Br. J. Pharmac. (1980), **70**, 545–554

MORPHINE DEPENDENCE AND DOPAMINERGIC ACTIVITY: TESTS OF CIRCLING RESPONSES IN RATS WITH UNILATERAL NIGRAL LESIONS

J.V. HALLIWELL[1] & R. KUMAR

Department of Psychiatry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

1 Rats with unilateral electrolytic lesions involving both parts of the substantia nigra show dose-related, ipsilateral circling responses to apomorphine which are stable over time.

2 In non-tolerant rats, morphine (up to 10 mg/kg) does not elicit any circling behaviour but as tolerance develops to morphine, initially 10 mg/kg daily and then 100 mg/kg daily for about 4 months, the rats show a progressive tendency to walk more towards the side of the lesion. This behaviour is qualitatively different from apomorphine-induced circling.

3 When apomorphine (0 to 1.0 mg/kg) and morphine (10 or 100 mg/kg) are tested together, the total amounts of 'circling' are increased in an additive manner. However, after 22 h withdrawal from morphine there is a more marked increase in apomorphine-induced circling which is related to the level of dependence.

4 It is suggested that the sensitivity of striatal dopamine receptors is not altered by morphine dependence and that the increased response to apomorphine in abstinence probably reflects changes in the modulating actions of other neurotransmitter systems in the striatum.

Introduction

Morphine interferes with dopaminergic neurotransmission in the striatum (Kuschinsky & Hornykiewicz, 1972), possibly through actions on presynaptic opiate receptors (Pollard, Llorens-Cortes & Schwartz, 1977). Although single doses of morphine result in apparently compensatory increases in the synthesis and turnover of dopamine in the striata of non-tolerant rats (Clouet & Ratner, 1970; Sugrue, 1974), analogous responses to chronic morphine medication are not seen in the brains of tolerant rats (Puri & Lal, 1974; Puri, Volicer & Lal, 1977). Tolerant rats, nevertheless, show marked behavioural changes for several hours after receiving their usual dose of morphine, such as increased locomotor activity and stereotyped responding (Fog, 1970; Ahyan & Randrup, 1972; Babbini & Davis, 1972), increased eating and drinking (Kumar, Mitchell & Stolerman, 1971) and reduced sexual activity (Mumford & Kumar, 1979). Aside from altered influences of other neurotransmitter systems, it has been suggested that there may be underlying and prolonged changes in the sensitivities of dopamine receptors themselves (Puri & Lal, 1973; Gianutsos, Hynes, Puri, Drawbaugh & Lal, 1974; Tarsy & Baldessarini, 1974). However, suggestions that striatal dopamine receptors are, in some way, rendered supersensitive by morphine have not met with universal support (e.g. Kuschinsky, 1975; Cox, Ary & Lomax, 1976). Disagreements may, to some extent, be ascribed to procedural variations between studies, e.g. in methods of inducing opioid dependence or abstinence, dosage regimes and, in the behavioural techniques for assessing striatal dopaminergic activity. Tests have included measures of stereotyped responses to dopamine agonsts (Scheel-Krüger, Golembiowska & Mogilnicka, 1977), catalepsy after treatment with antagonists (Kuschinsky & Hornykiewicz, 1972; Puri & Lal, 1974), the induction of aggressive behaviour in abstinent rats (Gianutsos *et al.*, 1974) and measures of circling behaviour in rodents with unilateral lesions of the nigro-striatal system (Iwamoto, Loh & Way, 1976a, b; Laschka, Herz & Bläsig, 1976; Halliwell & Kumar, 1977).

The experiments described here have attempted to clarify how morphine dependence affects dopaminergic activity in the striatum. Circling after doses of apomorphine, a direct dopamine agonist, was measured before and after chronic medication with increasing doses of morphine The rats had previously received unilateral electrolytic lesions involving both anatomical divisions of the substantia nigra and they turned in an ipsilateral direction when given apomorphine, thus reflecting an ascendancy of the intact striatum over the lesioned side (Andén, Dahlström,

[1] Present address: Department of Pharmacology, School of Pharmacy, Brunswick Square, London, WC1.

0007-1188/80/120545-10 $01.00

Fuxe & Larsson, 1966). An advantage of such preparations over animals with small electrolytic lesions of the pars compacta of the substantia nigra (Dray, Fowler, Oakley, Simmonds & Tanner, 1977) or with lesions induced chemically by 6-hydroxydopamine, is that analyses of any effects of morphine are not complicated by the manifestation of supersensitivity in the deafferented striatal receptors on the lesioned side (Ungerstedt, 1971) because the nigral output pathways which are considered to be responsible for the initiation of circling behaviour (Di Chiara, Olianas, Del Fiacco, Spano & Tagliamonte, 1977; Waddington & Cross, 1978) have also been destroyed.

Methods

Male hooded rats (Olac, U.K.), 200 to 300 g at the time of surgery, were used in this study; they were housed four to a cage with *ad libitum* access to food and water. The rats were kept in rooms with controlled temperature (21 ± 2°C) and an unreversed light–dark cycle (lights on between 08 h 00 min and 20 h 00 min.

Surgery

Following administration of a sedative dose of pentobarbitone (30 mg/kg i.p.), rats were anaesthetized with ether and placed in a stereotaxic apparatus (Stoelting, U.S.A.). A monopolar electrode (stainless steel wire 0.5 mm, insulated to the cut tip) was placed in the rostral region of the substantia nigra with the coordinates A 2.8, L 2.3, V 2.5 (De Groot, 1959). Unilateral lesions of the left or right substantia nigra were effected by the passage of 1.5 to 2 mA of direct anodal current for 10 s.

Drugs

The drugs used were apomorphine HCl (doses: 0, 0.25, 0.5, 0.75 and 1.0 mg/kg), morphine HCl (doses up to 100 mg/kg) and naloxone HCl 1 mg/kg. Apomorphine and naloxone were dissolved in distilled water (1 mg/ml) and injected subcutaneously and intraperitoneally respectively. Morphine was dissolved in distilled water to the required concentration such that animals receiving an injection obtained 2 ml/kg solution. Saline (0.9% w/v NaCl solution) injections 1 ml/kg were substituted for zero doses of apomorphine and naloxone and 2 ml/kg for zero doses of morphine.

Apparatus

After at least 2 weeks of post-operative recovery the lesioned rats were tested for circling behaviour in a specially constructed apparatus, which comprised four, square cages (30 × 30 × 46 cm), over each of which was suspended a counterbalanced swivel and harness. The cage walls were made of clear Perspex, the floors were wire grids and each cage was illuminated by a 30 W incandescent light. One rat was tested at any one time in each cage by securing it in a harness; the animal's circling movements were transmitted via a flexible tight-coiled spring which joined the harness to the swivel device. A single magnet, attached to a driven rotor within the swivel was able to trigger by its proximity three reed switches which were spaced equally around the periphery of the swivel stator. The sequence of contact closures from the reed switches was decoded by a simple logic network into the number of complete left and right hand rotations made by the animal. In addition, the number of times a single reed switch was triggered irrespective of the sense of rotation was counted to give a rough indication of gross activity. An animal's circling was automatically recorded by printing the frequency of left and right hand turns and 'activity' counts occurring during 5 min epochs. To economise on the amount of logic, pairs of cages 'time-shared' the decoding network and therefore a particular animal's circling was recorded during alternate epochs of 5 min. Cables from the cages were led to the logic and printout equipment in an adjacent room to minimize noise distraction.

Procedure

Sixteen rats were selected at random from a pool of lesioned animals which had previously been screened for circling behaviour after a dose of apomorphine 1 mg/kg. A minimum total count of 50 turns was required during the ten 5 min epochs obtained during 95 min of testing after injection. The cumulative count of circling during the alternative 5 min epochs was the main dependent variable; it was expressed as either ipsilateral minus contralateral turns or as ipsilateral turns alone. In every experiment a rat was attached to its harness and allowed to remain in the apparatus for at least 30 min before any drugs were given. The precise regimes of medication are described in the appropriate sections below, corresponding to different phases of the experiment. The main experimental group ($n = 8$) received a course of morphine injections; the remaining 8 rats were given matched saline injections; thus the effects of morphine administration and injections alone on apomorphine-induced circling could be assessed independently.

(A) *Stability of circling responses to apomorphine in control rats* (n = 8)

(1) *Effects of repeated injections* To assess the effects of repeated injections alone on apomorphine-induced

circling, dose–response curves were determined to this drug before, during and after a period of daily saline injection (37 days) in a group of 8 control rats. No further tests were done with these animals.

(2) *Replications of apomorphine-induced circling at intervals in non-tolerant rats* ($n = 8$) Dose–response curves for circling behaviour were determined after injections of apomorphine HCl (0, 0.25, 0.5 and 1.0 mg/kg; see Figures 1 and 2). Each rat received every dose according to a Latin-Square design with an interval of 2 to 3 days between doses. All tests lasted 90 min and were begun immediately after the injections of apomorphine. Three dose–response curves were obtained with an interval of one month between each series of tests. No other drugs were given between the first and second dose–response tests but there were intervening tests with morphine between the second and third dose–response estimations with apomorphine (see below, Section B). The same general procedure was used throughout in obtaining dose–response curves for apomorphine-induced circling.

(B) *Circling behaviour after injections of morphine in non-tolerant rats* (n = 8)

To begin with, three doses of morphine HCl (1.25, 2.5 and 5.0 mg/kg) were tested during the interval between the second and third apomorphine dose–response estimations. Each rat was randomly given two doses of morphine with one week between tests, thus providing at least five observations at each dose level. After the third apomorphine dose–response curve had been obtained, all the rats ($n = 8$) were tested after an injection of morphine 10 mg/kg and these scores were incorporated in the analysis of the effects of acute doses of morphine alone (0 to 10 mg/kg) on circling behaviour. All tests lasted 90 min and were begun immediately after injection; the results are illustrated in Figure 3.

(C) *Circling behaviour after chronic medication with morphine (10 mg/kg daily)*

(i) *Circling after morphine (10 mg/kg) alone* The final test of the previous part of the experiment (circling after 10 mg/kg of morphine) also served as the first measure in this part of the study where the rats were injected with morphine HCl, daily 10 mg/kg for 14 days. Circling was monitored for 90 min after the injection of morphine on the 1st, 4th, 8th and 12th days (see Figure 4).

(ii) *Apomorphine-induced circling after withdrawal from morphine or in combination with morphine, 10 mg/kg* After 14 days of medication with morphine (10 mg/kg) an apomorphine dose–response curve was determined as before but with the rats in about 22 h abstinence from morphine i.e. they received their daily dose of morphine *after* the circling tests had been completed. Following this phase, which lasted 8 days, rats were then tested in a similar manner with apomorphine (0 to 1 mg/kg) but were given their dose of morphine (10 mg/kg) 60 to 90 min *before* the circling test (see Figure 5).

(D) *Circling behaviour after chronic medication with morphine (100 mg/kg daily)*

The dose of morphine was then increased in rapid steps to 100 mg/kg daily and was maintained at this level for 136 days. During this period a series of tests were made of circling responses after morphine alone, and after apomorphine was given to rats which were either drugged as usual with morphine or were 22 h abstinent.

(i) *Circling after morphine alone (100 mg/kg)* The rats were tested on the 28th, 51st and 128th days after daily medication with morphine was begun (i.e. after 14, 37 and 114 days of daily treatment with 100 mg/kg of morphine) and the scores are illustrated in Figure 4 together with the analogous measures taken after the lower dose alone [procedure as for section C(i)].

(ii) *Apomorphine-induced circling after withdrawal from morphine or in combination with morphine 100 mg/kg* Procedure as for the tests with smaller dose of morphine 10 mg/kg, Section C(ii).

(iii) *Apomorphine-induced circling after precipitated abstinence from morphine* In this final test a dose–response determination was again attempted with apomorphine (0 to 1 mg/kg s.c.) followed immediately by an intraperitoneal injection of naloxone 1 mg/kg, both given 60 to 90 min after the usual daily dose of morphine 100 mg/kg.

Histology

Surviving rats were perfused transcardially with normal saline followed by 10% neutral formol saline. After 30 min their brains were removed and fixed further in 10% neutral formol saline. The brains of rats ($n = 7$) dying in the test following precipitated abstinence were quickly removed and transferred to the fixing solution without pre-fixation. Fixed brains were sectioned either frozen or embedded in celloidin and sections (33 or 50 μm) were stained with cresyl violet.

Statistical analysis

Counts of circling responses were compared by analyses of variance with unweighted means solutions for

unequal group sizes (Winer, 1970). Apart from the analyses of the effects of morphine (0 to 10 mg/kg) in non-tolerant rats and the tests in precipitated abstinence, all comparisons were based on $n = 8$/group. Individual group means were compared with paired or unpaired two-tailed t tests as appropriate. Regression lines for the dose–response curves were determined by the method of least squares. Differences in slope were compared by t tests based on the standard error estimates of the slope value (Colquhoun, 1971).

Results

Histology

In all rats both parts of the substantia nigra were shown to be damaged. Even when the lesion appeared to be placed predominantly in the reticular part of the nucleus, its influence was manifest in the compacta as evidenced by loss of cells in this region.

Damage to additional brain structures was minimal; slight incursions were occasionally observed onto the medial lemniscus and in one case the lesion extended to the cerebral peduncle. Anterior placements and the electrode track appeared to destroy a small part of the zona incerta.

Circling responses to apomorphine

In accordance with previous observations (e.g. Andén *et al.*, 1966; Costall & Naylor, 1975; Iwamoto *et al.*, 1976a) apomorphine induced ipsilateral circling in rats with lesions in the substantia nigra. Figure 1 shows that the circling behaviour lasted for about 90 min after the largest dose of apomorphine. The scores for the 60 min period before injection show a characteristic tendency for activity counts to habituate in the early part of the test. Injections of saline increased activity but caused little turning, whereas apomorphine systematically increased ipsilateral circling. At the time of the peak effect there was virtually no contralateral circling and nearly all the measured activity was in the form of ipsilateral circling.

Figure 2 shows the results of three dose–response estimations of apomorphine-induced circling obtained at intervals of a month. There is a linear relationship between the log dose of apomorphine and the number of net ipsilateral turns recorded in 10 epochs during 90 min. The stability of the response over time is also apparent ($F = 1.98$, d.f. 2.77) and it therefore becomes possible to make sequential comparisons from a stable base-line of responding. Additionally, in 8 other rats, a course of 37 days of saline injections failed to modify the level of circling behaviour during or after the treatment regime ($F = 0.65$, d.f. 2.77, $P > 0.05$).

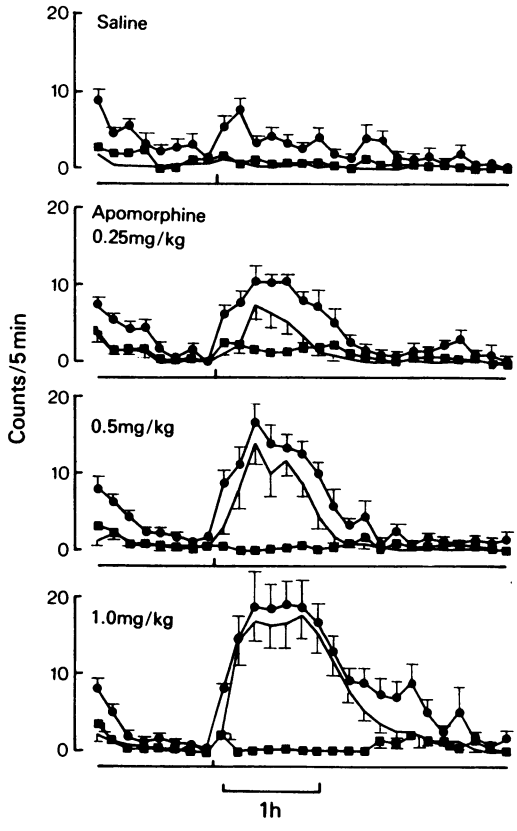

Figure 1 Rats ($n = 8$) with unilateral lesions of the substantia circle in an ipsilateral direction after injections of apomorphine. Mean counts of circling (—— ipsilateral, ■——■ contralateral) as well as of activity (●——●) in alternate 5 min epochs are shown, with standard errors. The vertical line on the abscissa scale indicates the time of injection. The amount of circling is related to the dose of apomorphine and when rats are circling at high rates very little other activity is measured.

Circling behaviour after acute doses of morphine in non-tolerant rats

Morphine in doses up to 10 mg/kg did not induce any consistent rotational behaviour (Figure 3) and the main effect was a slightly increased level of general activity (Babbini & Davis, 1972). However, Cowan, Detmar & Walter (1975) have reported that ipsilateral circling in chemically lesioned rats is increased by acute doses of morphine (0.3 to 3.0 mg/kg). Our results suggest that both ipsilateral and contralateral turning are induced by low doses of morphine in non-tolerant rats as part of a general locomotor stimulant effect. Such 'non-specific' effects may be contrasted

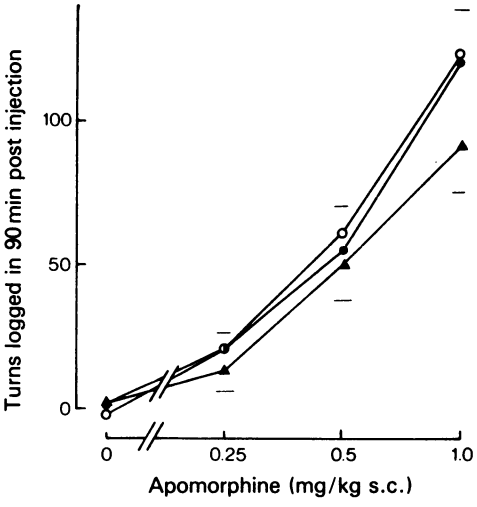

Figure 2 Stability of circling responses induced by apomorphine. 3 dose–response curves (DR1–3) were determined one month apart for the same group of 8 animals. Mean ipsilateral minus contralateral turns logged in the 90 min post injection (ordinate scale) are plotted against dose of apomorphine (abscissa scale). (○) DR1; (●) DR2; (▲) DR3. Horizontal bars indicate the magnitude of the standard errors for DR1 (upper set) and DR3 (lower set). For clarity s.e. mean for DR2 are not shown.

with the very clear circling responses to doses of apomorphine.

Circling behaviour after chronic medication with morphine (10 and 100 mg/kg)

Repeated tests of circling were made after the usual daily doses of morphine (10 mg/kg and 100 mg/kg) and Figure 4 shows that as the rats became tolerant to morphine there was a consistent and increasing tendency to circle towards the side of their lesions. The rats' behaviour was qualitatively different from that seen after doses of apomorphine; it was characterized by increased locomotion, sudden bursts of locomotion towards the side of the lesion which were often triggered by noise (cf. Jacquet, Carol & Russell, 1976) and interspersed bouts of immobility or gnawing (Ayhan & Randrup, 1972). Apomorphine, on the other hand, even when tested in combination with morphine, always induced a characteristic tightly hunched posture twisted towards the side of the lesion; rotational behaviour was usually regular although stereotyped responses were sometimes superimposed.

Circling responses to apomorphine in morphine-dependent rats

Figure 5a shows that ipsilateral turning was increased in the dependent rats which were tested with apomor-

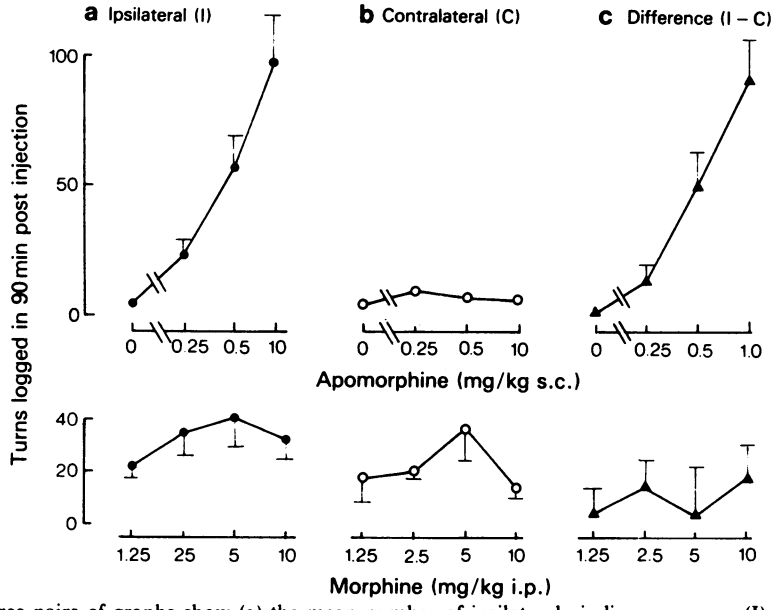

Figure 3 Three pairs of graphs show (a) the mean number of ipsilateral circling responses (I), (b) contralateral responses (C) and (c) the net difference (I–C). In contrast with apomorphine (0 to 1.0 mg/kg), morphine in doses of up to 10 mg/kg, does not elicit any ipsilateral circling in non-tolerant rats. Approximately equal numbers of ipsilateral and contralateral 'turns' are recorded reflecting a general locomotor stimulant effect of small acute doses of morphine.

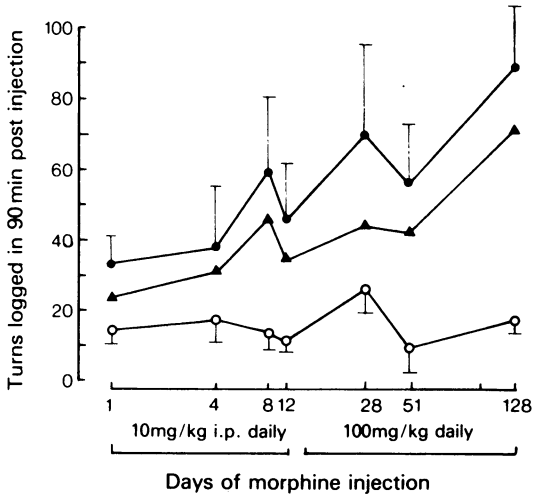

Figure 4 As rats (n = 8) with unilateral nigral lesions become tolerant to morphine they appear to show increased ipsilateral circling in the 90 min after their usual daily injections. Circling scores are plotted against the number of days, on a log scale, for which the rats were injected daily with morphine, initially 10 mg/kg and then 100 mg/kg; the scores are shown as numbers of ipsilateral (●), contralateral (○), and net ipsilateral (I–C, ▲) turns. There is much greater variability than after apomorphine (cf. Figure 3) indicated by the standard error bars.

phine in the presence of 10 or 100 mg/kg of morphine ($F = 7.24$ d.f. 1.35, $P < 0.01$) and ($F = 24.77$, d.f. 1.35, $P < 0.0001$) respectively. Examination of the three regression lines suggests that there was a parallel shift to the left of the dose–response curves. When the scores obtained with morphine alone were subtracted, the slopes of the dose–response curves remained unchanged and the absolute values were very similar to those obtained with apomorphine alone, before dependence was induced (Figure 5b).

In contrast, when the same rats were tested after 22 h of withdrawal from the two doses of morphine, they showed marked increases in rates of circling after apomorphine injections, resulting in progressively steeper slopes of the dose–response curves (Figure 5c). After withdrawal from 10 mg/kg morphine, ipsilateral turning was significantly increased when compared with the base-line dose–response curve which was obtained before the rats were made dependent ($F = 9.96$, d.f. 1.49, $P < 0.01$); however, the interaction term was not significant. When the rats were tested again after withdrawal from 100 mg/kg morphine there was a further increase in the rates of circling ($F = 20.03$, d.f. 1.49, $P < 0.001$). The interaction term was now significant, reflecting a change in the slope of the dose–response curve ($t = 2.21$, d.f. 21, $P < 0.05$).

Precipitated abstinence and circling behaviour after apomorphine

Circling responses to apomorphine were recorded for 90 min after the injections of both naloxone and apomorphine and the cumulative scores were found to be similar to, or greater than, those seen after 22 h of withdrawal. However, seven of the animals died during this series of tests.

Jumping behaviour was elicited by naloxone when it was administered alone or in combination with apomorphine. This took the form of well-coordinated attempts at escape from the test cages and its occurrence was rapidly 'occluded' (Lyon & Randrup, 1972) by rotational behaviour induced by apomorphine, especially at higher doses. At the zero dose of apomorphine, jumping was prolonged (up to 45 min) and the apparatus logged some of these movements as rotational behaviour; however, in contrast to the ipsilateral circling induced by morphine and apomorphine in the dependent animals, a naloxone challenge alone to the daily morphine elicited equal amounts of 'rotation' in both directions.

Discussion

Lesions involving both parts of the substantia nigra resulted in reliable and consistent dose-related ipsilateral circling to apomorphine (Andén *et al.*, 1966; see also Glick, Jerussi & Fleischer, 1976); such preparations appear particularly suitable for studies of chronic drug treatments because interpretations are made easier by having stable 'base lines' over time.

In non-tolerant rats, morphine by itself, in doses up to 10 mg/kg had no discernible effect on circling responses (see Figure 3), but as daily treatment with the drug was maintained, first 10 mg/kg and then 100 mg/kg, there was a definite and progressive tendency for ipsilateral circling to occur as shown in Figure 4. Tolerance develops to the cataleptic actions of morphine following repeated daily injections of the drug and there is a concomitant enhancement of stimulant actions on motor activity (Kumar *et al.*, 1971; Babbini & Davis, 1972). It seems possible that, as tolerance develops, there is a further disturbance of striatal compensatory mechanisms in the lesioned rats and, as a result, bias appears in the direction of locomotor behaviour. No such bias is seen after smaller doses of morphine, which also increase activity in non-tolerant rats (see Figure 3). Gross observation of the animals emphasized the qualitative difference between the morphine-induced rotations and circling elicited by apomorphine. After morphine there was no obvious postural asymmetry and rates of circling were irregular and subject to the disruptive effects of external disturbance.

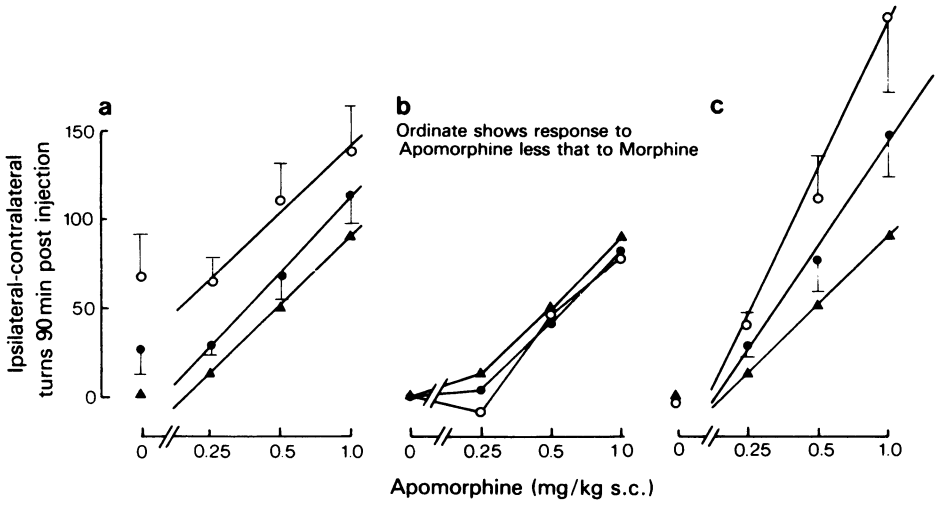

Figure 5 Effects of dependence and abstinence on apomorphine-induced circling. The graphs show the net ipsilateral circling responses of 8 dependent rats induced by different doses of apomorphine in the presence of 10 (●) and 100 (○) mg/kg morphine (a & b) and in withdrawal from 10 (●) and 100 (○) mg/kg morphine (c). In each graph these responses are compared with the baseline (▲) before morphine was administered chronically (DR3 of Figure 2). In (a) and (c), least squares linear regression lines have been fitted to the data; vertical bars denote s.e. mean. In (b) the data shown in (a) have been transformed by subtracting from each animal's individual score following administration of both drugs its score in the presence of morphine alone (i.e. at zero dose of apomorphine) so that the circling induced by apomorphine alone in the dependent states may be compared directly with baseline performance. (For clarity the s.e. mean for graph (b) and for the baseline (DR3) have been omitted.)

When tolerant rats were given morphine, 10 or 100 mg/kg, and then tested with apomorphine, their rates of circling were greater than after doses of apomorphine alone (Figure 5). Circling was increased more by 100 mg/kg of morphine than by 10 mg/kg, and although possible confounding effects of the sequence of testing cannot be excluded, it is striking that when circling scores to apomorphine are examined by subtracting the scores after morphine alone i.e. following apomorphine 0 mg/kg + morphine 10 or 100 mg/kg, there is almost total overlap of the three dose–response curves (Figure 5b). It seems, therefore, that there was an additive interaction between circling responses to apomorphine and a qualitatively different tendency for the tolerant drugged animals to walk more in an ipsilateral direction (cf. Pert & Sivit, 1977). Such an interpretation is consistent with the view that morphine tolerance does not affect the sensitivity of dopamine receptors. Although Cox *et al.* (1976) have reported that apomorphine-induced stereotypy is increased by morphine in tolerant rats, it is possible that tests of circling provide a more effective way of distinguishing between effects of drugs at postsynaptic receptors and elsewhere in the nigro–striatal system.

Figure 5 also shows that apomorphine-induced circling was increased when morphine-tolerant rats were tested after 22 h abstinence from morphine. The mechanisms underlying the increased circling were presumably different from the increased circling seen in the presence of morphine, because the slopes of the dose–response curves were changed. The most likely explanation is an altered modulating influence on the postsynaptic dopamine receptors and neurones, but the nature of such an interaction was not further examined in these particular tests. It has been suggested (Kelly & Moore, 1976; 1977; Pycock & Marsden, 1978) that circling behaviour comprises a postural imbalance coupled with activation of locomotor responses. If this is, indeed, the case the increased circling shown by morphine abstinent rats is consistent with the report by Iversen & Joyce (1978) that apomorphine-induced locomotor activity is increased when rats are tested in withdrawal from morphine. Iwamoto *et al.* (1976b) reported that morphine-dependent rats with unilateral lesions of the zona compacta of the substantia nigra showed contralateral rotation during precipitated abstinence and our discrepant observations may reflect differences in the site and size of the lesion (Andén *et al.*, 1966; Glick *et al.*, 1976) as well as procedural variations. Laschka *et al.* (1976) inactivated rats' corpora striata on one side with KCl and then recorded circling behaviour; in non-tolerant rats small subcutaneous doses of apomorphine (0.05 mg/kg) had no effect and large doses (3.0 mg/kg) produced marked ipsilateral circling. Similar tests were then done in morphine-

tolerant rats in which abstinence had first been precipitated with naloxone; consistent with our own findings, circling after the large dose of apomorphine was enhanced. However, after 0.05 mg/kg apomorphine the rats circled in a contralateral direction suggesting an enhanced interaction at presynaptic dopamine receptors on the intact side. In general, therefore, although the tests of circling behaviour differ among themselves, the results do not lend support to the view that morphine dependence alters the sensitivities of dopamine receptors.

There are many sites at which opiate receptors, could interact with dopaminergic transmission. A high density of opiate binding sites is found in the pars compacta and ventral tegmental area (Atweh & Kuhar, 1977a) and in the terminal projection regions of these dopamine containing cells, the neostriatum and nucleus accumbens (Atweh & Kuhar, 1977b). At the level of the striatum, coexistence of opiate and dopamine receptors has been demonstrated on presynaptic afferents (Celsen & Kuschinsky, 1974; Pollard *et al.*, 1977) and at postsynaptic locations on striatal cells (Pollard Llorens, Schwartz, Gros & Dray, 1978; Havemann & Kuschinsky, 1978; Minneman Quik & Emson, 1978). Functional interactions occur presynaptically (Celsen & Kuschinsky, 1974) and postsynaptically (Minneman, 1977) but are unlikely to be direct opiate effects at the dopamine receptor since morphine has little action on dopamine-sensitive adenylate cyclase activity in homogenates (Miller, Horn & Iversen, 1974; Iwatsubo & Clouet, 1975; Minneman, 1977). Morphine does not compete for striatal dopamine receptors of non-tolerant rats (Czlonkowski, Höllt & Herz, 1978; Puri, Spaulding & Mantione, 1978) and the ways in which the numbers and affinities of dopamine receptors are altered by morphine dependence are not yet clear (Puri *et al.*, 1978; Christie & Overstreet, 1979; de la Baume, Patey, Marcais, Protais, Costentin & Schwartz, 1979). Various behavioural tests of the sensitivity of dependent rodents to dopamine agonists so far have also given conflicting results (Iversen & Joyce, 1978; de la Baume *et al.*, 1979; Christie & Overstreet, 1979).

Opiate receptors are well-placed to modulate dopaminergic activity; one possible mechanism is via a 'second messenger': cyclic AMP or GMP (Minneman & Iversen, 1976). Thus, the change in occupation of 'dependent' receptors experienced in either abstinence or precipitated withdrawal might change the level of a restraining influence on dopaminergic activity. The speed of onset of the effects on circling is consistent with this hypothesis, being of the order of hours rather than days as in the case of presumed denervation supersensitivity.

Another possibility is that the withdrawal effects are secondary to changes in the level of serotoninergic or cholinergic activity in the dependent rats. Enhancements of both these neurotransmitter effects are observed in precipitated withdrawal (Way & Glasgow, 1978); since both acetylcholine and 5-hydroxytryptamine (5-HT) effects are antagonistic towards the striatal actions of dopamine, it is unlikely that changes in these substances are responsible for the present results. Furthermore, Marsden & Guldberg (1973) have noted that raphe lesions which cause a marked reduction in forebrain 5-HT fail to affect circling behaviour in rats with electrolytic lesions of the nigra.

At an empirical level, the enhancements in circling responses to apomorphine which were seen after 22 h abstinence (Figure 5) were related to the level of dependence. Since circling behaviour is easily quantifiable such tests may be a useful addition to existing methods for assessing morphine dependence in a quantitative way.

This work was supported by a grant from the Medical Research Council. We are very grateful to Professor C.D. Marsden and to Dr P. Jenner for their helpful comments.

References

ANDÉN, N.E., DAHLSTRÖM, A., FUXE, K. & LARSSON, K. (1966). Functional role of the nigro neo-striatal dopamine neurons. *Acta Pharmac. (Kbh)*, **24**, 263–274.

ATWEH, S.F. & KUHAR, M.J. (1977a). Auto-radiographic localisation of opiate receptors in rat brain. II The brain stem. *Brain Res.*, **129**, 1–12.

ATWEH, S.F. & KUHAR, M.J. (1977b). Autoradiographic localization of opiate receptors in rat brain. III The telencephalon. *Brain Res.*, **134**, 393–405.

AYHAN, J.H. & RANDRUP, A. (1972). Role of brain noradrenaline in morphine-induced stereotyped behaviour. *Psychopharmac. (Berl.)*, **27**, 203–212.

BABBINI, M. & DAVIS, W.M. (1972). Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. *Br. J. Pharmac.*, **46**, 213–224.

CELSEN, B. & KUSCHINSKY, K. (1974). Effects of morphine on kinetics of [^{14}C]-dopamine in rat striatal slices. *Naunyn-Schmiedebergs Arch. Pharmac.*, **284**, 159–165.

CHRISTIE, M.J. & OVERSTREET, D. (1979). Sensitivity of morphine-tolerant rats to muscarinic and dopaminergic agonists: relation to tolerance or withdrawal. *Psychopharmac.*, **65**, 27–34.

CLOUET, D. & RATNER, M. (1970). Catecholamine biosynthesis in brains of rats treated with morphine. *Science, N.Y.*, **168**, 854–856.

COLQUHOUN, D. (1971). *Lectures on Biostatistics*. Oxford: Clarendon Press.

COSTALL, B. & NAYLOR, R.J. (1975). A comparison of circling models for the detection of anti Parkinson activity. *Psychopharmac.*, **41**, 57–64.

COWAN, A., DETMAR, P.W. & WALTER, D.S. (1975). Analgesics and rotational behaviour in rats with unilateral substantia nigra lesions. Effects in the presence and absence of (+)-amphetamine. *Br. J. Pharmac.*, **55**, 316P.

COX, B., ARY, M. & LOMAX, P. (1976). Changes in sensitivity to apomorphine during morphine dependence and withdrawal in rats. *J. Pharmac. exp. Ther.*, **196**, 637–641.

CZLONKOWSKI, A., HÖLLT, V. & HERZ, A. (1978). Binding of opiates and endogenous opioid peptides to neuroleptic receptor sites in the corpus striatum. *Life Sci.*, **22**, 953–962.

DE GROOT, J. (1959). The rat forebrain in stereotaxic coordinates. *Trans. R. Neth. Acad. Sci.*, **52**, No. 4.

DE LA BAUME, S., PATEY, G., MARCAIS, H., PROTAIS, P., COSTENTIN, J. & SCHWARTZ, J.C. (1979). Changes in dopamine receptors in mouse striatum following morphine treatments. *Life Sci.*, **24**, 2333–2342.

DRAY, A., FOWLER, L.J., OAKLEY, N.R., SIMMONDS, M.A. & TANNER, T. (1977). Regulation of nigro-striatal dopaminergic neurotransmission in the rat. *Neuropharmac.*, **16**, 511–518.

DI CHIARA, G., OLIANAS, M., DEL FIACO, M., SPANO, P.F. & TAGLIAMONTE, A. (1977). Intranigral kainic acid is evidence that nigral non-dopaminergic neurones control posture. *Nature*, **268**, 743–745.

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

GIANUTSOS, G., HYNES, M.D., PURI, S.K., DRAWBAUGH, R.B. & LAL, H. (1974). Effect of apomorphine and nigro-striatal lesions on aggression and striatal dopamine turnover during morphine withdrawal: evidence for dopaminergic supersensitivity in protracted abstinence. *Psychopharmac.*, **34**, 37–44.

GLICK, S.D., JERUSSI, T.P. & FLEISHER, L.N. (1976). Turning in circles: the neuropharmacology of rotation. *Life Sci.*, **18**, 889–896.

HALLIWELL, J.V. & KUMAR, R. (1977). Influence of morphine dependence and withdrawal on circling behaviour in rats with unilateral nigral lesions. *Br. J. Pharmac.*, **59**, 454–455P.

HAVEMANN, U. & KUSCHINSKY, K. (1978). Effect of morphine on prostaglandin E_2 (PGE_2)-sensitive adenylate cyclase in corpus striatum of rats and its cellular localisation by using Kainic acid. *Brain Res.*, **150**, 441–445.

IVERSEN, S.D. & JOYCE, E.M. (1978). Effect in the rat of chronic morphine treatment on the behavioural response to apomorphine. *Br. J. Pharmac.*, **62**, 390–391P.

IWAMOTO, E.T., LOH, H.H. & WAY, E.L. (1976a). Dopaminergic–cholinergic interactions in naloxone-induced circling in morphine-dependent rats with nigral lesions. *Eur. J. Pharmac.*, **38**, 339–356.

IWAMOTO, E.T., LOH, H.H. & WAY, E.L. (1976b). Circling behaviour after narcotic drugs and during naloxone-precipitated abstinence in rats with unilateral nigral lesions. *J. Pharmac. exp. Ther.*, **197**, 503–516.

IWATSUBO, K. & CLOUET, D.H. (1975). Dopamine-sensitive adenylate cyclase of the caudate nucleus of rats treated with morphine or haloperidol. *Biochem. Pharmac.*, **24**, 1499–1503.

JACQUET, Y.F., CAROL, M. & RUSSELL, I.S. (1976) Morphine-induced rotation in naive, non-lesioned rats. *Science, N.Y.*, **192**, 261–263.

KELLY, P. & MOORE, K.E. (1976). Mesolimbic dopaminergic neurones in the rotational model of nigrostriatal function. *Nature*, **263**, 695–696.

KELLY, P.H. & MOORE, K.E. (1977). Mesolimbic dopamine neurons: effects of 6 hydroxydopamine induced destruction and receptor blockade on drug-induced rotation in rats. *Psychopharmac.*, **55**, 35–42.

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

KUSCHINSKY, K. (1975). Does chronic morphine treatment induce a supersensitivity of dopamine receptors in rat brain? *Psychopharmac.*, **42**, 225–229.

KUSCHINSKY, K. & HORNYKIEWICZ, O. (1972). Morphine catalepsy in the rat: relation to striatal dopamine metabolism. *Eur. J. Pharmac.*, **19**, 119–122.

LASCHKA, E., HERZ, A. & BLASIG, J. (1976). Activity of the nigrostriatal dopaminergic system during precipitated morphine-withdrawal investigated in rats with acute unilateral inactivation of the striatum. *Naunyn-Schmiedebergs Arch. Pharmac.*, **296**, 15–23.

LYON, M. & RANDRUP, A. (1972). The dose–response effect of amphetamine upon avoidance behaviour in the rat seen as a function of increasing stereotypy. *Psychopharmac.*, **23**, 334–347.

MARSDEN, C.A. & GULDBERG, H.C. (1973). The role of monoamines in rotation induced or potentiated by amphetamine after nigral, raphe and mesencephalic lesions in the rat brain. *Neuropharmac.*, **12**, 195–212.

MILLER, R.J., HORN, A.S. & IVERSEN, L.L. (1974). The action of neuroleptic drugs on dopamine-stimulated adenosine cyclic 3',5'-monophosphate production in rat neostriatum and limbic forebrain. *Mol. Pharmac.*, **10**, 759–766.

MINNEMAN, K.P. (1977). Morphine selectively blocks dopamine-stimulated cyclic AMP formation in rat neostriatal slices. *Br. J. Pharmac.*, **59**, 480–481P.

MINNEMAN, K.P. & IVERSEN, L.L. (1976). Enkephalin and opiate narcotics increase cyclic GMP accumulation in slices of rat neostriatum. *Nature, Lond.*, **262**, 313–314.

MINNEMAN, K.P., QUIK, M. & EMSON, P.C. (1978). Receptor-linked cyclic AMP systems in rat neostriatum: differential localisation revealed by kainic acid injection. *Brain Res.*, **151**, 507–521.

MUMFORD, L. & KUMAR, R. (1979). Sexual behaviour of morphine dependent and abstinent male rats. *Psychopharmac.*, **65**, 179–185.

PERT, A. & SIVIT, C. (1977). Neuroanatomical focus for morphine and enkephalin-induced hypermotility. *Nature, Lond.*, **265**, 645–647.

POLLARD, H., LLORENS-CORTES, C. & SCHWARTZ, J.C. (1977). Enkephalin receptors on dopaminergic neurones in rat striatum. *Nature, Lond.*, **268**, 745–747.

POLLARD, H., LLORENS, C., SCHWARTZ, J.C., GROS, C. & DRAY, F. (1978). Localisation of opiate receptors and enkephalins in the rat striatum in relationship with the nigrostriatal dopaminergic system: lesion studies. *Brain Res.*, **151**, 392–398.

PURI, S.K. & LAL, H. (1973). Effect of dopaminergic stimulation or blockade on morphine-withdrawal aggression. *Psychopharmac.*, **32**, 113–120.

PURI, S.K. & LAL, H. (1974). Tolerance to the behavioural and neurochemical effects of haloperidol and morphine in rats chronically treated with morphine or haloperidol. *Naunyn-Schmiedebergs Arch. Pharmac.*, **282**, 155–170.

PURI, S.K., SPAULDING, T.C. & MANTIONE, C.R. (1978). Dopamine antagonist binding: a significant decrease with morphine dependence in the rat striatum. *Life Sci.*, **23**, 637–642.

PURI, S.K., VOLICER, L. & LAL, H. (1977). Effect of apomorphine on dopamine turnover and adenosine 3',5'-monophosphate content in striatum of morphine dependent rats. *Neuropharmac.*, **16**, 205–209.

PYCOCK, C. & MARSDEN, C.D. (1978). The rotating rodent: a two component system. *Eur. J. Pharmac.*, **47**, 167–175.

SCHEEL-KRÜGER, J., GOLEMBIOSKA, K. & MOGILNICKA, E. (1977). Evidence for increased apomorphine-sensitive dopaminergic effects after acute treatment with morphine. *Psychopharmac.*, **53**, 55–63.

SUGRUE, M.F. (1974). The effect of acutely administered analgesics on the turnover of noradrenaline and dopamine in various regions of the rat brain. *Br. J. Pharmac.*, **52**, 159–165.

TARSY, D. & BALDESSARINI, R.J. (1974). Behavioural supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. *Neuropharmac.*, **13**, 927–940.

UNGERSTEDT, U. (1971). Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. *Acta physiol. scand. Suppl.*, **367**, 69–73.

WADDINGTON, J.L. & CROSS, A.J. (1978). Denervation supersensitivity in the striatonigral GABA pathway. *Nature*, **276**, 618–620.

WAY, E.L. & GLASGOW, C. (1978). Recent developments in morphine analgesia: tolerance and dependence. In *Psychopharmacology: A Generation of Progress*. ed. Lipton, M.A., Di Mascio, A. & Killam, K.F. pp. 1535–1555. New York: Raven Press.

WINER, B.J. (1970). *Statistical Principles in Experimental Design*. New York: McGraw Hill.

(Received October 16, 1979
Revised January 30, 1980.)