distinct. The behavior of d4-90 cells in response to sodium, as measured by duration of continuous ciliary reversal, also exhibits a significant range as indicated by the figures given in published reports (*5*). Stock d4-578 cells always have yielded plaque patterns readily distinguishable from the wild type. In a "blind" experiment with six coded cultures of wild-type cells and d4-578, we successfully identified each on ultrastructural bases alone.

Our finding that a mutation of a known excitable membrane characteristic is correlated with a clear ultrastructural abnormality of the membrane does not indicate the particular function or functions of the particles or the plaques, nor does it indicate a causal relation between plaque morphology and paranoiac phenotype. It strongly suggests an involvement of these structures in Na^+ influx or K^+ efflux but does not demonstrate whether this correlation is primary, that is, whether the membrane particles represent ion gates or channels, or is secondary. An eclectic approach, combining genetical, biochemical, and electrophysiological analyses may lead to a clarification of the meaning of ciliary plaque variation in the function of the excitable membrane.

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11. This figure is derived by comparing the number of observed plaques on the 40 cilia from d4-578 (27) to the number observed on the same sample size of wild-type cilia (56).
12. Supported by grants from Wells College and the Research Corporation to B.J.B. and B.C.B. and NIH grant R01GM29714-03 to C. Kung, University of Wisconsin. We acknowledge C. Kung's support and comments. We also thank M. V. Parthasarathy (Cornell University) and the Midwest Regional Primate Center (University of Wisconsin) for the use of facilities, A. M. Srb (Cornell University) for advice on the manuscript, R. Dute (University of Wisconsin) for the use of unpublished observations, and A. Shilepsky (Wells College) for assistance with statistical analyses.

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Endorphins: Naloxone Fails to Alter Experimental Pain or Mood in Humans

Abstract. *In 30 human subjects, experimental pain was produced by either ischemia or cold-water immersion. In a double-blind procedure, intravenous doses of up to 10 milligrams of naloxone hydrochloride in saline were indistinguishable from similarly administered saline alone. There were no effects on subjective pain ratings, finger plethysmograph recordings, or responses to mood-state questionnaires. These laboratory procedures do not activate any functionally significant pain-attenuating or mood-altering effect of endorphins.*

The existence of endogenous morphinelike substances (endorphins) in several species, including man (*1*), raises questions about their function. Narcotic antagonists, substances that competitively replace endorphin molecules at receptor sites, reverse and block opiate effects. The failure of the antagonist naloxone even at high doses to have any effect on individuals not addicted to opiates suggests that in the normal state endorphin receptors are unoccupied. However, the ability of naloxone to diminish (i) analgesia produced by electric brain stimulation (*2, 3*) and by acupuncture (*4, 5*) in both animals and man, and (ii) stress-induced analgesia in rats (*6*) suggests that endorphins are released during these procedures. On the other hand, naloxone has no effect on hypnotic analgesia in humans (*7*). Naloxone also does not alter the threshold for escape of trained animals from an electric shock (*8*), but it reduces latency to escape when mice and rats are exposed to a hot plate for the first time (*9, 10*). In humans, naloxone at low dosage does not alter the response to painful electric shocks (*11*). In 12 subjects, we found no effect of naloxone on experimentally induced ischemic pain, but we noted an apparent increase of anxiety after naloxone (*12*). In the two experiments reported here, with 30 additional subjects, we have used both ischemia and cold-water immersion to produce pain. Naloxone had no effect on pain or mood in either procedure.

Subjects for the cold-water (N = 18) and ischemic pain studies (N = 12) were male and female volunteers in equal numbers. Informed consent was obtained. Each subject experienced the painful stimulus at three sessions at 24-hour intervals.

In the cold-water technique (*13*), pain was produced by immersing the dominant hand in a circulating bath of water at 10°C. Subjects rated the pain every 30 seconds on a 10-point scale. A plethysmograph recorded the digital pulse from the index finger of the nondominant hand. As shown by Wolf and Hardy (*13*), pain increases for the first 2 minutes, then decreases. Pulse amplitude decreases (indicating vasoconstriction) within a few seconds after the hand is immersed, then gradually returns to baseline as the pain decreases. If endorphins are involved in these adaptive responses, both the decrease in reported pain and the return of the pulse amplitude to baseline should be blocked by naloxone. Double-blind intravenous injections of saline or naloxone (1 and 10 mg) were administered in a counterbalanced order. The sequence of events at each session was: (i) begin recording the digital pulse, (ii) administer the Profile of Mood States (POMS) questionnaire (*14*), (iii) inject the drug, (iv) wait 5 minutes, (v) immerse the hand for 5 minutes and record subjective pain ratings, (vi) administer the morphine-benzedrine scale (MBG) of the Addiction Research Center Inventory (*15*) (a scale measuring opiate effects), and (vii) repeat POMS administration.

Naloxone had no effect on any of the measures. Both the pain ratings (Fig. 1) and the digital pulse amplitude showed similar adaptive responses after saline and after naloxone. After all of the injections, hand immersion produced an initial acceleration of the pulse, then a slight deceleration. The pulse remained slightly elevated during the entire period the hand was immersed. Naloxone did not affect the scores on either the MBG or the POMS (Table 1). Subjects were not able to differentiate naloxone from saline.

Since we failed to find the same naloxone effect as we had reported (*12*) on the

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tension-anxiety scale of the POMS, the ischemic pain study was repeated with 12 new subjects. As in the original study, pain was produced by placing a blood pressure cuff above the elbow, inflating it to 250 mm-Hg, and having the subject exercise the hand by pulling a dynamometer (12-kg load) 20 times. For the subsequent 10 minutes, while the circulation remained blocked, the subject rated the pain every 30 seconds on a 10-point scale. Pain was produced twice using this procedure, first in the dominant arm, and then, 5 minutes after receiving an intravenous injection of either saline or naloxone (2 and 10 mg), in the nondominant arm. These injections were coded (double-blind), and the order was counterbalanced. The sequence of events at each session was: (i) produce pain in the dominant arm, (ii) administer the POMS, (iii) inject the drug, (iv) wait 5 minutes, (v) produce pain in the nondominant arm, and (vi) repeat administration of the POMS.

As is consistent with our previous report (*12*), naloxone had no effect on ischemic pain (Fig. 2). In addition, there was no effect on mood (Table 1); our previous finding of an effect of naloxone on "tension-anxiety" was not confirmed. Again, subjects were unable to differentiate naloxone from saline.

Table 1. Scores on the profile of mood states (POMS) questionnaire. Mean scores (±S.E.M.) for the seven scales of the POMS questionnaire (*14*). In the cold-water study the predrug POMS was administered before the injection (just prior to the cold-water pain), and the postdrug POMS was after the pain (N = 18). In the ischemic pain study the predrug POMS was before the injection (just after the first production of ischemic pain) and the postdrug POMS was administered just after the second production of ischemic pain (N = 12). The difference is the postdrug score minus the predrug score. A negative value indicates a decrease in the mood state. Repeated measures analyses of variance (*16*) failed to indicate a significant drug effect on the difference scores.

	Saline			Naloxone (1 mg)			Naloxone (10 mg)		
	Predrug	Postdrug	Difference	Predrug	Postdrug	Difference	Predrug	Postdrug	Difference
				Cold-water stress study					
Friendliness	16.1 ± 0.9	15.9 ± 1.1	−0.2 ± 0.5	16.7 ± 1.2	16.0 ± 1.3	−0.7 ± 0.6	16.6 ± 1.1	15.4 ± 1.1	−1.2 ± 0.6
Tension-anxiety	4.8 ± 0.7	4.1 ± 0.6	−0.7 ± 0.7	5.1 ± 1.0	4.0 ± 0.9	−1.1 ± 0.9	5.3 ± 0.8	4.3 ± 0.6	−1.0 ± 0.6
Confusion-bewilderment	4.1 ± 0.7	4.2 ± 0.6	0.1 ± 0.5	4.1 ± 0.9	4.2 ± 0.8	0.1 ± 0.3	3.8 ± 0.7	4.3 ± 0.7	0.5 ± 0.4
Vigor-activity	15.9 ± 1.6	15.3 ± 1.7	−0.6 ± 0.6	15.9 ± 1.6	15.3 ± 1.7	−0.6 ± 0.5	16.8 ± 1.3	15.6 ± 1.4	−1.2 ± 0.9
Depression-dejection	2.7 ± 1.1	1.8 ± 0.8	−0.9 ± 0.5	3.0 ± 1.3	2.7 ± 1.4	−0.3 ± 0.3	3.1 ± 1.1	2.9 ± 1.2	−0.2 ± 0.5
Anger-hostility	1.5 ± 0.5	1.0 ± 0.4	−0.5 ± 0.3	2.6 ± 1.6	2.4 ± 1.4	−0.2 ± 0.6	1.7 ± 1.1	1.3 ± 0.5	−0.4 ± 0.8
Fatigue-inertia	4.7 ± 1.1	4.5 ± 1.0	−0.2 ± 0.4	4.4 ± 1.3	4.8 ± 1.3	0.4 ± 0.4	3.3 ± 1.0	3.1 ± 0.9	−0.2 ± 0.5
	Saline			Naloxone (2 mg)			Naloxone (10 mg)		
				Ischemic pain study					
Friendliness	15.0 ± 1.9	14.2 ± 2.1	−0.8 ± 0.5	14.7 ± 1.7	14.3 ± 1.9	−0.4 ± 0.8	15.1 ± 1.7	13.8 ± 1.7	−1.3 ± 1.0
Tension-anxiety	4.5 ± 0.8	4.7 ± 0.8	0.2 ± 0.5	5.7 ± 1.0	5.3 ± 1.0	−0.4 ± 0.5	4.1 ± 1.0	4.8 ± 1.1	0.7 ± 0.8
Confusion-bewilderment	3.0 ± 0.3	2.7 ± 0.5	−0.3 ± 0.4	3.0 ± 0.3	3.6 ± 0.5	0.6 ± 0.3	3.4 ± 0.4	3.0 ± 0.4	−0.4 ± 0.6
Vigor-activity	14.3 ± 2.3	13.7 ± 2.4	−0.6 ± 0.8	14.3 ± 1.8	13.1 ± 2.3	−1.2 ± 0.8	14.1 ± 1.7	13.3 ± 2.0	−0.8 ± 0.8
Depression-dejection	0.7 ± 0.3	0.5 ± 0.3	−0.2 ± 0.4	0.8 ± 0.3	1.3 ± 0.5	0.5 ± 0.4	0.9 ± 0.3	1.0 ± 0.5	0.1 ± 0.6
Anger-hostility	0.3 ± 0.1	0.3 ± 0.1	0.0 ± 1	0.2 ± 0.1	0.6 ± 0.4	0.4 ± 0.4	0.4 ± 0.2	0.5 ± 0.2	0.1 ± 0.2
Fatigue-inertia	3.1 ± 0.7	2.8 ± 0.8	−0.3 ± 0.4	1.3 ± 0.6	2.0 ± 0.8	0.7 ± 0.4	2.0 ± 1.1	2.8 ± 1.0	0.8 ± 0.5

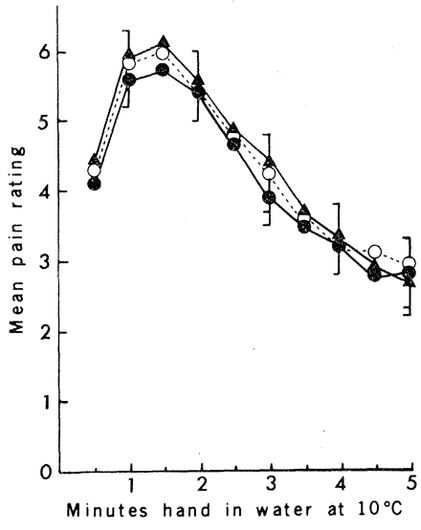

Fig. 1 (left). Failure of naloxone to affect ratings of cold-water pain: mean pain ratings (± S.E.M.) by 30-second intervals after intravenous injections of saline (▲−▲); 1 mg of naloxone (○−○); and 10 mg of naloxone (●−●). The scale ranged from zero (the hand did not hurt at all) to 10 (the pain was unbearable, and the subject wanted to end the experiment) (N = 18).

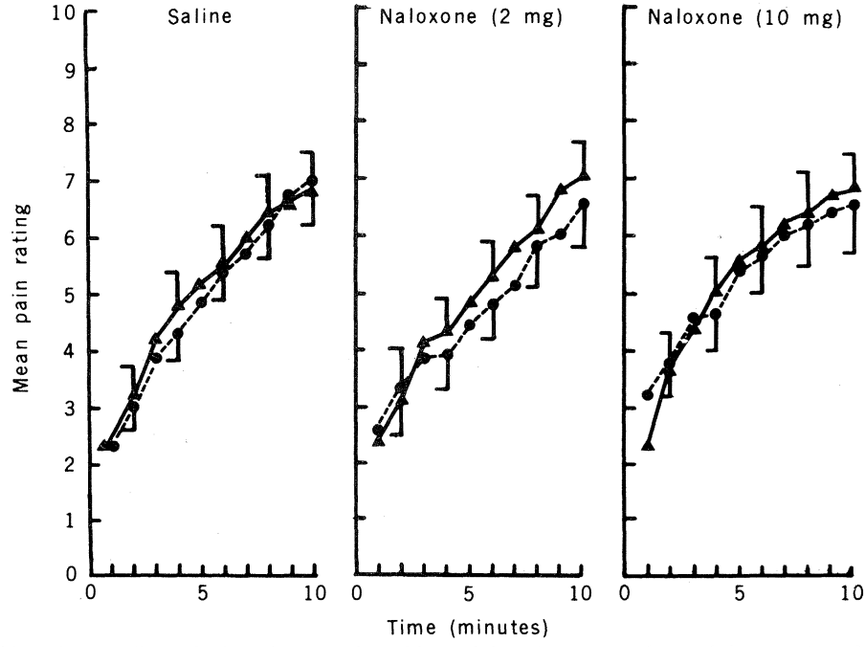

Fig. 2 (right). Failure of naloxone to affect ratings of ischemic pain: mean pain ratings (± S.E.M.) by 1-minute intervals, before (▲) and after (●) intravenous injections of saline or naloxone (2 mg and 10 mg, respectively). The scale ranged from zero (the arm did not hurt at all) to 10 (the pain was unbearable and the subject wanted to end the experiment) (N = 12).

The consistent failure to find an effect of naloxone on experimental pain in humans suggests that endorphin release did not occur during these procedures. The failure in both experiments to find an effect of naloxone on tension-anxiety scores suggests that our previous positive finding may have been significant only by chance.

The naloxone doses administered in our studies were more than adequate to occupy the endorphin receptors. The 10-mg dose is 13 times higher than the largest dose used in the experiment of El-Sobky *et al.* (*11*), and also 25 times higher than the usual dose of naloxone used to arouse a comatose person suffering from an opiate overdose. Moreover, 0.2 mg of naloxone diminishes analgesia produced by electric brain stimulation (*3*), and a dose of 0.8 mg diminishes acupuncture analgesia (*5*). To elicit endorphin release, more painful and stressful stimuli may be necessary, such as the inescapable intermittent shocks used by Akil with rats (*6*) and the lengthy (2-minute) exposure of mice and rats to a hot plate as reported by Jacob (*9*), and replicated by us (*10*). In humans, this may require the study of pain and stress under more realistic conditions than obtain in the laboratory.

Note added in proof: A 1-hour delay was introduced following a naloxone (10 mg) or saline injection. Then a supplemental injection of naloxone (5 mg) or of saline was given, followed by the ischemic pain procedure exactly as described. Again naloxone had no significant effect on pain ratings or mood.

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Anosmia in Male Rhesus Monkeys Does Not Alter Copulatory Activity with Cycling Females

Abstract. *Three adult male rhesus monkeys were tested daily with intact adult female partners over the course of four or five menstrual cycles. The males were made permanently anosmic by chemical ablation of the olfactory epithelium after the second or fourth cycle was completed. All males continued to display typical cycles of copulation with their partners after the anosmia procedures, with the shortest latencies to ejaculation occurring during the periovulatory phase of the partner's ovulatory cycle. Hence, female attractivity and cyclic copulatory performance of rhesus monkeys are not dependent upon olfactory signals.*

When heterosexual pairs of rhesus monkeys are tested in the laboratory for sexual behavior throughout one or more complete menstrual cycles, males ejaculate most frequently and with the shortest latencies during the fertile, periovulatory phase of their partner's ovarian cycle (*1*). Under similar testing conditions, changes in sexual invitations or sexual refusals by the female have not been found to be so well correlated with changes in her hormonal state. Specifically, many females do not show obvious refusals of the male at any time in the cycle, and stimulation of invitational postures of the female by hormonal manipulations such as systemic or intracerebral implants of testosterone do not reliably increase copulatory probabilities (*2*). These observations have led some investigators to conclude that the clear behavioral cyclicity of the male when paired with an intact female is the result of his responding to nonbehavioral changes in the sexual attractiveness of the female, and that the quality of sexual attractiveness of the female rhesus monkey consists, at least in part, of olfactory cues emanating from the vagina (*3*). By such an interpretation, the male's cyclic sexual behavior is affected or produced by changes in the levels or characteristics of these cues. A critical test of the need for olfactory cues for the maintenance of cyclic sexual responding of male rhesus monkeys is to deprive the male of the ability to smell while paired with a cycling female. We report the results of such a study here, showing that copulatory performance and cyclicity in male sexual responses are maintained even though the male is anosmic.

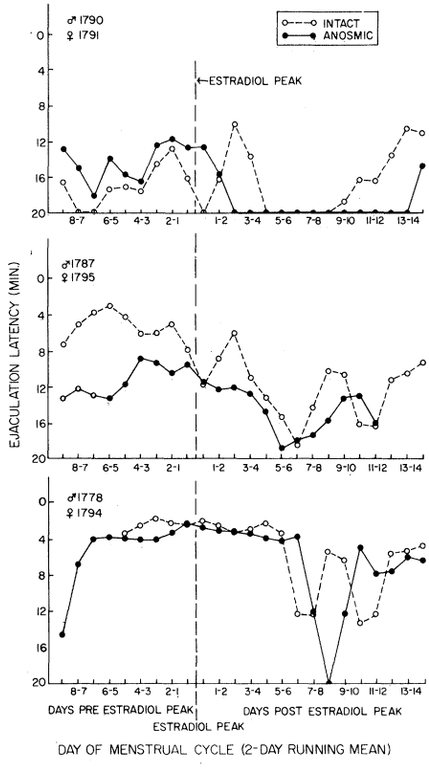

Fig. 1. Latency to first ejaculation during successive menstrual cycles of three rhesus pairs before and during peripheral anosmia of the males. Males 1790 and 1778 were made anosmic on the first menstrual day of their partners' cycle; male 1787, on the fifth day. Data are plotted relative to the estradiol peak (day 0) and are expressed as 2-day running means (the means for days 0 and 1, 1 and 2, 2 and 3, and so forth).

Three vasectomized adult male and three intact adult female rhesus monkeys were obtained 9 months before the study directly from a semifree-ranging troop

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