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Effects of 5,7-Dihydroxytryptamine Lesions of the Nucleus Accumbens on Rat Intravenous Morphine Self-Administration

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SMITH, J E , K SHULTZ, C CO, N E GOEDERS AND S I DWORKIN *Effects of 5,7-dihydroxytryptamine lesions of the nucleus accumbens on rat intravenous morphine self-administration* PHARMACOL BIOCHEM BEHAV **26**(3) 607–612, 1987 —The role of serotonergic innervations of the nucleus accumbens in the processes maintaining intravenous morphine self-administration were assessed Pairs of male rat littermates were implanted with intravenous jugular catheters and bilateral injection guide cannulae into the central medial nucleus accumbens, made physically dependent on morphine and then allowed to intravenously self-administer with continuous access When stable baselines of drug intake were obtained (2–3 weeks), one of each pair received bilateral microinjections of vehicle and the other 5,7-dihydroxytryptamine (5,7-DHT) into the nucleus accumbens Response independent infusions of morphine were delivered for 24 hours at the previous rate of self-injection and the animals were again allowed to self-administer while drug intake was monitored for thirteen days The littermate pairs were then sacrificed by immersion in liquid nitrogen, the brains removed at −20°C and frozen sections of the cannulae tract taken for histological assessment The nucleus accumbens, anterior caudate nucleus and pyriform cortex were removed at −20°C and biogenic monoamine content determined The 5,7-DHT lesions resulted in a significant increase in drug intake and significantly decreased the content of serotonin (5-HT) and 5-hydroxyindoleacetic acid in the nucleus accumbens (−49% and −30%, respectively) and 5-HT in the anterior caudate nucleus (−14%) and pyriform cortex (−17%) Dose-effect relationships were assessed in four additional animals before and after similar bilateral 5,7-DHT lesions The lesion resulted in similar rates of responding maintained by all drug doses including vehicle, thus eliminating the typical pattern of dose related decreases in responding as a function of increasing dose 5-HT innervations of the nucleus accumbens appear to participate in neuronal activity mediating intravenous morphine self-administration

Neurotoxin lesions 5,7-Dihydroxytryptamine Nucleus accumbens lesions Intravenous morphine self-administration Opiate reinforcement Opiate reward Serotonin Neurotransmitter content Biogenic amines

BEHAVIOR must in some way be controlled and/or influenced by events that take place in the central nervous system (CNS). Some drugs when delivered response contingently engender and maintain the behavior that resulted in their presentation—a process called reinforcement. Hypotheses proposing reinforcement to be mediated by specific neuronal pathways evolved from studies of responding maintained by intracranial electrical stimulation [23] The neuronal systems responsible for reinforcing drug effects have been investigated with pharmacological blockade and lesion techniques Agents that antagonized acetylcholine [7,12], dopamine [12, 27, 34] and norepinephrine [8] neurotransmission attenuated intravenous morphine self-administration indicating an involvement of these neurons in systems maintaining this behavior Electrolytic lesions of some brain regions also altered drug intake which further focused research on the discrete systems that participated in these processes [10, 11, 13–15] Innervations and projections of the nucleus accumbens were shown to be important since neurotoxin lesions modulated intravenous self-administration. Kainic acid lesions of this region selectively decreased the rate of morphine intake by rats responding on a concurrent schedule of food, water and drug presentation [9] while similar lesions also decreased intravenous cocaine intake [41]. 6-Hydroxydopamine (6-OHDA) lesions of dopaminergic inputs to the nucleus accumbens decreased intravenous co-

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caine [29–31] and amphetamine maintained responding [19] but had no effect [24] or moderately increased opiate intake [33]. Lesions of serotonergic innervations of this region with 5,7-dihydroxytryptamine (5,7-DHT) increased total intake of intravenous amphetamine but did not alter the rate of acquisition of self-administration [20] Since innervations of the nucleus accumbens may be differentially involved in the processes maintaining the self-administration of stimulants and opiates [24], this investigation was initiated to assess the effects of 5,7-DHT lesions of this structure on intravenous morphine self-administration

METHOD

Subjects

Eleven littermate pairs of adult male Fischer F-344 rats (90–150 days old) were used These animals were maintained on a reversed 12 hour light (1700 to 500 hr) and 12 hour dark (500 to 1700 hr) cycle with continuous access to food (Purina rat chow) and water The rats were implanted with a venous catheter, trained to self-administer morphine and then given either bilateral sham-vehicle or 5,7-DHT lesions of the nucleus accumbens. Four additional rats received bilateral 5,7-DHT lesions with intravenous morphine self-administration dose-effect relationships evaluated before and after

Surgical Procedures

The rats were implanted with jugular catheters and bilateral injection guide cannulae into the nucleus accumbens using previously described procedures [25] The catheter (0.76 mm o d. × 0 25 mm i.d., polyvinylchloride tubing) was inserted into the right posterior facial vein and pushed down into the jugular vein until it terminated outside the right atrium The catheter was anchored to tissue in the area and continued subcutaneously to the back where it exited just posterior to the scapulae through a plastic harness that was implanted under the skin for attachment of a leash. Bilateral 26 gauge stainless steel injection guide cannulae were then stereotaxically implanted into the nucleus accumbens (9 5 mm anterior to lambda, 1 2 mm from the midline, and 5.1 mm below the surface of the brain [18]) using previously described procedures [21] The guide cannulae were cemented to the skull with dental cement and 32-gauge stainless steel stylets inserted that extended 0 5 mm below the tip A stainless steel needle tubing-spring leash was then attached to the subcutaneous harness to protect the catheter which was connected to a fluid swivel suspended and counterbalanced above the self-administration cage The surgery was followed by 48 hours of hourly infusions of 200 μl of heparinized saline

Self-Administration

Each animal was housed in a self-administration cage inside a sound attenuated chamber containing a speaker which maintained a constant intensity of white noise Continuous access to food and water and the reversed light-dark cycle were continued Two days after surgery each rat was made physically dependent with hourly infusions of morphine sulfate in increasing doses (three days each of 1 25, 2.5, 5 0 and two days of 10 0 mg/kg in 0 2 ml of heparinized saline delivered over 5 5 seconds). Each morphine infusion was followed by a 30-sec time-out period during which tone and light stimuli were presented (when self-administration was initiated responding during the timeout was counted but had no schedule consequences). Non-contingent infusions were discontinued after two days of exposure to the 10 mg/kg dose and a response lever was introduced The animals were then allowed to self-administer morphine on a fixed-ratio schedule with 24-hr access and continued pairing of the tone and light stimulus During the first week the fixed-ratio requirement was increased from one lever press to 10

Lesions

When stable baselines of self-administration were obtained (2 to 3 weeks), one of each of the eleven littermate pairs received a bilateral 5,7-DHT lesion while the second littermate received sham-vehicle treatment The rats were pretreated with desmethylimipramine (30 mg/kg, IP) 30 min prior to being anesthetized with methohexital (1 mg/kg, IV) Forty-five minutes after desmethylimipramine treatment, 0 5 μl of either the vehicle (isotonic saline–0.02% ascorbic acid) or 5,7-DHT (6 μg in isotonic saline–0 02% ascorbic acid) were bilaterally injected over 7 1/2 min into the central medial nucleus accumbens through 32-gauge injection cannulae extending 0 5 mm below the guide cannulae The injection cannulae were left in place an additional 10 min, removed and the stylets replaced Response-independent morphine infusions were delivered for 24 hours since the 5,7-DHT lesions are potentially debilitating and could disrupt physical dependence resulting in an artifactual change in self-administration The response-independent infusions were delivered at equal intervals with the total number per day equal to the average for intake of each animal during the five days immediately prior to the lesion The rats were again allowed to self-administer morphine and drug intake was monitored for days 6–14 post-lesion and compared with prelesion for each group

Dose-Effect

The effects of 5,7-DHT lesions on the self-administration of different doses of morphine were evaluated in four additional rats After self-administration of the 10 mg/kg dose stabilized (2–3 weeks), these rats were exposed to duplicate 24-hr probes of vehicle, 2 5, 5 0 and 40.0 mg/kg of morphine sulfate A dose probe consisted of substitution of another drug concentration (or vehicle) for the training dose for 24 hours commencing at 5 a m (the beginning of the dark cycle) Dose probes were separated by 2 to 4 days and were not initiated until the 10 mg/kg intake had returned to baseline The animals then received bilateral 5,7-DHT lesions of the nucleus accumbens using the procedures outlined above After 24 hours of response-independent infusions of morphine (10 mg/kg) the animals were again allowed to self-administer the drug Duplicate 24-hr vehicle, 2 5, 5 0, 40 0 mg/kg dose probes were again evaluated with the same procedure used prior to the lesion

Sacrifice Procedure

The eleven littermate pairs were sacrificed by total immersion for 5 minutes in liquid nitrogen and the brains removed at −18°C and stored at −69°C The four dose-effect animals were sacrificed by decapitation and the brains frozen in methyl butane cooled with liquid nitrogen for histological analysis

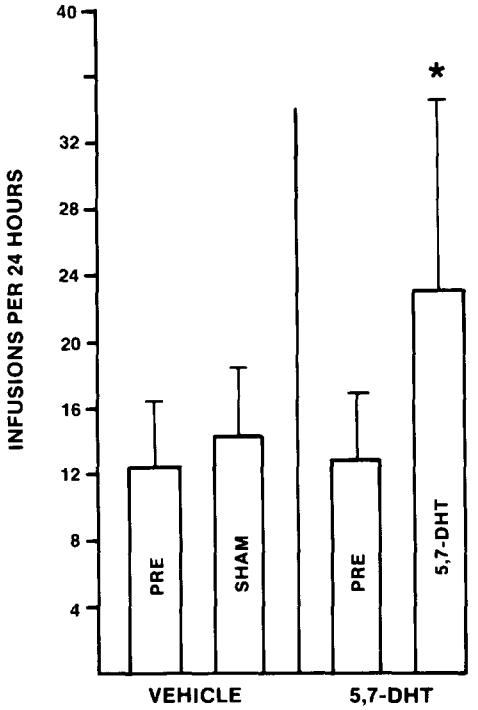

FIG 1 Effects of either sham-vehicle treatment or 5,7-DHT lesions of the nucleus accumbens on intravenous morphine self-administration Values are means and error measures standard deviations for 11 pairs of littermates The values represent drug intake for the 5 days just prior to the treatment and for days 6–14 post-treatment Post-lesion intake for each treatment group was compared with pre-lesion for that condition The significance of the differences between means determined with Students' t-tests were *$p<0$ 025

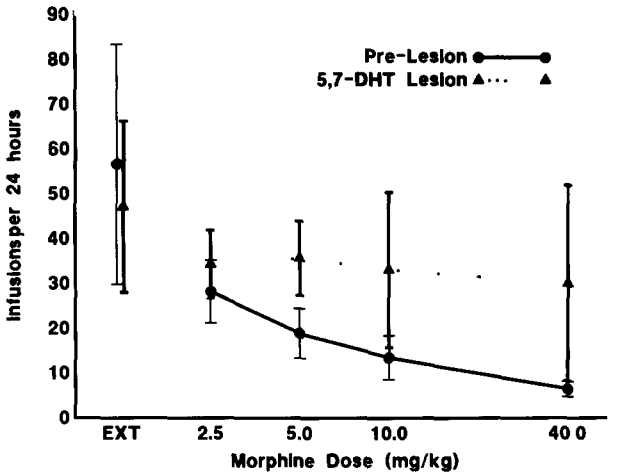

FIG 2 Dose-effect relationship for intravenous morphine self-administration prior to and after bilateral 5,7-DHT lesions of the nucleus accumbens. Values are means and error measurements one standard deviation on each side of the mean for four animals with two 24 hour probes at each dose (vehicle, 2 5, 5 0 and 40 0 mg/kg) and the mean intake at the 10 mg/kg training dose for each day prior to a dose probe "EXT" represents the vehicle infusion rates

Histological Procedures

Location of the guide cannulae and histological assessment of the lesions were determined in the eleven littermate pairs and the four dose-effect animals The brains were warmed to −18°C in a cryostat and a coronal cut made at the point of entry along the tract of the guide cannulae Four 16 μm frozen sections of the cannulae site were thaw-mounted on microscope slides and the remaining nucleus accumbens (95–98% of the total) and adjacent anterior caudate nucleus-putamen and pyriform cortex individually dissected at −18°C for assessment of biogenic monoamine content. The frozen sections were fixed in 70% ethanol, stained with luxol fast blue and cresylecht violet using previously reported procedures [17] and the location of the cannulae and lesion damage assessed by light microscopy.

Biogenic Monoamine Content

The content of dopamine (DA), dihydroxyphenylacetic acid, homovanillic acid, serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine (NE) were concurrently measured in extracts of the nucleus accumbens, anterior caudate nucleus-putamen and pyriform cortex from the eleven littermate pairs by high pressure liquid chromatography with electrochemical detection using a previously reported procedure [6]

Statistical Procedure

Students t-tests were used to compare the significance of differences in the intake of morphine pre- and post-lesion or sham treatment in the eleven littermate pairs. Analysis of variance with repeated measures was used to assess the effects of the lesion on the four dose-effect animals

RESULTS

Morphine intake stabilized after two or three weeks of self-administration. The mean number of infusions of the training dose (10 mg/kg) per 24 hours was 12 4 and 12.8 with 55% and 59% occurring during the dark cycle for the animals that were to receive sham-vehicle and 5,7-DHT treatments, respectively The sham-vehicle treatment did not alter self-administration, increase (15%), $t(20)=-1$ 03, p N.S. However, the 5,7-DHT lesion resulted in significant increases (79%) in drug intake compared to pre-lesion self-administration, $t(20)=-2$ 78, $p<0.025$ (Fig. 1). This increase in drug intake shortened interinfusion intervals during both the light and dark cycles In the four dose-effect rats the neurotoxin lesion increased drug intake and appeared to eliminate the dose effect relationship (Fig. 2). Morphine intake at various doses was significantly altered ($p<0.0341$) by the lesion (Table 1)

Body weights were not altered by the treatments [sham-vehicle lesion—303±5.7 g pre- and 321±10 g post-lesion, 5,7-DHT lesion—307±11 g pre- and 313±12 g post-lesion (means±standard deviations)]. Histological assessment indicated guide cannulae to be in the medial central nucleus accumbens of the eleven littermate pairs (Fig. 3) and in the four dose-effect animals (data not included) The 5,7-DHT lesion resulted in significant decreases in the content of 5-HT (−49%) and 5-HIAA (−30%) in the nucleus accumbens and in 5-HT in the anterior caudate nucleus (−14%) and pyriform

TABLE 1

REPEATED MEASURES ANALYSIS OF VARIANCE OF EFFECTS OF 5,7-DHT LESIONS OF THE NUCLEUS ACCUMBENS ON THE SELF-ADMINISTRATION OF VARIOUS DOSES OF MORPHINE*

Source	*df*	F Value	Probability	V^2
Overall Model	38	7 8	0 0001	0 77
Rat	3	17 3	0 0001	
Lesion	1	59 2	0 0001	
Dose	4	26 1	0 0001	
Lesion × Dose	4	7 1	0 0001	
Rat × Lesion × Dose	11	2 0	0 0341	

*Data obtained from N=4 rats exposed to several doses (0, 2 5, 5 0, 10 0 and 40 0 mg/kg) of morphine before and after bilateral 5,7-DHT lesions of serotonergic innervations of the nucleus accumbens

cortex (−17%) (Table 2), but did not effect DA or NE content in any of these regions

DISCUSSION

Behavior controlled by contingent drug presentation (self-administration) can be influenced by neurobiological manipulations of discrete brain regions. The nucleus accumbens appears to be a region that has a crucial role in these processes since damage to dopaminergic inputs attenuated intravenous amphetamine [19], cocaine [29–31] and morphine [33] self-administration. Furthermore, the conditioned reinforcing properties of stimuli paired with noncontingent administration of amphetamine [35] and heroin [37], were also attenuated by lesions of these dopaminergic inputs. The increase in morphine intake after the 5,7-DHT lesion reported here suggests the involvement of serotonergic innervations of the nucleus accumbens in the neuronal processes mediating responding maintained by contingent morphine presentation This increased drug intake was observed for more than 45 days in the four dose-effect animals indicating affects of long duration. The decrease in 5-HT content (−49%) and lack of changes in DA and NE reflects a substantial and specific lesion of the central nucleus accumbens The decrease in 5-HT in the anterior caudate nucleus (−14%) and pyriform cortex (−17%) most likely did not result from direct exposure to the neurotoxin since the small volume and slow rate of infusion decrease the likelihood of such diffusion artifacts Instead, these decrements may reflect loss of collateral fibers to the caudate nucleus and pyriform cortex from the damaged serotonergic innervations of the nucleus accumbens

Several factors could be responsible for the increase in morphine self-administration after the lesion since the behavioral mechanisms involved are of considerable complexity The lesion could have directly increased activity or altered the effects of morphine on activity and/or stereotypy, all of which could increase lever pressing and, thus, drug intake Similar lesions increased intravenous amphetamine self-administration indicating the rate increasing effects to not be specific to opiates [50]. Motor activity has been demonstrated to increase after chronic opiate administration [1] and after decrements in serotonergic neuronal activity resulting

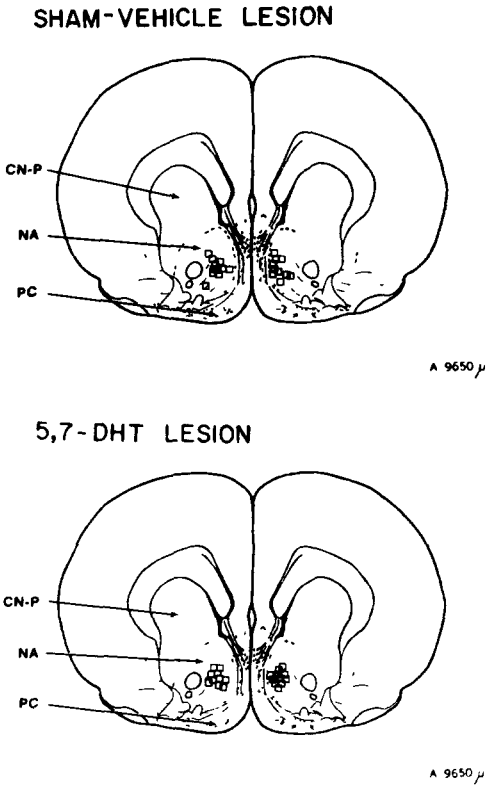

FIG 3 Location of the tips of the bilateral injection cannulae in eleven pairs of littermates each receiving either sham-vehicle treatment or 5,7-DHT lesions of the nucleus accumbens Abbreviations CN-P—caudate nucleus-putamen, NA—nucleus accumbens, PC—pyriform cortex

from either intracerebroventricular (ICV) 5,7-DHT [2] or electrolytic lesions of the raphe nuclei [39] These affects could be partially mediated by 5-HT innervations of the nucleus accumbens since direct injections of serotonin into this region decreased locomotion [26] and lesions of serotonergic neurons increased dopamine stimulated circling [16] Therefore, the lesion could have decreased serotonergic inhibition of nucleus accumbens dopamine neurons which are involved in the motor effects of stimulants [28]. Moreover, the increase in stimulant self-administration after this lesion may be a direct result of these activity effects and not a modulation of the reinforcing efficacy of the drug. Although, it is possible that similar mechanisms may be responsible for the changes in morphine self-administration, several factors are not consistent with this explanation First of all, the lesion did not increase responding during the 30 second time out which followed each injection by the four dose effect animals (16.8±7 0% versus 20.4±3 5% of total responding pre-lesion and post-lesion, respectively) Such increases would be expected if there was a general non-specific increase in lever pressing Secondly, dose-related effects on responding were also not observed which is demonstrated by the flat relationship after the lesion (Fig 2). If the lesion had non-specifically increased the stimulatory effects of morphine on motor activity, then some dose related relationship would be expected.

TABLE 2

CONTENT OF DOPAMINE, DIHYDROXYPHENYLACETIC ACID, HOMOVANILLIC ACID, 5-HYDROXYTRYPTAMINE, 5-HYDROXYINDOLEACETIC ACID AND NOREPINEPHRINE IN THE NUCLEUS ACCUMBENS AND ADJACENT ANTERIOR VENTRAL CAUDATE NUCLEUS-PUTAMEN AND PYRIFORM CORTEX OF LITTERMATE PAIRS OF INTRAVENOUS MORPHINE SELF-ADMINISTERING RATS FOLLOWING EITHER BILATERAL 5,7-DIHYDROXYTRYPTAMINE OR SHAM-VEHICLE LESIONS OF THE NUCLEUS ACCUMBENS

	Content (pmoles mg protein^{-1})					
Brain Region Treatment	DA	DOPAC	HVA	5-HT	5-HIAA	NE
Nucleus Accumbens						
Sham-Vehicle Lesion	615 9 ± 56 1	92 0 ± 16 4	40 5 ± 10 7	43 0 ± 4 3	22.7 ± 2.5	21 1 ± 12 9
5,7-DHT Lesion	580 4 ± 63 2	101 7 ± 26 9	40 7 ± 11 8	22 1 ± 4 6†	15 8 ± 2 6†	12 5 ± 4 3
Anterior Caudate-Putamen						
Sham-Vehicle Lesion	888.2 ± 81 4	82 2 ± 13 3	58 1 ± 13 6	43 9 ± 3 7	22 1 ± 7 6	11 3 ± 5 7
5,7-DHT Lesion	833.7 ± 136 7	80 5 ± 20 1	56 4 ± 12 8	37 7 ± 7 4*	21 5 ± 5 2	16 2 ± 8 7
Pyriform Cortex						
Sham-Vehicle Lesion	209 3 ± 96 4	32 0 ± 11 9	16 4 ± 4 2	57 3 ± 9 1	16 6 ± 2 0	22 3 ± 3 4
5,7-DHT Lesion	202 1 ± 80 1	31 6 ± 11 4	17 1 ± 4 5	47 4 ± 11 3*	14 9 ± 4 6	21 5 ± 3 9

Values are means ± S D. for eleven pairs of littermates
The significance of the difference between means determined with Student's t-tests were *=$p<0.05$, †=$p<0.001$

The increase in morphine intake could also have resulted from a modification in the intensity of the withdrawal response The lesion may have altered neuronal processes involved in withdrawal or the associated behavioral effects of withdrawal. The possibility exists that the increased intake resulted from enhanced negative reinforcing properties of the drug (the removal of aversive withdrawal stimuli). However, this was not likely since noradrenergic and not serotonergic systems are thought to be specifically involved in opiate withdrawal [3,40] Increased deprivation produced by decreased food and/or water intake after the lesion is another potential reason for the increase in drug self-administration that can be discounted [4,5] The 5,7-DHT lesions of the nucleus accumbens do not alter food or water consumption [19] and body weights assessed at the conclusion of this study were not different between lesioned and non-lesioned littermates The increase in responding post-lesion could have been the result of a decrease in the disruptive effects of morphine. The descending limb of the dose effect relationship is assumed to result from the disruptive effects of the drug. Higher doses may be more reinforcing but are also more behaviorally debilitating resulting in a net decrease in response rate The lesion may attenuate these disruptive effects resulting in the same number of daily infusions at all doses The rate of self-administration was similar at the 2.5 mg/kg dose for the dose effect animals both before and after the lesion (Fig. 2) Therefore, one could assume that this dose produced minimal disruptive effects and, perhaps, maximal other effects (reinforcing) The lesioned animals took 2.1, 3 9, and 14 0 times more drug at the 5 0, 10 0, and 40.0 mg/kg doses respectively, which could result from enhanced reinforcing efficacy of these higher doses.

The increased morphine intake following the lesion could also result from a change in central reinforcement processes. Serotonin appears to dampen these neurobiological mechanisms ICV infusions of 5-HT decreased intracranial electrical self-stimulation (ICSS) [38]. Moreover, injections of 5-HT directly into the nucleus accumbens decreased lateral hypothalamic ICSS [22]. The increased morphine intake could result from loss of this serotonergic modulation of reinforcement Electrolytic lesions of the raphe nuclei shift the dose-effect curve for intravenous morphine self-administration to the right which was interpreted as decreased reinforcing efficacy [13]. Furthermore, increased postsynaptic action of serotonin produced by the administration of a reuptake inhibitor decreased oral morphine intake [32] The increase in morphine intake reported here indicate that the serotonin inputs to the nucleus accumbens participate in the neuronal processes maintaining contingent morphine presentation. The elevated and flattened dose-effect relationship suggests a potential attenuation of the disruptive effects and enhancement of the reinforcing properties of morphine

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