mals by variations in their dorsal fins. During 35 of the 150 days, we obtained enough good fin photographs of each individual (at least four per animal) to be certain that all within the group had been recorded. In this manner we were able to study not only exact group size (as opposed to estimates of group size, which have traditionally been made on porpoises), but group composition over time as well. The size of the group varied between 8 and 22 ($\bar{X}$ = 15, standard deviation = 3.28). Throughout the 21-month study, five animals were always present when the group was sighted. Six others were present until October 1975, at which time they disappeared and five new porpoises took their place. The six no longer sighted near our study area after October 1975 were spotted 6 months later within a new group, at a distance from our study area of more than 300 km. Four of these six animals were again found in the study area in December 1976, 9 months after completion of the 21-month continuous study. Although we do not know when during the 9 months they moved back to their original site, they had covered an extremely long round-trip distance (at least 600 km), a distance previously unknown for these coastal porpoises. Other of the 53 identified individuals appeared with the original "core" unit of five for brief periods ranging from several days to several months and then disappeared again.

The evidence represents an interesting group structure for this species of marine mammal. Only five animals of a partial population of 53 recognized individuals were consistently present when a group was sighted. The interchange of 11 others during October 1975 and the fluidity with which additional animals appeared and disappeared (presumably to join other groups), far surpasses the individual interchanges between known "open" groups of most terrestrial mammals (*2*). It appears that a redefinition of "group" for this population may be more in line with the generally accepted group concept. Thus, we may regard the 53 known animals as part of the same group, while those sighted at any one time, numbering 8 to 22, actually represent subgroups or bands within the main unit. The composition of these subgroups, except for the relatively stable units described, changes greatly. Whether or not the more stable units of five and six animals represent kinship ties is not known.

Because many different porpoise species are periodically found close to shore in different parts of the world (*3*), we believe that this photographic technique would be useful in other areas.

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Hyperalgesia Induced by Naloxone Follows Diurnal Rhythm in Responsivity to Painful Stimuli

Abstract. *A diurnal rhythm was observed in the responsiveness of mice to nociceptive stimuli and in the hyperalgesic activity of naloxone. These rhythms may follow a diurnal rhythmicity in the activity of endogenous opioid peptides and may partly account for previous controversy over the direct action of naloxone in opiate-naive animals.*

Evidence is growing that the recently discovered endorphins, endogenous peptides with opioid activity (*1*), have physiological roles as hormones or neurotransmitters (*2*). They may function in nociception and neuroendocrine regulation and even influence complex mood and behavior (*2*). The suggestion of a physiological role for these substances seemed to demand that naloxone, a pure narcotic antagonist, should have direct pharmacological activity in opiate-naive subjects. Naloxone, however, has been generally considered to have no significant pharmacological activity other than what results from antagonism of exogenous opiates, and this presumed fact has been used as an argument against the likelihood of existence of endogenous opioid ligands (*3*). Even with the recent demonstration that endogenous opioid ligands do indeed occur in brain, the controversy concerning the hyperalgesic activity of naloxone has continued. The lack of such activity has been reported (*3, 4*), and the antagonism by naloxone of the analgesia produced by electrical stimulation of the periaqueductal gray matter (*5*) has also been questioned (*6*). Jacob *et al.* (*7*), nevertheless, have reported a lowering of response latencies by naloxone in the mouse hot-plate test, and we have also observed hyperalgesic activity of naloxone in both the rat flinch-jump (unpublished observations) and mouse hot-plate tests (*8*). We noticed, however, that positive results were not always reproducible but were dependent on the specific response measured and the conditions of the experimental situation. Others have also assumed that naloxone would be expected to have hyperalgesic effects only when the endogenous opioid peptide system was activated (*4, 9*).

In our studies we noticed that the baseline latencies in the mouse hot-plate test showed significant increases from early morning to late afternoon. Diurnal rhythms in the analgesic activity of morphine (*10*) and in the hypothalamic control of neuroendocrine activity (*11*) have been reported. Since endorphins have been implicated in the regulation of nociception and neuroendocrine activity, a rhythm in such endogenous opioids could provide an explanation for these observations and, furthermore, might provide an explanation for the conflicting results concerning the hyperalgesic activity of naloxone. We have observed a diurnal rhythm in responsivity of mice to nociceptive stimuli and in the hyperalgesic activity of naloxone, which might reflect a rhythm in endogenous opioids. A preliminary account of this work has been reported (*12*).

In our studies we used Cox standard mice (20 to 23 g, about 5 weeks old, Harlan Industries) that were raised and maintained on a lighting schedule which consisted of lights on from 0600 to 1800 hours, and lights off from 1800 to 0600 hours. The mice either had continued access to food and water or were deprived for 2 to 6 hours before testing. We detected no difference in the results obtained whether or not the animals had

free access to food and water up to the time of testing. The hot-plate test for analgesia utilized an apparatus with an electrically heated, thermostatically controlled metal plate (Technilab Instruments, model 475). A plexiglass cylinder (30.5 cm high, 12 cm in inner diameter, and open at the top) served to confine the mice to a defined area of the hot plate. The surface was maintained at a temperature of 52°C. The time in seconds from contact with the plate until a hind-paw lick occurred was recorded as a response latency. The latency until an escape jump occurred was also recorded. Each mouse was used only once. The opiate narcotics and also the enkephalins produce a dose-dependent, naloxone-reversible analgesia in this mouse hot-plate test (*2, 8*). The opioid compounds were all more effective at increasing the latency to jump response than the hind-paw lick response.

Opioid peptides are active in this test. Therefore, if they are released endogenously in brain, they should affect response latencies and naloxone should antagonize these effects. Indeed, naloxone, in the dose range 0.5 to 4 mg/kg, given subcutaneously, had hyperalgesic activity; that is, it lowered response latencies in the hot-plate test with opiate-naive mice. Higher doses were not extensively tested. The latency to the jump response was more definitely and reproducibly lowered than was that for the hind-paw lick response (Fig. 1) and the effect appeared maximal at 1 mg/kg. A marked lowering of the latency to jump response occurred also at a dose of 8 mg/kg, but we could not collect any data on the paw lick response at this dose since the animals all jumped before producing the latter response. The dose-response curve for naloxone appears to be bell-shaped since we no longer saw a decrease in jump latency after naloxone at a dose of 16 mg/kg. The results at this high dose were compromised, however, by the occurrence of leg weakness, tremors, and occasionally very mild convulsive behavior. Thus, we observed hyperalgesic activity produced by naloxone very similar to that reported by Jacob *et al.* (*7*); but our attempts to demonstrate the reproducibility of this activity proved variable. In some experiments the activity was seen, while in other presumably identical experiments it was not. Eventually, we noticed that control jump response latencies were significantly different between the early morning hours and late afternoon hours. This prompted us to examine the diurnal variation in control response latencies and in the effects of naloxone and morphine.

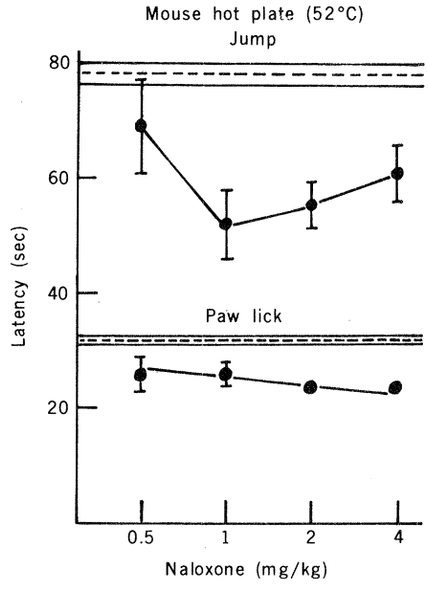

Fig. 1. Hyperalgesic activity of naloxone in the mouse hot-plate test. The plate was maintained at 52°C. The upper set of horizontal lines represent the latency [mean ± standard error (S.E.)] of control mice (saline-treated) to the jump response. The lower set of horizontal lines refer to the control latency (mean ± S.E.) for the hind-paw lick response. Naloxone was injected subcutaneously 15 minutes before testing.

Thus, groups of ten mice each were tested for hot-plate response latencies at 1-hour intervals throughout the day. Anywhere from one to ten groups were tested at each time point. The animals were tested either 15 minutes after subcutaneous administration of saline or naloxone or 30 minutes after administration of morphine. All testing was performed with lights on. These data were collected over a period of several months, and the results are summarized in Fig. 2. The data demonstrate a diurnal rhythm in the jump response latency of the controls. The control latencies are highest, representing a period of reduced irritability, during the hours 1530 to 0430 and lowest, representing a period of increased irritability, during the hours of 0430 to 1130. Treatment with naloxone (3 mg/kg, subcutaneously) damped this rhythm by significantly lowering the latencies during the later hours (1530 to 0430) when the latencies were naturally highest. The effects of naloxone were smallest and most variable during the hours 0630 to 1530, the period when experimental investigations are carried out in most laboratories. The rhythm in the effect of morphine on jump latency also appeared to follow the rhythm in control latency. Naloxone tended to decrease the paw lick latency also, but the effects were small compared to those on the jump latencies; they were only rarely statistically significant and no periodicity was readily apparent.

Thus, the apparent variability in our initial studies with naloxone was clarified when we examined the nociceptive response as a function of time of day. There was a diurnal variation in the baseline latencies to the jump response with lowest values during the early morning hours and a steady increase throughout the day to maximum values in the evening. There was also a diurnal variation in the hyperalgesic activity of naloxone. Naloxone was most effective during the times of highest control latencies. These results might be most readily explained by assuming a diurnal rhythm in the levels of endogenous opioid activity. Indeed, we observed endorphin levels to be occasionally higher in mouse brain at 1500 hours when jump latencies were near maximum than at 0800 hours when these latencies were near minimum (unpublished observations). A diurnal rhythm in the analgesic activity of morphine was reported earlier (*10*). In the present studies, we found this rhythm to follow that of the baseline latencies. Thus, the periods of apparent greater activity of morphine may be due to synergism with endogenous opioid whose activity differs depending on the time of day.

In our studies with the hot plate we measured two endpoints, the latency to hind-paw lick and the latency to escape jump. The hind-paw lick appears to be more a measure of the sensitivity of perception of nociceptive stimuli while the escape jump measures reaction to continued perception of a nociceptive stimulus and, therefore, appears to have more of an emotional component. A diurnal rhythm was not apparent in the paw lick response. Furthermore, both the opiate narcotics and the opioid peptides were more potent in increasing, and naloxone was more effective at reducing, the latency to the jump response than the latency to the paw lick response. This seems in agreement with the reports from studies in humans that morphine appears to obtund more the reaction (or tolerance) to pain than its perception (*13*). The two responses might be regulated by different neuronal systems, and the endorphins apparently have a more prominent role in modulating a system with a greater emotional component. This may be the limbic system, which is known to have a high level of enkephalin and also of stereospecific receptors (*14*), but is apparently not involved in the perception of pain.

The data presented here may provide an explanation for some of the dis-

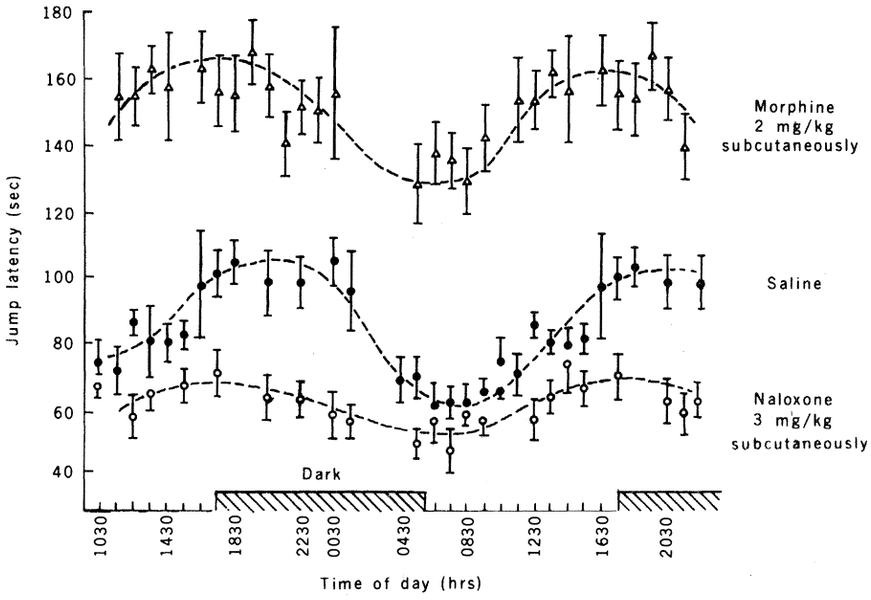

Fig. 2. Diurnal rhythm in latencies to the jump response on the mouse hot plate maintained at 52°C. Closed circles refer to animals treated with saline, subcutaneously, 15 minutes before testing. Open circles refer to animals treated with naloxone at 3 mg/kg, subcutaneously, 15 minutes before testing. Open triangles refer to animals treated with morphine at 2 mg/kg, subcutaneously, 30 minutes before testing. The latencies (mean ± S.E., N = 10 to 100) in seconds are plotted as a function of the time of day. The datum points at each ½ hour are the means of values collected over a 1-hour period. For example, the points plotted at 0930 hours are the means of measurements made from 0900 to 1000 hours. Lights were off during the time period marked by the hatched bars. Several points are repeated beyond 24 hours in order to better illustrate the rhythm.

crepancies of the results in different laboratories concerning the hyperalgesic action of naloxone. The baseline jump latencies are comparatively short and the effects of naloxone are small and variable during the morning and early afternoon hours when most studies are probably conducted. Of course, the occurrence of a diurnal rhythm has many implications beyond providing an explanation for some of the controversy concerning the direct actions of naloxone. One obvious implication concerns the possible variation in the necessary dose of analgesics at different times of day because of the differing levels of endogenous opioids if a diurnal rhythm occurs also in man. In fact, there have been reports of different sensitivities to pain and responses to analgesics depending on the time of day in several studies with man (*15*).

Circadian rhythms in release of various pituitary hormones and also levels and release of monoamines (*16*) have been reported. Since the endogenous opioids influence the release of these hormones and also of monoamines (*17*), it will be of interest to determine whether the endogenous opioids regulate the rhythm in their release or vice versa. Studies of the diurnal course of actual release of endorphins will be necessary to pursue this question.

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