remanent magnetic field decay observed in vivo can be attributed to intracellular motions within particle-containing macrophages.

Magnetometry can also be used in the study of cytoskeletal function and intracellular viscosity. Frey-Wyssling pointed out as early as 1938 that subcellular structures appear to be attached to each other by protoplasmic threads (*16*), and more recent data support the idea that the cytoplasm is a highly organized lattice of elements (*17*). The mechanisms responsible for cell organelle movement in motile nonmuscle cells is currently under close scrutiny (*13, 14, 18*), and the role of various cytoplasmic filaments has not been settled. With magnetometric techniques it is possible to detect noninvasively the motion of magnetic particles within isolated cells, and also external magnetic fields can be used to twist the particles and probe their viscous environment (*19*). Although intracellular particle motions can be observed in videomicroscopy on a cell-by-cell basis, magnetometric techniques provide a quantitative description of mechanical events and cytoplasmic motility within a large ensemble of cells. Thus, measurement and manipulation of magnetic particles within cells in vitro or in situ can be used to sense amoeboid motions, to probe cell rheology, and to assess the integrity of contractile elements.

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10. A maghemite ($\gamma$-$Fe_2O_3$) aerosol was produced by the controlled combustion of iron pentacarbonyl. The particles produced are crystalline in appearance and about 0.1 to 0.2 μm in diameter (*4*).
11. Each hamster was anesthetized, and the trachea was exposed and cannulated. The lungs were lavaged 13 times with Dulbecco's calcium- and magnesium-free phosphate-buffered saline (4 ml per wash). The absence of divalent cations promotes detachment of macrophages from the alveolar surface, and gentle massage of the chest further increases the cell yield [P. A. Valberg *et al.*, *Exp. Cell Res.* **141**, 1 (1982)].
12. The remanent magnetic field was sensed with a Förster (model 1.107) flux-gate magnetometer with two sets of special field and gradient probes wired in a second-order gradiometer configuration (see Fig. 1). The probes were oriented parallel to field lines from the local magnetic dipole to optimize their sensitivity to the field from the cells (the signals from the probes were added to give four times the signal from one probe) and to strongly reject the more uniform fields from distant sources. The output from this instrument was sent to a phase-sensitive amplifier, where it was combined with a reference signal synchronized to cell cuvette rotation.
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## Anatomically Distinct Opiate Receptor Fields Mediate Reward and Physical Dependence

Abstract. *Rats never before exposed to opioids rapidly learned to press a lever for microinjections of morphine into the ventral tegmental area. Challenge by a narcotic antagonist produced no signs of physical dependence. Dependence was not seen after long-term morphine infusions into the ventral tegmentum but was seen after similar infusions into the periventricular gray region. Thus a major rewarding property of morphine is independent of the drug's ability to produce physical dependence. These data challenge models of drug addiction that propose physical dependence as necessary for the rewarding effects of opioids.*

The rewarding effects of opioid injections in humans and laboratory animals (*1*) have been presumed by many investigators (*2*) to result from the ability of opioids to relieve the distress of withdrawal after long-term drug use is discontinued. This view does not explain, however, why opioids are initially taken or why they retain their potent rewarding effects after long periods of drug abstinence. Several workers (*3*) have argued that opioids can be rewarding independent of any ability to alleviate withdrawal stress, but in most demonstrations of opioid reward, repeated injections are given and some degree of dependence may play a role (*4*). We now report that opioids can be rewarding even when they are restricted to brain regions where they do not activate mechanisms involved in physical dependence. Nondependent rats learned to press a lever for morphine injections into the ventral tegmental area but not into the periventricular gray region; rats were made physically dependent on morphine by long-term infusions into the periventricular gray but not by infusions restricted to the ventral tegmental area.

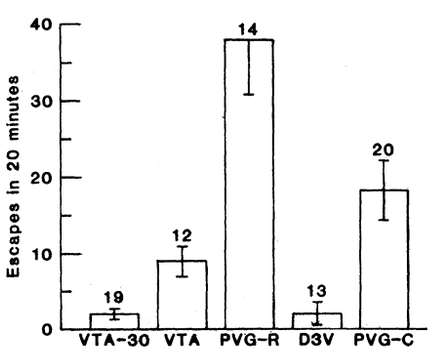


Fig. 1. Naloxone-precipitated escape responding after long-term morphine infusions into various brain regions. Abbreviations: *VTA-30*, ventral tegmental area with angled cannulas; *VTA*, ventral tegmental area with unangled cannulas; *PVG-R*, rostral aspect of the periventricular gray substance; *D3V*, dorsal aspect of the third ventricle; and *PVG-C*, caudal aspect of the periventricular gray substance. Error bars depict standard errors of the means. The number of subjects is shown above each bar.

To identify the opiate receptor fields involved in opioid reward and physical dependence, we microinjected morphine directly into brain tissue. Experimentally naïve rats learned to administer 100-ng infusions of morphine sulfate into the ventral tegmental area (*5*) but failed to learn to press for the same infusions into other opiate receptor fields (*6*). To deter-

mine whether physical dependence is produced by this regimen, we challenged six subjects with the opiate receptor antagonist naloxone (5 mg/kg, injected intraperitoneally) and assessed the traditional dependence signs—escape from the test box, chattering teeth, and "wet-dog" shakes. None of these behaviors were induced by the naloxone challenge. Although this test suggested that the rewarding effect of morphine in these animals was not accompanied by physical dependence, it was possible that undetected dependence was sufficient to motivate drug-taking behavior.

Since the severity of withdrawal signs is exacerbated by increased drug exposure (*7, 8*), we tested whether continuous, prolonged morphine infusions into the ventral tegmentum could produce physical dependence. Morphine was also infused into the periventricular gray substance and the ventricular space dorsal to this region. The drug (0.5 μg in 0.5 μl of vehicle per hour) was delivered for 72 hours through permanently implanted 21-gauge stainless steel cannulas connected by polyethylene tubing to osmotic minipumps (*9, 10*). The animals were then intraperitoneally injected with naloxone (5 mg/kg) and placed in a 25-cm Plexiglas cylinder. The number of escapes from the enclosure and the incidence of chattering teeth and of wet-dog shakes were noted for 20 minutes (Fig. 1). Long-term infusions into the periventricular gray produced clear signs of naloxone-precipitated withdrawal similar to that previously reported for both systemic and centrally administered morphine (*7, 10, 11*).

Some dependence signs were also seen after infusions into the ventral tegmentum, which lies 2 mm ventral to the injection site in the periventricular gray. Since intracranial drug injections can flow up the injection cannula to dorsal sites of action (*12*), the physical dependence produced by ventral tegmental morphine infusions may have resulted from the drug's reaching the periventricular gray. To assess this possibility, additional animals were tested with cannulas angled to avoid penetration of the periventricular gray (*13*). When morphine was infused through angled cannulas, no signs of dependence were precipitated by the naloxone challenge (Fig. 1). These data indicate that morphine injections into the ventral tegmental area that are sufficiently rewarding to establish the lever-pressing response in experimentally naïve laboratory rats (*5*) do not produce the dependence signs usually associated with opioid addiction in this species. Physical dependence does result from the same regimen of morphine infusions into the periventricular gray region, but this site does not support intracranial morphine self-administration at doses that are effective in the ventral tegmentum (*6*). Thus, at least one rewarding consequence of opioids does not involve the dependence mechanism; this result confirms the view of several investigators that physical dependence is not a necessary condition for opioid reward (*3*). It remains possible that relief of withdrawal distress can add to the rewarding effect of morphine when systemic drug intake is prolonged (*14*), but the existence of a primary rewarding effect independent of any relief of withdrawal stress suggests the need to deemphasize dependence in definitions of addiction and questions the utility of treatment programs aimed at simply alleviating withdrawal discomfort. The primary rewarding effect of morphine in the ventral tegmental area may explain two facts that are explained only with difficulty by homeostatic theories that stress the relief of withdrawal symptoms as the source of drug reward: opioids are potent rewards in naïve subjects (*3*), and drug-oriented behavior is prevalent in addicts and experienced laboratory animals even after prolonged periods of abstinence.

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5. Rats were tested in three 4-hour sessions. Each lever-press resulted in a 100-ng infusion of morphine sulfate (300 pmole) dissolved in 100 nl of Ringer solution. Animals in the yoked control condition rarely pressed the lever. The intracranial self-administration of morphine was blocked by naloxone; this blockade eliminates nonspecific drug action as an explanation of the rewarding effect [M. A. Bozarth and R. A. Wise, *Life Sci.* **28**, 555 (1981)].
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14. Animals will work to avoid naloxone injections that precipitate withdrawal symptoms [S. R. Goldberg, F. Hoffmeister, U. Schichting, W. Wuttke, *J. Pharmacol. Exp. Ther.* **179**, 268 (1971)]. Thus, the negatively reinforcing property of withdrawal distress can maintain operant responding. Also, the potency of an opiate in supporting intravenous self-administration is related to its potency in producing physical dependence [A. M. Young, H. H. Swain, J. H. Woods, *Psychopharmacology* **74**, 329 (1981)].
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