Selective, Naloxone-Reversible Morphine Depression of Learned Behavioral and Hippocampal Responses

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Abstract. *Morphine administered intravenously causes immediate and complete abolition of a simple learned response (classically conditioned nictitating membrane extension in rabbit) and of the associated learning-induced increase in hippocampal neuron activity. Both effects are completely reversed by low doses of naloxone. Morphine has no effect at all on behavioral performance of the unconditioned reflex response.*

Currently there is widespread interest in the effects of opiates and endogenous opioids on learning and memory processes (*1, 2*). At present, these effects are complex and not well understood (*3*). Indeed, the effects of the original opiate—morphine—on learning and memory processes are not clear (*2, 4*). A major source of difficulty is that, in most learning experiments, drug effects on memory and on performance cannot easily be distinguished. Classical conditioning has the advantage of permitting relatively direct and independent measurement of drug effects on the learned response—the conditioned response (CR)—and on performance—the unconditioned response (UR). If a drug abolishes the CR but does not affect the UR, performance variables relating to the execution of the behavioral response can be excluded. We now report such a selective action of morphine on a simple conditioned response.

In previous work, we have decribed a learning-induced increase in hippocampal unit activity that develops in simple learning situations and that invariably predicts behavioral learning. This response is independent of performance and relates only to the learned response: over a wide range of conditions that influence the development, maintenance and extinction of the learned behavioral response, alteration of the learning-induced hippocampal response precedes and accurately predicts subsequent alteration of the learned behavioral response (*5, 6*). In short, the hippocampal response seems to have the properties of a relatively direct measure of the inferred processes of learning and memory retrieval in the brain. The present experiment provides a further test of this hypothesis.

Methods of training and recording have been described in detail (*5*). Rabbits are restrained and given classical conditioning training with paired tone conditioned stimulus (CS) (1 kHz, 85 dB, 350 msec) and corneal airpuff unconditioned stimulus (US) (210 g/cm pressure, 100 msec, coterminating with CS) trials at a rate of approximately one per minute, eight paired trials and one CS-alone test trial per block, 13 blocks per day. Control animals are given the same number of stimuli but explicitly unpaired in a pseudorandom sequence with an interstimulus interval of approximately 30 seconds. The nictitating membrane extension response is measured with a micropotentiometer attached to the membrane and digitized for computer analysis. Multiple unit and isolated single unit activity is recorded from the CA1 pyramidal cell layer of the dorsal hippocampus using permanently implanted microelectrodes (or a permanently implanted microdrive system). The largest unit discharges (multiple unit recording) or all unit discharges (single unit recording) are detected with a discriminator circuit and stored in the computer in 3-msec time bins for each trial for analysis. Standard scores of the conditioned increase in unit activity are computed from the background and CS period activity.

In the present experiments, animals were trained to a criterion of eight CR's in any nine successive trials, given two blocks of additional training, and then injected with morphine intravenously (ear vein). A rough dose response was determined in pilot animals injected with 1, 5, or 10 mg of morphine per kilogram of body weight in constant volume (0.25 ml/kg). The 1.0 mg/kg dose had no effect on the learned behavioral response and served as a vehicle control. The 5 and 10 mg/kg doses both had profound effects on behavior. In these experiments, morphine doses were as follows: 13 paired

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animals were given 5 mg/kg; six paired animals, 10 mg/kg; and four unpaired control animals, 5 mg/kg. Naloxone (0.1 mg/kg) was injected five blocks later, and two additional blocks of training were given. Two additional animals were given paired training with unit recording electrodes in the central nucleus of the inferior colliculus and injected with 5 and 10 mg of morphine per kilogram, followed by naloxone.

Figure 1A shows the behavioral data for the 5 mg/kg dose of morphine. The CR was immediately and completely abolished, but the UR did not change at all. The CR's recovered slightly over the five blocks, and then immediately returned to initial level after naloxone. This morphine effect on the CR is statistically significant [$F(7, 84) = 30.50$, $P < .001$]. There was no effect on the UR [$F(7, 84)$, $P < 1$]. Results for the 10 mg/kg paired animals were identical except that the CR was even more profoundly depressed. This dose had no effect on the UR. The unpaired control animals permitted independent determination that morphine had no effects on the UR [$F(7, 21) = 1.58$, not significant]. The powerful effect of morphine on memory retrieval was for a task that had just been learned. The animals had been trained to a high level of conditioned responding but had not been overtrained—the memory is recent, not old.

For hippocampal unit recording, only those animals with electrode tips localized to the CA1 pyramidal cell layer and with acceptable unit recordings (*5*) were analyzed ($N = 6$). The effect of morphine on the learned hippocampal response (standard scores) for this group is shown in Fig. 1B. As with the learned behavioral response, the learned increase in hippocampal unit activity was immediately and completely abolished [$F(7, 35) = 3.51$, $P < .05$]. The recovery of this response is greater than and in fact predicts the recovery of the learned behavioral response. Naloxone immediately restores the learned hippocampal unit response to its initial level. An individual example of the action of morphine on the simultaneously recorded behavioral and hippocampal unit responses is shown in Fig. 2. Morphine had no effect on the number of tone CS evoked unit discharges in the inferior colliculus. That the marked morphine depression of the learned behavioral and hippocampal responses is immediately reversed by a low dose of naloxone suggests that it is due to a specific receptor action, possibly on the μ receptors, which preferentially bind morphine and for which naloxone has its highest affinity (*7*).

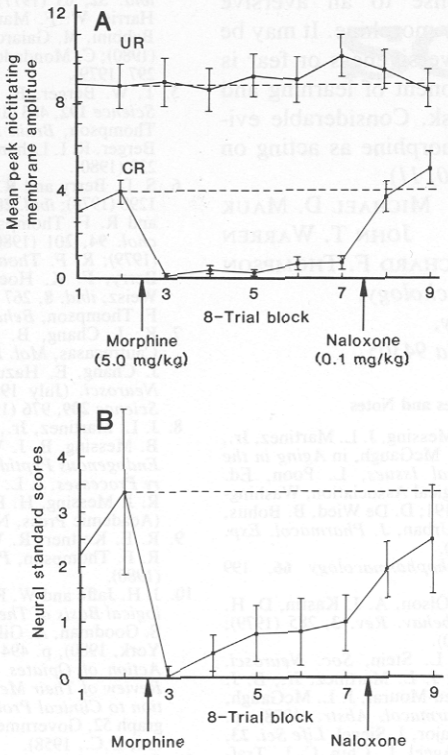

Fig. 1. (A) Mean ($N = 13$) nictitating membrane response peak amplitude during the conditioned stimulus period (for CR) and unconditioned stimulus period (for UR). Dashed lines represent the baseline before morphine was given. (B) Mean hippocampal unit standard scores during the conditioned stimulus period. Dashed line represents the baseline.

Important recent evidence suggests that some effects of administered brain opioids on memory may be mediated in part peripherally (*8*). We have completed preliminary studies with morphine and naloxone analogs administered both peripherally and intracerebrally that indicate strongly a direct, central action of morphine. The selective and reversible abolition of the CR by morphine provides a potentially useful tool for identifying the neuronal circuitry that codes the learned response—the memory system—as well as for understanding of the mechanisms of action of morphine. The absence of morphine action on neurons in the central nucleus of the inferior colliculus argues that the essential neuronal plasticity coding the learned response does not develop in the CS pathway—the primary auditory relay nuclei (*9*). By the same token, the UR is unaffected by morphine. Consequently, the reflex pathways that generate the UR are not a part of the essential neuronal plasticity coding memory.

The action of morphine on the learning-induced hippocampal response parallels its action on the learned behavioral response. Two effects of hippocampal unit activity support the general hypothesis that the learning-induced hippocampal unit response is a relatively direct measure of learning and memory processes in the brain. (i) It recovers more rapidly from the effects of morphine than the learned behavioral response does, and (ii) it predicts the recovery of the behavioral response over trials. It cannot be argued, however, that the effect of morphine on the learned behavioral and hippocampal responses is due to a direct action on the hippocampus. Although this is possible, it is also possible that morphine exerts its primary effects on other structures and circuits in the brain. Some portion of the neuronal circuitry essential for the learned response—for memory retrieval of a simple, classically

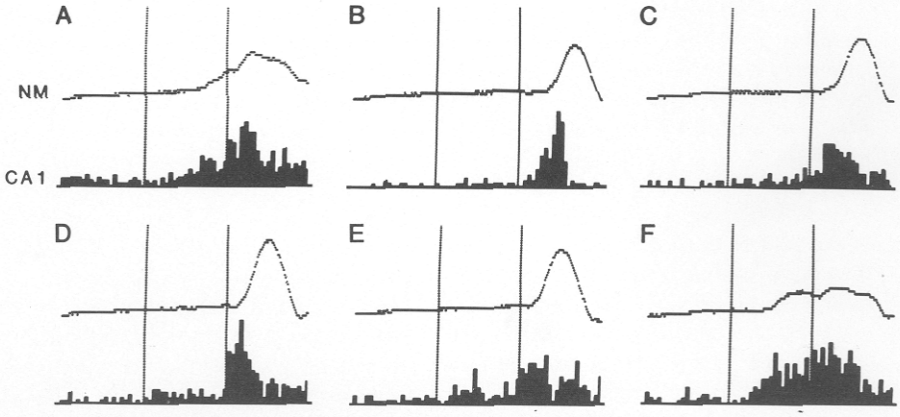

Fig. 2. Examples of eight-trial averaged behavioral nictitating membrane (*NM*) responses (upper trace) and associated multiple unit histograms of hippocampal activity (lower trace, 12-msec time bins) for a single animal. The early vertical line indicates tone onset, and the later line, airpuff onset. Total trace length equals 750 msec. (A) Block of eight trials immediately preceding the injection of morphine. Note the (conditioned) increase in hippocampal activity in the CS period (CS-US interval), which is completely absent immediately after the injection of morphine (B). The unit increase begins to redevelop in the later blocks 3 to 5 (C to E). Both the behavioral and unit conditioned responses recover fully after an injection of naloxone (F).

conditioned response to an aversive US—is impaired by morphine. It may be that conditioned aversiveness or fear is an essential component of learning and memory in this task. Considerable evidence implicates morphine as acting on conditioned fear (*10, 11*).

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References and Notes

1. R. A. Jensen, R. B. Messing, J. L. Martinez, Jr., B. J. Vasquez, J. L. McGaugh, in *Aging in the 1980's: Psychological Issues*, L. Poon, Ed. (American Psychological Association, Washington, D.C., 1980), p. 191; D. De Wied, B. Bohus, J. M. Van Ree, I. Urban, *J. Pharmacol. Exp. Ther.* **204**, 570 (1978).
2. I. Izquierdo, *Psychopharmacology* **66**, 199 (1979).
3. G. A. Olson, R. D. Olson, A. J. Kastin, D. H. Coy, *Neurosci. Biobehav. Rev.* **3**, 285 (1979); *Peptides* **1**, 365 (1980).
4. J. D. Belluzzi and L. Stein, *Soc. Neurosci. Abstr.* **3**, 230 (1977); J. L. Martinez, Jr., B. J. Vasquez, B. Soumireu-Mourat, J. L. McGaugh, *7th Int. Congr. Pharmacol. Abstr.* (1978), p. 560; N. White, R. Major, J. Siegel, *Life Sci.* **23**, 1967 (1978); A. M. Ageel, L. Chin, C. L. Trafton, B. C. Jones, A. L. Picchioni, *Psychopharmacologia* **46**, 311 (1976); W. M. Davis and T. P. Smith, *ibid.* **44**, 95 (1975); C. Castellano, *Psychopharmacology* **42**, 235 (1975); L. D. Byrd, *ibid.* **49**, 225 (1976); N. White, L. Sklar, Z. Amit, *ibid.* **52**, 63 (1977); D. M. McLendon, R. T. Harris, W. F. Maule, *ibid.* **50**, 159 (1976); M. Babbini, M. Gaiardi, M. Bartoletti, *ibid.* **70**, 73 (1980); C. Mondadori and P. G. Waser, *ibid.* **63**, 297 (1979).
5. T. W. Berger, B. E. Alger, R. F. Thompson, *Science* **192**, 483 (1976); T. W. Berger and R. F. Thompson, *Brain Res.* **145**, 323 (1978); T. W. Berger, R. I. Laham, R. F. Thompson, *ibid.* **193**, 229 (1980).
6. S. D. Berry and R. F. Thompson, *Science* **200**, 1298 (1978); *ibid.* **205**, 209 (1979); F. K. Hoehler and R. F. Thompson, *J. Comp. Physiol. Psychol.* **94**, 201 (1980); *Physiol. Psychol.* **7**, 345 (1979); R. F. Thompson, T. W. Berger, S. D. Berry, F. K. Hoehler, R. E. Kettner, D. J. Weisz, *ibid.* **8**, 267 (1980); T. W. Berger and R. F. Thompson, *Behav. Brain Res.*, in press.
7. K. J. Chang, B. R. Cooper, E. Hazum, P. Cautrecasas, *Mol. Pharmacol.* **16**, 91 (1979); K. J. Chang, E. Hazum, P. Cuatrecasas, *Trends Neurosci.* (July 1980), p. 160; S. H. Snyder, *Science* **209**, 976 (1980).
8. J. L. Martinez, Jr., H. Rigter, R. A. Jensen, R. B. Messing, B. J. Vasquez, J. L. McGaugh, in *Endogenous Peptides and Learning and Memory Processes*, J. L. Martinez, Jr., R. A. Jensen, R. J. Messing, H. Rigter, J. L. McGaugh, Eds. (Academic Press, New York, 1981), p. 305.
9. R. E. Kettner, R. V. Shannon, T. M. Nguyen, R. F. Thompson, *Percept. Psychophys.* **28**, 504 (1980).
10. J. H. Jaffe and W. R. Martin, in *The Pharmacological Basis of Therapeutics*, A. G. Gilman, L. S. Goodman, A. Gilman, Eds. (Macmillan, New York, 1980), p. 494; A. Wikler, *Mechanisms of Action of Opiates and Opiate Antagonists: A Review of Their Mechanisms of Action in Relation to Clinical Problems* (Public Health Monograph 52, Government Printing Office, Washington, D.C., 1958).
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