Pharmacology Biochemistry & Behavior, Vol 22, pp 101–105, 1985 © Ankho International Inc Printed in the U S A 0091-3057/85 $3 00 + 00

Neuroleptics Block the Positive Reinforcing Effects of Amphetamine but not of Morphine as Measured by Place Conditioning

W. BRUCE MACKEY AND DEREK VAN DER KOOY[1]

Neurobiology Research Group, Department of Anatomy, University of Toronto, Toronto, Ontario Canada M5S 1A8

Received 9 May 1984

MACKEY, W B AND D VAN DER KOOY *Neuroleptics block the positive reinforcing effects of amphetamine but not of morphine as measured by place conditioning* PHARMACOL BIOCHEM BEHAV 22(1) 101–105, 1985.—The role of dopamine brain systems in mediating the rewarding effects of opiates and stimulants was investigated using the conditioned place preference paradigm The effects of the neuroleptics α-flupentixol (0 8 mg/kg, IP) and haloperidol (1 0 mg/kg, IP) were tested against the place preferences produced by morphine sulphate (1 0 and 5.0 mg/kg, SC), d-amphetamine sulphate (1 0 mg/kg, IP) and cocaine hydrochloride (5 0 mg/kg, IP) Amphetamine place preference was successfully blocked but neuroleptic pretreatment had no effect on the place preferences produced by cocaine and morphine. α-Flupentixol alone produced no place conditioning These results support the hypothesis of dopamine involvement in amphetamine reward However, morphine reward, as measured by the conditioned place preference paradigm, appears not to be critically dependent on brain dopamine systems

Reward Morphine Neuroleptics Dopamine Amphetamine Cocaine Place conditioning

THERE is a great deal of evidence for the involvement of dopamine in the reward produced by many psychoactive drugs. Most convincing in this regard is the evidence for amphetamine and cocaine [17, 18, 21, 24, 25, 26, 27], two psychoactive drugs with known agonist effects at dopamine synapses [13] However, for the opiates, the involvement of dopamine in reward is less clear This evidence primarily consists of studies correlating intracranial morphine self-administration sites in the ventral tegmental area to the locations of dopamine fields [2, 3, 4] and two place preference studies in which heroin place preference was attenuated by the neuroleptics pimozide and haloperidol [5,22]. Other studies find little role for dopamine in morphine reward [10,19] and instead implicate other pharmacological systems [6, 8, 12].

The present experiments were performed to investigate the effects of the neuroleptics α-flupentixol and haloperidol on the conditioned place preferences produced by morphine, cocaine, and amphetamine. As a measure of drug reward, the conditioned place preference paradigm was chosen over other paradigms because of its sensitivity (particularly with morphine [15]) and because testing is performed drug-free. Testing in a drug-free state is particularly important since in other paradigms, in which animals are tested while under the effects of drugs, interpretation of a drug's motivational properties may be confounded by its other effects In our experiments the motoric effects of neuroleptics (which may have led to incorrect interpretation of data in at least one study [12,19]) were avoided by testing drug-free.

We now report that morphine (1.0 and 5.0 mg/kg SC), cocaine (5.0 mg/kg, IP) and amphetamine (1.0 mg/kg, IP) all produced significant place preferences, and that amphetamine place preference was blocked by α-flupentixol whereas cocaine and morphine place preferences were not. These results suggest that dopamine involvement is not critical in opiate reward.

METHOD

Seventy-eight adult male Wistar rats (Charles River) weighing 250–400 g were used. The rats were housed individually throughout all handling and conditioning procedures in a room kept at 22°C and lit from 0900 to 2100 hr. Purina rat chow and tap water were available ad lib.

The place conditioning procedures used were very similar to those used by Mucha *et al* [15]. Briefly, conditioning took place for each rat in one of two boxes which differed in colour, texture and smell. One had black walls and a black Plexiglas floor which was wiped with a 2% vinegar solution just prior to placing each rat inside it. The other box had white walls and a wood chip floor which gave off a slight smell of wood. Each rat received injections of a drug on one

[1]Requests for reprints should be addressed to D van der Kooy

day and vehicle on the next and this continued for a total of six days. Only six days were used to minimize tolerance to the neuroleptics When injected with the drug (morphine, amphetamine or cocaine) a rat was placed immediately in one of the boxes and on alternate days, when injected with saline vehicle, it was placed in the other box Each pairing lasted 30 minutes The order of drug and vehicle presentation and the choice of which environment rats received drug injections in was counterbalanced for the rats in each group

On the seventh day each rat was placed into a larger, rectangular test box which consisted of environments similar to the conditioning boxes at each end separated by a smaller grey area ("neutral zone") The time that each rat spent on each of the two ends was recorded over a ten minute period

We tested three drugs (morphine sulphate, cocaine hydrochloride and d-amphetamine sulphate) for their ability to produce place preferences The effect of neuroleptics flupentixol and/or haloperidol) were tested against each of these drugs In the first experiment two groups of rats (n=6) were given saline vehicle injections 2 5 hours prior to injections of 1 0 mg/kg or 5 0 mg/kg morphine sulphate SC on the opiate pairing days On alternate days they received two vehicle control injections 2 5 hours and immediately before being placed into the other box To test the effect of neuroleptics on the morphine conditioning two other groups were treated as above except that a 0 8 mg/kg IP dose of α-flupentixol was given instead of the vehicle 2 5 hours prior to each of the six conditioning trials Neuroleptic injection was given prior to both drug and vehicle conditioning trials in order to maximize its pharmacological blockade properties and minimize any possible motivational properties of neuroleptics which could be specifically paired with one environment The pretreatment time of 2 5 hours was chosen so that the peak neuroleptic effect would occur when the test drug of interest was administered [9] More recent evidence suggests that four hours may be required for α-flupentixol to reach peak effectiveness, but other studies and our own observations confirm that at this high dose the α-flupentixol is very effective 2 5 hours post-injection [7] In order to test if α-flupentixol itself produced place conditioning, an additional group of rats (n=6) was run This group received α-flupentixol 2 5 hours before being placed in one box and on alternate days received saline vehicle 2 5 hours before being placed in the other conditioning box Each day these rats received saline vehicle injections just prior to being placed in a conditioning box

In the second experiment one group of rats (n=8) was given 1 0 mg/kg SC morphine and a second group (n=8) was treated identically but with a 1 0 mg/kg IP dose of haloperidol 2 5 hours prior to each conditioning trial Our third and fourth experiments were identical to the one above but used d-amphetamine sulphate (1 0 mg/kg, IP) and cocaine hydrochloride (5 0 mg/kg, IP) respectively to establish a place preference and 0 8 mg/kg α-flupentixol to attempt to block it

The data were analyzed for effects of the test drug and effects of the neuroleptic using analyses of variance and a students t-test in one instance The accepted level of significance was $p<0$ 05 Analyses of variance were used on the assumption that the time spent on one side of the test box during the 10 min test period was independent of the time spent on the opposite side This assumption was possible because during the test each rat could also spend time in the grey "neutral zone" and, therefore, time spent on one side of the test box did not necessarily predict time spent on the other side

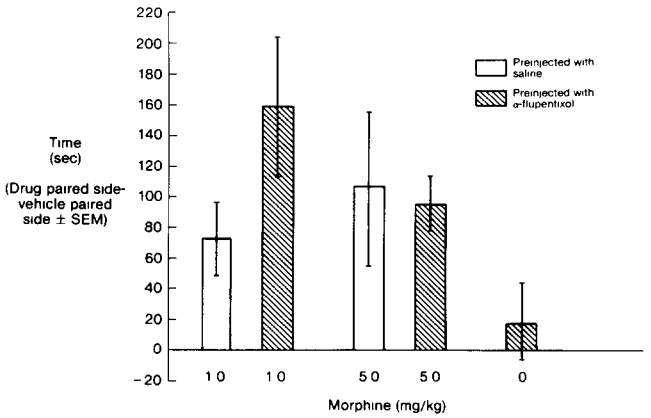

FIG 1 Conditioned place preference in groups of rats receiving either saline (n=6) or 0 8 mg/kg IP α-flupentixol (n=6) injections 2 5 hours prior to injections of morphine (1 0 or 5 0 mg/kg SC) One group (n=6) received α-flupentixol only Data represent mean difference±S E M in time that each group spent on the drug paired side of the test box vs the time spent on the vehicle paired side

RESULTS

Morphine

Figure 1 shows the conditioned place preference produced in groups of rats given morphine alone (1 0 or 5 0 mg/kg, SC) or given morphine 2 5 hours after injection with α-flupentixol (0.8 mg/kg, IP) The final group shown was injected with α-flupentixol only Rats injected with morphine showed typical catelepsy which was quickly overcome when they were startled or touched α-Flupentixol produced a substantial immobility 2 5 hours post-injection which was not affected by startling or touching Rats injected with both drugs were identical in appearance to α-flupentixol only injected rats ANOVA showed that 1 0 mg/kg SC morphine resulted in more time spent on the drug paired than vehicle paired side of the test box, $F(1,10)=23$ 75, $p<0$ 01 No significant effect of α-flupentixol on this place conditioning was seen, $F(1,10)=0$ 89, $p>0$ 25, and no interaction was found to exist between the effects of morphine and the α-flupentixol, $F(1,10)=3$ 32, $p>0$ 10 Similarly, the 5 0 mg/kg dose of morphine produced place preferences, $F(1,10)=8$ 39, $p<0$ 01 No significant effect was seen for α-flupentixol, $F(1,10)=0$ 70, $p>0$ 25, and no interaction was seen between the two drugs, $F(1,10)=0$ 005, $p>0.25$ The group of rats given only α-flupentixol showed no place conditioning at all, $t(6)=0$ 815, $p>0$ 05

Figure 2 shows the conditioned place preference produced in a group of rats given morphine (1 0 mg/kg, SC) and a group of rats injected with haloperidol (1 0 mg/kg, IP) 2 5 hours prior to receiving morphine Haloperidol injected rats appeared identical to α-flupentixol injected rats Again morphine produced place preferences, $F(1,14)=9$ 37, $p<0$ 01 Pretreatment with haloperidol failed to have any significant effect itself on place conditioning, $F(1,14)=3$ 60, $p>0$ 10, and there was no significant interaction between the two drugs, $F(1,14)=1$ 95, $p>0$ 05

Amphetamine

Figure 3 shows the conditioned place preferences produced in a group of rats given amphetamine (1 0 mg/kg,

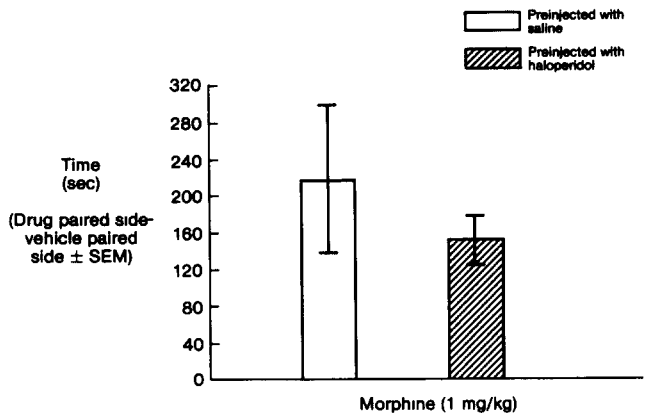

FIG 2 Conditioned place preferences in groups of rats receiving either saline (n=8) or 1 0 mg/kg IP haloperidol (n=8) injections 2 5 hours prior to injection with morphine (1 0 mg/kg SC) Data represent mean differences±S E M in time that each group spent on the drug paired side of the test box vs the time spent on the vehicle paired side

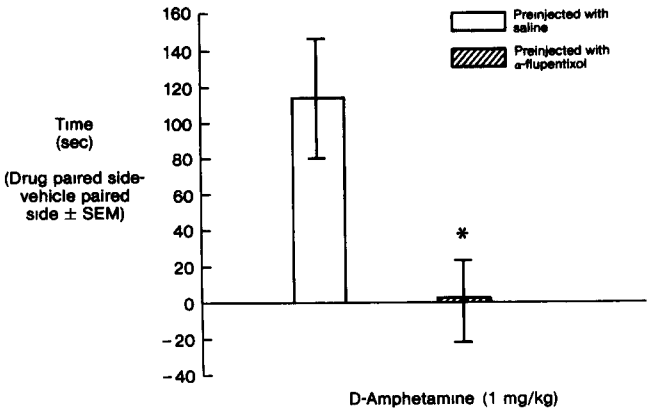

FIG 3 Conditioned place preference produced in a group of rats (n=8) receiving saline injection 2 5 hours prior to d-amphetamine injection (1 0 mg/kg IP) but not in the group receiving 0.8 mg/kg IP α-flupentixol (n=8) prior to d-amphetamine Data represent mean difference±S E M in time that each group spent on the drug paired side of the test box vs the time spent on the paired side. The asterisk indicates a significant block of place preference by α-flupentixol ($p<0$ 01)

IP) and a group of rats injected with α-flupentixol (0.8 mg/kg, IP) 2.5 hours prior to receiving amphetamine. Amphetamine injected rats showed hyperactivity and explored their environments much more than vehicle injected rats. On the early conditioning trials the behaviour of the amphetamine injected rats that were pretreated with α-flupentixol seemed identical to their behaviour when they were injected with α-flupentixol only. However, by the final amphetamine conditioning day, the rats injected with amphetamine and α-flupentixol were slightly more active than when injected with α-flupentixol only, suggesting that tolerance was beginning to develop to the α-flupentixol. Like morphine, amphetamine was seen to produce a significant place preference, $F(1,14)=7$ 94, $p<0$ 05 α-Flupentixol, however, significantly interacted with amphetamine, $F(1,14)=11$ 89, $p<0.01$, and seemed to completely eliminate the place preference caused by amphetamine alone

Cocaine

Figure 4 shows the conditioned place preferences produced in a group of rats given cocaine (5 0 mg/kg, IP) and a group of rats injected with α-flupentixol (0 8 mg/kg, IP) 2.5 hours prior to receiving cocaine. Rats injected with cocaine behaved identically to amphetamine injected rats Cocaine alone produced place preferences, $F(1,14)=22.06$, $p<0$ 01, and no effect of α-flupentixol, $F(1,14)=0.003$, $p>0.05$, or interaction between the drugs, $F(1,14)=0.99$, $p>0.05$, was found.

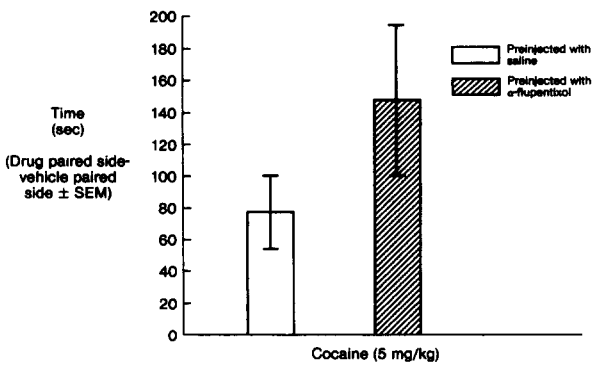

FIG 4 Conditioned place preferences in groups of rats receiving either saline (n=8) or 0 8 mg/kg IP α-flupentixol (n=8) injections 2 5 hours prior to injection with cocaine (5 0 mg/kg SC) Data represent mean difference±S E M in time that each group spent on the drug paired side of the test box vs the time spent on the vehicle paired side

DISCUSSION

The present results confirm the results of previous studies showing conditioned place preference produced by morphine [5,15], amphetamine [21] and cocaine [20]. Also confirmed was the effective neuroleptic blockade of amphetamine but not cocaine induced place preference [20,21]. This supports the widely held view that amphetamine reward is mediated by a dopaminergic substrate [21, 24, 25, 26, 27] but fails to support this same view for cocaine. This failure to implicate dopamine in cocaine reward stands in stark contrast to experiments in the intravenous self-administration literature [24, 26, 27] An explanation for the apparent lack of neuroleptic effect on cocaine place preference was put forth by the group who first observed this effect They suggested that place conditioning with cocaine is the result of cocaine's dopamine mediated central stimulant effects and also of cocaine's local anesthetic properties which are not dopamine mediated [20] To test this they attempted place conditioning with procaine, a local anesthetic presumably with no central stimulant effects at the doses used Since positive place conditioning was obtained with procaine they hypothesized that the central rewarding effects of cocaine were indeed blocked by neuroleptic treatment and that the place preferences still seen were the result of cocaine's local anesthetic properties [20]. However, it is not clear why these local anesthetic properties do not maintain normal response rates

after neuroleptics in the cocaine intravenous self-administration paradigm and why much higher doses of procaine than cocaine were needed to produce place preferences since procaine is at least as effective as cocaine in producing local anesthesia [16]. Moreover, if the central aspects of cocaine reward were blocked by neuroleptic pretreatment then one may have expected to see at least an attenuation of place preference conditioning This, however, was not seen in either the former [20] or the present study.

With both the neuroleptics α-flupentixol and haloperidol we obtained no attenuation of the conditioned place preferences produced by morphine In fact in one group we seemed to get a potentiation of conditioning with α-flupentixol pretreatment although this was not statistically significant (see Fig 1). The failure to block the reinforcing effects of morphine with neuroleptics is particularly striking given the low doses of morphine used (just above the threshold doses for producing morphine SC place preferences [14], Bechara and van der Kooy, unpublished observations) and the high doses of neuroleptics used (which did block amphetamine place preference)

Our inability to block morphine conditioned place preference with neuroleptics is in contrast to earlier studies showing pimozide and haloperidol attenuation of heroin induced place conditioning [5,22] In one of these studies [22], however clear place preferences were evident even after haloperidol One possibility for this discrepancy may be differences in the drugs used in each study although we believe it unlikely that morphine and heroin would act via different pharmacological substrates and also unlikely that there is any significant difference in the effectiveness of dopamine blockade by the neuroleptics at the high doses used Another possible explanation may involve differences between the versions of the conditioned place preference paradigm used Our version (the "balanced" version) involves complete counterbalancing of the order of morphine presentations and the environment morphine is paired with This is possible because prior studies have established that the two environments we use are equally preferable to well handled rats [15] The "unbalanced" version, on the other hand, employs two environments (one of which is greatly preferred by naive rats) and pairs the non-preferred side with the drug under investigation for all of the rats in the study [5, 20, 21, 22] A recent investigation [14] examining this version of the paradigm in measuring morphine reward suggests that something more than the rewarding effects of morphine is measured (possibly an anti-anxiety effect [21]) Whether or not these differences are sufficient to explain the differences in neuroleptic effect is at present unknown. It should be noted however, that our results with amphetamine and cocaine were identical with previous studies which employed the "unbalanced" version of the paradigm [20,21]

Given our results with cocaine and the explanation proposed by others [20] of why neuroleptics do not block cocaine induced place conditioning, it seems possible that our results with morphine could be via a similar mechanism (i e , neuroleptic pretreatment may have blocked the central rewarding effects of morphine but spared a peripheral non-dopaminergic opiate system which was itself able to cause a place preference) This appears unlikely, however, given recent results from our lab demonstrating that the peripheral effects of opiates are in fact aversive and that peripherally acting opiate antagonists are rewarding [1] If dopamine blockade was preventing morphine's central effects and leaving the peripheral systems unaffected, then one would expect a place avoidance instead of the observed place preference

A further possible explanation for our ability to block amphetamine but not morphine place preference with neuroleptics lies in the different time courses of the two drugs Since the effects of morphine last considerably longer than amphetamine's effects, it is conceivable that morphine's effects may have outlasted the effects of the neuroleptics used If this were the case, and morphine reward was indeed blocked by neuroleptic pretreatment, conditioning to morphine's rewarding aspects could still have occurred after the neuroleptics had worn off and produced similar place preferences to those rats receiving only morphine This is unlikely, however, because the neuroleptics used act for many hours and the morphine injections were given at or even slightly before the peak neuroleptic effect (see Method section) This explanation is also unlikely since morphine place perferences are successfully blocked by naloxone which has a much shorter time course than morphine itself [15] Furthermore, near the end of our amphetamine experiments there was some evidence that the locomotor effects of amphetamine were starting to partially overcome neuroleptic blockade, and yet a complete block of amphetamine place preference was seen

Although several studies support the view that opiate reward is mediated by dopaminergic substrates and in particular the cells of the ventral tegmental area [2, 3, 4, 12], some of these studies have been challenged and other studies show no dopamine involvement at all [6, 8, 10, 19] For example, self-administration of morphine was decreased by preinjection with haloperidol and this was taken as evidence for a potentiation of reward [12] Results from another investigation, however, suggest this to be the result of neuroleptic motor impairment [19] In conclusion, morphine reward as measured by the conditioned place preference paradigm is not affected by pretreatment with either α-flupentixol or haloperidol Amphetamine reward is blocked by neuroleptic pretreatment These results suggest that there may be a critical dopaminergic link in amphetamine but not opiate reward

ACKNOWLEDGEMENTS

Supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Medical Research Council of Canada We are indebted to Ms Karen McMurray for typing the final manuscript

REFERENCES

1 Bechara, A and D van der Kooy Endogenous opiods Opposite motivational effects in brain and periphery *Soc Neurosci Abstr* **10:** 312, 1984

2 Bozarth, M A Opiate reward mechanisms mapped by intracranial self-administration In *Neurobiology of Opiate Reward Mechanisms*, edited by J E Smith and J D Lane New York Raven Press, 1982

3 Bozarth, M A and R A Wise Intracranial self-administration of morphine into the ventral tegmental area in rats *Life Sci* **28:** 551–555, 1981

4 Bozarth, M A and R A Wise Localization of the reward relevant opiate receptors In *Problems of Drug Dependence* edited by L S Harris Washington, DC National Institute on Drug Abuse, 1982, pp 158–164

5 Bozarth, M A and R A Wise Heroin reward is dependent on a dopaminergic substrate *Life Sci* **29:** 1881–1886, 1981
6 Brown, Z W , Z. Amit, D Siayor, G. E Rockman and S O Ogren Supression of voluntary ingestion of morphine by inhibition of dopamine-beta-hydroxylase *Arch Int Pharmacodyn Ther* **232:** 102–110, 1978
7 Corbet, D , J R Stellar, L Stinus, A Kelley and G Fouriezos Time course of α-flupentixol action explains "response artifacts" of neuroleptic action on brain stimulation reward *Science* **222:** 1251–1252, 1983
8 Davis, W M , S G Smith and J H Khalsa Noradrenergic role in the self-administration of morphine and amphetamine *Pharmacol Biochem Behav* **3:** 477–484, 1975
9 Ettenberg, A , G F Koob and F E Bloom Response artifact in the measurement of neuroleptic-induced anhedonia *Science* **213:** 357–359, 1981
10 Ettenberg, A , G F Koob, H O Pettit and F E Bloom Heroin and cocaine intravenous self-administration in rats Mediation by separate neural systems *Psychopharmacology (Berlin)* **78:** 204–209, 1982
11 Glick, S D and R D Cox Self-administration of haloperidol in rats *Life Sci* **16:** 1041–1046, 1975
12 Glick, S D and R D Cox Dopaminergic and cholinergic influences on morphine self-administration in rats *Res Commun Chem Pathol Pharmacol* **12:** 17–24, 1975
13 Moore, K E , C C Chieuh and G Zelds Release of neurotransmitters in vivo by amphetamine, methylphenidate and cocaine In *Cocaine and Other Stimulants*, edited by E H Ellinwood, Jr and M M Kilbey New York Plenum Press, 1977, pp 143–160
14 Mucha, R F and S D Iverson Reinforcing properties of morphine and naloxone revealed by conditioned place preferences A procedural examination *Psychopharmacology (Berlin)* **82:** 241–247, 1984
15 Mucha, R F , D van der Kooy, M O'Shaughnessy and P Bucenieks Drug reinforcement studied by the use of place conditioning in rat *Brain Res* **243:** 91–105, 1982
16 Ritchie, J. M , P J Cohen and R D. Dripps. Cocaine, procaine and other synthetic local anesthetics. In *The Pharmacological Basis of Therapeutics*, edited by L. S Goodman and A Gilman Toronto Macmillan, 1970, pp 371–402
17 Roberts, D. C S , G F Koob, P Klonoff and H C Fibiger Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens *Pharmacol Biochem Behav* **12:** 781–787, 1980.
18 Roberts, D C S , M. E Corcoran and H. C Fibiger On the role of ascending catacholaminergic systems in intravenous self-administration of cocaine *Pharmacol Biochem Behav* **6:** 615–620, 1977
19 Smith, S G and W M Davis. Haloperidol effects on morphine self-administration Testing for pharmacological modification of the primary reinforcement mechanism *Psychol Record* **23:** 215–221, 1973
20 Spyraki, C , H C Fibiger and A G Phillips Cocaine-induced place preference conditioning· Lack of effects of neuroleptics and 6-hydroxydopamine lesions. *Brain Res* **253:** 195–203, 1982
21 Spyraki, C , H C Fibiger and A. G Phillips Dopaminergic substrates of amphetamine induced place preference conditioning *Brain Res* **253:** 185–193, 1982
22 Spyraki, C , H C Fibiger and A G Phillips Attenuation of heroin reward in rats by disruption of the mesolimbic dopamine system *Psychopharmacology (Berlin)* **79:** 278–283, 1983
23 van der Kooy, D. Place conditioning. A simple and effective method for assessing the motivational properties of drugs. In. *Methods of Assessing the Reinforcing Properties of Abused Drugs*, edited by M A Bozarth Brunswick Frederick Haer and C O , in press
24 Wise, R A Action of drugs of abuse on brain reward systems *Pharmacol Biochem Behav* **13:** Suppl 1, 213–223, 1980
25 Wise, R A and M A Bozarth Action of drugs of abuse on brain reward systems An update with specific attention to opiates *Pharmacol Biochem Behav* **17:** 239–243, 1982
26 Wise, R A Catacholamine theories of reward A critical review *Brain Res* **152:** 215–247, 1978
27 Yokel, R A and R. A Wise Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats *Psychopharmacologia* **48:** 311–318, 1976