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# Regional Brain Morphine Injections Selectively Attenuate Aspects of Septal Hyperreactivity: A Multivariate Assessment

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VALDES, J. J., W. R. CAMERON, S. EVANS AND F. H. GAGE. *Regional brain morphine injections selectively attenuate aspects of septal hyperreactivity: A multivariate assessment.* PHARMAC. BIOCHEM. BEHAV. 12(4)563–572, 1980.—Rats with septal lesions show quantitative and qualitative changes in responsivity to, respectively, noxious and non-noxious stimulation, responding as if both were noxious. Morphine analgesia is characterized by decreased responsivity to noxious stimulation without a concomitant change in sensory threshold. Thus, both of these phenomena are thought to involve neural mechanisms related to the affective component of behavioral responsivity, and the septal lesion-induced affective changes may be mediated by the endogenous opiate system. Rats given either septal or sham lesions were injected with either morphine or vehicle into either the corticomedial amygdala, posterior hypothalamus, or the ventral hippocampus, and were tested for responsivity to noxious and non-noxious stimuli. Naloxone injections were given to determine the opiate specificity of the effects. The data were submitted to discriminant function analyses to determine which measures were most sensitive to the manipulations. Morphine's greatest effect was a site-dependent attenuation of reactivity to non-noxious stimuli. Naloxone showed site-dependent antagonism of the morphine effect as well as unspecified independent effects of its own. The data suggest the mediation of the affective component of septal hyperreactivity by opiate receptors, and a functional differentiation between naloxone-sensitive and insensitive receptors.

Septal hyperreactivity  Morphine  Discriminant function analysis  Endogenous opiate system
Behavioral responsivity  Limbic system

THE identification of endogenous opiate peptides subsequently named enkephalins [20] has raised the question of the function of such morphine-like factors in mammalian brain. The distribution [39] and multiplicity [25, 34, 44] of endogenous opiate binding sites, and the differential localization and effects [30,39] of these peptides in specific brain regions, suggest that the enkephalins subserve a number of functions which vary among brain regions.

Among these functions is the mediation of both somatic and affective responses to noxious stimulation [6, 11, 12]. It follows that the periaqueductal gray (PAG), an area of high opiate receptor density [39], is involved with behavioral responsivity to both noxious and non-noxious stimuli [22] and is thought to be a primary site of opiate analgesia [49]. Despite this, neither lesions [3] nor anesthetic drugs [48] applied to the PAG appreciably alter nociceptive thresholds, although in some cases lesions of the PAG result in a decrease in pain responsiveness [2]. These results suggest that PAG-mediated opiate analgesia results from diaschitic [43] influences on distal structures.

Distal structures influenced by the PAG may be either rostral or caudal to this region, and morphine attenuation of the tail flick response has both spinal [47] and supraspinal [26] components, and the full expression of opiate analgesia is dependent upon the integrity of the supraspinal system [19]. Morphine analgesia within the PAG has been shown to be mediated by independent ascending supraspinal, and descending spinal mechanisms [50]. These systems may be differentially involved with the sensory and affective components of behavioral response to stimulation. Along these lines, a number of studies [10,18] suggest that manipulations of the PAG which result in analgesia achieve these effects by either influencing rostral structures involved with nociception, by altering affective aspects of behavioral responsivity not directly related to nociceptive thresholds, or both. The presence of dense concentrations of enkephalins in the rostral limbic areas to which the PAG projects, and the relative paucity of enkephalin along the primary sensory pathways [23], suggest that the role of the enkephalins in these rostral structures is to modulate the affective component of behav-

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ioral responsivity. This observation is in accord with clinical findings [13,45] that morphine does not alter the sensory threshold for noxious stimulation but does alter the patient's behavioral response to the stimulus, and that human subjects can distinguish between the sensation of a noxious stimulus and their affective response to it [27].

In order to test the hypothesis that the endogenous opioid systems in rostral limbic structures mediate behavioral responsivity independent of the sensory input, it was necessary to inject an opiate directly into the appropriate rostral structures in a preparation in which behavioral responsivity to noxious and to non-noxious stimuli could be assessed. The septal-lesioned rat was chosen as the preparation ideally suited for these requirements.

Rats with septal lesions show heightened reactivity to stimuli [4,5], and respond to noxious and non-noxious stimuli as if both were noxious. These induced behavioral changes provide, respectively, a quantitative and qualitative assessment of behavioral responsivity. Several limbic structures interconnected with the septal area have been shown to modulate septal hyperreactivity as well as opiate analgesia. Three such areas which share complex neuroanatomical interconnections with the septal area and which differ widely in opiate receptor density are the corticomedial amygdala (CMA), with a dense distribution of receptors, the posterior hypothalamus (PHYPO), with a moderate distribution, and the ventral hippocampus (VHIP), which is lightly populated with opiate receptors. The fact that these three rostral areas are all involved with opiate analgesia and behavioral responsivity [16, 21, 24, 35, 36] while differing so widely in their distribution of opiate receptors suggests a functional differentiation of the endogenous opioid system across these limbic regions.

In summary, the purpose of the present study was to assess the involvement of the endogenous opiate system within rostral limbic structures, which differ widely in receptor density and type, on behavioral responsivity to various noxious and non-noxious stimuli and motor ability. To accomplish this morphine was injected into either the CMA, PHYPO, or VHIP of septal- and sham-lesioned rats, and the rats were tested on a battery of behavioral tests at various times after injection. The naloxone reversibility of the morphine effects was also assessed. The data were submitted to discriminant function analyses (DFA), a multivariate procedure which weights the dependent behavioral measures according to their ability to discriminate among the experimental groups. This provided an assessment of the differential drug effects, with respect to the brain region injected, on behavioral responsivity to each of the several classes of measures used (i.e., noxious, non-noxious, motor). Univariate statistics were employed to determine whether there were any lesion and drug effects on the measures not represented well by the DFA variates.

## METHOD

### *Subjects*

Subjects were 66 male Sprague-Dawley albino rats weighing between 225 and 250 g at the beginning of the study. They were individually housed and fed ad lib Purina laboratory chow and tap water.

Initially, 22 rats of the original 66 were randomly assigned to each of the three experimental groups on the basis of whether they would receive cannula implants in the CMA, PHYPO or VHIP. Each of these three groups was further divided into five treatment groups described below.

(1) SMN (N=5). Rats receiving septal lesions and injected with morphine at Time 1 (T1) and naloxone at Time 2 (T2). (2) SM (N=5). Rats receiving septal lesions and injected with morphine at T1 and Krebs buffer at T2. (3) SP (N=5). Rats receiving septal lesions and Krebs injections at both T1 and T2. (4) ShMN (N=5). Rats receiving sham lesions and injected with morphine at T1 and naloxone at T2. (5) SH (N=2). Rats receiving sham lesions and Krebs injections at T1 and T2.

The two rats in each of the three sham treatment groups (SH) were combined across the three experimental groups to serve as sham controls in each of the three DFA's which made up the statistical analyses.

### *Surgical*

Animals were injected with 0.2 cc atropine sulfate, anesthetized with ketamine chloral hydrate [28], and mounted in a stereotaxic device. A lesioning electrode constructed from a No. 1 insect pin and insulated to within 1.0 mm of the tip was lowered into the septal area (+1.8 A/P, ±0.5 M/L, −5.0 D/V) and a bilateral radiofrequency lesion was inflicted (14 mA, 14 sec.). All coordinates were taken from deGroot System B as described by Pellegrino and Cushman [31]. Rats in the sham lesion group had the electrode lowered into the septal area without the passage of current. The trephine holes were then packed with gelfoam and bone wax.

Stainless steel 21 ga guide cannulae were then implanted into one of three brain regions: the CMA (−0.8 A/P, ±4.0 M/L, −9.5 D/V), the VHIP (−5.5 A/P, ±5.1 M/L, −5.0 D/V) or the PHYPO (−1.2 A/P, −0.5 M/L, −8.3 D/V). Placements were bilateral in the CMA and VHIP and unilateral in the PHYPO and were anchored to the skull with four 0.08 in. stainless steel screws and dental acrylic. The wound was then sutured closed and an obdurator was placed into the guide cannula to keep it free of debris.

### *Reactivity Testing*

Animals were tested for reactivity to a variety of stimuli at various times postoperatively before and after drug treatment. This provided assessments of both the lesion and drug effects on behavioral reactivity. Double-blind procedures were instituted with the tester being ignorant of both the surgical intervention and the drug injection. Only one tester was used as previous experiments (unpublished data) revealed that the inter-tester reliability was extremely high (r=0.95).

The rats were tested for reactivity to non-noxious stimuli, these being nose poke, body poke, handling and air puff, and their motor ability was assessed on a cage climbing task. The latter test required the rat to locomote on a screen inclined at a 45° angle. Following this battery, they were tested for reactivity to the hotplate and to footshock, these latter tasks representing assessments of reactivity to noxious stimuli.

The responses to handling, nose and body pokes, and air puff were rated on a scale of zero to three as described elsewhere [17], a score of three representing the extreme in reactivity. The cage climbing test was rated on the reverse of this scale, with three representing intact motor abilities (i.e., an ability to locomote on the tilted screen) and zero representing complete motor disability (i.e., an inability to even cling to the screen). The footshock apparatus has been recently developed and tested in our laboratory and enables

the calculation of an objective, quantified response measure to footshock [15]. The test cage is mounted on a grid connected to a force transducer which records the amplitude of the response to footshock on a polygraph. Five shocks were administered at 10 sec intervals, shock parameters being 0.5 mA for 300 msec. These values were chosen because previous behavioral kinetic studies in our laboratory [15] have shown that these parameters yield responses which are half maximal, allowing for either an increase, or a decrease in response amplitude as a function of lesion and drug treatment effects. Response to heat was assessed via a thermostatically controlled hot plate with an automatic latency timer, and the data were expressed as latency in seconds to pawlick after being placed on the heated (51°C) surface.

*Drug Administration and Testing*

One day following surgery, rats were tested on the full battery of behavioral tests in order to assess the effects of the septal lesion on reactivity (To). One hour after testing, rats in the three morphine-treated groups were injected with a total of 1.0 $\mu$l morphine (10 $\mu$g/$\mu$l in Krebs buffer), and rats in the two Krebs-treated groups were injected with 1.0 $\mu$l Krebs buffer. Thus, all morphine-treated rats received a total of 10 $\mu$g morphine. Injection was accomplished by lightly anesthetizing the animals with ether and infusing the solutions through the 27 ga injector cannula via a 10 $\mu$l Hamilton syringe at a rate of 4 $\mu$l/min. The injector cannula was then removed and replaced by an obdurator, and the rat was returned to its cage. Light anesthesia was necessary because of the extreme hyperreactivity of the septal-lesioned rats.

The rats were tested on the behavioral battery at 10 min after injection (T1). At 60 min after this initial injection the rats were injected again, the morphine-treated rats receiving intraperitoneal injections of either naloxone-HCL (10 mg/kg) or Krebs buffer, and the Krebs-treated rats receiving injections of Krebs buffer. They were then tested on the behavioral battery 10 min after this second injection (T2).

*Histology*

Following the last test session, the rats were administered an overdose of chloral hydrate and intracranially injected with 1.0 $\mu$l neutral red dye to serve as a marker for the location of the cannula tip. The rats were then perfused with saline followed by 10% neutral buffered formalin. The brains were fixed in Formalin, embedded in celloidin, sectioned (30 $\mu$), stained with thionin, and mounted for microscopic examination. Representative sections of the cannula tip placements were mounted on slides prior to staining in order to better visualize the extent of the dye marker. Photographs were taken of representative lesion and cannula placements.

*Statistical Analysis—Multivariate*

The experimental question as to which behavioral measure, or combination of measures, best described the lesion and drug effects was addressed via DFA [42]. Used as an analytic tool, the DFA identifies the linear combination of variables which best separates the treatment groups and provides weights for these variables according to their contribution to this discrimination. The linear combination which best separates the treatment group centroids is the first discriminant function, the linear combination orthogonal to the previous function with the next best discriminative ability is the second discriminant function, and so on. The discrimination of the treatment groups can be visualized and statistically evaluated by plotting the group centroids with respect to the discriminant functions. The weights of the dependent measures can be used to name or describe the functions with respect to these measures. Statistical tests on discriminant variates defined from the data have certain aspects of *a posteriori* tests. To be conservative in the use of these tests, an alpha level of 0.01 was required for rejection of the null hypothesis.

Separate DFA's were computed for each of the three placement groups, i.e., groups defined by cannula placement in one of the three brain areas. The four treatment groups included in each of the three DFA's were: a group composed of data, recorded after naloxone injection, from animals receiving septal lesions and both morphine and naloxone injections (SMN); a group composed of data, recorded after morphine injection, from animals receiving septal lesions and morphine injections (SM); a group composed of data recorded from animals receiving septal lesions and Krebs injections (SP); and a group composed of data recorded from animals receiving sham lesions and Krebs injections (SH). A fifth treatment group, composed of data from animals receiving sham lesions and both morphine and naloxone injections (ShMN), was originally included in the design to assess drug effects on normal animals. A DFA comparing this group with the untreated shams found no differences, hence the group was not included in the overall DFA's.

In summary, the four treatment groups to be discriminated within each of the three DFA's were groups defined in terms of the lesion and drug treatment regimen in effect at the time the data were recorded. The three dependent measures providing the data for these analyses were reactivity to footshock, latency to pawlick on a hotplate and reactivity to non-noxious somatosensory stimuli. The measure of motor ability, the cage climbing task, was not included in the DFA's because virtually every score (173 of 180) recorded was a three, the maximum, and so no discriminative ability would be added by including this measure.

*Statistical Analysis—Univariate*

The DFA's defined the variable which best separated the experimental groups. Univariate statistics were employed to determine whether there were any lesion and drug effects on the measures not represented well by the DFA variates. The appropriate comparisons were evaluated via univariate *t*-tests for independent means.

## RESULTS

*Multivariate Data Analysis: CMA*

The four data groups—SP, SM, SMN and SH—representing the four lesion and drug regimens to be discriminated by the somatosensory, hotplate, and footshock reactivity measures were submitted to DFA. Eighty-nine percent of the variance on the first discriminant function was between-groups variance with a discriminant criterion of 8.49, and this function showed significant separation of the groups, $\chi^2(9)=94.7$, $p<0.001$. The measures of somatosensory reactivity were weighted most heavily on this function. Thirty-one percent of the variance on the second discriminant function was between-groups variance with a discriminant criterion of 0.46, and this function showed significant separation of the groups, $\chi^2(4)=14.8$, $p<0.01$. On this function, both the measures of somatosensory and footshock reactivity were heavily weighted, albeit in opposite directions.

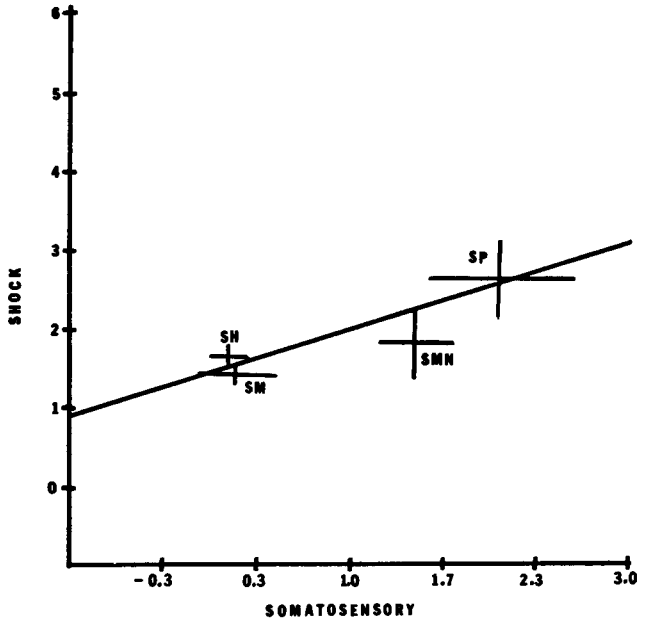


FIG. 1. Centroids and standard deviations of the groups receiving sham (SH) or septal lesions (SP), septal lesions and either morphine (SM) or morphine and naloxone (SMN) injections into the corticomedial amygdala (CMA) plotted with respect to the regression line calculated for the somatosensory and shock measures.

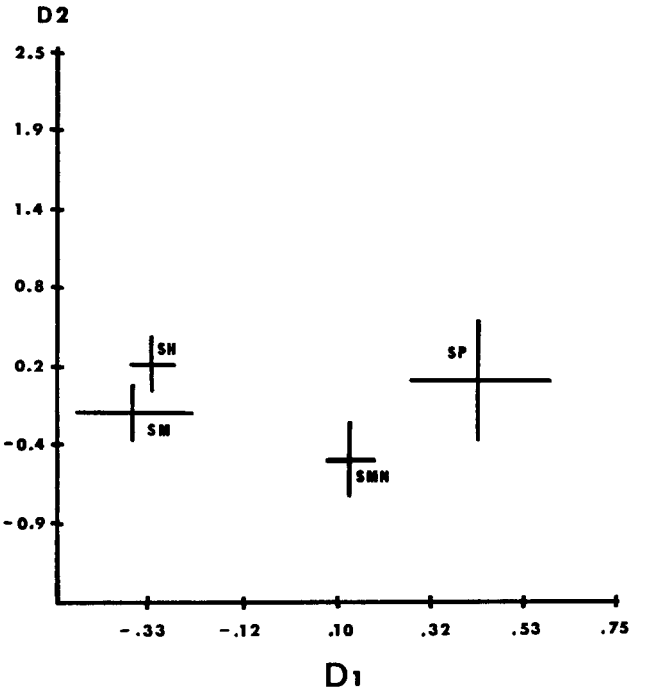


FIG. 2. Centroids and standard deviations of the four experimental groups with CMA injections plotted within the space defined by the first two discriminant functions.

The third and last discriminant function was unable to statistically separate the groups, $\chi^2(1)=1.2$, $p<0.24$.

In order to interpret the weights of the somatosensory and the footshock variables on the second discriminant function, the data obtained from these variables for groups SP, SH, SM and SMN were subjected to regression analysis and plotted on the scatterplot shown in Fig. 1. The X axis represents the somatosensory measure and the Y axis represents the footshock measure, with the regression plotted as shown. The correlation coefficient between the two variables was high (r=0.84). The centroids and standard deviations of the four treatment groups are plotted in this space. Treatment of the septal-lesioned rats with morphine alters the behavior of these rats. Naloxone shows a greater reversal of the morphine attenuation of somatosensory, relative to footshock, reactivity as evidenced by the position of the SMN group below the regression line. In summary, the first discriminant function reflects mainly somatosensory reactivity and the second discriminant function represents the disproportionate naloxone effect on the reversal of the morphine attenuation of somatosensory relative to footshock reactivity.

The data from the experimental groups involved in the comparisons are plotted in the space defined by the first two discriminant functions as shown in Fig. 2. These functions were plotted because both were statistically able to separate the groups. The first discriminant function, representing mainly somatosensory reactivity, best separates the experimental groups. A comparison of the septal lesion and sham groups (SP vs. SH) shows that they are significantly different along this dimension, $t(23)=13.3$, $p<0.0001$. Morphine injected into the amygdala of septal-lesioned rats (SM) significantly attenuates the lesion-induced reactivity such that this group is significantly different from the untreated septal-lesioned rats (SP), $t(23)=12.4$, $p<0.0001$, and statistically indistinguishable from the untreated shams (SH), $t(18)=0.81$, $p<0.43$, along this dimension. A comparison of septal-lesioned rats treated with morphine (SM) versus those treated with both morphine and naloxone (SMN) shows that naloxone antagonized the effect of morphine on the animals with septal lesions such that these two groups are statistically discriminable, $t(13)=7.72$, $p<0.0001$, along this dimension. Along the second discriminant function, morphine injected into septal-lesioned rats has a slight though statistically insignificant, $t(23)=1.34$, $p<0.19$, effect on the septal lesion-induced hyperreactivity.

In summary, the measures of somatosensory reactivity best discriminated among the treatment groups. Morphine completely reversed the septal lesion-induced reactivity, and the greatest morphine effect was on somatosensory reactivity. Naloxone showed complete antagonism of this morphine attenuation of somatosensory reactivity.

*Multivariate Data Analysis: PHYPO*

The data representing the four lesion and drug regimens to be discriminated were submitted to DFA as with the CMA data. Eighty-five percent of the variance on the first discriminant function was between-groups variance with a discriminant criterion of 5.48 and this function was statistically able to separate the groups, $\chi^2=40.4$, $p<0.0001$. As is immediately obvious, the somatosensory measures are weighted most heavily on this function. Forty-five percent of the variance on the second discriminant function was between-groups variance with a discriminant criterion of 0.81 and this function was statistically able to separate the groups, $\chi^2=28.1$, $p<0.001$. Both the somatosensory and shock measures are weighted heavily, albeit in opposite directions. The third discriminant function was also statistically significant, $\chi^2=7.0$, $p<0.01$, and showed the same weighting pattern as the second function.

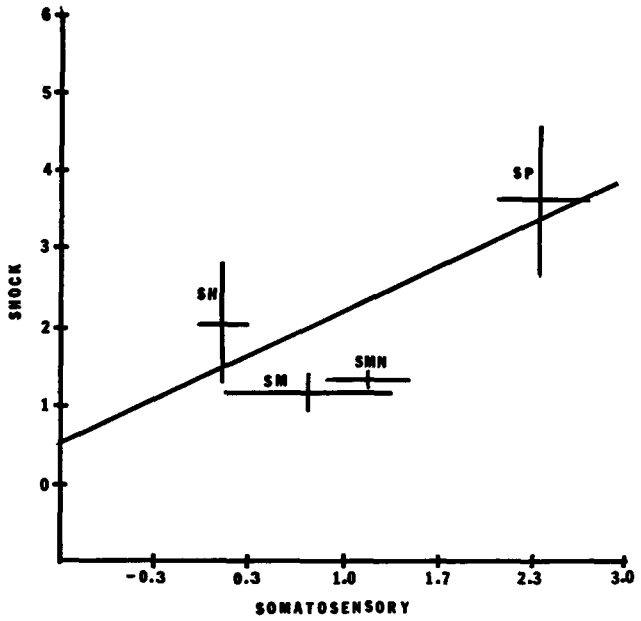


FIG. 3. Centroids and standard deviations of the experimental groups with injections into the posterior hypothalamus (PHYPO) plotted with respect to the regression line calculated for the somatosensory and shock measures.

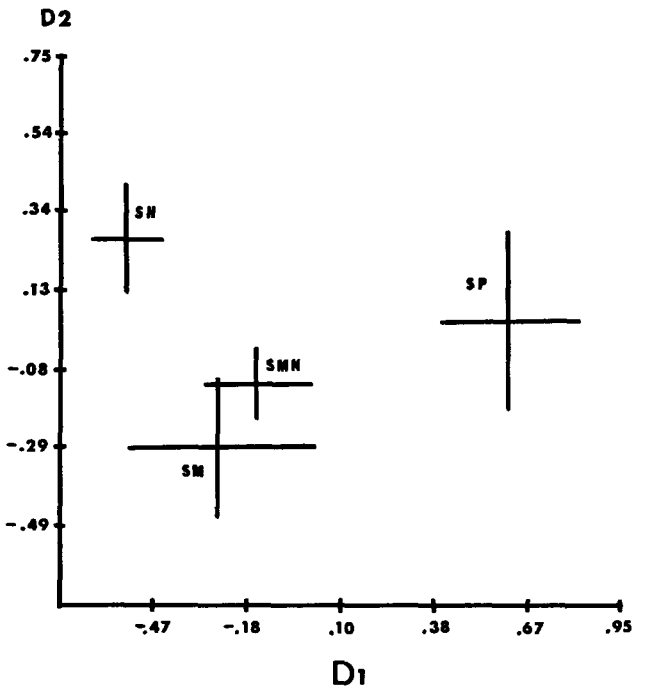


FIG. 4. Centroids and standard deviations of the four experimental groups with PHYPO injections plotted within the space defined by the first two discriminant functions.

In order to interpret the weights of the variables on the second discriminant function, a scatterplot for the data of the two most heavily weighted variables was plotted in Fig. 3 using the data obtained from groups SP, SH, SM and SMN. The X axis represents data from the somatosensory measures and the Y axis represents data from the shock measure with the regression line plotted as shown. The correlation coefficient computed for the two variables was high (r=0.64). The centroids and standard deviations for the four experimental groups are plotted in this space. Treatment of a septal-lesioned rat with morphine attenuates both somatosensory and shock reactivity. Naloxone shows a greater reversal of the morphine attenuation of somatosensory reactivity relative to shock reactivity. Thus, the opposite weightings of these two variables on the second discriminant function represents the disproportionately greater naloxone reversal of the morphine attenuation of somatosensory, relative to shock reactivity. This naloxone effect is similar to that seen in the CMA group.

The experimental groups involved in the comparisons are plotted in Fig. 4. The first two discriminant functions were plotted because both were statistically significant and although the third function was significant the pattern of weighting did not add any additional information to the analysis. As is readily apparent, the first discriminant function, representing mainly the somatosensory measures, best separates the experimental groups. SP and SH are significantly different, $t(23)=15.4, p<0.0001$, along this dimension. Morphine injected into the PHYPO of septal-lesioned rats (SM) significantly attenuates the lesion-induced reactivity, $t(23)=8.8, p<0.0001$, such that the septal-lesioned rats are more nearly similar to shams on this dimension. In addition, morphine-treated septal-lesioned rats (SM) may be discriminated from both untreated shams (SH) and septals (SP) along the second discriminant function, $t(18)=6.0, p<0.0001$ and $t(23)=3.3, p<0.003$. Naloxone shows slight but insignificant antagonistic action on morphine attenuation of septal lesion-induced reactivity along the first discriminant function, $t(13)=0.83, p<0.42$, and somewhat greater antagonism of the morphine effect along the second discriminant function, $t(13)=1.9, p<0.07$.

In summary, the measures of somatosensory reactivity were best able to discriminate among the treatment groups. Morphine significantly, but incompletely reversed the septal lesion-induced reactivity and had an additional unspecified effect which was unrelated to the symptoms of the lesion. Naloxone did not antagonize this unspecified effect and had no effect on the morphine attenuation of septal lesion-induced reactivity.

*Multivariate Data Analysis: VHIP*

The data representing the four lesion and drug regimens to be discriminated were submitted to DFA as with the CMA data. Seventy-eight percent of the variance on the first discriminant function was between-groups variance with a discriminant criterion of 3.40 and was statistically significant, $\chi^2(9)=65.3, p<0.001$. The measures of somatosensory and footshock reactivity were most heavily weighted on this function. Twenty-four percent of the variance on the second discriminant function was between-groups variance with a discriminant criterion of 0.31 and this function was statistically significant, $\chi^2(4)=11.9, p<0.02$. Both somatosensory and shock reactivity are heavily weighted on this function, though in opposite directions. The third discriminant function was not statistically significant, $\chi^2(1)=2.4, p<0.12$.

In order to interpret the weights of these two variables on the second discriminant function, the data obtained from these two variables for the four groups were subjected to regression analysis and plotted on the scatter-plot shown in Fig. 5. The X axis represents the somatosensory measure and the Y axis represents the shock measure, with the regression line plotted as shown. The correlation coefficient

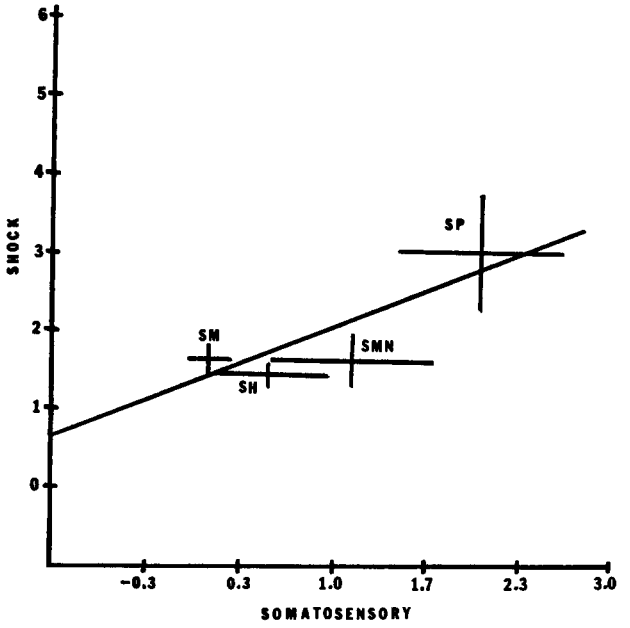


FIG. 5. Centroids and standard deviations of the experimental groups with injections into the ventral hippocampus (VHIP) plotted with respect to the regression line calculated for the somatosensory and shock measures.

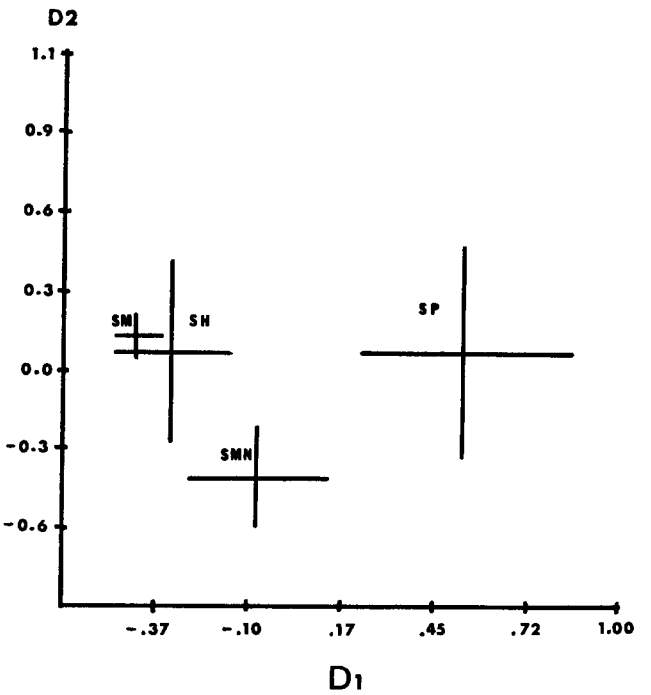


FIG. 6. Centroids and standard deviations of the four experimental groups with VHIP injections plotted within the space defined by the first two discriminant functions.

computed for the two variables was high (r=0.74). The centroids and standard deviations of the four experimental groups are plotted in this space. Treatment of rats with septal lesions with morphine (SM) alters the behavior of rats in this group along both dimensions, as evidenced by their reduced reactivity to both somatosensory and footshock stimuli. Naloxone shows a greater reversal of the morphine attenuation of somatosensory, relative to shock, reactivity as evidenced by the position of the SMN group below the regression line. In summary, the first discriminant function reflects mainly somatosensory reactivity and the second discriminant function represents the disproportionate naloxone reversal of the morphine attenuation of somatosensory reactivity relative to shock reactivity.

The experimental groups are plotted in the discriminant space defined by the first two discriminant functions as shown in Fig. 6. These two functions were plotted because they were significantly able to separate the groups. The first discriminant function best separates the experimental groups. A comparison of the septal and sham-lesioned groups (SP vs. SH) shows that they are significantly different along this dimension, $t(23)=9.0$, $p<0.0001$. Morphine injected into the VHIP of septal-lesioned rats (SM) significantly attenuated the septal lesion-induced reactivity such that this group was significantly different from untreated septal-lesioned rats, $t(23)=7.5$, $p<0.0001$, along this dimension. Although this group appeared to be nearly identical to the shams (SH) in terms of its position in the discriminant space, sufficient disparity remained between these groups to indicate that morphine reversal of the septal lesion effect was not complete, $t(18)=1.75$, $p<0.09$. Naloxone significantly antagonized the morphine attenuation of septal lesion-induced reactivity (SM vs. SMN) on the first discriminant function, $t(13)=2.16$, $p<0.04$.

The second discriminant function is the better indicator of the naloxone effects. There are no differences among the septal or sham-lesioned groups and the morphine-treated septal-lesioned group (SP vs. SM and SH vs. SM) on this dimension, $t(23)=0.6$, $p<0.90$ and $t(18)=0.58$, $p<0.57$, but the septal-lesioned group treated with both morphine and naloxone is significantly different from the septal-lesioned group treated with morphine (SMN vs. SM) along this dimension, $t(13)=2.75$, $p<0.01$.

In summary, both somatosensory and footshock measures were able to discriminate among the treatment groups. Morphine completely reversed the septal lesion-induced reactivity and this effect was only incompletely antagonized by naloxone. In addition, naloxone had effects which were independent of the morphine effects.

*Univariate Data Analysis*

The DFA's indicated that the measures of somatosensory reactivity were the measures best able to discriminate among the treatment groups. Univariate *t*-tests employing the appropriate comparisons were computed to determine whether there were any lesion and drug effects on the remaining measures, in this case, footshock and hotplate reactivity.

The question of the behavioral effects of the septal lesion could be addressed independently of the cannulae placements since the data for this comparison were collected prior to any drug injection. Therefore, footshock data recorded one day post-operatively from all rats with septal lesions regardless of cannulae placements were combined, as were footshock data from all the sham-lesioned rats. This same procedure was followed for the hotplate data. *T*-tests revealed significant increases in reactivity to both footshock, $t(63)=6.18$, $p<0.0001$, and hotplate, $t(63)=3.13$, $p<0.003$, in rats with septal lesions relative to shams. The remaining comparisons were made within each brain region and will be considered separately. All comparisons were made with between-groups data.

*Univariate Data Analysis: CMA*

In order to assess the morphine-induced attenuation of septal hyperreactivity comparisons were made between two groups of rats with septal lesions tested ten minutes after either morphine or Krebs injection into the CMA. Morphine attenuated the septal lesion-induced reactivity to both footshock, $t(13)=5.53$, $p<0.0001$, and hotplate, $t(13)=4.71$, $p<0.0001$. In order to assess the naloxone-reversibility of this morphine-induced attenuation of reactivity, comparisons were made between two septal-lesioned morphine-treated groups tested ten minutes after either intraperitoneal injection of naloxone or Krebs. Naloxone did not antagonize the morphine attenuation of footshock reactivity, $t(8)=1.64$, $p<0.13$, but did antagonize the morphine attenuation of hotplate reactivity, $t(8)=2.6$, $p<0.03$. In order to determine whether the morphine attenuation of the septal lesion-induced reactivity could be classified as complete reversal, comparisons were made between the septal-lesioned morphine-treated group and the sham-lesioned Krebs-treated group ten minutes after injection. Morphine completely reversed the septal lesion-induced reactivity to both footshock, $t(13)=1.54$, $p<0.14$, and hotplate, $t(13)=1.98$, $p<0.06$, reactivity as these groups did not differ significantly from the shams.

*Univariate Data Analysis: PHYPO*

In order to assess the morphine-induced attenuation of septal hyperreactivity, comparisons were made between two groups of rats with septal lesions tested ten minutes after either morphine or Krebs injection into the PHYPO. Morphine attenuated the septal lesion-induced reactivity to both footshock, $t(13)=5.96$, $p<0.0001$, and hotplate, $t(13)=2.98$, $p<0.01$. In order to assess the naloxone-reversibility of this morphine-induced attenuation of reactivity, comparisons were made between two septal-lesioned morphine-treated groups tested ten minutes after intraperitoneal injection of either naloxone or Krebs. Naloxone did not antagonize the morphine attenuation of septal lesion-induced reactivity to either footshock, $t(8)=1.29$, $p<0.23$, or hotplate, $t(8)=1.8$, $p<0.10$. In order to determine whether the morphine attenuation of the septal lesion-induced reactivity could be classified as complete reversal, comparisons were made between the septal-lesioned morphine-treated group and the sham-lesioned Krebs-treated group ten minutes after injection. Morphine did not completely reverse the septal lesion-induced reactivity to footshock stimulation, $t(13)=2.18$, $p<0.04$, although it did completely reverse the septal lesion-induced reactivity to the hotplate, $t(13)=1.99$, $p<0.06$.

*Univariate Data Analysis: VHIP*

In order to assess the morphine-induced attenuation of septal hyperreactivity comparisons were made between two groups of rats with septal lesions ten minutes after either morphine or Krebs injections into the VHIP. Morphine attenuated the septal lesion-induced reactivity to footshock, $t(13)=6.65$, $p<0.0001$, but had no effect on the septal lesion-induced reactivity to the hotplate, $t(13)=0.86$, $p<0.40$. In order to assess the naloxone reversibility of the morphine attenuation of footshock reactivity, a comparison was made between two septal-lesioned morphine-treated groups tested ten minutes after intraperitoneal injection of either naloxone or Krebs. Naloxone did not antagonize the morphine attenuation of footshock reactivity, $t(8)=0.90$, $p<0.39$. Finally, in order to determine whether the morphine attenuation of the septal lesion-induced reactivity to footshock could be classified as complete reversal, a comparison was made between the septal-lesioned morphine-treated group and the sham-lesioned Krebs-treated group ten minutes after injection. Morphine completely reversed the septal lesion-induced footshock reactivity, $t(13)=1.05$, $p<0.31$, as these groups did not differ significantly from each other.

*Histology*

Neutral red dye (1 $\mu$l) was injected into the animals before sacrifice. Brains were imbedded in celloidin and sectioned. Representative unstained sections were mounted for visual inspection of the dye marker, and the other sections were stained with thionin. All coordinates were taken from deGroot System B [31].

Damage to the septal area extended from 1.6 to 2.4 in the rostral-caudal plane, with some slight damage extending as far as 3.2 rostrally and 1.0 caudally. Damage was confined mostly to the lateral septal nuclei throughout the rostral-caudal extent of the lesions, with overlapping but incomplete damage to the medial septal nucleus and occasional damage to the medial extent of the caudate-putamen complex and the nucleus accumbens septi.

Cannula placements in the amygdala ranged from 0.4 to $-1.4$ in the rostral-caudal plane, with most of the placements lying between 0.0 and $-1.0$. Placements fell between 3.5 and 4.5 in the medial-lateral plane, and between 9.0 and 9.5 in the dorsal-ventral plane. Both dye injection and inspection of the cannula tip tracks revealed that most injections were within the area of the medial and cortical amygdaloid nuclei, with a few instances of overlap onto the intercalated nucleus in the more rostral placements. Cannula tip placements in the VHIP ranged between $-3.0$ and $-3.4$ in the rostral-caudal plane, between 5.5 and 7.0 in the dorsal-ventral plane, and between 4.75 and 5.25 in the medial-lateral plane. Inspection of the dye marker in unstained sections revealed no spread into the ventricles. Cannula tip placements in the PHYPO ranged between $-0.8$ and $-1.6$ in the rostral-caudal plane, with most of the placements lying between $-1.0$ and $-1.2$, between 7.5 and 8.25 in the dorsal-ventral plane, and between 0.5 and 0.75 in the medial-lateral plane. Inspection of the dye marker revealed some overlap of the injection onto the mammillothalamic tract in a few instances. Representative lesion and cannula placements are shown in Fig. 7.

## DISCUSSION

The three DFA's consistently assigned the heaviest weights to the somatosensory measures indicating that the most pronounced lesion and drug effects were alterations in reactivity to non-noxious stimuli. This observation does not imply an absence of lesion or drug-induced alterations in responsiveness to noxious stimuli. It merely indicates that the measures of reactivity to noxious stimuli were less sensitive to the treatments. In fact, univariate statistics revealed smaller, but significant, lesion-induced increases in reactivity to both footshock and hotplate stimuli. Thus, these measures might be made more sensitive by modification which reduce their error variance.

Morphine injected into either the CMA or the VHIP reversed the septal lesion-induced hyperreactivity to non-noxious stimuli without debilitating motor function. This apparently similar effect of morphine in areas so dissimilar in opiate receptor density is nevertheless consonant with elec-

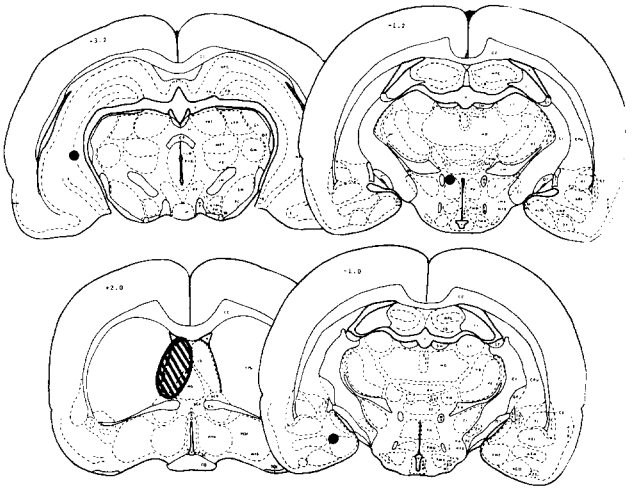

FIG. 7. Representative histological sections for the septal lesion and cannulae placements in the CMA, PHYPO, and VHIP.

trophysiological data which showed similar effects of morphine on neuronal activity in these areas [8]. One difference between these areas was that the morphine effect in the CMA predominantly represented somatosensory effects, while the effect in the VHIP also included a substantial effect on footshock reactivity.

The dual involvement of the VHIP with both noxious and non-noxious stimuli was not surprising given its known functions in the interpretation of nociceptive stimuli and in responsivity to stimulation [9, 36, 40], as well as the multiple effects of opioids on neuronal activity in this area [14, 37, 50]. Similarly, the total reversal of somatosensory reactivity by morphine injected into the CMA is consonant with the fact that the CMA has a dense population of opiate receptors and is involved with emotional behavior [29] and with the affective-motivational interpretation of noxious stimulation [35]. Further, the specificity of the morphine effect to somatosensory reactivity suggests the existence of a functionally homogeneous population of opiate receptors in the CMA.

Morphine injected into the PHYPO incompletely reversed the septal lesion-induced hyperreactivity to non-noxious stimuli and in addition had an unspecified effect on behavior which made these rats statistically discriminable from both untreated shams and septal-lesioned rats. The incomplete reversal of somatosensory reactivity as well as the unspecified morphine effect suggest the existence of multiple opiate receptor sites in this area. Because of the descending hypothalamic influence on the PAG, and thence on the spinal cord [1], the final morphine effect on responsivity may be qualitatively different from effects achieved in more rostral structures and may be independent of septal lesion-induced hyperreactivity. In fact, some work has suggested that the ascending and descending systems are independent and may mediate different aspects of opiate analgesia [46].

Reversal by naloxone is the usual criterion to determine the specificity of an opiate analgesic effect, although the identification of opiate receptors which are not naloxone-sensitive [41] suggests a functional differentiation among these receptors. Thus, the question was not whether the morphine reversal of hyperreactivity was modulated by opiate receptors, but whether or not it was modulated by naloxone-sensitive morphine receptors.

Naloxone completely reversed the morphine attenuation in the CMA of somatosensory reactivity, an observation which supports the hypothesis that this structure possesses a uniform group of naloxone-sensitive opiate receptors which modulate affective responsivity [35] to stimulation. Naloxone reversed the unspecified lesion-independent, but not the lesion-dependent morphine effect in the PHYPO, suggesting that the morphine attenuation of somatosensory reactivity was mediated by naloxone-insensitive receptors. Naloxone partially reversed the morphine attenuation in the VHIP of reactivity and since this effect represents both somatosensory and footshock reactivity the possibility that they were mediated by different opiate receptors was suggested. Correlations computed between the measures of somatosensory and footshock reactivity within each group defined by brain area and scatterplots constructed from these data revealed the disproportionate naloxone reversal of the morphine effect on somatosensory relative to footshock reactivity in all brain areas tested. In addition, the greatest naloxone effect was a reversal of the attenuation of reactivity to non-noxious stimuli induced by morphine injected into the CMA, again suggesting that the naloxone-sensitive opiate receptors are involved with affective responsivity.

Although the DFA's indicate that the best description of the lesion and drug effects is in terms of increased reactivity to non-noxious stimuli, univariate analyses revealed that septal lesions increased reactivity to both the hotplate and footshock measures. Thus, the septal lesion increased reactivity to two qualitatively different noxious stimuli, although these effects were not as powerful as that observed for somatosensory reactivity. The morphine attenuation of the lesion effect was most pronounced in the CMA and PHYPO, areas of respectively high and moderate opiate receptor density, and less pronounced in the VHIP, an area of low opiate receptor density. More specifically, morphine reversed septal lesion-induced reactivity to both footshock and hotplate stimuli when injected into the CMA or the PHYPO, and reversed footshock but not hotplate reactivity when injected into the VHIP. Naloxone antagonism of the morphine attenuation of reactivity was only evidenced in the CMA group and only on the hotplate measure. Thus, with this one exception, the effects of regional micro-injection of morphine which attenuated septal lesion-induced reactivity to noxious stimuli were not mediated by naloxone-sensitive morphine receptors.

The ability of morphine to selectively attenuate, in a site-dependent manner, specific components of septal hyperreactivity suggests that at least these components of septal hyperreactivity are mediated by the endogenous opiate system. This is a neural system which selectively binds both exogenous and endogenous opioid compounds, and shows a density distribution which parallels systems involved with the affective, rather than the primary sensory, responsivity to stimulation [33]. Thus, areas involving paleospinothalamic and spinoreticular terminations of limbic circuitry are high in endogenous opiate receptor content, while those areas receiving primary sensory or nonsomatosensory input are low in receptor content [33], prompting speculation that opiates ease the diffuse, or affective, component of response to noxious stimuli [13, 32, 45].

In summary, several lines of evidence suggest the existence of an affective component of septal hyperreactivity which is modulated at least in part by the naloxone-sensitive endogenous opiate system. First, septal hyperreactivity to

non-noxious stimuli suggests an affective component of this syndrome. Second, the close association of the endogenous opiate system with limbic areas and non-primary fibers conveying somatosensory information [33], as well as clinical response of humans [13,45], and the electrophysiological [38,40] and pharmacological [7] response of animals to opiates and selective antagonists, suggest an opiate role in affective responsiveness to stimulation [32]. Third, the comparatively more potent morphine attenuation of septal lesion-induced reactivity to non-noxious, relative to noxious, stimulation, as well as the considerably greater naloxone-sensitivity of the former, suggests a more specific opiate involvement with this aspect of septal hyperreactivity. Finally, the DFA was shown to be a sensitive statistical tool to assess subtle drug and lesion effects on behavior as measured by multiple dependent measures.

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