concentrations by about 20 percent (control concentrations were 0.023 μmole per milligram of protein). The magnitude of the change observed was compatible with the decrease in $^{45}Ca^{2+}$ uptake obtained in the earlier experiments.

We then studied the effects of tolerance development on synaptosomal Ca^{2+} uptake. Male Sprague-Dawley rats (150 to 200 g) were rendered tolerant by the repeated intraventricular injection of a relatively high dose of β-endorphin. The rats, housed two to a cage, were anesthetized with ether and a stainless steel cannula was implanted in each in the fourth ventricle. Twenty-four hours later they were divided into three groups and given one of three treatments twice daily for three successive days: 5 ml of physiologic saline; 9.84 μg of morphine sulfate in 0.9 percent saline, or 9.0 μg of β-endorphin in saline. The dose of each opiate produced catalepsy that diminished with each injection and was absent after the sixth and final injection. The animals were killed 30 minutes after the last injection and synaptosomal uptake of $^{45}Ca^{2+}$ was determined.

The results indicate that the development of tolerance to β-endorphin is accompanied by enhanced synaptosomal $^{45}Ca^{2+}$ uptake. The mean uptake (± standard error) by the control groups for 10 minutes was found to be 23.9 ± 0.34 μmole per gram of protein, whereas that of the β-endorphin-treated group was 32.2 ± 0.47 μmole/g. The morphine-treated group likewise exhibited increased $^{45}Ca^{2+}$ uptake with a value of 28.9 ± 0.45 μmole per gram of protein ($P < .01$).

These data are consistent with our previous findings that morphine decreases the synaptosomal uptake of $^{45}Ca^{2+}$ prior to the development of tolerance and increases uptake when tolerance has developed (*12*). These effects are mimicked by β-endorphin and the similarities between β-endorphin and morphine suggest that β-endorphin may participate in the regulation of calcium flux and neurotransmitter release. Although the relative changes effected by β-endorphin in $^{45}Ca^{2+}$ uptake by the synaptosomes were modest, this is not surprising considering that the experiments were performed on synaptosomes derived from whole-brain homogenates. Subcellular studies indicate that long-term morphine administration increases the binding of calcium in synaptic vesicles and increases in Mg^{2+}-dependent adenosine triphosphatase activity in the same organelle (*11*, *17*). Data showing that the secretion of neurotransmitters caused by excitation is coupled with entry of Ca^{2+} (*18*) and that vesicular Mg^{2+}-dependent adenosine triphosphatase may be involved (*19*) support the concept that β-endorphin may inhibit the release of transmitters by inhibition of Ca^{2+} influx in the manner reported for morphine (*8*).

Since Ca^{2+} is necessary for the release of the enkephalins (*2*), it is reasonable to suppose that this should also hold true for the endorphins. Thus, it appears that Ca^{2+} and brain opiate peptides are interdependent and may function as part of a common system in regulating each other and the release of neurotransmitters (or neurohormones).

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14. The scintillation fluid consisted of 2,5-diphenyloxazole (PPO; 18 g) and 1,4-bis[2-4-methyl-5-phenyloxazolyl]benzene (POPOP; 0.5 g) dissolved in ethylene glycol monomethyl ether (750 ml) and toluene (2250 ml).
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Long-Term Analgesic Effects of Inescapable Shock and Learned Helplessness

Abstract. *Although exposure to inescapable shocks induced analgesia in rats, the analgesia was not manifest 24 hours later. A brief reexposure to shock, however, restored the analgesia. This reexposure to shock had an analgesic effect only if the rats had been shocked 24 hours previously. Further, long-term analgesic effects depended on the controllability of the original shocks and not on shock exposure per se. Implications of these results for learned helplessness and stress-induced analgesia are discussed.*

Whether or not an organism can control aversive events has widespread behavioral and physiological consequences. Exposure to inescapable, unavoidable shocks leads to subsequent failure to learn to escape in a different situation, decrements in shock-elicited aggression, and severe stress symptomology. None of these follow exposure to controllable (escapable) aversive events (*1*). Of the various consequences of exposure to uncontrollable aversive events, the greatest empirical and theoretical attention has been given to interference with subsequent escape learning, termed the learned helplessness effect (*1*). The mechanism or mechanisms producing it are a matter of controversy, however (*2*). Under the learned helplessness hypothesis (*1*), an organism exposed to uncon-

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Table 1. Mean latency to lick a paw or to flick the tail in rats that had received inescapable shock and escape training (IE), restraint and escape training (RE), or inescapable shock or restraint and no escape training (I no E and R no E).

Group	Latency (sec)	
	Paw lick	Tail flick
IE	25.55	11.63
I no E	15.87	8.26
RE	12.18	7.69
R no E	14.86	8.63

trollable events learns that they are independent of its voluntary responses. This expectation produces later failure to learn to escape by (i) reducing the organism's motivation to attempt to escape and (ii) interfering with the organism's propensity to associate or perceive the relationship between shock termination and the new escape response.

Other theorists reject the existence of motivational and associative deficits induced by inescapable shock. They argue that exposure to inescapable shock interferes with subsequent active escape learning because inactivity in the presence of shock is established either as a learned response to shock or because the stress of inescapable shock depletes neurochemicals necessary for locomotion (*2*).

Inescapable shock has recently been demonstrated to induce deficits in both association and activity, which seem to be independent of one another (*3*, *4*). Only the helplessness hypothesis predicts the occurrence of an associative deficit, but all current explanations address the activity deficit. Each of these explanations has difficulty with some aspect of the existing activity data, however (*4*). Thus, the source of the activity deficit is not known.

Inescapable exposure to foot shock, cold water stress, or chemical agents has been found to produce an analgesic reaction (*5*, *6*). This finding implies that inescapably shocked subjects may be less active in the presence of shock because shock is not as painful as it would otherwise be. The difficulty with this argument is that stress-induced analgesia and the helplessness effect occur at different times. Stress-induced analgesia dissipates within 2 hours after exposure to the stressor (*6*), whereas failure to escape can be observed 24 hours after inescapable shock (*1*).

Those experiments which have mapped the time course of stress-induced analgesia, however, have not reexposed the animal to the stressor before the analgesia test. Reexposure does occur in the typical helplessness experiment. For example, in our laboratory, the test requires the animal to cross a shuttle box twice to escape shock. This test is preceded by five trials in which only a single crossing is required to escape (*1*, *3*). It is possible that reexposure to shock in the course of escape training reinstates analgesia that would otherwise be dissipated. We now report that reexposure to shock will induce analgesia 24 hours after exposure to inescapable shock.

Rats were either first inescapably shocked through fixed body electrodes ($N = 16$) or only restrained ($N = 16$) in a restraining device. After 24 hours, half of each group received five single-crossing shuttle-box shock escape trials, while the other half were individually confined in the shuttle box with no shock for an equivalent period. Five such trials were used because this is what usually precedes the measurement of the learned helplessness effect in our laboratory. Shuttle-box treatment was immediately followed by three analgesia test trials on a hot plate maintained at 50.0° ± 0.5°C. The procedures, variables, and apparatus used have been described elsewhere (*3*).

There were no differences in the amount of shock received by inescapably shocked ($\bar{X} = 8.03$ seconds) and restrained rats ($\bar{X} = 8.24$ seconds) on the five single-crossing shuttle-box trials [$t(14) < 1.0$]. Table 1 shows the mean latency to lick a rear paw during the hot plate test trials. Only the group receiving both inescapable shock during preliminary treatment and escape training before testing (group IE) differed from any of the other groups. Analysis of variance revealed a reliable effect of preliminary treatment [$F(1,24) = 16.76$, $P < .001$] and a reliable interaction of preliminary treatment with shuttle escape [$F(1,24) = 12.37$, $P < .002$]. Newman-Keuls post hoc comparisons indicated that group IE was slower to respond than the remaining three groups, which did not differ among themselves. Thus, reductions in nociceptive responding can persist for at least 24 hours after exposure to stress, but must be reinstated by a brief reexposure to shock.

A second experiment was conducted using a different index of analgesia to corroborate these findings. The procedure was identical to that used in experiment 1, except that latency to flick the tail from radiant heat served as the measure of pain sensitivity. Trials terminated automatically when the animal moved its tail about 5 mm laterally or when a maximum of 20 seconds without a response had elapsed. Inspection of column 3 of Table 1 shows the mean latency to remove the tail from the heat during the test trials. This measure of pain sensitivity revealed a pattern of results identical to those of the hot plate test. Animals first exposed to inescapable shock and then tested 24 hours later showed reduced pain sensitivity only if reexposed to shock. Analysis of variance revealed an unreliable group effect [$F(1,28) = 3.15$, $P < .08$] and, crucially, a reliable interaction of group and shuttle escape [$F(1,28) = 4.66$, $P < .05$]. Newman-Keuls comparisons ($P = .05$) showed that group IE differed from the other three groups; which, in turn, did not differ among themselves.

Table 2. Mean latency to flick tail from radiant heat as a function of whether, during preliminary treatment, rats received escapable shock (escape), an equivalent amount of inescapable shock (yoke), or no shock (naïve).

Group	Latency (sec)
Escape	9.52
Yoke	14.41
Naïve	8.05

These data encourage the view that the activity deficit induced by exposure to inescapable shock is caused by nociceptive changes. If, however, differences in analgesia are to account for this activity decrement and hence, poor shuttle escape performance, parallels should exist between the conditions producing interference with shuttle escape and those producing analgesia. Shuttle escape deficits have occurred only when organisms have received prior treatment with inescapable shocks (*1*, *3*). Thus, decrements in pain sensitivity 24 hours after shock treatment should be a function of the controllability of that shock.

We examined this possibility. Rats were run in squads of three. One member of each of seven triads could terminate tail shock by turning a wheel with its paws. Shock began at the same time for a second member of the triad and terminated only when the first member responded. Thus rats received identical shocks on each trial but only the first had control over the shock. The third was merely restrained in the apparatus. Details of apparatus and escape training procedure have been described (*3*). After 24 hours, all rats received five trials of shuttle-box escape training followed by analgesia testing in the tail-flick apparatus. Groups not reexposed to shock before testing were not used, because

the first two experiments found no changes in nociceptive responding 24 hours after inescapable shock exposure in the absence of reexposure. We thus examined the effects of inescapable shock under those conditions in which long-term analgesia can be observed.

As in experiments 1 and 2, we found no differences in the single-crossing shuttle-box trials (Table 2). The effect of shock on tail-flick latency does depend upon whether the subject can control shock. The group that could originally escape shock did not differ from the unshocked controls, whereas the yoked group exhibited analgesia. Analysis of variance revealed a reliable effect of preliminary treatment [$F(2,18) = 12.97$, $P < .001$]. Newman-Keuls tests ($P = .05$) showed that the escape and no-shock groups did not differ, and both responded more rapidly than the yoked group.

These results have implications for the mechanisms that produce the effects of learned helplessness and stress analgesia. With regard to learned helplessness experiments, our results potentially explain why inescapably shocked subjects are less active in the presence of shock: Shock may simply be less painful. Antinociceptive processes will not explain all of the effects of uncontrollable shock, however. In particular, they cannot explain why an associative deficit of the sort predicted by the learned helplessness hypothesis occurs (*3*, *4*).

The implications of our results for the mechanism or mechanisms producing analgesia are also important. The results of the final experiment indicate that long-term analgesic effects may not be a simple consequence of exposure to an aversive stimulus. Only inescapable shock produced a nociceptive change 24 hours later. Thus the psychological dimension of uncontrollability may determine stress-induced analgesic reactions. The same need not be true of the short-term stress-induced analgesias, however, which do not require reexposure to the stressor for their occurrence. There may be two or more different analgesic effects produced by different mechanisms.

It might appear that there are poor grounds for arguing that the reinstatement of analgesia occurs only after inescapable shock. Only experiment 3 varied control over shock, and reexposure groups were not compared with no-reexposure groups. But in the first two experiments we found no long-term analgesic reactions after inescapable shock unless brief reexposure to shock was given. Moreover, groups exposed to inescapable shock of fixed duration and yoked controls exposed to inescapable shock have not been found to differ on any measure (*1*). Thus, it is likely that, had we used no reexposure groups in experiment 3, there would have been no analgesic reaction in yoked subjects.

The nature of the mechanisms whereby uncontrollable shock produces changes in nociception remains open. A number of investigators (*5*, *6*) have suggested that the short-term stress-induced analgesia is mediated by the release of endorphins. Our longer-term effects may be produced because exposure to uncontrollable events sensitizes the system or systems responsible for controlling endorphins. Thus reexposure to shock might lead to the release of these analgesia-producing substances. It should be emphasized that our data indicate that if the opiate peptides are involved, the systems controlling the opiate peptides are regulated by the controllability of the stressor. This possibility suggests a potentially important psychological role for the endorphins. When an organism encounters aversive stimuli, pain motivates coping behaviors. If the aversive stimulus is uncontrollable, the continuation of active coping attempts may not be beneficial, since it would deplete bodily energy resources. If escape is not possible, it would be more adaptive to conserve these resources until active coping might be successful (*7*). Endogenous pain regulatory systems could facilitate conservation by reducing coping attempts directly by reducing the motivation to engage in these behaviors.

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Learning in Normal and Mutant *Drosophila* Larvae

Abstract. *Adult* Drosophila melanogaster *have previously been conditioned with shock to avoid various odors. In these experiments, larvae also sensed airborne odorants, responded to electric shock, and learned. Larval behavior paralleled adult behavior for (i) a mutant, smellblind, which failed to respond to odorants; (ii) three mutants, dunce, turnip, and cabbage, which were deficient in olfactory learning ability; and (iii) a mutant heterozygote, turnip/+, which learned but also forgot rapidly.*

Populations of fruit flies can be trained to avoid an odor by being shocked in its presence (*1*, *2*). We wondered whether the larva, with its simpler brain, could also learn. An earlier report of *Drosophila* larval conditioning retained through metamorphosis (*3*) was shown to be attributable to simple habituation (*4*). Nevertheless, larvae of another insect, *Tenebrio molitor*, have been successfully trained to several tasks (*5*, *6*). The experiments reported here indicate that *Drosophila* larvae sense and discriminate odorants, respond to electric shock reinforcement, and learn approximately as well as adult flies. Furthermore, several mutations that block learning or memory in adults cause comparable deficiencies in larvae.

Genetic stocks used were the Canton-Special (C-S) wild-type strain and four mutants derived from it. The mutant smellblind (smb^{PS542}) (*7*) and the learning-deficient mutants dunce (*8*), turnip (tur^{PS274}) (*7*, *9*), and cabbage (cab^{PS264}) (*7*) were isolated according to the mutagenesis and screening procedure of Dudai *et al.* (*8*). All flies and larvae were raised in half-pint milk bottles at 25°C on standard cornmeal medium (*10*).

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