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Brain Areas Involved in Production of Morphine-Induced Locomotor Hyperactivity of the C57Bl/6J Mouse

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STEVENS, K. E., G. A. MICKLEY AND L. J. McDERMOTT. *Brain areas involved in production of morphine-induced locomotor hyperactivity of the C57Bl/6J mouse.* PHARMACOL BIOCHEM BEHAV 24(6) 1739–1747, 1986.—Previous studies reveal a dose-dependent increase in locomotor activity of the C57Bl/6J mouse after administration of morphine or amphetamine. Concurrent partial lesions of both the dorsomedial caudate and lateral septal nuclei resulted in a significant decrease in morphine-induced, but not amphetamine-induced, hyperactivity. Concurrent partial lesions of the nucleus accumbens and stria terminalis produced only a nonsignificant decrease in the morphine-induced hyperactivity. Lesions of the individual brain structures did not significantly affect the morphine-induced locomotor hyperactivity. Microinjections of the opiate antagonist naloxone into discrete portions of the caudate and septal nuclei produced suppression of the morphine-induced hyperactivity response without affecting the hyperactivity caused by amphetamine injections. Only a slight suppression of morphine-induced locomotion was produced when naloxone was injected into the nucleus accumbens and stria terminalis. These data suggest that portions of the caudate and septum may be involved in the mediation of morphine-induced hyperactivity in the C57Bl/6J mouse.

Morphine Caudate nucleus Septal nucleus Amphetamine Locomotion Mice

MORPHINE, in high doses, produces immobility in most species [4]. However, when administered systemically to certain strains of mice, it evokes instead a dose-dependent locomotor hyperactivity [12,26]. This hyperactivity is characterized by a stereotypic "running fit" [16], usually around the perimeter of the cage, and is accompanied by an elevated or "straub" tail [12,16].

Several studies have attempted to identify brain areas that might mediate opioid-induced locomotor hyperactivity. Some of these experiments have implicated the nucleus accumbens as a primary locus of both morphine and endorphin actions. For example, locomotor hyperactivity follows the injection of morphine or enkephalin into the nucleus accumbens of the rat [5,17]. Naloxone reverses both of these effects [24]. Similarly, lesions involving the nucleus accumbens partially block the morphine-induced locomotor hyperactivity of the C57Bl/6J mouse [26]. The behavioral findings that have implicated the nucleus accumbens as a partial mediator of morphine-induced locomotion are further supported by immunohistochemical studies. Experiments have isolated endorphins as well as large numbers of enkephalin receptors, and beta-endorphin receptors in this brain area [2, 7, 8, 20, 24, 25, 28–30].

However, the failure of the lesions of nucleus accumbens to completely eliminate morphine-induced hyperactivity [26] suggests that other brain areas may also be partially involved in the production of this behavior. Candidate nuclei include several brain structures that are connected to the nucleus accumbens and also contain both beta-endorphin and enkephalin and their receptors [3, 7, 20, 22, 24, 25, 28–30]. For example, some opioid-containing cells in the nucleus accumbens project to the stria terminalis, including its bed nucleus [14, 15, 31]. The accumbens is also anatomically connected, both directly and indirectly (through the cells of the A9 group), to the caudate nucleus [14,31], which is known to contain endorphins and enkephalins [1]. Massive nucleus accumbens and septal connections have also been reported as part of the brain's endogenous opiate system [18].

Since the nucleus accumbens projects to the caudate, septum and stria terminalis (areas which contain opioid peptides and their receptors), one or more of the brain structures in these systems may also be involved in the mediation of morphine-induced locomotor hyperactivity. The present investigation sought to specify other brain areas involved in the production of this behavior in the C57Bl/6J mouse.

EXPERIMENT 1

Ablation of brain structures that mediate morphine-induced hyperactivity should reduce or eliminate this behav-

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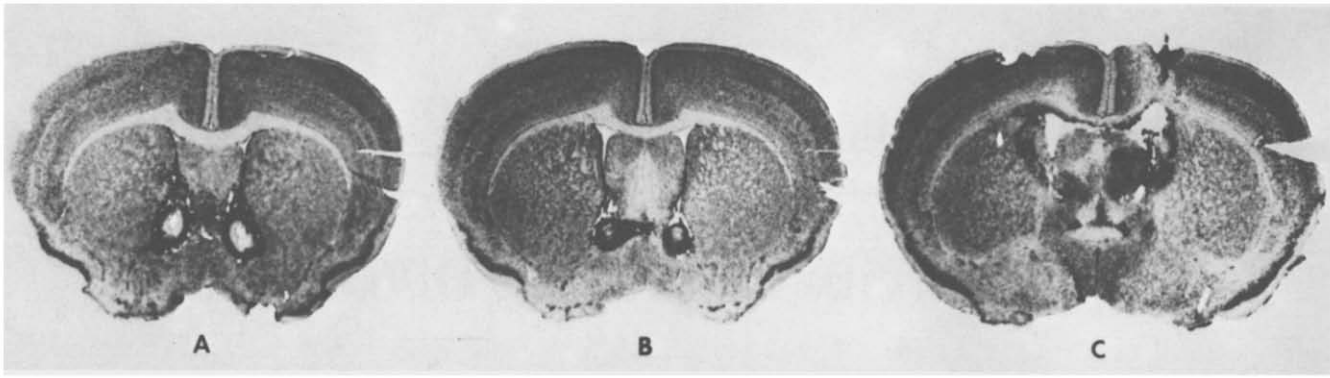

FIG. 1. Representative micrographs of lesioned brains. A and B are two different sections from the same brain, and show the lesion in both the nucleus accumbens and stria terminalis target areas. C shows combined lesions of the dorsomedial caudate and lateral septal nuclei.

ioral response. In this experiment, bilateral lesions were made in the nucleus accumbens, the stria terminalis, or a combination of these areas. In other animals, bilateral lesions were made in the dorsomedial portion of the caudate nucleus, the lateral septal nucleus, or a combination of these two areas.

Method

The subjects were C57Bl/6J male mice (Jackson Laboratories, Bar Harbor, ME), weighing between 16 and 22 grams. The mice were individually housed and were maintained on a 12-hour light/dark cycle (lights on at 6:00 a.m.). Purina rodent chow and water were continuously available.

Bilateral lesions were placed either in (a) nucleus accumbens (ACCUM), (b) stria terminalis (STRIA), (c) nucleus accumbens and stria terminalis (ACCUM + STRIA), (d) dorsomedial portion of the caudate nucleus (CAUD), (e) lateral septal nucleus (SEPT), or (f) dorsomedial caudate and lateral septal nuclei (CAUD + SEPT). Stereotaxic surgery was accomplished using atropine sulfate (0.4 mg/kg, IP) as a pre-anesthetic treatment and also sodium pentobarbitol (75 mg/kg, IP) anesthetic. Methoxyflurane was used as an auxiliary anesthetic. Bilateral lesions were placed using the following coordinates: *ACCUM*—anterior-posterior (AP) +1.0 mm from bregma, lateral (LAT) ±0.9 mm from midline, and depth (DEP) −4.2 mm from skull; *STRIA*—AP bregma, LAT ±0.6 mm, and DEP −4.0 mm; *ACCUM + STRIA*—AP +0.6 mm, LAT ±0.6 mm and DEP −4.5; *CAUD*—AP bregma, LAT ±1.5 mm DEP −2.5 and 3.3 mm; *SEPT*—AP bregma, LAT ±0.5 mm DEP −2.5 and −3.3 mm; and *CAUD + SEPT*—AP bregma, LAT ±1.0 mm DEP −2.5 and −3.3 mm. All coordinates were determined by using a mouse stereotaxic atlas [23]. The lesions were made at two different depths in animals with CAUD, SEPT, and combined CAUD + SEPT lesions in order to achieve an elliptical lesion rather than a spherical lesion. Lesions were produced by passing a 1.5 mA current through a 0.27-mm enamel-coated nickel-chromium wire for 7 seconds. This produced a lesion approximately 0.6 mm in diameter. Animals were allowed to recover for a minimum of 1 week before postsurgical testing began.

Activity was measured on Columbus Instruments' Animal Activity Monitors Automex D and Automex 2 SDII (Columbus Instruments, Columbus, OH). Prior to surgery, baseline activity was measured in the following manner: on the first day of baselines, each animal received an injection (IP) of saline followed by a 5-minute waiting period and two 30-minute periods of recorded locomotor activity. Immediately following the baseline activity readings, mice received a second IP injection of 30 mg/kg morphine sulfate, or 4 mg/kg amphetamine sulfate (which produced nonopiate mediated hyperactivity), or saline. Again, there was a 5-minute waiting period followed by two more 30-minute locomotor activity recordings. On the two following days, each animal received one of the other remaining drug solutions as the second injection. The order of drug presentation was randomized for each animal. Activity data from these three combinations of saline and drug injections (saline/amphetamine, saline/morphine, saline/saline) constituted the baseline measurements.

Postsurgical testing was performed in the same manner as presurgical baselines. Each animal received the three drug solutions (IP) following the saline injection (IP), one each

TABLE 1

MEAN CHANGES* IN LOCOMOTOR ACTIVITY "DIFFERENCE SCORES"† AFTER BRAIN LESION(S): SECOND 30-MINUTES OF RECORDING

Brain Lesion Site	Morphine	Amphetamine	Saline
Nucleus Accumbens	926	−142	370
Stria Terminalis	−214	−783	35
Nucleus Accumbens and Stria Terminalis	−48	160	−138
Caudate Nucleus (dorsomedial)	798	951‡	−44
Lateral Septal Nucleus	576	813‡	−147
Caudate (dorsomedial) and Lateral Septal Nuclei	−1062‡	360	−237

*Post-surgical locomotion minus pre-surgical locomotion.

†Difference score=activity recorded after an injection of saline, morphine or amphetamine minus activity after an initial saline injection (baseline).

‡$p<0.05$, Newman-Keuls. For purposes of this table we present only the results of the Newman-Keuls analysis of pre- vs. post-surgical locomotor activity (here represented by the change statistic). The actual Newman-Keuls comparison included pre- and post-surgical activity after all 3 drug treatments within each lesion group only (therefore, a total of 6 groups were involved in each post-hoc analysis).

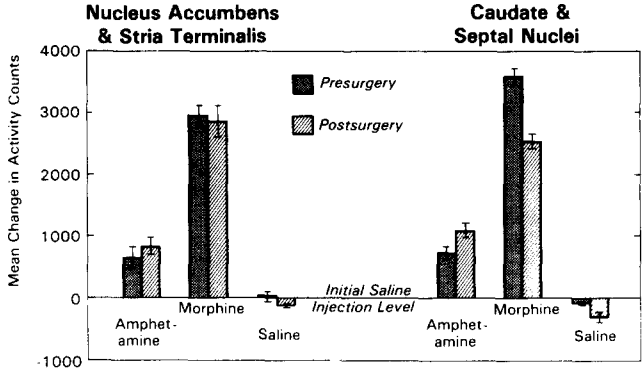

FIG. 2. Average change in locomotor activity counts after experimental drug injections (counts after the drug injection minus counts after initial saline injection) for each drug used (amphetamine 4.0 mg/kg, morphine 30 mg/kg or saline). Data presented are for the second 30 minutes of activity recorded. Bars show average activity changes both before and after surgery for the two combination brain lesion areas: (1) nucleus accumbens and stria terminalis (ACCUM + STRIA), and (2) dorsomedial caudate and septal nuclei (CAUD + SEPT). Variability indicators are the standard error of the means.

day, over the course of 3 days of testing. A randomized pattern of order of drug presentation was used for each animal.

After completion of testing, animals were sacrificed by perfusion, and brain tissue from each was sliced. The tissue was stained with thionin and lesion placements were verified. Only animals with well-defined bilateral lesions were retained in the experiment which resulted in the following distribution of lesion placements: ACCUM, (N=10); STRIA, (N=15); ACCUM + STRIA, (N=13); CAUD, (N=12); SEPT, (N=16); and CAUD + SEPT, (N=22). The lesions were, for the most part, discrete, but in some cases slight damage to adjacent structures had occurred. However, damage to other structures was random and slight, relative to the bulk of the lesioned area (see Fig. 1).

Data were analyzed using difference scores (activity after second injection of saline, morphine, or amphetamine minus activity after the initial saline injection). This was done to control for any hyperactivity caused by the injection procedures. Difference scores were computed for both time periods (first and second 30 minutes of activity recorded after injections) for presurgical baselines and postsurgical testing. The data were analyzed separately for the first and second 30 minutes of activity by 2, 3-way analyses of variance: (F)—lesion site by drug (second injection) by pre- or postsurgery. Newman-Keuls a posteriori tests were performed to locate specific differences between means [11]. In some instances, t-tests were performed if they were more applicable to the data. An alpha level of 0.05 was adopted throughout.

Results

Statistically significant differences occurred with respect to the interaction of lesion site and drug injection at both time frames: $F(12,170)=2.59$, $p<0.003$, for the first 30 minutes of activity, and $F(12,170)=2.905$, $p<0.003$, for the second 30 minutes of activity (see Table 1).

During the first 30 minutes of behavioral testing, amphetamine-induced increases in activity were observed postsurgically in CAUD, SEPT, and CAUD + SEPT animals ($p<0.05$, Newman-Keuls). These were the only statistically significant changes in locomotion observed in this early time period.

During the second 30 minutes of activity, morphine-injected animals with CAUD + SEPT lesions showed a significant decrease ($p<0.05$, Newman-Keuls) in locomotor activity, compared to morphine-stimulated activity before lesioning. In these same animals, amphetamine induced a small nonsignificant increase in activity postsurgically compared to presurgical amphetamine-induced hyperactivity, the drug's effect was potentiated after CAUD, SEPT, lesions ($p<0.05$).

ACCUM + STRIA lesioned mice displayed a slight nonsignificant postsurgical reduction in morphine hyperactivity, which was not reflected in amphetamine-induced hyperactivity. Individual lesions of the STRIA, and ACCUM produced nonstatistically significant changes in both morphine- and amphetamine-induced locomotor hyperactivity.

To verify that systemic injections of morphine and amphetamine caused an increase in locomotor activity in the C57Bl/6J mouse, t-tests were performed on the baseline (presurgical) difference score activity counts for morphine and amphetamine compared to saline control injections. As expected, the results show a significant increase in locomotor activity for both drugs ($p<0.001$ for both).

Discussion

This study reveals two different brain regions that may be involved in the mediation of morphine-induced hyperactivity: the dorsomedial caudate and lateral septal nuclei. Combined lesions of the dorsomedial caudate and lateral septal nuclei (CAUD + SEPT) caused a reduction in morphine-induced hyperactivity. This reduction cannot be described as a generalized lethargy since no similar postsurgical decrease was seen in the saline or amphetamine responses; indeed, in these animals a trend was seen towards an increase in amphetamine hyperactivity, although statistical significance was not achieved. The lesioned brain areas that produced decrements, corresponded very closely to areas known to contain opiate receptors (primarily enkephalin) [1,28]. It may be that the CAUD + SEPT lesions destroyed the endorphinergic neurons that partially mediate the morphine-induced locomotor response. These areas are probably not solely responsible for the mediation of morphine-induced hyperactivity, since ablation did not totally eliminate the morphine response, but only reduced it.

Combined partial CAUD + SEPT lesions produced a statistically significant reduction in morphine-induced activity while similarly-sized lesions of the individual areas did not. It may be the case that lesions of either the CAUD or SEPT alone leave enough opioid receptors intact (in other brain areas) to maintain the hyperactivity observed after morphine administration. Alternatively, the two brain areas may work in a synergistic way to produce this drug-induced locomotor hyperactivity response.

ACCUM + STRIA lesions produced a small decrease in morphine-induced hyperactivity and failed to block amphetamine-induced locomotion. This latter result is similar to that described in rats after anterior nucleus accumbens lesions [32]. In addition, large kainic acid lesions, which destroyed portions of the striatum and nucleus accumbens, also apparently spared amphetamine-induced locomotion

while attenuating morphine-stimulated activity [21]. Thus, it may be the case that the present lesions spared dopaminergic neurons while damaging at least a portion of the endorphinergic neurons in the region. The results of the present study are somewhat inconsistent with other data [10,26] that have demonstrated a significant reduction in amphetamine-stimulated locomotion after lesions of the posterior nucleus accumbens. However, direct comparisons between the 2 studies are not justified since the lesions of the present study were more posterior (consistently involving the stria terminalis) than those previously reported [26].

EXPERIMENT 2

Experiment 1 established two brain areas which seemed to be involved in mediation of morphine-induced hyperactivity: the dorsomedial caudate and the lateral septal nuclei. In addition, the nucleus accumbens and stria terminalis area may be involved [21,26]. As further verification of the role of these areas in the mediation of morphine-induced activity, naloxone, an opiate antagonist, was microinjected into the caudate and septal nuclei or the nucleus accumbens and stria terminalis of mice that had also received an intraperitoneal injection of morphine.

Method

The subjects were again C57Bl/6J male mice (Jackson Laboratories, Bar Harbor, ME) and were housed in the same manner as Experiment 1.

The mice were surgically implanted, bilaterally, with 23-gauge stainless-steel guide cannulas aimed at both the nucleus accumbens and stria terminalis (ACCUM + STRIA), or at both of the dorsomedial caudate and lateral septal nuclei (CAUD + SEPT). Because of the proximity of the lateral ventricles to both implant areas, a third group of mice was added in which guide cannulas were directed into only the lateral ventricles (VENT). This was done to control for the effect of possible backwash of intracerebrally injected solutions up the guide cannulas into the lateral ventricles and traveling to other brain structures. Stereotaxic coordinates for the placement of cannulas were: *ACCUM + STRIA*—anterior-posterior (AP) +0.6 mm from bregma, lateral (LAT) ±0.6 mm from midline; and depth (DEP) −4.0 mm from skull; *VENT*—AP +0.6 mm, LAT ±0.6 mm and DEP −3.0 mm and *CAUD + SEPT*—AP +0.6 mm, LAT ±2.8 mm and DEP −2.5 mm. Coordinates were determined using a mouse stereotaxic atlas [23]. In order to effect intracerebral injections to both the caudate and the septal nuclei, an angular placement of guide cannulas was used as well as two different lengths of injection cannula. The guide cannulas were placed at an angle of 30 degrees to the vertical midline plane. Surgery was accomplished using atropine sulfate (0.4 mg/kg, IP) presurgically and sodium pentobarbitol (75 mg/kg, IP) anesthetic. Methoxyflurane was also used as an auxiliary anesthetic. Mice were allowed a minimum of 1 week recovery from surgery before testing.

Activity was measured using Columbus Instruments' Animal Activity Monitors Automex D and Automex 2 SDII. Intracerebral injections were made using a 30-gauge injection cannula attached to a Hamilton microliter syringe inserted into a Stoelting syringe assembly with a Starrett Micrometer head. Injection cannulas for mice with ACCUM + STRIA and VENT implants extended 0.5 mm beyond the end of the guide cannulas. Intracerebral injection volumes for these two groups were 1 μl per cannula (hemisphere). Intracerebral injections in the CAUD + SEPT group were made at two depths (0.5 mm and 1.5 mm beyond the end of the guide cannula) to introduce the drug into both the caudate and septal nuclei. Injections in this group were 0.5 μl per injection per depth, or a total of 1 μl per hemisphere. Injection cannula were left in the guide cannulas for 30 seconds following injections of the solution to allow complete delivery of the dose.

On the first day of testing, each animal received an intraperitoneal injection of 30 mg/kg morphine sulfate, 4 mg/kg amphetamine sulfate or saline, followed immediately by 30, 1-minute activity readings. Then an intracerebral injection of 1 μg naloxone in 1 μl saline or saline alone was administered [12], and 45 1-minute activity readings were taken immediately following completion of the injection. On each of the following 5 days, each animal received one of the following combinations of intraperitoneal plus intracerebral injections: saline plus saline, saline plus naloxone, morphine plus saline, morphine plus naloxone, amphetamine plus saline, or amphetamine plus naloxone. Each animal received all six combinations of intraperitoneal and intracerebral injections during the course of the testing period. The order of presentation was randomized for each animal.

At the completion of testing, 1 μl of Evans Blue dye was injected into each cannula. Mice were sacrificed by decapitation, and the brain quickly dissected out and frozen in freon 12. The frozen tissue was sectioned, and drawings and photographs of the spread of dye were taken to verify the cannula placement and the relative diffusion of fluid into the brain (see Figs 3–5). Only animals with "dye-marked" tissue corresponding closely to target areas were retained in the experiment. After elimination of animals with inaccurate "dye-marked" areas, the following animals were retained in the experiment: ACCUM + STRIA, (N=10); CAUD + SEPT (N=7); and VENT (N=12). Examination of ACCUM + STRIA placements showed an area of dye approximately 0.5 mm in diameter centering in the ACCUM + STRIA area, with some extension into the anterior commissure. VENT implant animals had dye only in the ventricles, primarily the lateral, but with some diffusion into the third ventricle. CAUD + SEPT implant animals had areas of dye extending angularly from the medial caudate into the lateral septal area but, surprisingly, no dye in the lateral ventricles. The entire length of the dye area in the CAUD + SEPT group was approximately 1.5 mm with a diameter of about 0.4 mm. The most posterior portions of the "dye-marked" tissue occasionally extended into the edges of the STRIA.

Data analysis was performed on individual activity scores for each fifth minute (from 5 to 45 minutes after injection). Data were analyzed by 2-way analysis of variance (F), drug (intracerebral) by time (after intracerebral injection) for each implant area, for each intraperitoneal drug injection. Newman-Keuls a posteriori tests [11] were used to locate specific differences between means. In order to compare the effects of IP saline plus IC naloxone against IP saline plus IC saline (to ascertain any depressant effects of naloxone alone), *t*-tests were performed on data accumulated in the following way. For each animal, under each drug condition, scores for the 5th, 10th, 15th . . . 45th minute of activity were summed to yield two total scores (saline versus naloxone effects) for each animal. These scores were then analyzed by a *t*-test for matched pairs. Again, an alpha level of 0.05 was adopted throughout.

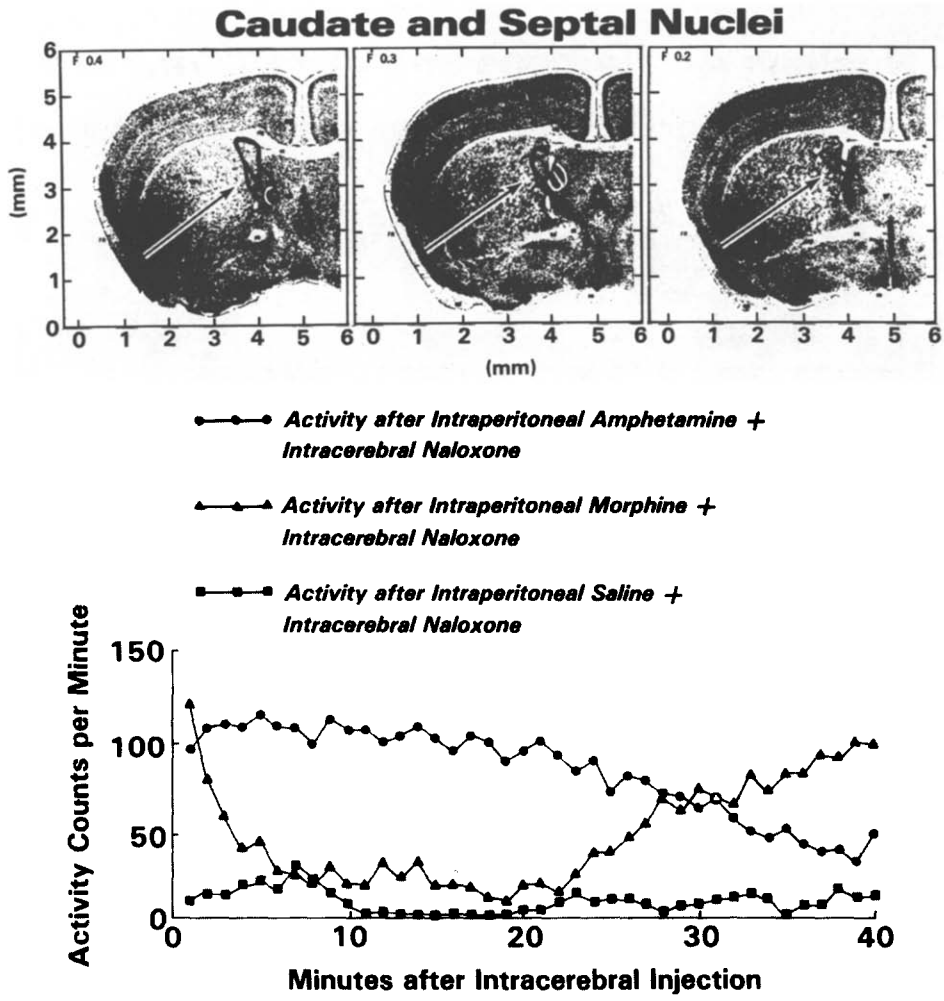

FIG. 3. Locomotor activity counts for each minute after bilateral injections of naloxone (1 μg/hemisphere) into the dorsomedial caudate and lateral septal nuclei. Lines on the brain sections (photographs from Slotnick and Leonard [23]) indicate probable diffusion areas as imaged by postexperimental dye injections. Intracerebral naloxone injections challenged the locomotion observed after IP amphetamine (4 mg/kg), morphine (30 mg/kg), or saline injections. Mean activity counts for IC injections of saline after IP amphetamine, morphine or saline injections were 90, 127, and 29, respectively.

Results

Naloxone, injected in the CAUD + SEPT area, produced a significant suppression of peripherally induced morphine hyperactivity, $F(8,102)=5.149$, $p<0.01$, but not amphetamine-induced hyperactivity (see Fig. 3). This decrease in activity following morphine plus naloxone injections was apparent at the 5th minute following intracerebral injection, and it continued through the 25th minute ($p<0.001$ for the 5th, 10th, and 15th minutes, $p<0.002$ for the 20th minute, and $p<0.001$ for the 25th minute; Newman-Keuls). At the 30th minute, and minutes thereafter, morphine plus naloxone activity was not significantly different from activity after morphine plus saline. Naloxone injections in the ACCUM + STRIA or VENT failed to significantly alter morphine-induced locomotion (see Figs. 4,5).

Intracerebral naloxone did not significantly alter the peripherally induced amphetamine hyperactivity of any group (as compared to IC saline), $F(3,442)=0.756$, $p=0.84$. Intracerebral naloxone, in and of itself, did not alter spontaneous (IP saline injection-induced) activity, and intracerebral saline also did not. As expected, intraperitoneal injections of morphine or amphetamine significantly increased locomotor activity over that of saline controls, $F(2,52)=48.445$, $p<0.001$.

Discussion

Intracerebral injections of naloxone significantly suppressed morphine-stimulated locomotor activity in animals with the CAUD + SEPT implants but not in the subjects with ACCUM + STRIA or VENT implants animals. However, a small (not statistically significant) decrease was observed in the ACCUM + STRIA lesioned group. These quantitative representations of activity were corroborated by behavioral observations. After intracerebral naloxone challenges to morphine-induced locomotion, VENT implant mice contin-

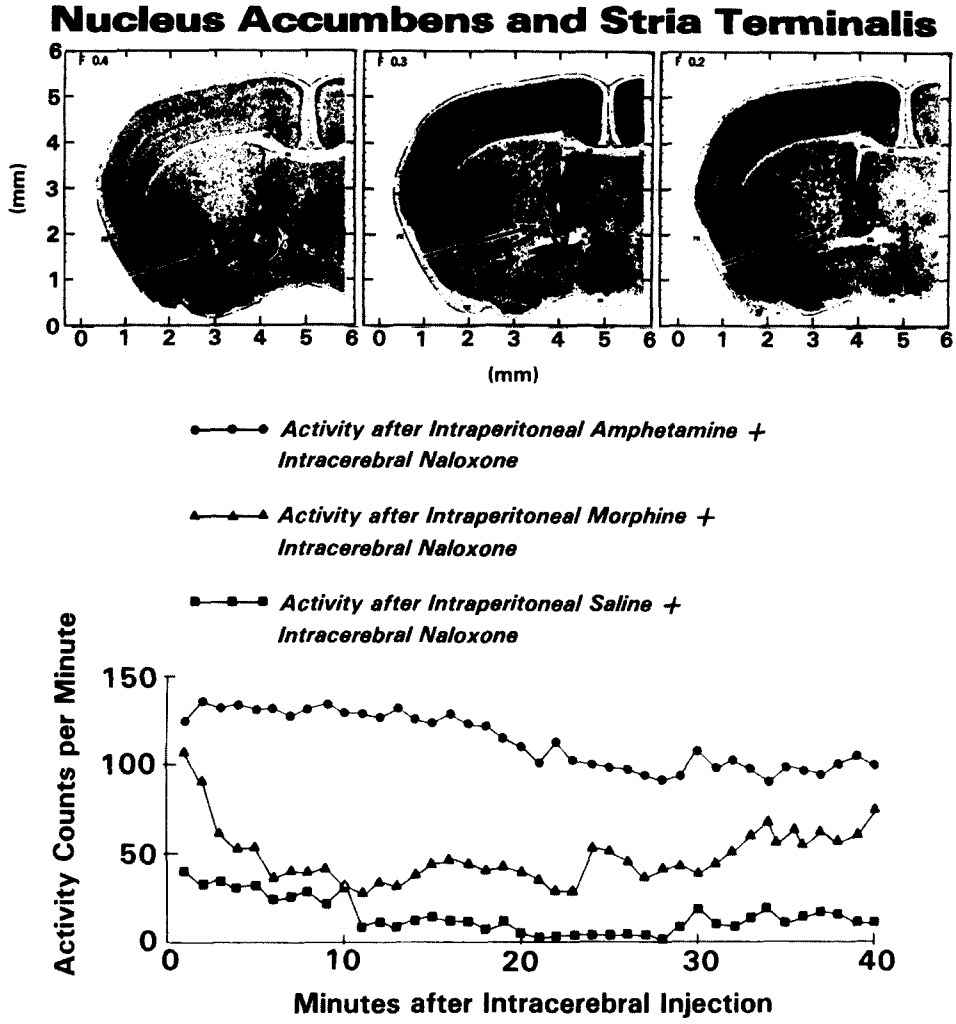

FIG. 4. Locomotor activity counts for each minute after bilateral injections of naloxone (1 μg/hemisphere) into the nucleus accumbens and stria terminalis. Lines on the brain sections (photographs from Slotnick and Leonard [23]) indicate probable diffusion areas as imaged by postexperimental dye injections. Intracerebral naloxone injections challenged the locomotion observed after IP amphetamine (4 mg/kg), morphine (30 mg/kg), or saline injections. Mean activity counts for IC injections of saline after IP amphetamine, morphine or saline injections were 110, 145, and 35, respectively.

ued to exhibit the "running fit" and straub tail associated with morphine hyperactivity; they just ran a little more slowly. ACCUM + STRIA animals showed a decrease in morphine stereotypic behavior, with short periods of rearing and grooming interspersed throughout the "running fit." During the period of greatest suppression of activity, the CAUD + SEPT implanted mice exhibited almost no stereotypic behavior at all. They groomed, reared, and sometimes explored but they did not exhibit the elevated straub tail response. Some mice rested with eyes closed in the nest area of the home cage. Later (approximately 25 to 30 minutes), the stereotypic running returned, so that by the end of the 45-minute period they were behaviorally indistinguishable from the mice injected with intraperitoneal morphine plus intracerebral saline.

The suppressant effect of naloxone was limited to the morphine response and did not affect the amphetamine-induced hyperactivity in any group. The small decrease in amphetamine activity seen late in the CAUD + SEPT implant animals was also observed after intraperitoneal amphetamine plus intracerebral saline injections, and may represent a wearing off of the amphetamine hyperactivity. These animals seemed to slow down from the amphetamine hyperactivity and spend more time in the nest area, but did not go to sleep.

The possibility that naloxone alone is a depressant was eliminated by the lack of difference in activity between intraperitoneal saline and intraperitoneal saline plus intracerebral naloxone activity counts. Intracranial pressure caused by microinjections into the brain was also eliminated as a possible explanation for the reduction in morphine hyperactivity, since no difference was seen between the lo-

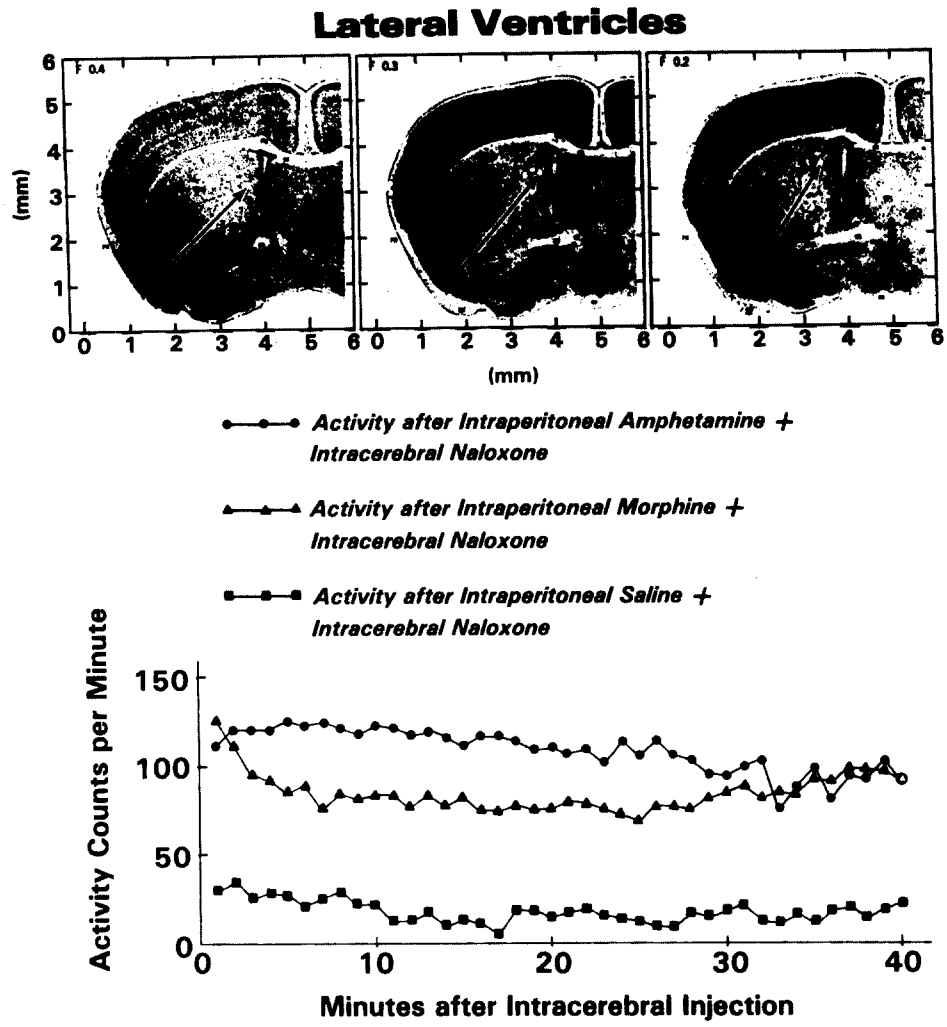

FIG. 5. Locomotor activity counts for each minute after bilateral injections of naloxone (1 μg/hemisphere) into the lateral ventricles. Lines on the brain sections (photographs from Slotnick and Leonard [23]) indicate probable diffusion areas as imaged by postexperimental dye injections. Intracerebral naloxone injections challenged the locomotion observed after IP amphetamine (4 mg/kg) morphine (30 mg/kg), or saline injections. Mean activity counts for intracerebral injections of saline after IP amphetamine, morphine or saline injections were 119, 130, and 15, respectively.

comotor activity of mice injected with intraperitoneal saline alone and those that received intraperitoneal saline plus intracerebral saline.

GENERAL DISCUSSION

Destruction of caudate and septal neurons as well as injection of naloxone to the same sites produced significant decreases in morphine-induced locomotor hyperactivity, although neither procedure completely eliminated this response. It remains unclear whether ablation of a greater number of endorphinergic neurons in this area, or injection of more naloxone, would reduce morphine-induced locomotion to premorphine levels. It is known that morphine-induced hyperactivity in C57 mice may depend on dopaminergic mechanisms. For example, morphine injections stimulate dopamine receptors in C57Bl/6J mice [27] and increase dopamine release in the striatum [19]. The results from experiment 1 indicate that dopamine and norepinephrine receptors were functional since amphetamine activity was not diminished significantly by any of the lesions. Perhaps this may account for the inability of any lesion to fully eliminate the morphine-induced hyperactivity.

The present experiments did not demonstrate significant reductions in morphine- or amphetamine-induced hyperactivity after either lesions of the ACCUM + STRIA or naloxone injections in these brain areas. However, the literature suggests a role for these structures in opiate-stimulated locomotion [26]. The current studies may not be directly comparable with those of Teitelbaum *et al.* since the present lesions were more caudal.

It is possible that all four areas (the nucleus accumbens,

the stria terminalis, the dorsomedial caudate, and the lateral septal nuclei, which are interconnected [1, 14, 15, 18] and all contain endorphins) are necessary for full mediation of the morphine-induced hyperactivity effect. This suggestion is in consonance with the findings of a recent experiment in which kainic acid lesions involving portions of the striatum and nucleus accumbens apparently produced a near-complete reduction in morphine-induced locomotion while sparing amphetamine-stimulated activity [21]. Another study has proposed, for example, that activity is divided into two parts—fine and gross motor activity—and that these two types of activity are mediated by different opiate receptor subpopulations [6]. The current data are consistent with this theory. The areas of caudate and septal nuclei studied contain primarily enkephalineregic neurons, which could indicate high concentrations of delta receptors [28,29]. The nucleus accumbens and stria terminalis areas contain more endorphinergic neurons than enkephalinergic neurons [2, 8, 28, 29]. This may indicate large numbers of mu receptors. Perhaps both regions are involved in the mediation of the hyperactivity induced by morphine, with the neurons of one region controlling predominantly fine activity and the neurons in the other controlling predominantly gross activity. This speculation concerning the possibly different role of opiate receptor subtypes is consistent with a recent report revealing that selective mu recepor antagonism produces only a partial reduction of morphine-induced hyperactivity in the C57Bl/6J mouse [9].

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