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Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment

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Summary

1. Effects of morphine sulphate (1·25, 2·5, 5, 10, 20 and 40 mg/kg i.p.) on locomotor activity of male rats were observed for 8 h after single doses in non-tolerant rats. The lower three doses had only an excitatory effect, whereas the higher three doses caused initial depression followed by a delayed excitatory effect.

2. The same doses of morphine were administered daily for 30 days. No tolerance developed within this time to the excitatory effect. The locomotor excitatory effect of the higher three doses of morphine became progressively more pronounced over treatment periods of 30 days (and 48 days for 20 mg/kg), while the latency to peak activity decreased.

3. An explanation of these results is suggested on the basis of two different central drug-receptor interactions affecting motility.

Introduction

Despite the considerable research directed to the effects of morphine on more complex behaviour, the actions of this drug on spontaneous motor activity of the rat have not been extensively or systematically characterized. In some studies on the production of tolerance and physical dependence to morphine, gross observations of increased motility were mentioned, but attempts to quantify the results were not reported (Kaymakcalan & Woods, 1956; Martin, Wikler, Eades & Pescor, 1963). During chronic administration of increasing doses of morphine to rats, Kaymakcalan & Woods (1956) found a 'disappearance of the sedation which follows the first administration'. Instead, more and more stimulation was exhibited, often consisting of continuous movement lasting 4–5 h after the injection. Similarly, Kumar, Mitchell & Stolerman (1971) found that tolerant rats showed a locomotor stimulant response to a high dose (120 mg/kg) of morphine. The neurochemical basis of increased motor activity after morphine (10 mg/kg) in tolerant rats was studied recently by Eidelberg & Schwartz (1970).

In several studies which attended primarily to other behavioural parameters, changes in motor activity after morphine were recorded secondarily (Beach, 1957; Collins, 1965; Sloan, Brooks, Eisenman & Martin, 1962; Gunne, 1963; Fog, 1970). Such observations were usually limited to one or two dose levels and only a few schedules of treatment. In an earlier study, the observations of Kaymak-

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calan & Woods (1956) were confirmed by one of us (M. B.), by means of an actographic technique that could give semiquantitative results, but the research was limited to only two doses and only one schedule of treatment (Tonini, Missere & Babbini, 1959). In repeating and enlarging that investigation, we have conducted a series of experiments with a battery of photocell actometers. As the course of the later experiments was determined by the results of the previous ones, the detailed procedure and the results will be described in sequential order. The general procedure was the same for all experiments.

Methods

Apparatus. Twenty photocell actometers were used for all the experiments. They were of a circular track type (Pickens & Crowder, 1967) that allows a selective record of the locomotor activity of the animals, only large movements being registered. Each actometer was equipped with 4 digital counters which could be operated successively for 1 h intervals by a motor-operated stepper switch to total the movements of each animal. As a pilot experiment showed that variability among actometers was very small, a completely randomized experimental design was adopted in their use. Each actometer included a food container and water bottle. A 6 W, 120 V lamp in the centre provided a low level of illumination in the actometer and served as light source to four peripherally placed photocells. The actometers were on racks in a darkened and partially sound-isolated room. Room temperature was maintained at 24° C.

Subjects. Male Holtzman albino rats about 180–200 days old were used. They were housed in community cages with food and water available *ad libitum.* The experiments were performed from 9.30 a.m. to 6 p.m., a period in which the activity level of rats was low and fairly constant when housed under an artificial light cycle of 8 a.m. to 8 p.m. All injections were made intraperitoneally in volumes of 0·5 to 1·0 ml/kg.

Procedure of Experiment I

Nine rats were placed in the actometers from 10 a.m. to 6 p.m. on 2 consecutive days without injection. On the 3rd day they were injected with 0·9% NaCl solution (saline) and were placed in the apparatus just after the injection. Their motility was recorded every hour from 10 a.m. to 6 p.m. Beginning on the 4th day they received 20 mg/kg of morphine sulphate by injection every 24 h for 48 days while activity was recorded on drug day 1, day 4, and every 4th day thereafter following the same schedule as on control day 3.

Procedure of Experiment II

For this acute experiment 140 rats were used. After receiving an injection of saline, they were placed in the actometers at 10 a.m. for 24 h. The next day they were injected with one of the following doses of morphine sulphate: 1·25, 2·5, 5, 10, 20 and 40 mg/kg. Twenty rats were used for each dose and for the saline-injected control group. Motility again was recorded hourly for 8 h following injection.

Procedure of Experiment III

Because Experiment II had shown that after adaptation for 24 h to the actometers the animals still showed higher values of activity during the 1st h after the treatment, a separate experiment was performed to clarify this. Twelve rats were injected with saline and their motility was recorded for 4 h (6 rats from 9.30 a.m. to 1.30 p.m., the others from 2 p.m. to 6 p.m.) for 8 consecutive days.

Procedure of Experiment IV

The design was similar to that of Experiment I but 6 groups injected with different doses of morphine and a saline-treated control group were used as in Experiment II. However, activity was recorded for only 4 h after injection; the totals for the first and second 2-h periods were used in the analysis of results, the values thus obtained tending to represent two different phases of the action of morphine. The 80 rats used were divided randomly into 8 equal groups. Each treatment group was then subdivided into two equal subgroups for morning and afternoon observations as in Experiment III in order to balance possible differences between periods of day. As the whole experiment was performed in 2 sessions of 40 days each, one morphine dose (5 mg/kg) was repeated in the second session to check for any possible systematic change in response to the drug over this time interval. Each group received a saline injection on 8 consecutive days before motility was recorded in the usual way. On the 9th day the groups were each injected with one of the following doses of morphine sulphate: 1·25, 2·5, 5, 10, 20, 40 mg/kg. Another group treated with saline served as control. These treatments were repeated for 30 consecutive days. On the 31st day all groups were treated again with saline in order to check possible carry-over effects of morphine on motility. Some animals died during the experiment, apparently from peritoneal infection. Five animals of the group treated with 40 mg/kg died after the first injection of morphine from respiratory depression.

Statistical analysis

The general design of most of the experiments involved repeated measurements on the same subjects. We have chosen a simple analysis which may be regarded as approximate, but which successfully reveals the main features of the data. On each day the mean motility score for a given treatment and a given hour of recording has been considered as a single value. Thus, at the end of a period of days we had a series of scores, one for each day, representing the pattern of the mean response of rats to the treatment. This series of scores was analysed for the presence of trends using the method of curve-fitting by the orthogonal polynomials of least squares (Graybill, 1961). Such analysis was always preceded by a test of non-randomness using the distribution of the ratio of the mean square successive difference to the sample variance (Von Neumann, 1941). Only when this test gave very significant results ($P<0·01$), and thus indicated that a trend of some degree was present in the data, have we fitted the curve. As was suggested by Graybill (1961), we have continued until finding two non-significant results before deciding on the degree of polynomial to be used in curve-fitting. The validity of this procedure is related to the statistical model underlying it, which assumes no interaction between subjects and days; however, as the models of analysis of variance

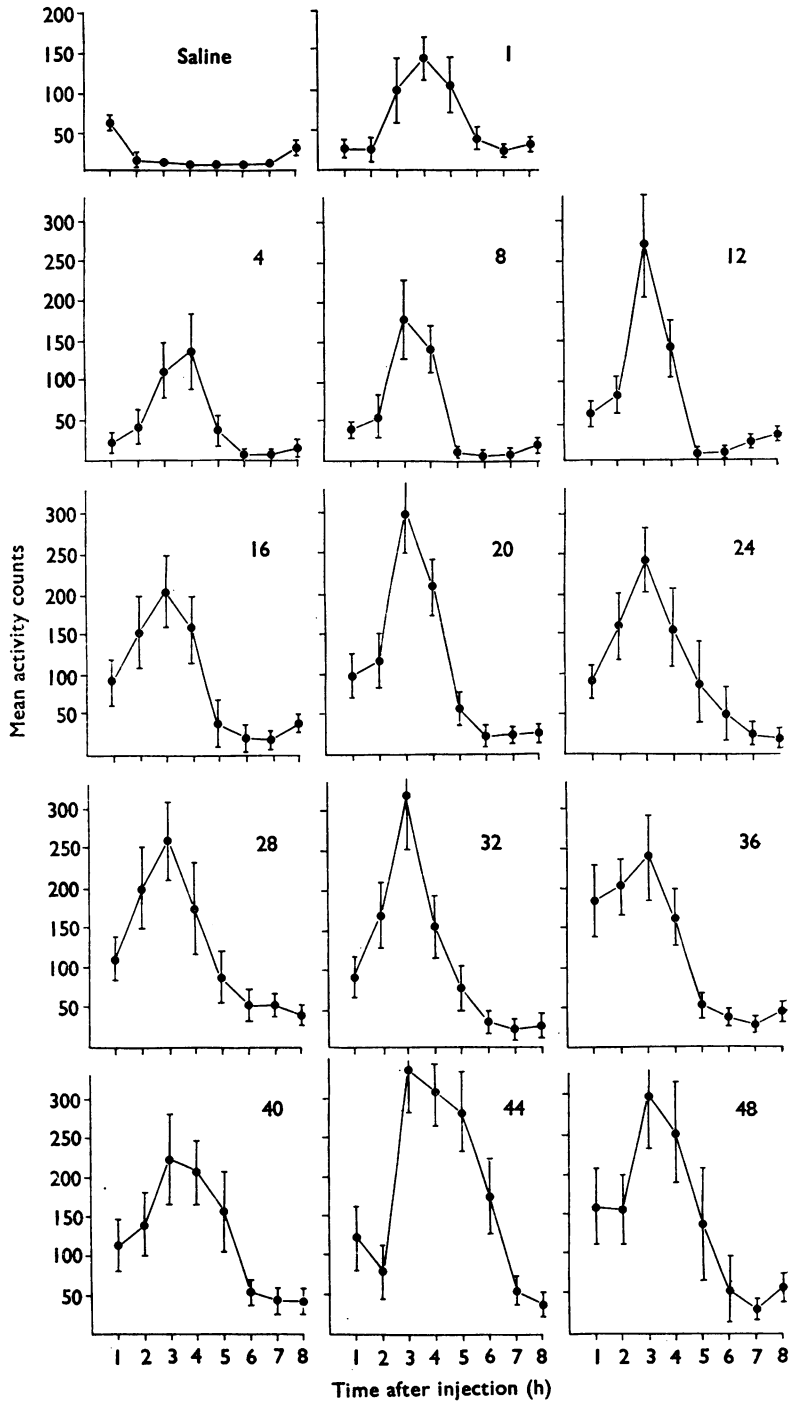

FIG. 1. Time curves for locomotor activity of rats. Saline or morphine sulphate (20 mg/kg, i.p.) was injected daily for 48 days. Vertical bars represent S.E. of mean. The numbers indicate the days of treatment.

are known to be robust regarding departures from their assumptions, the procedure should be considered a valid one. In other cases, Student's *t* test, analysis of variance and regression analysis have been applied.

Results

Experiment I

The mean hourly motility scores for 1–8 h after saline and morphine (20 mg/kg) treatments for days 1–48 are shown in Figure 1. On the first day of treatment the effect of morphine was clearly diphasic. During the first hour following injection motility was depressed; in fact, the moderate activity found in the controls (saline) was entirely absent, the difference from controls being significant ($P<0{\cdot}01$). The length of the depressive action cannot be determined exactly because the activity of saline controls during the second hour was very low. By the third hour the depressive effect was replaced by an excitatory one. Chronic treatment with the same dosage did not change the diphasic response pattern. However, the level of activity increased from day to day, and the time of the peak excitation occurred earlier. When the regression of activity on days was calculated either for the first or third hours or for the cumulative scores over the 8 h, it was even more evident that the motility in the morphine-treated rats increased from day to day, and that this increase still occurred after 48 days.

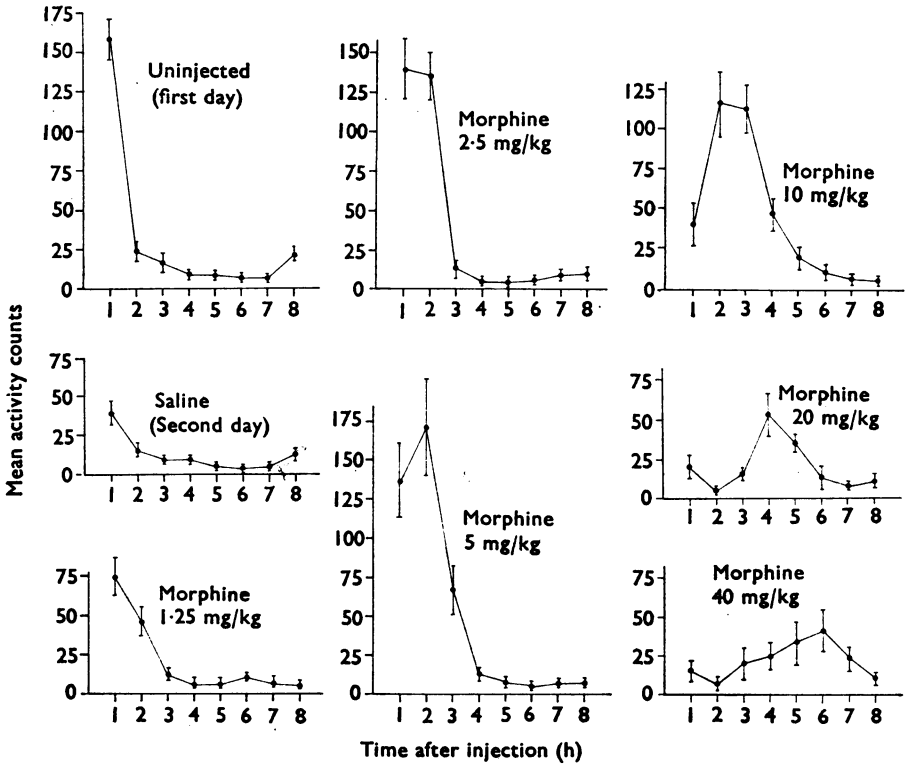

FIG. 2. Time curves for locomotor activity of rats for different doses of morphine given acutely. Vertical bars represent 95% fiducial limits of the means obtained from 20 rats except in the group given 40 mg/kg in which only 14 rats survived.

A similar experiment with 40 mg/kg, performed at the same time, is not reported here but the results were fully in agreement with those of Experiment IV.

Experiment II

In this study an initial adaptation period of 24 h in the actometers was introduced to obtain a low and stable baseline. This aim was achieved only in part. Although the mean score of the control group for the first hour decreased greatly from the first to the second day, it was still higher than for subsequent hours. This difference was taken to result from the injection procedure and regarded as unavoidable.

Figure 2 shows time-effect curves for single doses of morphine. At 40 mg/kg the time-effect curve includes only 14 rats since 6 died from respiratory depression. After 1·25, 2·5 or 5·0 mg/kg of morphine was given the effect was only excitatory. The increased motility lasted for about 2 h with the lower two doses and about 3 h with the third. The peak effect was during the first hour with 1·25 and 2·5 mg/kg and during the second hour with 5 mg/kg. Regression analysis of the totals obtained for the first 3 h indicated that the relationship between log dose of morphine and log activity counts was essentially linear, i.e., the magnitude of the effect increased monotonically with these three doses. The diphasic effect seen in Experiment I again became evident with 10, 20 and 40 mg/kg of morphine. For 10 mg/kg the mean score of the first hour equalled that for saline, but for 20 and 40 mg/kg these means were lower, reflecting a depressive effect. The delay in the excitatory effect was evident with all 3 doses but the peak of the effect decreased from 10 to 40 mg/kg. A similar experiment performed later with 80 mg/kg of morphine also showed the diphasic effect.

Experiment III

In saline-treated rats there was not a substantial decrease in the motility during the first hour beyond the fifth day, the mean score still being higher than that for the later hours (Fig. 3). This finding confirmed that the higher level of activity

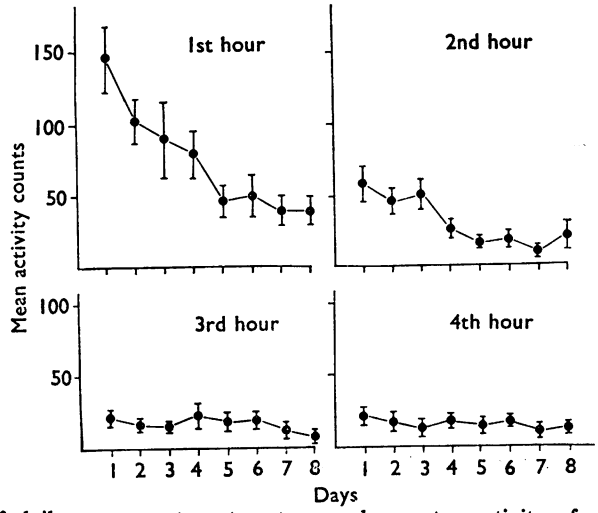

FIG. 3. Effects of daily exposure to actometers on locomotor activity of saline-treated rats. The graphs represent 4 successive hours of recording. The vertical bars indicate S.E. of the mean.

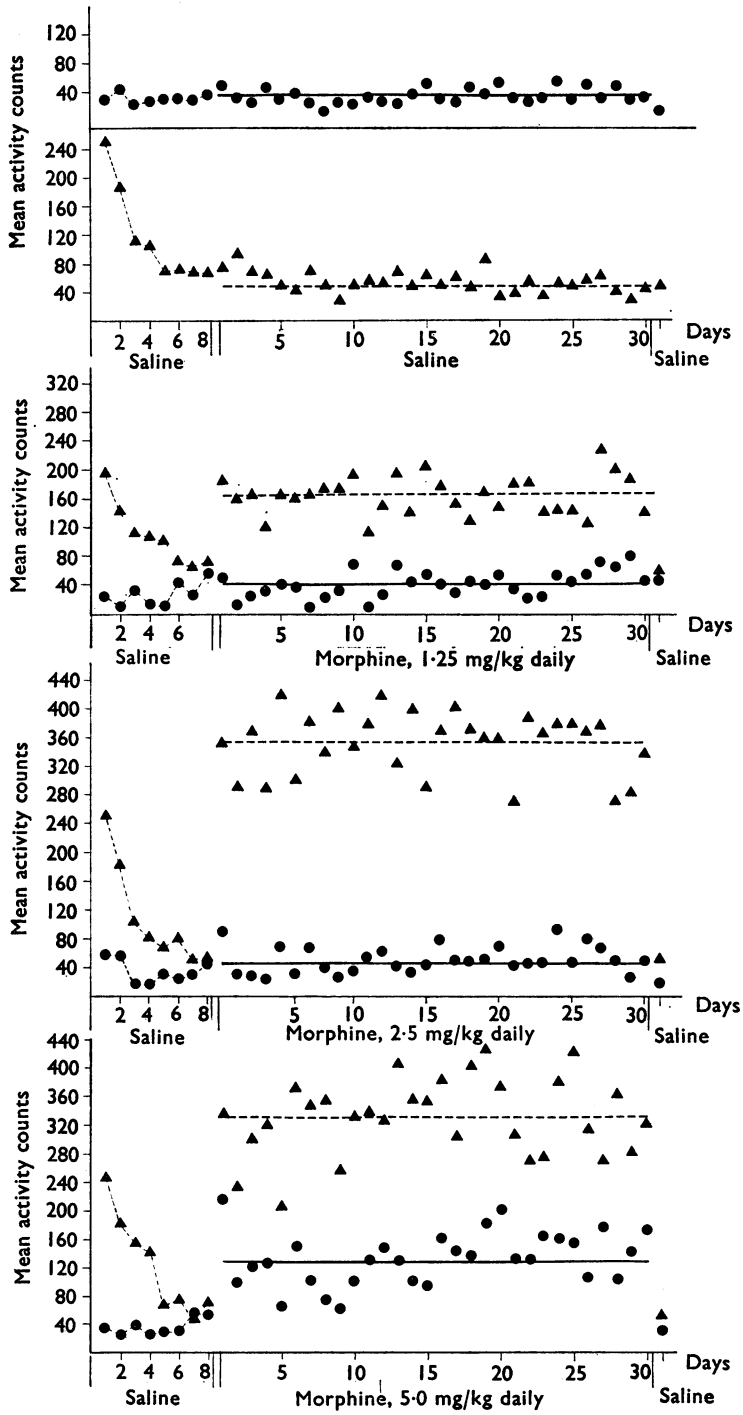

FIG. 4. Effects of daily injections of saline, 1·25, 2·5 and 5 mg/kg of morphine on locomotor activity. ▲, Mean motility counts 0–2 h after injection; ●, mean motility counts 2–4 h after injection. Solid and dashed lines are the means of motility counts during 30 days of treatment.

was related to the handling of the animals for injection and therefore could not be avoided. The results also suggested that 8 days of adaptation are sufficient for the animals to be quite accustomed to the apparatus.

Experiment IV

The principal pharmacological finding was that the course of action of chronic morphine treatment followed a pattern which was completely different for the three lower and the three higher dose levels. With 1·25, 2·5, and 5·0 mg/kg of

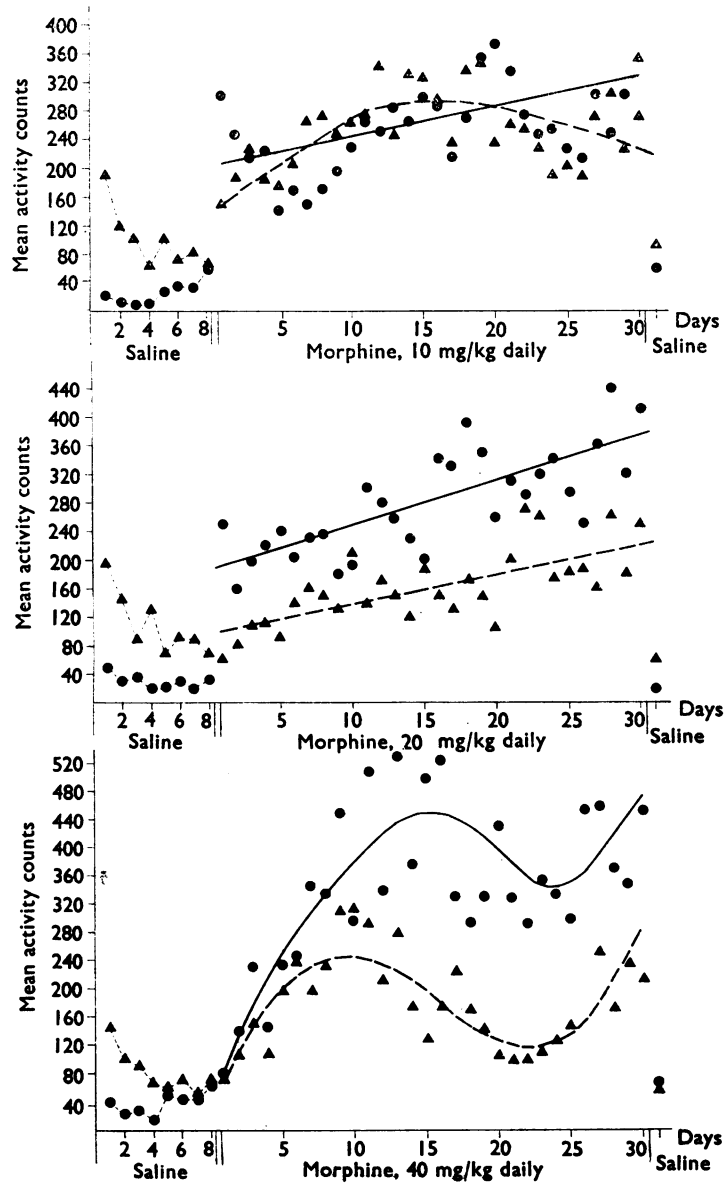

FIG. 5. Effects of daily injections of 10, 20 and 40 mg/kg of morphine on locomotor activity. Symbols as in Fig. 4. Solid and dashed lines are calculated regression lines.

morphine there was no substantial change during the 30 days of treatment (Fig 4). Smaller day-to-day fluctuations in response could have occurred, but if this were the case, the experimental design was not sensitive enough to detect it. Only with 2·5 mg/kg were the scores for the first 2-h period after injection not randomly distributed (P, 0·05–0·01); some cyclic short-term influence might possibly have affected the data. Comparative analysis of the results for the lower three doses confirmed the dose-effect relationship found in Experiment II. With 1·25 mg/kg, the excitatory effect was limited to the first 2-h period; the mean for the second 2-h period after injection was not statistically different from the control. With 2·5 and 5·0 mg/kg, the excitatory effect lasted more than 2 h and, on the whole, was greater with the latter dose.

A contrasting pattern was evident with 10, 20, and 40 mg/kg of morphine (Fig. 5). Mean scores for both the first and second 2-h periods increased from day to day, confirming the results of Experiment I. Because motility after saline injections was very low at the end of the 8-day adaptation period, morphine could produce a starting point only slightly below the control level, even with higher doses. Motility increased gradually in the following days, the rate of increase being less with 20 mg/kg than with 40 mg/kg. However, with the former dose the increase was still evident at the end of the month, whereas with the latter it reached a maximum about the 10th day and then seemed to undergo a cyclic oscillation. The pattern was less clear after 10 mg/kg of morphine, but there was an increase of activity from day to day within at least the first 15 days of treatment. The excitatory effect that was evident in the later hours during treatment with the highest doses also increased from day to day. This increase did not reach a maximum within a month in the rats treated with 10 and 20 mg/kg doses, whereas in those treated with 40 mg/kg a maximum was apparently reached by about the 15th day of treatment. It appeared that a direct relationship existed between dose and rate of increase in excitation during chronic treatment with a constant dose of morphine.

Two findings of Experiment IV indicate that the experimental control over the procedure was satisfactory. When the rats were treated once with saline after a month of chronic morphine treatment, the motility of the animals on all the doses returned to the initial pre-drug values. This observation suggested that there was no day-to-day carry-over effect of morphine on activity. Moreover, since the control group showed a fairly stable baseline during a month of saline treatment, the basal motility did not change within this period. In later experiments an injection with saline was given after 15 days of treatment in rats receiving 10, 20 and 40 mg/kg of morphine daily. Here also, the motility, while very high under morphine, returned to control values when the rats received saline. The repetition of the 5 mg/kg dose of morphine during the second 40-day experimental session that was to check for any alteration in the response to the drug between sessions, gave results which were extremely close between the two replications. Therefore, there was no evidence of any change in conditions which might have exerted a systematic influence on the results.

Discussion

Our demonstration of the biphasic pattern of action on locomotor activity by certain doses of morphine substantiates a pattern suggested by the data of other

workers (Sloan *et al.*, 1962; Gunne, 1963; Martin, 1963). The data from our Experiments I and IV agree with observations of Kaymakcalan & Woods (1956) and Gunne (1963), who reported that a schedule with increasing dosage induced an increase of activity in rats and that this increase appeared progressively sooner after the injection of morphine as the course of treatment continued. Our results showed the same pattern although a constant dosage schedule was used.

Another feature of the action of morphine was the purely excitatory effect of lower doses of morphine in non-tolerant rats. In the rat, this fact has not been well documented previously, although it has been frequently demonstrated for the mouse. Sloan *et al.* (1962) reported that, in non-tolerant rats, doses of 15 to 30 mg/kg produce excitant effects only after an initial depression. In rats, Fog (1970) observed a slight excitation 10 min after a subcutaneous injection of 1·0 mg/kg of morphine.

Few attempts have been made to explain the biphasic pattern of locomotor activity or the increasing excitation found with repeated doses of morphine. We shall consider whether the 'dual action hypothesis' of physical dependence on morphine might provide a basis for such an explanation. According to the concept of dual action (Tatum, Seevers & Collins, 1929; Seevers & Woods, 1953; Seevers & Deneau, 1962, 1963), morphine combines with receptors at two sites: on or near the surface of certain medullated axons of internuncial neurones and in the cell body of the same or other neurones (Seevers & Woods, 1953). Such drug-receptor combinations at the axon are characterized by rapidity of reaction, ease of reversal and rapid return of function when the drug is displaced, the resulting effect being one of inhibition. In contrast, the cell body receptor combination is characterized as firm, long-lasting and leading to cellular excitation.

In terms of these concepts, an early decrease in motility as described above might be seen as the consequence of morphine effects at axon receptors. Since long-lasting excitatory effects from morphine action at cell body receptors could not be manifested until the former action waned, a delayed excitation, and thus a biphasic action at certain doses, would be expected to occur. That depressive effects of morphine can conceal 'underlying' stimulant effects is supported by observations of Seevers & Deneau (1963) who found that, when sufficient nalorphine had been given to non-tolerant monkeys, even small doses of morphine, normally very depressant in this species, elicited only excitation. Kayan, Woods & Mitchell (1971) found that in rats repeated administration of morphine could reverse even the analgesic effect of morphine, apparently by an 'unmasking' effect.

The fact that only excitatory effects are observed after sufficiently low doses of morphine (1·25 to 5·0 in Experiment II) is consistent with the concept of dual action if one assumes that the threshold for activation of cell body receptors is lower than that for axon receptors. This seems reasonable since the effective dosage for excitatory effects of morphine can be lower than that for depression of the vomiting centre (Wang & Glaviano, 1954) and of body temperature (Martin *et al.*, 1963). It would follow that with higher dosage the stimulant action could be progressively antagonized by an increasing depressant action, although incompletely because excitation persists longer than depression. Our data are in line with this idea in several regards, such as the direct relationship between dosage

and peak time of delayed excitation and the inverse relationship between dosage and magnitude of delayed excitation.

The hypothesis of Seevers & Deneau (1963) that tolerance develops to the depressant but not to the excitatory effects of morphine in the central nervous system is supported for the rat by the data of Experiment IV, although results obtained in mice are not in agreement with this hypothesis (Shuster, Hannam & Boyle, 1963). Assuming that in the rat tolerance does not develop to excitatory effects, we would expect that there would be no change in the locomotor responses to doses sufficiently low to have an excitatory but not a depressant action. Moreover, the excitatory effects of higher doses should be progressively 'unmasked' as tolerance develops to the depressant actions. Schedules with progressively increasing doses are not so well suited for the detection of this 'unmasking' as is a constant dosage schedule as used by us, because the level of 'potential' excitatory activity would not be held constant, but would increase with dosage.

It is well known that development of tolerance is time-dependent (Seevers & Deneau, 1963) and that tolerance develops more rapidly on a constant dosage schedule than on a schedule with increasing dosage (Miller & Cochin, 1968). Therefore, the apparent direct relationship between dose level and rate of increase in excitation on treatment with daily injections of morphine (Experiment IV) would be expected if the change in the delay of excitation is taken to be an index of tolerance development.

In no instance does it seem that the excitatory effects of morphine we measured may be attributable to consequences of a state of partial withdrawal. The high motility levels we observed during chronic administration of morphine are in accord with the type of activity described by Martin *et al.* (1963) for morphine-treated rats rather than the activities seen in morphine-withdrawn rats.

This study has shown that several features of the effects of morphine on locomotor activity in the rat are explainable in terms of the 'dual action' concepts of Seevers and his associates. Shuster *et al.* (1963) have advocated actometric studies in the mouse for the study of opiate tolerance and dependence. Because for certain doses of morphine both inhibitory and excitatory effects on motility can be measured in the rat, this species may be even more suited for some such applications than the mouse.

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REFERENCES

BEACH, H. D. (1957). Effect of morphine on the exploratory drive. *Can. J. Physiol.*, **11**, 237–244.

COLLINS, L. G. (1965). Effect of morphine sulfate on spontaneous activity level of the albino rat. *Psychol. Rep.*, **16**, 693–696.

EIDELBERG, E. & SCHWARTZ, A. S. (1970). Possible mechanisms of action of morphine on brain. *Nature, Lond.*, **225**, 1152–1153.

FOG, R. (1970). Behavioural effects in rats of morphine and amphetamine and a combination of the two drugs. *Psychopharmacologia*, **16**, 305–312.

GRAYBILL, F. A. (1961). *An Introduction to Linear Statistical Models*, Vol. 1, McGraw-Hill Series in Probability and Statistics. New York: McGraw-Hill Book Company.

GUNNE, L. M. (1963). Catecholamines and 5-hydroxytryptamine in morphine tolerance and withdrawal. *Acta physiol. scand.*, **58**, suppl. 204, 1–91.

KAYAN, S., WOODS, L. A. & MITCHELL, C. L. (1971). Morphine-induced hyperalgesia in rats tested on the hot plate. *J. Pharmac. exp. Ther.*, **177**, 509–513.

KAYMAKCALAN, S. & WOODS, L. A. (1956). Nalorphine-induced 'abstinence syndrome' in morphine-tolerant albino rats. *J. Pharmac. exp. Ther.*, **117**, 112–116.

KUMAR, R., MITCHELL, E. & STOLERMAN, I. P. (1971). Disturbed patterns of behaviour in morphine tolerant and abstinent rats. *Br. J. Pharmac.*, **42**, 473–484.

MARTIN, W. R. (1963). Strong analgesics. In: *Physiological Pharmacology*, Vol. 1, ed. Root, W. S. & Hofmann, F. G., pp. 275–312. New York: Academic Press.

MARTIN, W. R., WIKLER, A., EADES, C. G. & PESCOR, F. T. (1963). Tolerance to and physical dependence on morphine in rats. *Psychopharmacologia*, **4**, 247–260.

MILLER, J. M. & COCHIN, J. (1968). Dose-related aspects of tolerance to the analgesic effect of chronically administered morphine sulfate (MS) in mice. *Pharmacologist*, **10**, 189.

PICKENS, R. W. & CROWDER, W. F. (1967). A recorder of locomotor activity. *Am. J. Psychol.*, **80**, 442–445.

SEEVERS, M. G. & DENEAU, G. A. (1962). A critique of the 'dual action' hypothesis of morphine physical dependence. *Archs int. Pharmacodyn.*, **140**, 514–520.

SEEVERS, M. G. & DENEAU, G. A. (1963). Physiological aspects of tolerance and physical dependence. In: *Physiological Pharmacology*, Vol. 1, ed. Root, W. S. & Hofmann, F. G., pp. 565–640. New York: Academic Press.

SEEVERS, M. G. & WOODS, L. A. (1953). The phenomena of tolerance. *Am. J. Med.*, **14**, 546–557.

SHUSTER, L., HANNAM, R. V. & BOYLE, W. E. JR. (1963). A simple method for producing tolerance to dihydromorphinone in mice. *J. Pharmac., exp. Ther.* **140**, 149–154.

SLOAN, J. W., BROOKS, J. W., EISENMAN, A. J. & MARTIN, W. R. (1962). Comparison of the effects of single doses of morphine and thebaine on body temperature, activity, and brain and heart levels of catecholamines and serotonin. *Psychopharmacologia*, **3**, 291–301.

TATUM, A. L., SEEVERS, M. H. & COLLINS, K. H. (1929). Morphine addiction and its physiological interpretation based on experimental evidence. *J. Pharmac. exp. Ther.*, **36**, 447–475.

TONINI, G., MISSERE, G. & BABBINI, M. (1959). Richerche actografiche ed elettroforetiche sul morfinismo nel ratto. *Archo ital. Sci. farmac.*, **9**, 425–455.

VON NEUMANN, J. (1941). Distribution of the ratio of the mean square successive differences to the variance. *Ann. math. Statist.*, **12**, 367–395.

WANG, S. C. & GLAVIANO, V. V. (1954). Locus of emetic action of morphine and hydergine in dogs. *J. Pharmac. exp. Ther.*, **111**, 329–334.

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