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Task-Dependent Effects of Intra-amygdala Morphine Injections: Attenuation by Intra-amygdala Glucose Injections

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Intraseptal injections of morphine impair learning and memory in rats, and these impairments are reversed by intraseptal injections of glucose. With evidence that injections of morphine into the amygdala also impair memory for some tasks, the present experiment determined whether (1) intra-amygdala morphine injections impair performance in inhibitory avoidance and spontaneous alternation tasks, and (2) intra-amygdala glucose injections attenuate the effects of intra-amygdala morphine injections. Rats receiving bilateral injections of morphine (4.0 nmol) into the amygdala, 30 min prior to training in inhibitory avoidance, had retention latencies significantly lower than those of unoperated and CSF controls when tested 24 hr later. Bilateral morphine injections (4.0 or 8.0 nmol) 30 min prior to testing in a spontaneous alternation task did not alter performance. The morphine-induced impairment observed in inhibitory avoidance was not due to diffusion up the cannulas, altered sensitivity to shock, or seizure activity. A glucose dose of 16.67 nmol, but not 8.33 nmol, injected into the amygdala attenuated the morphine-induced deficit in inhibitory avoidance. Rats receiving CSF into the amygdala exhibited decreased retention latencies in inhibitory avoidance compared to those of unoperated controls. This decrease was not attenuated by glucose at doses of 8.33 and 16.67 nmol. Therefore, these findings suggest that the amygdala is another brain region in which glucose affects brain functions, possibly by interacting with the opioid system and/or other neurotransmitter systems.

[Key words: memory, glucose, morphine, amygdala, inhibitory avoidance, spontaneous alternation]

Numerous experiments indicate that glucose modifies learning and memory (for reviews, see Gold, 1991, 1994; White, 1991). Glucose administration enhances memory in rodents, healthy elderly subjects, and Alzheimer's patients (Gold, 1986; Messier and White, 1987; Messier and Destrade, 1988; Hall et al., 1989; Manning et al., 1990, 1992, 1993; Packard and White, 1990; Fernandez, 1992). Furthermore, glucose injections into the lateral ventricles enhance memory, suggesting that glucose may have direct central actions on mnemonic processes (Lee et al., 1988).

To begin a pharmacological analysis of glucose effects on mnemonic processing, a series of experiments, using systemic injections, examined glucose interactions with drugs that affect learning and memory. One set of findings indicate that glucose attenuates memory impairments induced by muscarinic and nicotinic cholinergic antagonists (Stone et al., 1988a, 1991; Messier et al., 1990; Ragozzino and Gold, 1991). Glucose also attenuates the effects of the opioid agonist, morphine, on memory and other measures (Stone et al., 1990, 1991; Arankowsky-Sandoval and Gold, unpublished observations). Thus, when injected systemically, glucose interacts with drugs directed at both the cholinergic and opioid systems.

More recently, we began to examine the effects of direct brain injections with glucose and other drug treatments on learning and memory. Findings from numerous experiments indicate that lesions or direct pharmacological manipulations of the septal area affect learning and memory (Winocur and Mills, 1969; Bostock et al., 1988; Chrobak et al., 1989; Givens and Olton, 1990; Mizumori et al., 1990; Decker et al., 1992; Izquierdo et al., 1992). The septal area may be one brain site in which glucose has modulatory effects to influence learning and memory processes. We found that intraseptal injections of morphine impair performance in spontaneous alternation and inhibitory avoidance tasks (Ragozzino et al., 1992). Furthermore, systemic and intraseptal injections of glucose attenuate impairments resulting from intraseptal morphine injections. These findings suggest that the septal area may be one brain region susceptible to glucose modulation.

Another brain area of interest in studying glucose modulation of mnemonic processing is the amygdala. Similar to the medial septum, manipulations of the amygdala, that is, lesions, chemical or electrical stimulation, produce changes in learning and memory (Gold et al., 1975; Gallagher and Kapp, 1978; Kesner and Andrus, 1982; Hitchcock and Davis, 1987; Brioni et al., 1989; Izquierdo et al., 1992). The amygdala binds high levels of opioid ligands (Zamir et al., 1985). Behavioral and pharmacological studies indicate that glucose interacts with opioids (Brase et al., 1987; Stone et al., 1990, 1991). Because glucose reduces the mnemonic deficits induced by intraseptal morphine treatment (Ragozzino et al., 1992), glucose may interact with opioids in other brain areas to alter mnemonic functioning.

The present experiment determined whether intra-amygdala morphine injections impaired spontaneous alternation and inhibitory avoidance performance, as observed previously with intraseptal morphine injections. The experiment also determined whether intra-amygdala glucose administration attenuated impairments induced by intra-amygdala morphine injections. The findings indicate that intra-amygdala injections of morphine impair performance in an inhibitory avoidance but

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not a spontaneous alternation task. Furthermore, intraamygdala injections of glucose attenuate the inhibitory avoidance deficit produced by intraamygdala morphine injections.

Materials and Methods

Subjects

Male Sprague–Dawley rats (Charles River Breeders, Wilmington, MA), weighing between 300 and 400 gm at the time of surgery, served as subjects. Rats were housed individually with food and water available ad libitum. They were maintained on a 12 hr light/dark cycle (lights on 0700 hr).

Surgery

Rats received atropine sulfate (0.2 cc of a 540 μg/cc solution, i.p.) 20 min prior to being anesthetized with sodium pentobarbital (65 mg/kg, i.p.). After being anesthetized, stainless steel guide cannulas (22 gauge; Plastics One, Inc., Roanoke, VA) aimed dorsal to the central nucleus of the amygdala were implanted bilaterally. The stereotaxic coordinates were 0.4 mm posterior from bregma, ±4.5 lateral, and 7.0 ventral from dura. The nose bar was set at 5.0 mm above the interaural line according to the atlas of Pellegrino et al. (1979).

In another set of rats, cannulas were implanted in the striata, using the stereotaxic coordinates as above except the cannulas were lowered 4.5 mm below dura.

Intracranial injections

Bilateral injections into the amygdala or striatum were administered through an inner cannula that extended 1.0 mm below the guide cannula. The 28 gauge inner cannula was attached by a polyethylene tube to a 25 μl Hamilton syringe. The syringe was driven by an infusion pump with solutions administered in a volume of 0.5 μl at a rate of 0.5 μl/min. Prior to removal, the injection cannula was left in place for an additional minute to allow diffusion. All drugs were mixed in artificial cerebrospinal fluid (CSF) that contained 3.33 nmol of glucose and pH to 7.4. The doses of morphine and glucose, specified below, were chosen because previous experiments demonstrated effects in memory tests at these doses (Ragozzino et al., 1992).

Behavioral procedures

Inhibitory avoidance. One week after surgery, rats were trained in an inhibitory avoidance task between 0800 and 1200 hr. Cannulated rats received injections 30 min prior to training, the same as in previous experiments with systemic and intracranial injections of morphine (Stone et al., 1991; Ragozzino et al., 1992). Rats implanted with cannulas in the amygdala were assigned to one of the following groups: (1) CSF (N = 13), (2) morphine sulfate (4.0 nmol; N = 16), (3) morphine sulfate (4.0 nmol) and glucose (8.33 nmol; N = 10), (4) morphine sulfate (4.0 nmol) and glucose (16.67 nmol; N = 13), (5) glucose (8.33 nmol; N = 8), (6) glucose (16.67 nmol; N = 8). Combined morphine and glucose treatment was administered in a single "cocktail" injection. Rats implanted with cannulas in the striata were assigned to one of the following groups: (1) CSF (N = 7), (2) morphine sulfate (4.0 nmol; N = 6). Another group of rats served as unoperated controls (N = 12). Inhibitory avoidance training was conducted in a trough-shaped alley (90.0 cm long, 15 cm high, 20 cm ceiling width, and 5.5 cm floor width) divided into two compartments by a sliding door. On the training trial, each rat was placed into a well-lit compartment (30 cm long). After 20 sec the door was opened and the rat was allowed to enter a dark shock compartment. Following entry into the dark compartment, the door was closed behind the rat and a footshock (2.0 mA, 1 sec) was delivered by a shock apparatus (900 kΩ resistor; Lafayette Instruments) through metal plates that comprised the floor and walls of the trough. Retention was tested 24 hr later by placing the rat in the start compartment and measuring the latency to cross into the dark compartment (600 sec maximum).

Separate groups of rats with amygdala cannulas and unoperated controls were used to assess whether morphine injections altered footshock sensitivity. This was determined by measuring flinch-jump thresholds using an ascending series of shock intensities. Cannulated rats received one of the following treatments, (1) CSF (N = 4), (2) morphine sulfate (4.0 nmol; N = 4), (3) glucose (16.6 nmol; N = 3), (4) morphine sulfate (4.0 nmol) and glucose (16.6 nmol; N = 3), 30 min prior to testing. Shock intensity began at 30 μA (1 sec duration) and was increased by 30 μA every 30 sec. The lowest shock intensity at which a rat exhibited a behavioral response was considered the flinch threshold. The lowest shock intensity at which a rat's paws left the floor was recorded as the jump threshold.

Spontaneous alternation. Three to five days following inhibitory avoidance testing, the same rats were tested in a Y-maze for spontaneous alternation behavior. Testing occurred between 0800 and 1200 hr. Rats were assigned to a different group so that no rat received the same treatment in the spontaneous alternation task as on the inhibitory avoidance task. All cannulated rats were injected 30 min prior to testing. The treatment groups were the same as in inhibitory avoidance with the addition of a group that received a higher dose of morphine (8.0 nmol) injected into the amygdala. The size of the groups ranged from 5 to 10.

The testing procedure was based on that of Sarter et al. (1988). The Y-maze consisted of three trough-shaped arms radiating from a central triangle. The length of each arm was 60 cm, the height of the maze was 17.5 cm, the width of the floor was 3.5 cm, and the width of the ceiling was 14 cm. The central triangular area was 4 cm along its longest axis. The ceiling was covered with translucent black Plexiglas. The rats were placed in one arm and allowed to traverse the maze freely for 8 min while the number and sequence of entries were recorded. An alternation was defined as the consecutive entry into all three arms on overlapping triplet sets. Using this procedure, possible alternation sequences are equal to the number of arm entries minus 2, and the percentage of alternation behavior is equal to the ratio of (actual alternations/possible alternations) × 100.

Electroencephalographic recordings. To determine whether intra-amygdala morphine injections produced seizures or other abnormal electroencephalographic activity, three rats were implanted with amygdala and cortical electrodes. For intra-amygdala recordings, stainless steel wire was soldered to each guide cannula (11 mm). Guide cannulas were insulated except at the tip (1 mm), thus being used for both recording and injections. The guide cannulas were implanted bilaterally at the following coordinates: −0.4 AP, ±4.5 ML, and 7.0 ventral from dura. The nose bar was set at 5.0 mm. Cortical recordings were obtained from small screws, one positioned rostral to bregma (2.0 mm AP from bregma and −3.0 mm ML) and the other caudal to lambda (−1.0 from lambda and 3.0 ML). One to two weeks following surgery, each rat was habituated to the EEG procedure for 2 d in a recording room. This involved attaching a recording cable to a connector strip while each animal remained in its home cage for 3 hr. All habituation and recording procedures were carried out between 0800 and 1200 hr. Baseline EEG recordings were made for 30 min. Continuing EEG recordings, rats then received intra-amygdala injections of morphine or CSF, using the same procedure as above, with recordings assessed up to 150 min after injections.

Histology

After the completion of testing, rats received an overdose of sodium pentobarbital followed by a 0.5 μl injection of ink into each cannula. Intracardial perfusions were performed with 0.9% saline followed by a 10% formalin solution. Brains were removed and placed in a 30% sucrose/formalin solution.

In preparation for sectioning, brains were frozen at −20°C and mounted on a Reichert-Jung cryostat. Forty-micrometer sections were taken beginning at the anterior amygdala, mounted onto slides, dried, and stained with cresyl violet. Figure 1 illustrates the location of injection sites and diffusion of ink in the amygdala and striatum of rats considered to have correct placements. Behavioral scores for rats with cannulas intended for the amygdala or striatum but not found in these areas, respectively, were excluded from the data analyses. In the amygdala, cannulas were aimed for the central nucleus because of the high density of opioid receptors (Zamir et al., 1985); however, data from rats with cannulas found in other amygdala nuclei (i.e., lateral, basolateral, and medial nuclei but not the cortical nucleus or anterior amygdala area) were included in the analyses. The data from these rats were included because ink injections with cannulas located in the central nuclei indicated that the ink spread to these other amygdala nuclei.

Statistical analysis

Inhibitory avoidance results were analyzed with two-tailed Mann–Whitney U tests. The percentage alternation scores and flinch-jump thresholds were analyzed by independent two-tailed t tests.

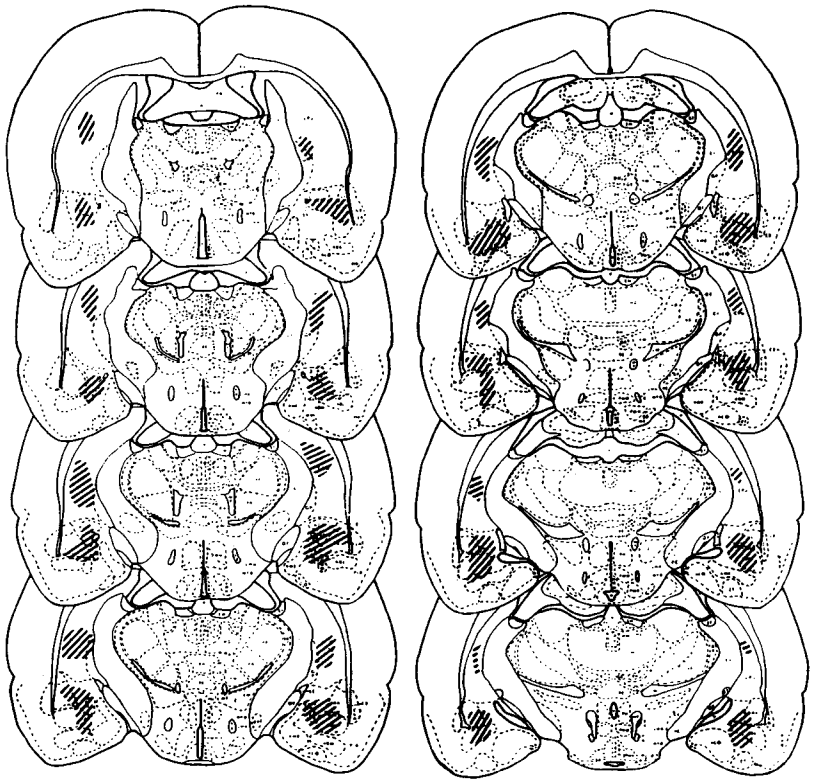

Figure 1. Location of the injection cannula tips and diffusion of ink in the amygdala and striatum for all rats included in behavioral analyses. Rat brain sections were taken from the stereotaxic atlas of Pellegrino et al. (1979).

Results

Inhibitory avoidance

Intraamygdala injections of morphine impaired inhibitory avoidance learning. This impairment was attenuated by coadministration of glucose. As shown in Figure 2, rats that received morphine injections into the amygdala 30 min prior to training had latencies significantly lower than those of unoperated [$U(12,16) = 13$, $p < 0.0001$] and CSF controls [$U(13,16) = 48$, $p < 0.02$] when tested 24 hr after training. At the higher glucose dose (16.67 nmol), glucose attenuated the morphine-induced impairment. The combined treatment of morphine and glucose (16.67 nmol) produced latencies that were significantly higher than the latencies following morphine treatment [$U(10,16) = 40$, $p < 0.05$], and were similar to those of the CSF group [$U(10,13) = 56, p > 0.05$]. At the lower dose (8.33 nmol), glucose treatment resulted in latencies intermediate to those obtained after morphine or CSF treatment. The rats coadministered morphine and glucose (8.33 nmol) into the amygdala had scores not significantly different from those of morphine-treated rats or CSF controls [$U(13,16) = 69$ and $U(13,13) = 59$, respectively, $p > 0.05$]. CSF controls had significantly lower scores than those of unoperated controls [$U(12,13) = 39, p < 0.05$]. Intraamygdala injections of glucose (8.33 nmol) or glucose (16.67 nmol) resulted in latencies similar to those following CSF injections. The latencies of the glucose (8.33 nmol) group were not significantly different than those of the CSF [$U(8,13) = 39$, $p > 0.05$] or unoperated [$U(8,12) = 24$, $p > 0.05$] controls. Rats receiving glucose (16.67 nmol) injections had scores not significantly different from those of CSF controls [$U(8,13) = 51, p > 0.05$], but were significantly lower than the scores of unoperated controls [$U(8,12) = 18$, $p < 0.02$]. Thus, at neither dose did glucose reverse the inhibitory avoidance deficit produced by cannula implantation. Moreover, the two morphine-glucose groups approached the scores of the CSF group but not the unimplanted controls. The morphine-glucose (16.67 nmol) group had latencies significantly different from those of unoperated controls [$U(10,12) = 22$, $p < 0.01$]. Similarly, rats coadministered morphine and glucose (8.33 nmol) had significantly lower latencies

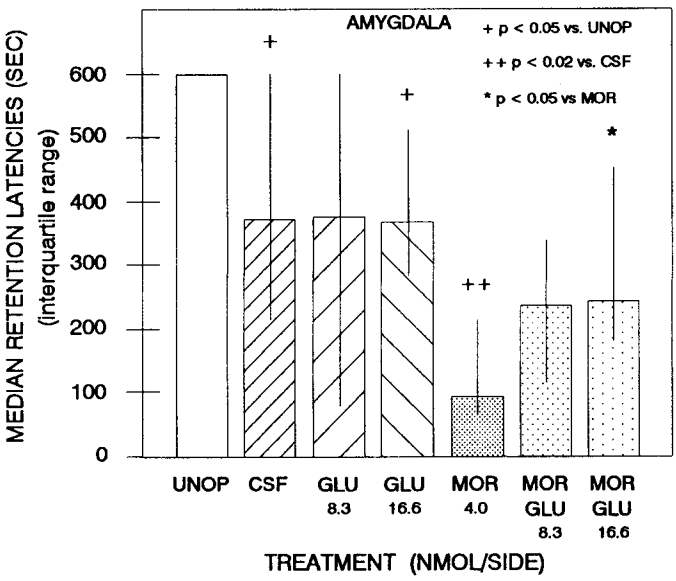

Figure 2. Intraamygdala glucose injections attenuate inhibitory avoidance deficits induced by morphine injections into the amygdala. Bilateral morphine (4.0 nmol) injections into the amygdala, prior to training, significantly reduced latencies. Intra-amygdala glucose injections alone (8.33 and 16.67 nmol), prior to training, did not modify latencies, but when glucose (16.67 nmol) was coadministered with morphine, latencies were significantly greater than those after morphine treatment alone. Cannulated controls had significantly lower latencies than those of unoperated controls.

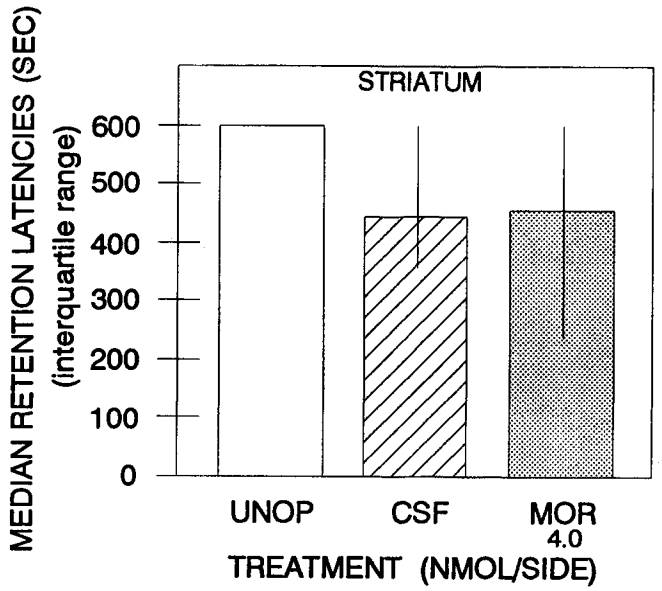

Figure 3. Bilateral morphine injections into the striatum did not impair inhibitory avoidance performance. Prior to training, morphine (4.0 nmol) injected dorsal to the amygdala, in the overlying striatum did not significantly reduce latencies compared to those of unoperated and CSF controls. Similarly, CSF injections did not reduce latencies compared to those of unoperated controls.

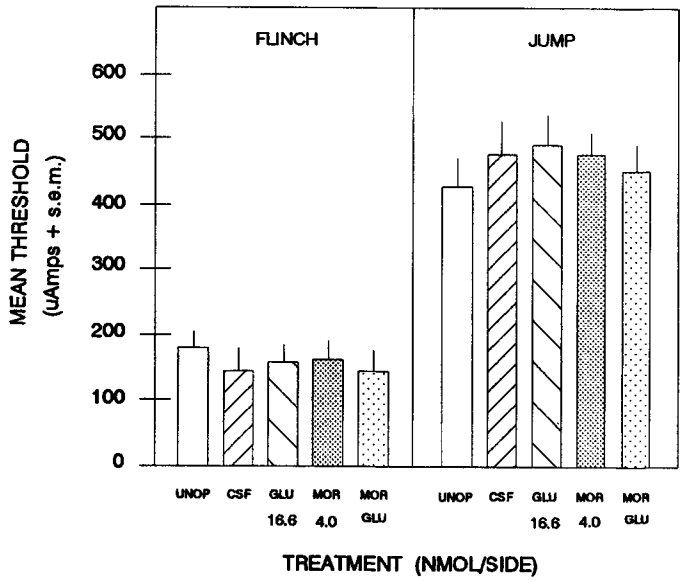

Figure 4. Bilateral amygdala injections of morphine, glucose, or morphine-glucose did not modify flinch-jump thresholds. Rats receiving morphine, glucose, or morphine-glucose injections into the amygdala resulted in flinch-jump thresholds not significantly different from those of unoperated and CSF controls.

compared to those of unoperated controls [$U(8,12) = 17$, $p < 0.05$].

Figure 3 illustrates that latencies to cross following morphine injections into the striatum were similar to those following intrastriatal CSF injections and somewhat lower than those of unoperated controls. The scores of rats receiving morphine injections were not significantly different than those of CSF [$U(6,7) = 20$, $p > 0.05$] or unoperated [$U(6,12) = 24$, $p > 0.05$] controls. CSF controls had latencies comparable to those of unoperated controls [$U(7,12) = 22$, $p > 0.05$].

Figure 4 illustrates that there were no differences in flinch-jump thresholds between any of the groups. The flinch thresholds to footshock for unoperated (mean = 172.5 μA, SEM = $\pm$22.5) and intra-amygdala CSF (mean = 150 μA $\pm$ 12.2) controls did not differ from each other [$t(6) = 0.88$, $p > 0.05$]. Flinch thresholds for the groups receiving morphine (mean = 165 $\pm$ 19.4 μA), morphine-glucose (mean = 150 $\pm$ 17.3 μA), or glucose (mean = 160 $\pm$ 20 μA) were comparable to those of unoperated and CSF controls ($p > 0.05$). Similar to flinch thresholds, jump thresholds were not significantly different between those of unoperated (mean = 435 $\pm$ 35.7 μA) and CSF (mean = 480 $\pm$ 53.4 μA) controls [$t(6) = 0.70$, $p > 0.05$]. The jump thresholds for the groups receiving morphine (mean = 480 $\pm$ 30 μA), morphine-glucose (mean = 460 $\pm$ 36.1 μA), or glucose (mean = 500 $\pm$ 40 μA) did not differ from those of unoperated and CSF controls ($p > 0.05$).

Spontaneous alternation

The findings shown in Figure 5 indicate that intra-amygdala morphine injections did not impair spontaneous alternation performance. Similar to previous observations (e.g., Parsons and Gold, 1992; Ragozzino et al., 1992), unoperated and CSF controls had alternation scores near 70% (means = 66.9 $\pm$ 4.2 and 66.7 $\pm$ 3.0, respectively). Rats receiving intra-amygdala morphine injections (4.0 nmol) had alternation scores (mean = 62.4 $\pm$ 4.2) similar to those of unoperated and CSF controls [$t(17) = 0.49$ and $t(16) = 0.50$, respectively, $p > 0.05$]. As seen with the lower dose, the 8.0 nmol dose of morphine (mean = 67.6 $\pm$ 4.5) did not modify spontaneous alternation performance compared to that of unoperated and CSF controls [$t(13) = 0.10$ and $t(12) = 0.12$, respectively, $p > 0.05$]. The scores of CSF controls were not significantly different from those of unoperated controls [$t(15) = 0.05$, $p > 0.05$].

As shown in Figure 6, morphine injections into the striatum did not impair spontaneous alternation performance. Rats re-

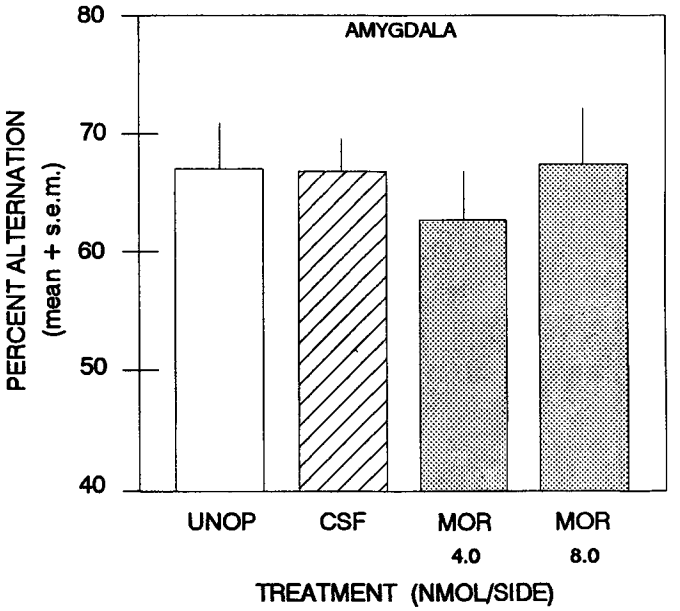

Figure 5. Intra-amygdala morphine injections did not impair spontaneous alternation performance. Bilateral morphine (4.0 and 8.0) injections into the amygdala did not significantly modify spontaneous alternation performance compared to that of unoperated and CSF controls

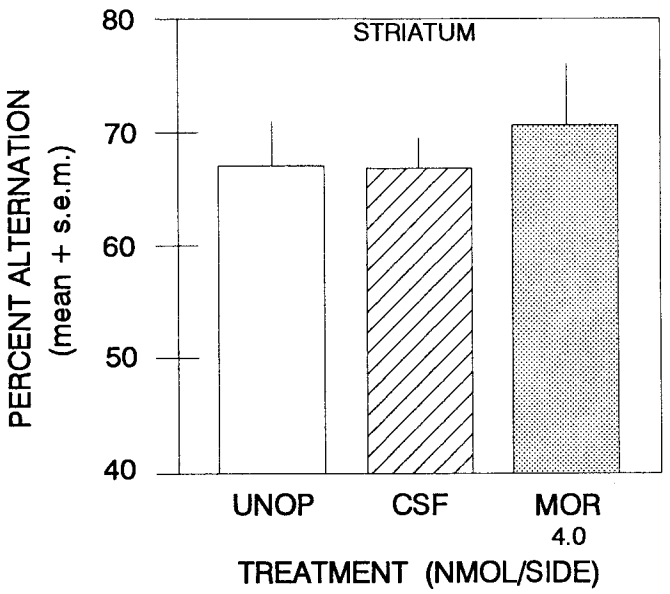

Figure 6. Bilateral morphine injections into the striatum did not impair spontaneous alternation performance. Morphine (4.0) injected dorsal to the amygdala, in the overlying striatum, did not significantly change spontaneous alternation scores compared to those of CSF and unoperated controls.

ceiving morphine injections into the striatum had alternation scores not significantly different from those of unoperated and CSF controls [$t(12) = 0.67$ and $t(11) = 0.54$, respectively, $p > 0.05$]. The alternation scores of CSF-injected controls were comparable to those of unoperated controls [$t(15) = 0.27, p > 0.05$].

Similar to previous experiments (Ragozzino and Gold, 1991; Ragozzino et al., 1994; Ragozzino, Hellems, Lennartz, and Gold, unpublished observations), minor differences in the number of arm choices between the groups occurred but did not appear related to the alternation scores. Unoperated controls (mean = 19.2 ± 1.1) made significantly fewer arm choices than did CSF controls (mean = 24.1 ± 1.4) [$t(15) = 2.78$, $p < 0.02$]. The number of arms entered by the 4.0 nmol morphine group (mean = 19 ± 3.1) was not significantly different from those of unoperated and CSF controls [$t(17) = 0.06$ and $t(16) = 1.38$, respectively, $p > 0.05$]. The 8.0 nmol morphine group (mean = 26.5 ± 2.6) chose significantly more arms than unoperated controls [$t(13) = 2.93$, $p < 0.02$], but not CSF controls [$t(12) = 0.87, p > 0.05$]. Rats receiving intrastriatal morphine treatment (mean = 22.2 ± 3.5) made a similar number of arm choices as rats administered CSF into the striatum (mean = 23.6 ± 2.1).

Inaccurate placements

Several rats were excluded from the data analyses because histological examination revealed that either one or both cannulas were outside the amygdala or ink was in the ventricles. There were only one to four rats excluded from each treatment group. Therefore, it was often difficult to determine any pattern of inaccurate placements by examining a single treatment group. However, because the results were similar independent of the treatment, including morphine, the data were combined across groups.

Analysis of the inaccurate placements in the inhibitory avoidance task indicated that rats with placements in the striatum or internal capsule had latencies (median = 470) that were not significantly different from those of unoperated [$U(12,20) = 71$, $p > 0.05$] or CSF controls [$U(13,20) = 110$, $p > 0.05$]. The latencies of rats with ink in the ventricles (median = 326) were significantly lower than those of unoperated controls [$U(12,16) = 39$, $p < 0.01$] but not CSF-treated rats [$U(13,16) = 39$, $p > 0.05$]. However, rats with cannula placements that penetrated through the lateral nucleus into the juxtaposed cortical area, essentially lesioning the amygdala, had latencies (median = 99) that were significantly lower than those of unoperated [$U(12,7) = 0.01$, $p < 0.001$] and CSF controls [$U(13,7) = 14$, $p < 0.05$]. These findings are consistent with substantial evidence that amygdala lesions impair avoidance learning (McGowan et al., 1972; Kemble and Davis, 1981; Dunn and Everitt, 1988; Cahill and McGaugh, 1990). Importantly, the distribution of placements for rats included in the behavioral groups was quite similar across groups. No rats were included in any treatment group for analyses of drug effects in inhibitory avoidance if their placements penetrated through the lateral amygdala to the adjacent cortex. Thus, differences in cannula placement did not contribute to the drug effects described here.

In contrast to inhibitory avoidance, rats with cannulas that were in the cortex or too ventral had spontaneous alternation scores (mean = 65.4 ± 3.2) not significantly different from those of unoperated and CSF controls [$t(11) = 0.32$ and $t(10) = 0.25$, respectively, $p > 0.05$]. Combining the scores from rats with dorsal placements or ink in the ventricles revealed that their performance in the spontaneous alternation task (mean = 66.8 ± 4.1) was similar to that of unoperated and CSF controls [$t(16) = 0.03$ and $t(15) = 0.02$, respectively, $p > 0.05$].

Electroencephalographic recordings

Analysis of the EEG records indicated that intra-amygdala injections of morphine or CSF did not result in seizure-like activity within 150 min after injection. Immediately following injections of morphine and CSF, there was a transient increase in amplitude and frequency lasting approximately 4 min. However, no spiking or other irregular EEG activity was found during the rest of the recording period.

Discussion

The findings of the present experiment indicated that morphine administered into the amygdala impairs performance in an inhibitory avoidance but not spontaneous alternation task. The amnesia resulting from intra-amygdala morphine injections is consistent with previous findings that intra-amygdala injections of opioid agonists cause mnemonic deficits in shock avoidance tasks (Gallagher and Kapp, 1978; Flood et al., 1992). Because morphine injections into the overlying striatum did not impair learning of the avoidance response, the inhibitory avoidance deficit after intra-amygdala morphine injections did not appear because of diffusion up the cannulas. Rather, the inhibitory avoidance deficit seemed to result from activation of opioid receptors in the amygdala. Furthermore, intra-amygdala morphine injections did not alter the rats' sensitivity to footshock or induce seizures. The 4.0 nmol morphine dose injected into the amygdala was lower than the morphine doses injected into the amygdala previously found to modify shock thresholds (13 nmol) and induce behavioral and EEG seizures (25–200 nmol) (Rodgers, 1978; Ikonomidou-Turski et al., 1987).

Besides the impairment with intra-amygdala morphine injections, rats injected with CSF into the amygdala exhibited an inhibitory avoidance deficit compared to unoperated controls, apparently the result of tissue damage within the amygdala and

overlying areas. Similar results have been observed following implantation of electrodes into the amygdala (Gold et al., 1975, 1978). Thus, the deficit may result because of cannula implantation alone but also may be due to the CSF injection. The intrastriatal controls also had latencies lower than those of unoperated controls, but these were not significantly different. Injections of glucose at 8.33 and 16.67 nmol did not reverse the deficit observed with implanted amygdala controls. Previously, we found that systemic glucose did not reverse the effect of medial septal lesions (McGlynn, Lennartz, Gold, and Gold, unpublished observations). Furthermore, Liang and McGaugh (1983) demonstrated that lesions of the stria terminalis block the memory-enhancing effect of epinephrine. These results suggest that cannula implantation or lesioning that partially damages multiple neurochemical systems in brain areas blocks the effects of modulators, such as glucose and epinephrine. However, in amygdala cannulated rats, a specific pharmacological-induced deficit is amendable to glucose treatment. In contrast to the inhibitory avoidance deficits, intra-amygdala morphine injections did not impair spontaneous alternation performance. This was also the case for morphine injections into the striatum. One possibility is that higher doses of morphine injected into the amygdala would impair spontaneous alternation performance, although the seizures likely at higher doses would confound an interpretation based on direct morphine actions in the amygdala.

The lack of an effect of intra-amygdala morphine injections on spontaneous alternation performance is consistent with previous experiments demonstrating that lesions or pharmacological manipulations of the amygdala do not impair performance in tasks involving spatial processing (Aggleton et al., 1989; Kesner et al., 1990; Peinado-Manzano, 1990; Stackman and Walsh, 1992). However, there are spatial components in inhibitory avoidance, as used here, and Y-maze discriminated avoidance tasks for which memory is susceptible to amygdala lesions, electrical stimulation, or drug manipulations (Gallagher and Kapp, 1978; McGaugh et al., 1988; Brioni et al., 1989; Introini-Collison et al., 1989; Cahill and McGaugh, 1990; Flood et al., 1992). The differential effects of intra-amygdala morphine treatment in inhibitory avoidance versus spontaneous alternation tasks may be the result of different levels of affect elicited by the tasks (Kesner and Andrus, 1982; Kesner, 1992; LeDoux, 1992). Moreover, the differences observed in the memory tasks between morphine injections into the septum and amygdala indicate that these two anatomical systems are involved in different mnemonic functions. Consistent with the idea of multiple memory systems, findings from McDonald and White (1993) suggest that the septohippocampal system is involved in processing relationships between stimuli while the amygdala processes information that has affective properties.

The present study also demonstrated that intra-amygdala glucose injections attenuate the inhibitory avoidance deficit resulting from intra-amygdala morphine injections. The morphine-glucose treatment had a higher osmolarity than the morphine treatment alone, raising the possibility that the glucose attenuation resulted because of osmolarity changes. However, intra-amygdala injections of glucose did not attenuate inhibitory avoidance deficits induced by intra-amygdala infusions of propranolol, a β-noradrenergic antagonist (Lennartz and Gold, unpublished observations). These findings suggest that the bases of glucose effects are pharmacological rather than changes in osmolarity.

The effective dose of glucose (16.67 nmol) was similar to the glucose dose injected into the septum that attenuated impairments of both inhibitory avoidance and spontaneous alternation performance after intraseptal morphine injections (Ragozzino et al., 1992). Thus, glucose modulation of brain function is not limited to the septal region, but also includes the amygdala. Although morphine injections into the septum and amygdala impair performance for different sets of tasks, the pharmacological bases for glucose interactions may be the same. The findings from our studies, as well as others (Izquierdo et al., 1992; Stackman and Walsh, 1992), suggest that the neurotransmitter systems responsible for learning and memory are redundantly organized but their involvement in specific tasks varies depending on the neural system being manipulated.

The attenuation of the effect of intra-amygdala morphine injections by intra-amygdala glucose treatment adds to other findings, indicating that glucose attenuates the effects of morphine on several measures (Simon and Dewey, 1981; Shook et al., 1986; Stone et al., 1990, 1992; Arankowsky-Sandoval and Gold, unpublished observations). Glucose attenuation of the behavioral effects of morphine may be due to a decrease in opioid-receptor affinity because increasing glucose concentrations decrease receptor affinities for ^{3}H-naloxone and ^{3}H-dihydromorphine in the brain (Brase et al., 1987). If glucose acts by decreasing opioid receptor affinity, then glucose should produce effects similar to those of an opioid antagonist. We found that systemic injections of glucose and naloxone, an opioid antagonist, produce similar effects on several behavioral measures (Stone et al., 1987, 1988a,b,; Walker et al., 1991).

Another possibility is that the effects of glucose are due to direct actions on the cholinergic system. The amygdala receives an extensive cholinergic innervation (Mesulam et al., 1983) and opioid agonists decrease the release of ACh in amygdala slices (Frankhuijzen et al., 1991). Under certain conditions, including pharmacological treatments, hypoxia, and aging, the availability of acetyl-CoA appears to regulate ACh synthesis (Gibson and Blass, 1976; Gibson and Peterson, 1981; Dolezal and Tucek, 1982; Ricny et al., 1992). Brain acetyl-CoA is principally derived from glucose, and therefore circulating glucose levels may regulate ACh levels under certain conditions (Tucek and Cheng, 1974; Tucek, 1978).

It is also possible that glucose has multiple actions or acts through another neurotransmitter system involved in learning and memory processes. For example, work by McGaugh et al. (1994) demonstrated that in addition to opioid and cholinergic systems, the GABAergic system within the amygdala modulates mnemonic processes. Thus, glucose may also interact with one or more of these neurotransmitter systems in the amygdala. Future investigations measuring neurochemical changes in the amygdala following glucose treatment may elucidate the role of this modulator on brain functions.

Overall, these findings indicate that opioid receptors in the amygdala are involved in learning of an avoidance response. In contrast, activation of opioid receptors in the amygdala does not affect performance in a spontaneous alternation task. The pattern emerging suggests that the opioids, as well as other neurotransmitter systems, are involved in several types of memory or memories that are defined by the relevant neural system and task. Furthermore, intra-amygdala glucose injections attenuate the inhibitory avoidance deficit induced by intra-amygdala morphine injections. This finding indicates another brain region where glucose affects brain functions, possibly by direct inter-

actions with the opioid system and/or other neurotransmitter systems, supporting the view that circulating glucose can modulate different brain systems.

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