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Facilitation of Memory Processing by Posttrial Morphine: Possible Involvement of Reinforcement Mechanisms?

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Abstract. Posttrial administration of 40 mg/kg and 100 mg/kg, but not of 1 mg/kg, of morphine hydrochloride facilitates learning of a one-trial passive avoidance task in drug-naive mice. The effect does not depend on the punishing properties of the morphine injection, since an injection of LiCl (a strong punisher) fails to enhance learning in a similar way. After the establishment of tolerance by several morphine administrations, the 100 mg/kg, but not the 40 mg/kg, dose level resulted in memory facilitation.

The data are discussed in connection with the hypothesis that morphine acts directly on reinforcement mechanisms by activating the opiate receptor.

Key words: Memory – Facilitation – Morphine – Reinforcement mechanisms – Opiate receptor – Mice

Behavior which is controlled by its consequences is termed operant behavior; the controlling consequences are reinforcers. Morphine as the controlling consequence for the operant behavior which leads to its self-administration is therefore, by definition, a reinforcer. The reinforcing properties of morphine are considered to be responsible for the establishment of morphine addiction (Schuster and Thompson, 1969; Spealman and Goldberg, 1978). These properties are mostly attributed to the ability of this drug to induce affective states, i.e., either to terminate the characteristic withdrawal syndrome in addicted subjects (negative reinforcement) or to induce euphoria (positive reinforcement). Both properties may account for specific phases in the development of addiction, but fail to give an explanation of the initial phase of morphine self-administration. The initial application of morphine does not lead to euphoria, nor does it terminate a withdrawal-induced discomfort. In particular, the first morphine injection is reported to be aversive in man (Lasagna et al., 1955) and in animals. Jacquet (1973) and Capell et al. (1973) demonstrated that morphine injections can punish saccharin drinking in conditioned taste aversion paradigms. Evidently an event which at least initially has punishing properties may act as a reinforcer. This obvious paradoxical effect of morphine (similar effects are known for amphetamine and apomorphine; Wise et al., 1976) raises the question of whether the reinforcing effects (of morphine) may be initially independent of the affective properties of the drug, i.e., that the drug may act directly on reinforcement/memory facilitation mechanisms.

Other reinforcers, such as food (in hungry animals) or electrical brain stimulation (at rates which approximate that at which the animals are found to self-administer when given a chance to self-stimulate), have already been shown to improve retention of different learning tasks if given posttrial (Huston et al., 1974; Huston and Mondadori, 1975; Mondadori et al., 1976; Mondadori et al., 1977; Müller et al., 1977). Major and White (1978) observed memory facilitation after posttrial self-stimulation in the lateral hypothalamus.

The following experiments were performed in order to find out if morphine, given posttrial, acts on memory processing, i.e., retention of a passive avoidance task.

General Methods

The animals were male albino mice, weighing 20–25 g, of the ICR-Z[1] strain, outbred from Charles River Mouse Farms ICR-COBS. They were housed in 42 × 26 × 15 cm Makralon cages with free access to food and water under a natural day/night regime.

The step-down apparatus for testing passive avoidance consisted of a 50 × 50 × 50 cm box with an electrifiable grid floor (6-mm stainless steel rods placed 13 mm apart). A 10-mm-high, 67-mm-diameter round wooden platform was situated in the middle of the grid. Enclosing this platform was a 20-cm-long, 68-mm inner diameter plastic tube. The foot shock was a scrambled 1-mA 50-Hz sine wave current of 1 s duration. The current was limited by a constant current unit.

A trial consisted of placing an animal on the wooden platform within the enclosing tube. After 10 s the tube was removed and the

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step-down latency recorded. As soon as the animal's four paws touched the grid, the foot shock was delivered. Within 10 s after the footshock the animals received the drug i.p. The test for learning, i.e., recording of latencies for a second step-down was performed 24 h later. Retest step-down latency (SDL) recording was cut off after 150 s. All experiments were performed under double-blind conditions.

Three experiments with different treatments and procedures were run; detailed methodological points pertaining to each of these three experiments are mentioned under the respective headings.

Experiment 1

Influence of a Single Posttrial Injection of Morphine on Retention of a Passive Avoidance Task

Methods. In the first part of this experiment, 72 drug-naive mice were subjected to the passive avoidance procedure and immediately after having received the foot shock they were injected with 40 mg morphine hydrochloride/kg. Another 72 mice were subjected to the same procedure, but received saline instead of morphine. In a second part, 25 animals each received either 1 mg/kg or 100 mg/kg of either morphine or saline.

Results and Discussion. A single injection of 40 mg/kg or 100 mg/kg, but not 1 mg/kg, of morphine facilitated learning of the step-down avoidance. This is shown by an increase of retest step-down latencies (Table 1). The memory facilitation effect seems to be dose-dependent. Belluzzi and Stein (1977b) reported a similar facilitation after posttrial *intracerebral* application of morphine and enkephalin. Using low doses of morphine (1 and 3 mg/kg), Messing et al. (1978) observed an *impairment* of retention after posttrial application. This is of interest, as in our experiments there was also a slight trend toward impairment at a dose level of 1 mg/kg. The same authors also investigated the effects of 30 mg morphine/kg but their abstract contains no results.

Experiment 2

Control for the Aversive Effects of the Morphine Injection

The results of the first experiment do not militate against a 'direct' action of morphine on memory processing. Nevertheless an alternative interpretation must be considered: As we mentioned earlier, a single morphine injection can be punishing. The punishing properties are reported to be dose-dependent: a single dose of morphine of 100 mg/kg was found to be more effective than 50 mg/kg or 25 mg/kg (Jacquet, 1973). Thus, we may ask whether it is the punishing effect of morphine that is responsible for the increase of the retest step-down latencies. One may indeed argue that receiving a drug punishment immediately after a foot shock punishment possibly leads to a simple addition of punishments resulting in longer step-down latencies.

Table 1. Effects of posttrial morphine. Means and standard errors for step-down latencies (SDL) during baseline and retest trials for the experimental (posttrial morphine) and control (posttrial saline) group

		SDL (s) baseline	SDL (s) retest	*n*
Part 1	Saline	7 ± 1	25 ± 5	72
	Morphine (40 mg/kg)	7 ± 1	50 ± 7*	72
Part 2	Saline	9 ± 1	26 ± 8	25
	Morphine (1 mg/kg)	9 ± 1	22 ± 5	25
	Morphine (100 mg/kg)	9 ± 1	43 ± 9*	25

* $P < 0.05$ Mann-Whitney *U*-test (two-tailed)

To investigate this assumption an experiment was performed in which LiCl was injected, a compound which is thought to be even more punishing than morphine (Gorman et al., 1978), but presumably does not have reinforcing properties. If the punishing effect of the morphine injection causes the improvement of learning, the one would expect a similar or even more pronounced facilitatory effect after posttrial LiCl injection.

Methods. One hundred and twenty mice were subjected to the passive avoidance procedure. They were divided into two groups, which received either 50 mg LiCl/kg (1 % of body weight) or saline i.p. This dose of LiCl has been found to induce a strong (comparable to the effects of 100 mg morphine/kg) conditioned taste aversion against saccharin in mice, i.e., it has comparable punishing properties (unpublished pilot study).

Results and Discussion. In this experiment the mean latencies of the saline-injected control groups and the LiCl-injected experimental animals did not differ in a statistically significant way. The results are shown in Table 2; they make it unlikely that the memory-facilitatory effect of posttrial morphine injection in Experiment 1 is due to the aversive effects of the treatment.

Experiment 3

The Effects of a Posttrial Morphine Injection on Retention of a Passive Avoidance Task in Morphine-Pretreated Mice

The results of Experiment 1 indicate that morphine facilitates learning if injected posttrial. The data obtained in Experiment 2 suggest that this effect does not depend on the aversive properties of this drug. However, the facilitatory effect of morphine could also be independent of its rewarding properties (euphoria,

Table 2. Effects of posttrial LiCl. Means and standard errors for step-down latencies (SDL) during baseline and retest trials for the experimental (posttrial LiCl) and the control (posttrial saline) group

	SDL (s) baseline	SDL (s) retest	*n*
Saline	10 ± 1	35 ± 6	59
LiCl (50 mg/kg)	9 ± 1	38 ± 6	59

pleasure) and thus would be the result of a direct influence on memory processing. One may indeed argue that the first morphine experience may be aversive because of a preponderance of the punishing properties. But this does not necessarily mean that no rewarding effects may be present. They may be masked by the strong punishing effects.

In man and animals, the rewarding properties of morphine injections are reported to increase, whereas the punishing properties decrease, with repeated administration (Lasagna et al., 1955; LeBlanc and Cappell, 1974). One may therefore argue that if the rewarding (affective) properties were instrumental in causing the observed memory facilitation it is likely that an even more pronounced effect would appear after repeated treatment with morphine. In turn, if morphine were to act directly on memory processing (independently of its rewarding properties), a reduced effect would be expected, due to tolerance.

Methods. To test this assumption, mice were given repeated injections of morphine; twice daily (at 8 a.m. and 4 p.m.) for 4 consecutive days. On the first day they received 2 × 5 mg/kg; on the second day 2 × 10 mg/kg; on the third day 2 × 20 mg/kg; and on the fourth day 2 × 40 mg/kg s.c. Control animals received saline according to the same schedule. On day 5 the animals were subjected to the passive avoidance task. The experiment was performed in two parts.

In the first part, 180 mice were used; one-half ($n = 90$) of these were morphine pretreated, the other half ($n = 90$) saline pretreated. Of the morphine-pretreated animals one-half ($n = 45$) received an injection of 40 mg morphine/kg immediately after the learning trial, the other half ($n = 45$) received saline. Of the saline-pretreated mice one-half ($n = 45$) received morphine (40 mg/kg) and the other half ($n = 45$) posttrial saline. Thus we had four experimental conditions: morphine-pretreated mice receiving posttrial saline (MS), morphine-pretreated animals receiving posttrial morphine (MM_{40}), saline-pretreated mice receiving posttrial morphine (SM_{40}), and, finally, saline-pretreated animals receiving posttrial saline (SS). All injections were made i.p.

In the second part, we used 90 mice. Of these, 60 were morphine pretreated (see above), 30 received saline pretreatment. After being subjected to the passive avoidance procedure, one-half ($n = 30$) of the morphine-petreated mice received 40 mg morphine/kg, the other half ($n = 30$) 100 mg morphine/kg i.p. The saline-pretreated mice ($n = 30$) received posttrial saline i.p. Thus we had three experimental conditions: morphine-pretreated animals receiving 40 mg/kg (MM_{40}) or 100 mg/kg (MM_{100}), and saline-pretreated mice receiving posttrial saline (SS).

Results and Discussion. In the first part of Experiment 3, a single dose of 40 mg morphine/kg (which had been effective in drug-naive animals) did not cause facilitation of memory in morphine-pretreated mice. The mean SDL (step-down latency) corresponded closely with the mean SDL of the SS control group. The SM_{40} animals showed an increase in step-down latencies not unlike the findings in Experiment 1. Since the mean retest SDL of the control group (SS) was unusually high, it may mask a weak facilitatory effect in the MM_{40} group and account for the relatively small difference between the SS and the SM_{40} group. In the second part of the experiment, a facilitatory effect was observed after application of either 40 mg/kg (though not significant, $0.05 < P < 0.06$) or 100 mg/kg ($P < 0.01$).

Table 3. The effects of posttrial morphine after different pretreatments. Means and standard errors for step-down latencies (SDL) during baseline and retest trials for the different experimental groups. SS: saline-pretreated animals receiving posttrial saline. SM: saline-pretreated animals receiving posttrial morphine. MS: morphine-pretreated animals receiving posttrial saline. MM: morphine-pretreated animals receiving posttrial morphine. The indices refer to the given dose in mg/kg)

	Group	SDL (s) baseline	SDL (s) retest	*n*
Part 1	SS	12 ± 1	61 ± 6	44
	SM_{40}	12 ± 1	84 ± 7*	44
	MS	15 ± 1	50 ± 5	43
	MM_{40}	18 ± 1	57 ± 6	43
Part 2	SS	13 ± 1	38 ± 9	29
	MM_{40}	19 ± 2	60 ± 10	30
	MM_{100}	16 ± 2	72 ± 10**	28

* $P < 0.05$ Mann-Whitney *U*-test (one-tailed)
** $P < 0.01$ Mann-Whitney *U*-test (two-tailed)

The data suggest that morphine probably affects memory in a direct way. But the alternative possibility of an involvement of the affective properties of the drug in the memory facilitation cannot be ruled out. Our morphine application schedule causes tolerance (Lienhard et al., 1975). The dynamics of reward and punishment after several morphine applications may be very different in man and animals, and in either may be subject to tolerance in a similar or completely different way.

General Discussion

Our results indicate an improvement of retention in retest after posttrial application of morphine. The classical way of interpreting such data is in terms of an influence on posttrial memory processes or memory consolitation (McGaugh and Herz, 1972).

Some years ago we proposed that reinforcers in general act (as least in part) on memory processing (Huston and Mondadori, 1975). This hypothesis is based on findings of food reinforcement facilitating passive avoidance learning if given posttrial (Huston

et al., 1974) and supported by other experiments using other reinforcers and other tasks (Mondadori et al., 1976, 1977; Müller et al., 1978; Major and White, 1978). Morphine, may thus affect one or more of those inputs that influence the reinforcement system. There is also the possiblity that morphine affects directly the network constituting the reinforcement system per se, and there is some evidence to support this suggestion.

It has been suggested that endogenous compounds, which have reinforcing properties, are somehow involved as network links in the reinforcement apparatus. Belluzzi and Stein (1977a) observed self-administration of enkephalins (the endogenous opiate agonists). Yet, while self-injection behavior may demonstrate reinforcing properties of the injected compound, it does not give any information on the mode and locus of the functional involvement of the endorphine system in reinforcement processing. However, the same authors also observed that electrical self-stimulation (of the central gray area) is inhibited by naloxone (a potent opiate antagonist); this implies a more central role for the opiate system(s) in reinforcement processing.

On the other hand, the locus of stimulation appears to be of paramount importance: van der Kooy et al. (1977) failed to inhibit electrical self-stimulation of the lateral hypothalamus and of the caudatus, with naloxone. Hence, at least some reinforcement circuits seem to be independent of the endorphergic link.

Moreover, there is at least comparable evidence for an involvement of catecholamines in reinforcement mediation (Wise, 1978; Wise et al., 1978; Yokel and Wise, 1975). Baxter et al. (1974) and Wise (1978) have shown that catecholamine agonists are self-administered, and, interestingly, catecholamines also facilitate learning if given posttrial (Haycock et al., 1977; Stein et al., 1975). These findings also support our hypothesis that memory processing may be facilitated via reinforcement mechanisms.

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