4$) stimuli and unstressed rats exposed to paired ($n = 8$) and unpaired ($n = 4$) stimuli.

The CRs evoked by the stressed rats were also of greater magnitude, that is, the EMG activity mediating the CR was longer in duration and reached a greater amplitude than that evoked from control rats (Fig. 2). Although exposure to the paired stimuli for several days significantly increased the magnitude of the CR ($P < 0.01$), exposure to the stressor and paired stimuli together increased the magnitude of the CR more than training alone ($P < 0.05$).

To obtain an independent measure of the response to the stressor and the potential stressful nature of the task, we measured serum corticosterone concentrations in rats immediately after the fourth and final day of training. Concentrations were obtained from two additional groups, one exposed only to the stressor and training apparatus and the other a group of naïve controls. Concentrations (mean ± SEM) obtained by radioimmunoassay were 52.1 ± 9.7 μg/dl for unstressed-paired rats, 73.8 ± 2.3 μg/dl for stressed-paired rats, 74.1 ± 3.7 μg/dl for the unstressed-unpaired group, and 66.0 ± 5.5 μg/dl for the stressed-unpaired group. The two untrained groups, stressed and naïve, had concentrations of 59.8 ± 3.5 and 18.2 ± 10 μg/dl, respectively. All stressed and trained groups exhibited corticosterone levels significantly greater than naïve controls ($P < 0.005$), but these groups did not differ significantly amongst each other. Apparently, classical conditioning in the freely moving rat is sufficient to strongly activate the hypothalamic-pituitary adrenal axis, and therefore corticosterone concentrations at the time of killing did not appear to be critical to the increase in performance after 4 days of training.

Exposure to inescapable tail shock has been shown to increase the binding of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) subclass of glutamate

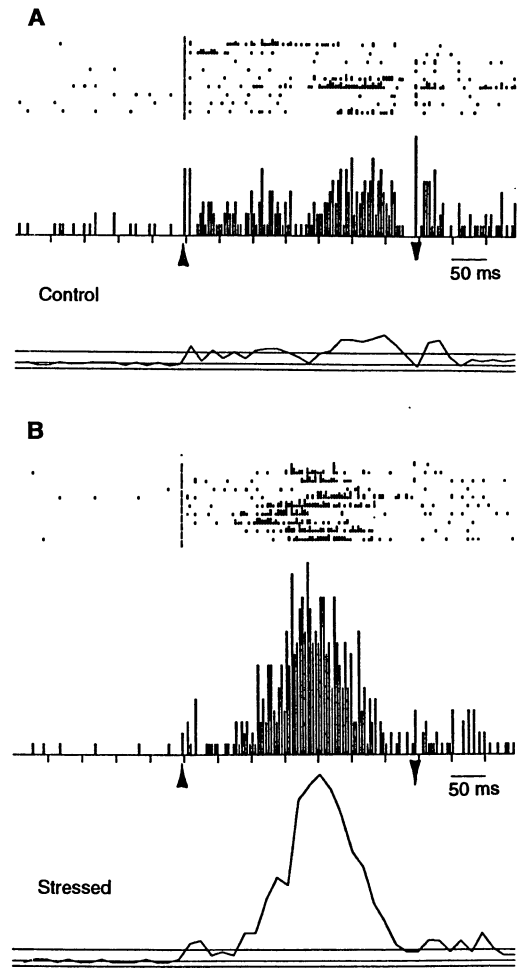

Fig. 2. EMG activity recorded from (**A**) a control and (**B**) a stressed rat on the fourth and last day of paired conditioning. Data obtained from the CS-alone test trials are presented, that is, when the CR is not contaminated by the unconditioned response. CS onset and offset are indicated by the upward and downward pointing arrowheads, respectively. The top panels of (A) and (B) are a raster display, with a dot present whenever the EMG activity from the upper eyelid was greater than a threshold voltage set just above baseline activity. The bin width equals 4 ms, and each line represents the activity from one CS-alone trial. The middle panels of each figure represent the same data pooled into a histogram format, that is, the data from each bin are summated among all CS-alone trials. The bottom panels display the same data after conversion to standard scores and normalization to the amount of activity before presentation of the CS for all CS-alone trials. Bin widths equals 16 ms. The top, middle, and bottom horizontal lines in the last panels of (A) and (B) reflect the mean + 2 SDs, the mean, and the mean − 1 SD, respectively. Standard scores greater than 1.96 SD were considered significantly different from baseline scores.

receptors in the hippocampus (5). Long-term potentiation (LTP) in the hippocampus—a long-lasting form of neuronal plasticity and putative neurobiological substrate for learning in the mammalian brain (6)—produces a similar effect (7). Moreover, the induction of LTP before training increased the acquisition of the eyeblink response to a differential CR (8), and conditioning alone increased the amplitude of the monosynaptic granule cell population spike in response to perforant path stimulation (an increase resembling LTP) (9). Therefore, if these changes in AMPA receptor binding are functionally significant, stress should facilitate the acquisition of the conditioned eyeblink response, as reported here.

A wide range of evidence supports the notion that the cerebellum and its associated brain stem neuronal network form the essential circuitry for the basic CR (10). The hippocampus, however, plays a key modulatory role in eyeblink conditioning (11) and contributes a major source of afferents to the cerebellum by way of the retrosplenial cortex and pontine nuclei (12). Alternatively, stress effects could act more directly on the cerebellar circuit, for example, by way of the locus ceruleus (13).

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3. The EMG activity from the upper eyelid muscle was amplified and then electronically filtered to pass signals ranging from 300 Hz to 3 KHz to a window discriminator. The discriminator digitized activity that was greater than a threshold set just above the envelope of background activity. The digitized data were collected by computer and stored into 4-ms bins. CRs were determined trial by trial with the use of chi-square analysis with Yates's correction. A response was considered a CR if the number of counts recorded 100 ms before shock onset was greater than that predicted by a random distribution of counts recorded 100 ms before CS onset. In white noise–alone trials, analysis windows were extended to 200 ms. Trials with a significant difference between two contiguous 100-ms time windows in the time period before the CS were excluded. Unconditioned responses (URs) were not analyzed owing to the shock artifact associated with the US. Data were analyzed by ANOVA with repeated measures and planned comparisons with two between-group levels and four within-subject levels. To quantify EMG activity, we standardized the number of counts per bin relative to the period before the CS. The ANOVA was performed on the number of bins with significant Z scores (>1.96) during the period of the animal's response to the CS without presentation of the US for the four groups over 4 days.
4. As determined by the standard delay paradigm, rabbits typically acquire at least 80% CRs by the third day of training, whereas the results from rats are more variable. In the present study, rats within a cohort shipment were randomized before stress and training, and treatment was counterbalanced within and between training days. The relatively low number of CRs attained by both trained groups compared to other rat eyeblink conditioning studies [R. Skelton, *Behav. Neurosci.* **102**, 586 (1988); N. A. Schmajuk and B. A. Christiansen, *Physiol. Behav.* **48**, 755 (1990)] is therefore most likely attributable to cohort, strain, or methodological differences. For example, higher rates were obtained from the restrained Long-Evans rat [R. M. Adams, A. A. Zhang, D. Lavond, *Soc. Neurosci. Abstr.* **15**, 890 (1989)], notably a stressful condition. Under freely moving conditions, F1 hybrids between the Fischer 344 and Brown Norway strains exhibited more CRs than Fischer 344 rats [C. Weiss and R. F. Thompson, *Neurobiol. Aging* **13**, 319 (1992)].
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Establishment of Stable, Cell-Mediated Immunity That Makes "Susceptible" Mice Resistant to *Leishmania major*

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Cell-mediated, but not antibody-mediated, immune responses protect humans against certain pathogens that produce chronic diseases such as leishmaniasis. Effective vaccination against such pathogens must therefore produce an immunological "imprint" so that stable, cell-mediated immunity is induced in all individuals after natural infection. BALB/c mice "innately susceptible" to *Leishmania major* produce antibodies after substantial infection. In the present study, "susceptible" mice injected with a small number of parasites mounted a cell-mediated response and acquired resistance to a larger, normally pathogenic, challenge. This vaccination strategy may be applicable in diseases in which protection is dependent on cell-mediated immunity.

Many nonreplicating antigens can induce either delayed-type hypersensitivity (DTH) or antibody-mediated responses, depending on quantitative variables such as antigen dose (1). A concentration of antigen that is subimmunogenic for the induction of antibody can induce DTH (1). Chronic administration of such low doses results in "low-zone paralysis" (2, 3), in which animals do not produce as strong an antibody response to subsequent challenge as do untreated animals. This state of unresponsiveness for the induction of antibody is associated with the expression of DTH to the antigen (4) and is therefore more appropriately referred to as "low-zone immune deviation." Low-zone immune deviation is probably associated with the induction of antigen-specific

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