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BRIEF COMMUNICATION

Intravenous Self-Administration of Morphine by Naive Mice

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CRISWELL, H E AND A RIDINGS *Intravenous self-administration of morphine by naive mice* PHARMACOL BIOCHEM BEHAV 18(3)467–470, 1983 —A simple method for IV self-administration of drugs by mice is described When morphine (0 5 mg/kg) was made contingent on a nosepoke response, naive mice increased their rate of nosepoking when compared either with animals receiving contingent saline vehicle injections or yoked control animals receiving noncontingent morphine

Morphine Opiate Self-administration Nosepoke Mouse

SINCE the pioneering work of Spragg in 1940 [12] there has been increasing interest in development of an animal model of opiate abuse in humans The use of intravenous (IV) self-administration has received attention because it parallels the preferred mode of human administration It is also consistent with known rules of reinforcement which require that the psychoactive effect of the drug must occur within a reasonably brief time after the response which is to be reinforced [7]

Using IV self-administration, rats [2, 3, 9], cats [8], dogs [6], rhesus monkeys [10], and baboons [4] have all been shown to self-administer opiates under some conditions We now describe a simple method for drug self-administration studies in mice based upon Moran and Straus' [11] chronic IV infusion technique and the use of a nosepoke response We show that mice will self-administer morphine using this paradigm without previously having been made dependent upon opiates To our knowledge, this is the first demonstration of IV self-administration of a substance by mice

METHOD

Animals

Twenty adult, female ICR Swiss-Webster mice (30 to 35 grams) were earmarked and group housed four to a cage with food and water available ad lib for the duration of the study All animals were naive to the drugs and experimental manipulations at the beginning of the study

Apparatus

Mice were tested in a pair of Plexiglas cages 8 cm square and 15 cm high with two 1 2 cm holes on opposite sides of the cage and 0 5 cm above the floor (see Fig 1) The mouse was restrained by placing it inside the cage with its tail extending through one of the holes The tail was then taped to a horizontal surface outside the cage allowing access to the lateral tail veins [11] To afford the largest contact area possible between the tail and tape, a piece of tape was wrapped around a tongue depressor and taped to the table outside the cage The tail was then held against this tape by another piece of masking tape near the base of the mouse's tail The tape was, therefore, in contact with the entire diameter of the tail The mouse was held by the stickiness of the tape not by its tightness

The wall opposite the tail hole held a manipulandum consisting of a 1 2-cm hole with a grain of wheat light (Radio Shack #272-1144) and a photodarlington (Poly Paks #92 CU 3276) mounted outside the hole so that when the tip of the mouse's nose extended 1 mm through the hole, it interrupted the light beam to the photocell and was counted as a nosepoke response The photodarlington was interfaced directly to the input port of a SYM-1 microcomputer which monitored responses, activated a Camden Instruments cumulative recorder and operated a Sage Instruments syringe pump to deliver solution contingent on a nosepoke response

An IV injection needle was made by removing the steel cannula from a disposable 26-gauge $^5/_8$ inch hypodermic needle and press fitting that cannula into a length of 28-gauge Teflon tubing

Procedure

At the beginning of each session, the mice were removed from their home cages and placed 0 5 meters below a 250-watt heat lamp for approximately 2 min This produced vasodilation of the tail and eased insertion of the IV needle into a lateral vein [11] During insertion, the needle was held as

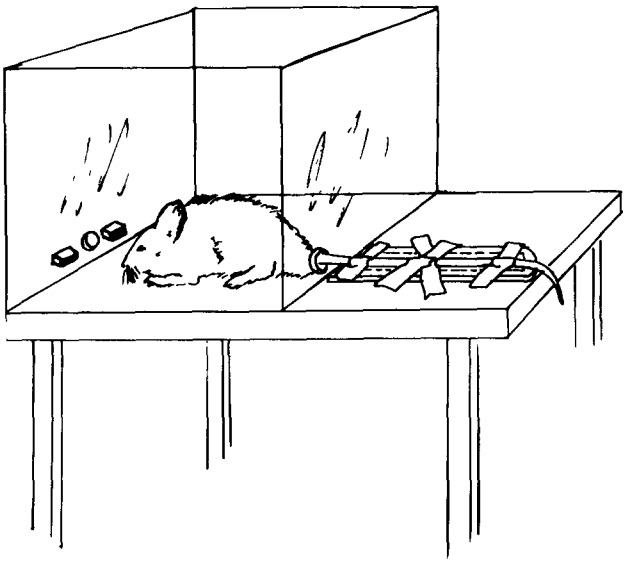

FIG 1 Mouse test cage for drug self-administration Dimensions are 8 cm long, 8 cm wide and 15 cm high Nosepoke and tail holes are 1 2 cm in diameter and centered 0 5 cm above the floor A photocell and light are mounted on the outside of the box so that a nosepoke interrupts the light beam A piece of masking tape is wrapped around a thin object (such as a tongue depressor) which is taped to the table top The mouse is placed in the cage with its tail protruding through the hole The tail is then taped to the object with masking tape, allowing room to insert the IV needle distal to the tape

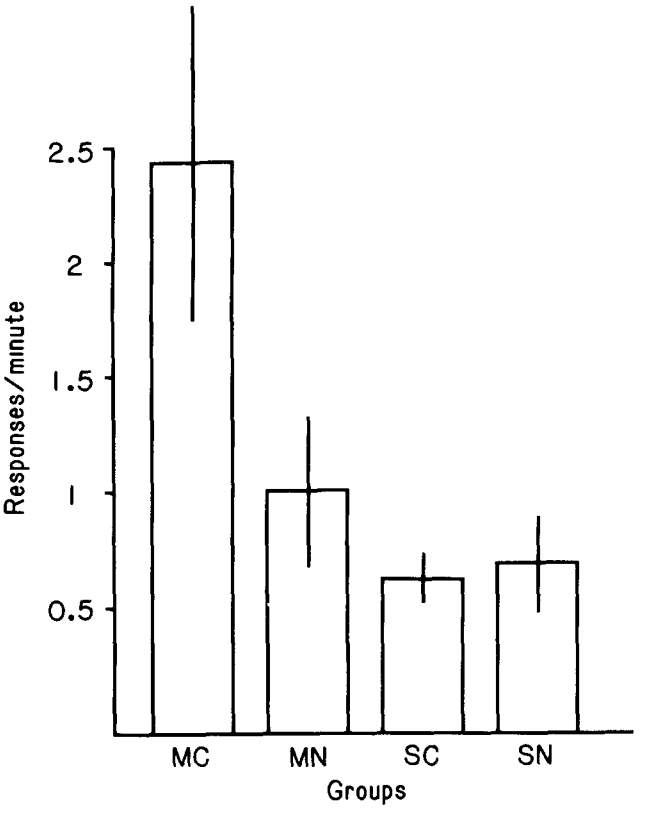

FIG 2 Means (wide bars) and standard errors (narrow bars) are shown for mice receiving contingent saline—SC, noncontingent saline—SN contingent morphine—MC, and noncontingent morphine—MN Responses were made during a 45-min test period

near parallel to the tail as possible with the beveled side of the needle out A test injection of 5 μl was then made and proper placement verified by observing the bolus of drug moving through the tail vein Improper insertion was accompanied by blanching of the tail at the site of injection Proper insertion occurred on the first attempt approximately 50% of the time If the needle was not properly placed, it was removed, reinserted and retested In all cases, proper insertion was achieved with 4 or fewer attempts At the end of a 45-min trial, the needle was removed and the animal was returned to its home cage Mice adapt rapidly to this form of immobolization and some investigators have maintained IV injections continuously for several days using the technique [11]

Animals were divided into 10 pairs at the beginning of the study One animal of each pair received either morphine sulfate (0 5 mg/kg disolved in 5 μl of saline) or the saline vehicle alone (5 μl delivered during $^1/_2$ sec) contingent upon a nosepoke response The other animal served as a noncontingent yoked control and received either morphine or saline as appropriate when its partner responded

Each pair was tested for two 4-day periods with 7 days separating the test periods Days 1 and 2 were training days where morphine or saline was available and days 3 and 4 were extinction days to return the animals to their original operant levels IV needles were not inserted during extinction trials Training trials lasted 45 min or until the animal had self-administered 20 mg/kg of morphine or an equivalent number of injections of saline Extinction trials lasted 30 min each and the 2 training and 2 extinction trials were conducted during 4 consecutive days Following a 7-day rest period each pair repeated the training and extinction procedure with animals which received saline now receiving morphine and vice versa Each animal therefore, received both morphine and saline in counterbalanced order The 10 pairs of animals were divided into 2 groups of 5, one group receiving morphine sulfate during the first two training sessions, while the other group received saline first Two pairs of animals from the group receiving morphine first were dropped from the study following malfunction of a photocell during training Complete data were analyzed for the remaining mice

RESULTS

Mean response rates of the four groups during the second drug day are shown in Fig 2 Paired comparison ANOVAs [1] showed a statistically reliable increase in response rate when morphine injections were contingent on the responses as compared to the noncontingent control $F(1,7)=13\ 25$ $p<0\ 01$, and to the saline control, $F(1,7)=13\ 87$, $p<0\ 01$ During the first day of extinction response rates were 0 78 responses/min for the mice which had previously received contingent morphine, 0 85 for noncontingent morphine and 0 83 for contingent saline There were no significant differences, $F(2,7)<1$, $p>0\ 5$, between these response rates

Cumulative records depicting acquisition of the nosepoke

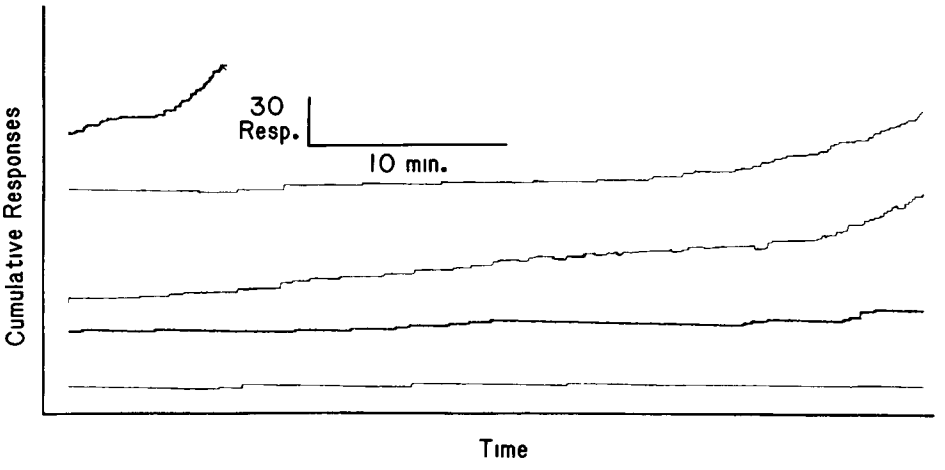

FIG 3 Cumulative records for Day 1 are shown for the 5 animals which received contingent morphine during their second test period Response rates are low during the first part of the test for 4 of 5 animals One animal responded rapidly from the start and received 20 mg/kg of morphine within the first 10 min 3 of the remaining 4 animals showed increases in response rate late in the training session This pattern is typical of animals acquiring a reinforced response

response are shown in Fig 3 These animals had previously experienced 2 days of saline followed by 2 days of extinction and 7 days rest They, therefore, had relatively low operant levels

DISCUSSION

The nosepoke response is a useful response in drug self-administration studies It is a more natural operant for a mouse than the traditional lever-press response and is less vulnerable to drug-induced motor impairment [5] The relatively high operant level of this response also eliminates the need for shaping The response needs only to be brought under the control of the experimental contingencies

When delivery of a small dose of morphine was made contingent on a nosepoke response, mice had higher rates of responding when compared to control groups which received either saline or noncontingent morphine This suggests that morphine was acting as a reinforcer for the nosepoke response Further evidence of the reinforcing properties of morphine injections comes from the positively accelerating slopes of the cumulative response records shown in Fig 3 The use of tail vein injections into mice, thus, appears to represent a viable method of demonstrating reinforcing properties of an experimental drug The use of noncontingent yoked controls is important in this procedure since changes in activity level due to the drug might alter the operant level of the nosepoke response independently of the reinforcing effects of the drug

It is especially interesting that these animals acquired the response without prior drug experience In the past, many studies [2, 3, 4, 9] have used animals previously made dependent The reinforcing effect of the morphine injections in those instances can thus be explained by negative reinforcement through reduction of aversive withdrawal effects The present study suggests that naive mice will self-administer morphine and that morphine acts as a positive reinforcer The lack of differential responding during extinction is somewhat troubling but may simply be due to the minimal training or to the differing procedure between training and extinction sessions The animals were not placed under the heat lamp and IV catheters were not inserted during extinction These are possible discriminative stimuli which were absent during extinction sessions Also, the shorter sessions during extinction eliminated the time period (the last 15 minutes) during which greatest differences were observed between experimental and control sessions This suggests that session length may be an important variable using this procedure The methodology used in the present study does not appear to be stressful to the mice [11] and allows comparison of the several available mouse strains It should be usable with any water soluble drug, offering a simple and inexpensive methodology for examining drug self-administration

REFERENCES

1 Bruning, J L and B L Kintz *Computational Handbook of Statistics* Atlanta Scott, Foresman and Company, 1968
2 Carroll, M E and R A Meisch Determinants of increased drug self-administration due to food deprivation *Psychopharmacology (Berlin)* **74** 197–200, 1981
3 Davis, W M and S G Smith Alpha-methyltyrosine to prevent self-administration of morphine and amphetamine *Curr Ther Res* **14** 814–819, 1972
4 Elsmore T F, G V Fletcher, D G Conrad and F J Sodetz Reduction of heroin intake in baboons by an economic constraint *Pharmacol Biochem Behav* **13** 729–731, 1980
5 Gerhardt, S and J M Liebman Differential effects of drug treatments on nose-poke and bar-press self-stimulation *Pharmacol Biochem Behav* **15** 767–771, 1981

6 Jones, B E and J A Prada Characteristics of chronic self-administration of morphine by dogs *Psychopharmacology (Berlin)* **74** 204–207, 1981
7 Kelleher, R T Characteristics of behavior controlled by scheduled injections of drugs *Pharmacol Rev* **27** 307–323, 1976
8 Kilbey, M M and E H Ellinwood Self-administration of morphine in the cat *Int J Addict* **15** 447–460, 1980
9 Lukas, S E, J E Moreton and N Khazan Effects of levoalpha-acetyl-methadol (LAAM) on morphine self-administration in the rat *Psychopharmacology (Berlin)* **73** 12–16, 1981
10 Mello, N K and J H Mendelson Self-administration of an enkephalin analog by rhesus monkey *Pharmacol Biochem Behav* **9** 579–586 1978
11 Moran, R E and M J Straus A method for establishing prolonged intravenous infusions in mice *Lab Anim Sci* **30** 865–867, 1980
12 Spragg, S D S Morphine addiction in chimpanzees *Comp Psychol Monogr* **15** 1–131, 1940