

returning to normal Ringer solution, thus suggesting that sodium is the pre-dominant ion mediating the GABA and glutamic acid depolarizations. The GABA response usually returned within 10 minutes of washing whereas the glutamic acid response often took much longer to return (approximately 1 hour).

In summary, we have treated the isolated spinal cord of the frog with magnesium in order to minimize synaptic activity and permit better investigation of the effects of putative transmitters on primary afferent terminals. Both GABA and glutamic acid increased the excitability of the terminals and concomitantly depolarized the dorsal root. Furthermore, both bicuculline and picrotoxin antagonized the GABA-induced depolarizations but had little effect on either the glutamic acid or glycine responses. Since both bicuculline and picrotoxin attenuate the synaptically developed dorsal root potential (3-5) and block GABA-mediated events elsewhere in invertebrate and vertebrate nervous tissue (5, 6), our evidence supports the hypothesis that GABA is the transmitter mediating primary afferent depolarization (1).

The investigation of the ionic mechanism(s) underlying the amino acid-induced depolarization of the dorsal root has provided evidence to support the hypothesis that sodium is the predominant ion involved in these responses (5). This sodium-dependent, GABA-induced depolarization of primary afferent terminals differs from its well-established action as a chloride-dependent inhibitory transmitter and suggests that GABA may mediate synaptic events by utilizing different ions at different sites.

Note added in proof: Since this report was submitted for publication Davidoff has reported an irreversible, specific antagonism of dorsal root potentials and both GABA-induced increases in primary afferent excitability and depolarizations of the dorsal root (17). This evidence complements our report and further strengthens the notion that GABA or some closely related analog is the natural transmitter mediating the presynaptic inhibition.

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9. The Ringer solution consisted of: 114 mM NaCl, 2.5 mM KCl, 1.9 mM CaCl₂, and 2.5 mM dextrose buffered to pH 7.4 with 10 mM tris-maleate-NaOH. The MgSO₄ (20 mM) was added to this solution after assessing the synaptic potentials present. The flow was adjusted so that approximately tenfold the bath volume was exchanged every minute. The ambient temperature of the experimental room and preparation was monitored by a thermometer in the bath and was maintained at 15°C. A calomel-cottonwick electrode in the bath served as a ground.
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14. By direct injection through a syringe we were able to supply three times the chamber volume within 5 seconds. This permitted a quick examination of pharmacologic responses. Repeated applications produced responses that were within 15 percent of each other, which indicates a good degree of reliability by this technique. Since this procedure occasionally resulted in small artifacts, Ringer solution was also injected with each series of drug tests. Ringer-produced artifacts that were large enough to alter the responses eliminated the drug responses from further study. An alternative method consisted of switching from the Ringer solution to a drug-containing solution merely by turning a stopcock. Since the flow was controlled between the stopcock and the bath, the flow rate was constant. Results obtained by this technique were similar to those derived from syringe injection. The drug concentrations used in the present study did not alter the pH of the solutions. The dorsal root responses to application of the amino acids were similar in normal Ringer solution and in high magnesium Ringer.
15. Chloride-free Ringer solutions consisted of either 114 mM sodium isethionate, or 57 mM Na₂SO₄ and 57 mM sucrose, 1.25 mM K₂SO₄, 1.9 mM CaSO₄, 2.5 mM dextrose, and 10 mM tris-maleate-NaOH buffered to pH 7.4. Sodium-free solutions consisted of: either 114 mM choline chloride, 114 mM LiCl, 114 mM tris-Cl, or 57 mM MgCl₂ and 57 mM sucrose, 2.5 mM KCl, 1.9 mM CaCl₂, 2.5 mM dextrose, and 10 mM tris-HCl buffered to pH 7.7. The 20 mM MgSO₄ was added to each solution in order to reduce synaptic activity.
16. The synaptically developed depolarization of primary afferent terminals remained and was markedly prolonged after 5 hours of chloride-free bathing, thus confirming earlier work [B. Katz and R. Miledi, *J. Physiol. London* **168**, 389 (1963)]. The amino acid-induced depolarizations might possibly reflect an outward chloride current. If 5 hours of chloride-free washing is not sufficient to entirely remove internal chloride, then part of the remaining depolarization might be due to a residual chloride current. These chloride-free experiments thus do not rule out the possibility, but those with sodium-free solutions would appear to eliminate chloride as the predominant ion involved in the drug-induced depolarizations.
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Depression and Later Enhancement of the Critical Flicker Frequency during Prolonged Monocular Deprivation

Abstract. *One eye was visually deprived for 1 day, and the critical flicker frequency in the other eye was determined at the start of the deprivation period and then at intervals of 3, 6, 9, 15, and 24 hours. There was an initial depression in performance, followed by an enhancement effect. No significant changes in the critical flicker frequency were observed in the occluded eye at corresponding times; thus the depression-enhancement phenomenon is specific to the nonoccluded eye.*

In 1923, Allen (1) reported that 3 hours of monocular light deprivation produced a decrease in the critical flicker frequency (CFF) of the non-occluded eye, a result confirmed by Hollenberg (2). In contrast, we demonstrated (3) a negatively accelerating

improvement in the CFF of the non-occluded eye during 1 week of monocular deprivation, the first measurement being taken 8 hours after deprivation was begun and the remainder at daily intervals. Furthermore, a sizable after-effect in this eye was still present 1

week after the removal of the black patch from the other eye. These two sets of results suggest that prolonged monocular deprivation may initially produce a depression of the CFF in the nonoccluded eye, and that the depression is followed by an enhancement effect of negatively accelerating magnitude. We report that this hypothesis was tested and confirmed.

Thirty male university students, all with normal vision, were divided into experimental and control groups, each containing 15 subjects. All subjects were required to live for 1 day in a large room (3.66 by 14.02 m), which was furnished with sofas, comfortable chairs, and study desks, and contained a radio, a television set, playing cards, and reading material. The mean ambient illumination, measured at desk height in eight different positions in the room, was 550 lu/m². A washroom, a kitchenette, and sleeping quarters were adjacent to this furnished room. The subjects were confined to these apartment-like quarters in groups of three, two from one group and one from the other group. During the day spent in the room, each experimental subject wore a black patch over the dominant eye.

The CFF determinations were made before the patch was put in place and then at intervals of 3, 6, 9, 15, and 24 hours, the first measurement being made between 8:30 and 9:30 a.m. Subjects were permitted to sleep for approximately 8 hours after completion of the 15-hour test and were awakened 1 hour before the start of the 24-hour test (4). In order to control for possible effects of changes in blood sugar level on the CFF, each of the six determinations was made after the subject had eaten a meal or a snack that included a chocolate bar.

Before the CFF was measured at each test period, the nonoccluded (nondominant) eye of each experimental subject was dark-adapted for 15 minutes (the other eye was already covered by a patch). This duration was felt to be sufficient for testing with a small, centrally fixated visual target (4). The CFF was then taken from the nonoccluded eye only, since the presentation of a bright light to one eye can affect the CFF in the other eye (1, 2, 5). For each confirmed control subject, the CFF of the nondominant eye at each interval was determined after 15 minutes of binocular dark adaptation.

The stimulus consisted of a white

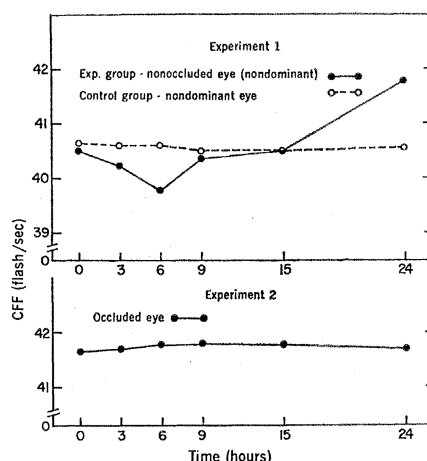


Fig. 1. Temporal changes in mean CFF values of subjects exposed to 24 hours of monocular deprivation. (Top) Measurements were taken on the nonoccluded (nondominant) eyes of the experimental subjects and on the nondominant eyes of control subjects. (Bottom) Measurements were taken on the occluded (dominant) eyes.

light, at an initial flicker frequency considerably higher than the fusion frequency. The light was presented monocularly by a cold cathode modulating lamp (Sylvania, type R1131c), mounted at the rear of a standard viewing chamber (Lafayette, model 1202C). The angle subtended by the centrally fixated stimulus was 2°10', a value assuring full foveal stimulation. The flicker-generating apparatus (Grason-Stadler, model E622) was set at a light to dark ratio of 0.50 and a lamp current

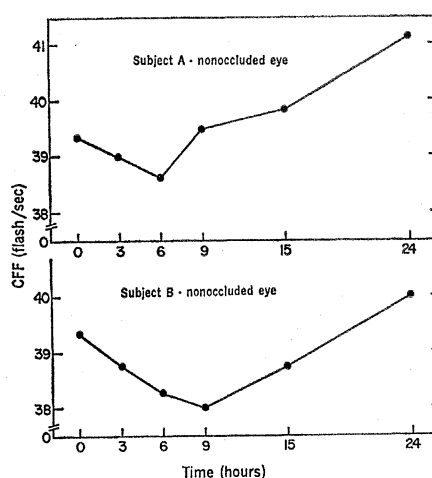


Fig. 2. CFF patterns in individual subjects. In subject A, the CFF for the nonoccluded eye is enhanced relatively early in the experiment. (Bottom) In subject B, the enhancement effect is seen only at the end of 24 hours of monocular deprivation.

reading of 22.6 ma. Eight trials, separated by 5-second intervals, were presented to the nondominant eye at each test period. The descending method of limits was used.

The CFF of the nonoccluded eyes shows an initial depression, as reported by Allen (1) and Hollenberg (2), a reversal toward the baseline level at 9 hours, and an enhancement effect at 24 hours (Fig. 1, top). On the other hand, the CFF of corresponding eyes of the confined controls does not change during the 24-hour session. An analysis of variance revealed a significant change over hours ($F = 21.82$, $P < .001$) and a significant interaction effect ($F = 20.29$, $P < .001$). A series of two-tailed *t*-tests comparing the relative performances of the two groups of subjects at the various time periods indicated that the decrease in the CFF at 3 and 6 hours, the reversal between 6 and 9 hours, and the increase at 24 hours were all statistically significant (P 's $< .01$).

An examination of the individual performance patterns of the 15 experimentals suggested the presence of two main types of "reactors" (Fig. 2). The first type, comprising a third of the sample, showed a prolonged period of depression with the enhancement effect appearing only at the 24-hour test, while the second type exhibited a relatively brief period of depression (at 3 and 6 hours only), and then an enhancement effect of progressively increasing magnitude. These two general response patterns may reflect possible differences in the degree of stress experienced by the experimental subjects resulting from the novelty of wearing an eye patch. Although no measures of stress or affect were made, we felt that subjects of the first type were somewhat more apprehensive and complained more often during the confinement period than did those showing the second type of response.

To determine whether a similar type of depression-enhancement phenomenon could be shown in the occluded eye, we did a second experiment. In each of nine subjects, the dominant eye was occluded for 1 day, and measurements were taken from this eye before the patch was put in place and then at intervals of 3, 6, 9, 15, and 24 hours. Before each test period, the nonoccluded eye was dark-adapted for 15 minutes, but it was not tested. (For the first measurement, both eyes were dark-

adapted since the black patch had not been applied.) There was no change in the CFF of the occluded eye at any time (Fig. 1, bottom). Thus, the depression-enhancement phenomenon is specific to the nonoccluded eye (4).

Although numerous variables affect the CFF, it is difficult to understand how any of them can account for both our present and earlier (3) results for the nonoccluded eye. The unusual time course, together with the persistence of the phenomenon for many days, suggests the disturbance of some interocular mechanism in the higher levels of the visual system. We believe that prolonged monocular deprivation may be producing changes in certain areas of the primary sensory system, changes similar to the denervation supersensitivity that occurs in the higher neural centers after partial surgical deafferentation at lower levels of the central nervous system (6). For example, Spiegel and Szekely (7) reported that lesions in the posteroventral nucleus of the thalamus (relay nucleus for touch) are followed, after an initial period of depression of the somesthetic cortex, by a hyperexcitability of this region. [More than a century ago, Hall (8) observed that "the first effect of injury done to the nervous system is a diminution of its functions, whilst the second or ulterior effect is the augmentation of these functions."] Occlusion of one eye, therefore, may be producing a state of temporary partial deafferentation of the visual system, a condition that is reflected behaviorally in the production of our CFF phenomenon. However, in contrast to surgically induced deafferentation, this deafferentation is functional, that is, it is produced by depriving the normal, intact organism of some of its accustomed visual experience.

This hypothesis is consistent with Sharpless's (9) revision of the law of denervation (6), which has as its main thesis that supersensitivity results from prolonged disuse of neural pathways. Sharpless states, "Disuse may be the result of drugs, privation of sensory experience, or, most commonly, injury produced by severance of nervous pathways." Further, he says that supersensitivity is a compensatory process that occurs as a consequence of "a radical and sustained change in the level of input to an excitable structure." This explanation of disuse of neural pathways, with which we concur, has the

merit of bringing together our results, the increased cutaneous sensitivity that occurs in human subjects after prolonged partial occlusion of the skin (10), and the various supersensitivity phenomena induced by surgery or by drugs. This explanation does not, however, adequately account for the presence of the CFF phenomenon in only one eye, nor does it indicate the specific neural locus of the interocular effect. Only future behavioral and electrophysiological research can provide satisfactory answers to these two problems.

Finally, our results are important in two general respects. First, they indicate that the monocular deprivation technique may provide a new method of attacking the complex problem of the physiological mechanisms underlying sensory isolation effects (11), an approach that can be used both in studies of humans and in electrophysiological studies in animals. Second, they suggest that many of the apparently contradictory results from isolation chamber studies (11), particularly those involving periods of 1 day or less and employing various sensory and perceptual-motor measures, may be accounted for by differences in the duration of experimental conditions. (The most commonly used periods have been 3, 9, 12, and 24 hours.) As we have demonstrated, performance on the same measure may be either impaired, improved, or not affected, the specific effect being dependent upon the duration of deprivation. It has been assumed by most previous investigators in the sensory

deprivation area that this experimental variable is probably not too important and therefore can be ignored. This assumption is no longer valid.

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Gratings Mask Bars and Bars Mask Gratings: Visual Frequency Response to Aperiodic Stimuli

Abstract. *Gratings and bars produce unexpected mutual visual masking. A grating masks a bar much less than a bar masks a bar; and a bar masks a grating uniformly over the grating field. These effects suggest that neural populations selective for size and orientation may be involved in frequency analysis rather than in simple feature detection.*

After a subject observes a square-wave grating, the apparent contrast of an identical grating will be greatly reduced (1, 2). A similar effect occurs when a half cycle of a grating—a dark bar—is presented and then presented again at a shorter duration (3). The reduction in apparent contrast of a stimulus when it is presented after another stimulus is called a forward masking

effect. The stimulus that loses apparent contrast is called the target; the other stimulus is called the mask.

These masking effects have been taken as evidence that the human visual system contains neural populations that are selective for size and orientation and that lose sensitivity after prolonged stimulation. The psychophysical evidence suggests this: The effects attenuate