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Elevation of Aversive Threshold in Rats by Intra-Amygdaloid Injection of Morphine Sulphate

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RODGERS, R. J. *Elevation of aversive threshold in rats by intra-amygdaloid injection of morphine sulphate.* PHARMAC. BIOCHEM. BEHAV. 6(4) 385–390, 1977. Bilateral micro-injection of morphine sulphate (10μg, 20μg) into the cortico-medial amygdala produced a dose-dependent increase in aversive threshold. Similar injections into the basolateral amygdala or caudate-putamen failed to have any consistent effect on aversive thresholds. Whilst overall activity levels remained unaffected by morphine injection into either amygdaloid site, caudate animals exhibited a significant decrement in total activity in response to both morphine and control injections. Results are discussed with reference to a possible role for limbic mechanisms in morphine analgesia.

Morphine Aversive thresholds Activity Amygdala Caudate

THE CENTRAL mediation of responsiveness to aversive stimulation has been subject to considerable research effort in recent years. Focal electrical stimulation of sites extending from the septum [21], through the hypothalamus [2,6] to the ventral midbrain tegmentum [21] yields varying degrees of analgesia. However, by far the most potent analgesic effect is produced in response to electrical stimulation of the ventro-lateral periaqueductal gray matter [1, 19, 20, 21, 22, 28].

The importance of such brain sites, in central pain mechanisms, has recently been emphasized by studies involving microinjection of morphine into specific subcortical loci. In the rhesus monkey two regions, the periaqueductal gray (PAG) and a lateral midbrain area, have been identified in which morphine produces antinociception [24]. In the rat the PAG has been shown to be an important site of both morphine analgesia [13, 14, 33, 38] and tolerance [14]. However, in both the monkey and the rat, direct morphine injections into forebrain areas such as the amygdala have failed to produce significant analgesia [24,38].

Paradoxically, whilst the PAG exhibits high levels of opiate receptor binding, the greatest amount of binding has been localized within the amygdaloid region of the limbic system [35]. These results have received considerable support from studies of the local distribution of the morphine-like factor, enkephalin, in the mammalian nervous system [32].

The present paper explores this apparent contradiction concerning opiate activity in the amygdaloid complex. Aversive thresholds were measured using a modification of the flinch-jump threshold test [10]. This test was selected for two main reasons: (a) it has previously been found to be responsive to both peripheral [10,34] and central [13] injections of morphine, and (b) since supraspinal mechanisms were implicated, a test was required that did not measure a simple spinal reflex. The central sites chosen for study were the caudate-putamen, the basolateral and corticomedial aspects of the amygdala. Since motor side effects, which could affect performance on this test, have been reported following central injections of morphine [5, 14, 33] a control activity test was carried out to assess the possibility of morphine-induced motor impairment.

METHOD

Animals

Seventy-eight adult male Sprague-Dawley rats, from Bradford University colony were used in these studies. All animals were individually housed with food and water available ad lib. The animals were maintained on a 12 hr light/dark cycle (7 a.m.- 7 p.m.) and all testing was performed under red light during the dark phase of the cycle, ie 7 p.m. onwards.

Surgery

Experimental animals (mean weight 300 g) were bilaterally implanted with guide cannulae under Equithesin anaesthesia (Jensen-Salsbury Lab. Inc). Cannulae consisted of a 0.6 mm o.d. stainless steel guide fitted with a 0.3 mm o.d. stylet. The guides were constructed such that each terminated 2 mm dorsal to desired injection site.

The animals were randomly assigned to 3 surgical groups (n in each = 26); the caudate-putamen (CPU), the basolateral amygdala (BLA) and the cortico-medial amygdala

(CMA). Implantation coordinates for the caudate-putamen were 8.0 mm anterior to stereotaxic zero, 3.6 mm lateral to the sagittal sinus and 5.0 mm below the surface of the brain (+8.00; ±3.6; 5.0 down). Coordinates for the amygdala were: baso-lateral (+5.2; +5.0; 8.5 down); cortico-medial (+5.0; +4.0; 9.5 down). These values were based on adjusted calculations from the atlas of Konig and Klippel [17]. After surgery animals received an intramuscular injection of pencillin (50,000 units) and at least 10 days postoperative recovery was allowed before commencement of behavioural testing.

Injection Technique and Drugs

During injection, stylets were replaced with 0.3 mm injection cannulae which extended 2 mm ventral to the tip of the guide. This allowed for (a) more precise localization of injection sites and (b) only minimal tissue damage at these sites. Whilst the animal was hand-held, bilateral microinjections were made, via polythene tubing, from a 10 μl Hamilton microsyringe (Model 701N). Injections were made at the rate of 0.5 μl/15 sec and total time taken to make bilateral injections was approximately 60 sec.

Morphine sulphate was used at two dose levels, 20 μg/μl and 40 μg/μl. The vehicle for morphine, which also served as injection control, was distilled water. All microinjections were made bilaterally in a volume of 0.5 μl and each animal was used once only.

Histology

Before sacrifice, animals received bilateral microinjection of 0.1 μl trypan blue to aid localization of injection sites. Animals then received an overdose of Nembutal and were perfused with normal physiological saline followed by 10% formal saline. Brains of animals used in the pain experiment were removed, embedded in wax and serial sections cut at 10 μ. Sections were stained for myelin (Luxol Fast Blue), counter-stained for cell bodies (Pyronin Y), mounted, and examined for injection sites. Brains of animals used in the activity tests were removed, hardened in formal saline and examined for injection sites following sectioning on freeze-microtome.

Apparatus

Flinch-jump test. A modified rat operant chamber, measuring 23½ × 22 × 22 cm served as the test chamber. An Aims Bioscience shock generator (Model 507) supplied scrambled electric shock of variable intensity to the grid floor of the chamber. The total number of shocks, shock duration and shock frequency were held constant by relayed programming equipment (Aims Biosciences).

Activity test. The activity apparatus has previously been described [4]. Briefly each activity chamber consisted of a perspex box (30 × 20 × 15 cm) with a wire mesh top. Aligned off centre on the longtitudinal axis, was a single photocell which was connected to an externally mounted counter. A bank of 5 similar chambers, each connected to a separate counter, was used in present experiment.

Procedure

Flinch-jump test. Fourteen animals for each surgical category (CPU, BLA, CMA) were randomly assigned to three injection groups; water control (n = 4), 10 μg morphine sulphate (n = 5) and 20 μg morphine sulphate (n = 5).

Aversive thresholds were measured using a modified flinch-jump threshold test [10,20]. Operated rats were individually placed in the test chamber and received 6 series of 8 shocks (0.5 sec duration) delivered at 10 sec intervals to the grid floor. Shock series were administered in alternating ascending and descending order with intensities ranging between 0.13 1.3 mA in eight steps. Since it has previously been demonstrated [10] that the flinch response does not provide a reliable aversive threshold, only the jump threshold (the intensity at which the animals hind feet leave the grid floor) was recorded. Jump thresholds were estimated for each series and an overall mean calculated to provide a preinjection baseline. The animal was then removed from the chamber, injected and immediately retested following an identical procedure.

Activity test. Twelve animals from each surgical category were randomly assigned to two injection groups; control (n = 5) and 20 μg morphine sulphate (n = 7). To obtain an activity baseline, individual animals were placed in the activity chambers for a period of 14 min, corresponding approximately to test periods in the flinch-jump test. Animals were then removed, injected and immediately replaced in the chambers for a further 14 min session.

Results

Flinch-jump test. Two-tailed correlated *t*-tests were used in the data analysis. In the graphic representation of results, the abscissa refers to 6 series of preinjection shocks and 6 series of postinjection shocks. For statistical purposes, overall averages of the 6 preinjection means were compared to averages of the postinjection mean jump thresholds. Figure 1 shows the results for the BLA group. Neither control injections ($t = 0.00$), 10 μg morphine sulphate ($t = -0.90$), nor 20 μg morphine sulphate ($t = 0.14$) produced any significant alteration in the jump threshold. However, when injected into the cortico-medial amygdala, morphine produced a dose dependent increase in the jump threshold (Fig. 2); Control ($t = 0.68$), 10 μg morphine sulphate ($t = 7.02$, $df = 4$, $p < 0.01$), 20 μg morphine sulphate ($t = 7.88$, $df = 4$, $p < 0.01$).

Although threshold elevations in response to both control and morphine injections were also apparent in immediate postinjection in CPU animals, these did not reach statistical significance (Fig. 3); control ($t = 1.72$), 10 μg morphine sulphate ($t = 0.44$), 20 μg morphine sulphate ($t = 1.79$). Casual observation of these animals suggested that sedation/motor impairment occurred during the immediate postinjection period.

Histological analysis indicated that all animals were implanted within the correct subcortical region. Figure 4 illustrates the areas which were found to contain implanted cannulae, as revealed by trypan blue injection. Four cortico-medial amygdaloid cannulae were found to be positioned close to the ventral hippocampus. However, since the behavioural results from these animals were in the same direction as the rest of the group, it seemed appropriate to include them in data analysis.

This experiment clearly demonstrates that micro-injection of morphine sulphate into the cortico-medial aspect of the amygdala results in significant elevations in the aversive threshold. That this effect is specific to the

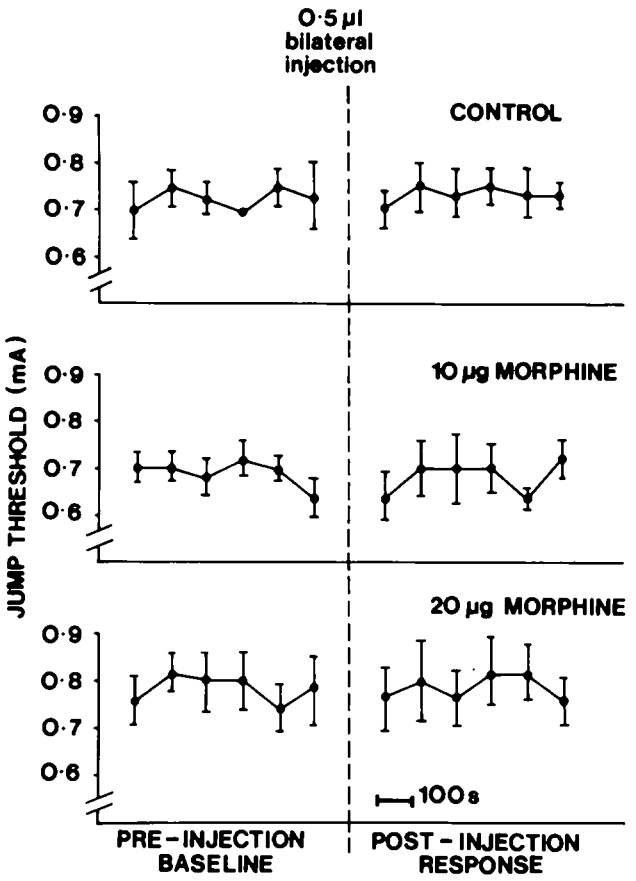

FIG. 1. The effect on aversive thresholds (+SEM) of control and morphine injections into the basolateral amygdala.

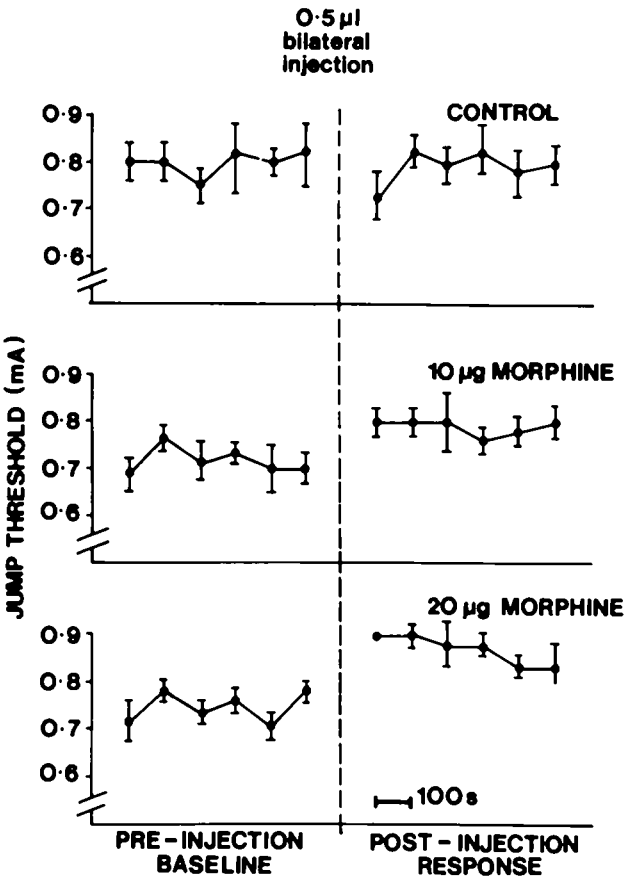

FIG. 2. The effect on aversive thresholds (+SEM) of control and morphine injections into the cortico-medial amygdala.

more medial regions of this complex is in agreement with data on the distribution of opiate binding [35].

Activity test. Since habituation of activity occurred between pre- and postinjection periods in all groups (Fig. 5), difference scores between these two periods were used for comparisons. A Kruskal-Wallis one-way analysis of variance revealed an overall significant effect ($H = 29.02$, $df = 5$, $p < 0.001$). A series of Mann-Whitney U tests were then employed in order to determine the nature of this significance.

No significant differences were found between activity depressions produced by morphine compared to control injections within each category. However, morphine depressed activity to a significantly greater extent in CPU than in CMA ($p < 0.02$) or BLA ($p < 0.03$). Also, control injections produced a larger activity decrement in CPU than in CMA ($p < 0.028$). No such differences were found between CPU and BLA or between CMA and BLA.

Location of injection sites were identified by histological analysis and found to be virtually indistinguishable from those reported for flinch-jump sites in all 3 surgical groups.

The results of this experiment do not support the hypothesis that threshold elevations in the CMA group in Experiment 1 were due to a motor deficit. However, the casual observation that CPU animals appeared sedated/ataxic immediately following morphine or control injections was supported by the present data. The decrease in activity between pre- and postinjection phases was significantly greater in the CPU animals than in either of the other two groups.

GENERAL DISCUSSION

Morphine (10 µg, 20 µg bilateral) produced a dose-dependent increase in aversive thresholds when injected into the cortico-medial aspect of the amygdaloid complex. This effect was not a consequence of drug-induced sedation since morphine injections at this site failed to affect activity scores (activity test). Comparable injections into more lateral amygdaloid sites were ineffective in altering aversive thresholds or motor activity, thus demonstrating site specificity within the amygdaloid complex. Although the possibility exists that morphine may have gained entry into the inferior horn of the lateral ventricles, this seems rather unlikely in view of the ventral distribution of most cortico-medial injection sites.

These results are at variance with those previously reported for the rhesus monkey and rat. In the rhesus monkey study [24] a possible species difference existed, a different procedure was used in assessment of aversive threshold and the injection sites did not correspond with those in the present study. More difficult to reconcile are differences between the present study and that of Yaksh *et al.* [38] on the rat. These authors used an extensive battery of behavioural tests, (tail flick, hot plate, pinch and shock titration) yet failed to find any effect with intra-

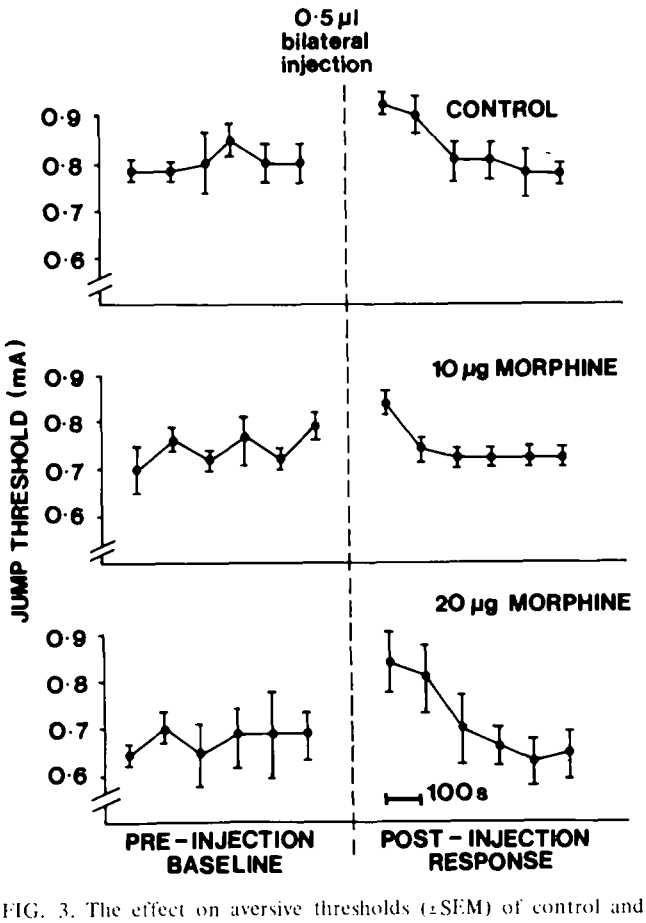

FIG. 3. The effect on aversive thresholds (±SEM) of control and morphine injections into the caudate-putamen.

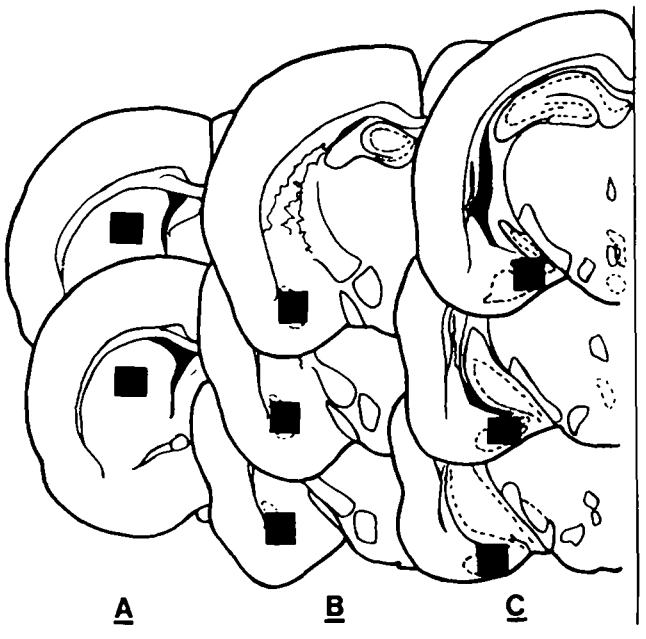

FIG. 4. Schematic summary of injection sites in (A) Caudate-putamen (A6670-A7470*), (B) basolateral amygdala (A5150-A4110), and (C) corticomedial amygdala (A3290-A2790). *Corresponding to A/P plates in Konig and Klippel [17].

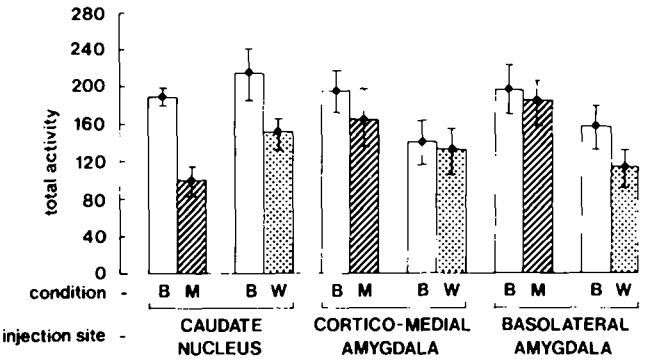

FIG. 5. Activity effects (±SEM) of control (W) and morphine (20 µg, M) injections into the caudate-putamen, cortico-medial amygdala and the basolateral amygdala.

amygdaloid morphine injections. Several possibilities are suggested for this discrepancy: firstly, the test battery did not include a test comparable to the flinch-jump test used in present study; secondly, the highest morphine dose injected into the amygdala was only 5 µg, compared to 20 µg (bilateral) used in present study; finally and perhaps most importantly, all microinjections in the Yaksh *et al* study [38] were made unilaterally.

Nonetheless, indirect support exists for involvement of amygdala in morphine activity. It has been reported that increasing doses of morphine (IP) decreases behavioural effects of amygdaloid stimulation, including vocalization [12], and that direct morphine injections exert profound effects upon amygdaloid EEG [36].

Morphine injections into the caudate-putamen initially appeared to result in a greater increase in aversive threshold than that observed in amygdala. However, casual observation suggested that these animals were sedated for a post-injection period of 2–5 min, an effect that could have rendered the animals incapable of performing the appropriate motor response. That a similar pattern of results was obtained following control injections indicated that motor impairment, perhaps related to nonspecific mechanical disruption of CPU tissue, was responsible for the raised aversive threshold. Indeed, the activity test revealed that, compared with other groups, the CPU animals under morphine or water exhibited less motor activity. It is suggested that this motor effect contributed to the reduced responsivity to aversive stimulation and that once this initial effect had dissipated, thresholds returned to predrug baseline levels. The effects on aversive threshold previously reported [13] following morphine injection into the caudate-putamen may therefore have been secondary to changes in motor activity.

The present paper provides evidence for an antinociceptive activity of morphine outside classical pain pathways, yet in an area rich in opiate receptor binding. It has earlier been noted that the amygdala possesses the greatest amount of opiate binding in the rat, monkey and human brain [18,35]. Further phylogenetic investigation has revealed that the olfactory nuclear regions (forerunners of the mammalian limbic system) in goldfish, dogfish and chick brain are highly enriched in opiate receptors [25]. This may provide a clue to the evolutionary importance of limbic sites in responsiveness to environmental stimulation, including pain. In support of this proposition, the amygdaloid complex has frequently been shown to exert a modulatory

influence on emotional responding; stimulation can produce attack, flight and defense [16], or escape responses and attempts to turn off stimulation [8]; lesions result in a paucity of emotional responses [9].

Clinical observations suggest that the primary action of morphine is not to inhibit pain perception per se but to attenuate emotional reactivity to pain [15]. Under influence of morphine, patients report that they still feel pain but are no longer worried or anxious about it's presence [3]. This flattening of affect is characteristic of amygdalectomy in both animals and man [9]. Present data confirm the importance of the amygdala to normal manifestation of responses to aversive stimuli. Since responses are attenuated, not totally blocked by intra-amygdaloid injection of morphine, it is suggested that this limbic site may function to modulate the intensity of responses to aversive stimulation. It is tempting to speculate that this function may correspond to the limbic motivational-affective control of pain, suggested by Melzack and Casey [23].

As reviewed above, morphine exerts it's most potent analgesic effect when injected into the periaqueductal gray matter [1, 19, 20, 21, 22, 28]. This analgesia has generally been attributed to a descending serotonergic inhibitory influence on the dorsal horn transmission cells in lamina V of the spinal cord [2, 7, 19, 21, 27, 30, 31]. However, the neurochemical mediation of a forebrain pain system has not yet received much research attention. Preliminary results suggest the involvement of dopaminergic mechanisms [1, 26, 31, 34], a finding that is consistent with the proximity of the morphine-sensitive lateral midbrain area to the cell origin of the ascending dopamine system in the substantia nigra [24]. This reticular area in the rat has recently been shown to be activated by noxious stimulation and that morphine decreases this activation [11].

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REFERENCES

1. Akil, H. and J. C. Liebeskind. Monoaminergic mechanisms of stimulation-produced analgesia. *Brain Res.* **94:** 279–296, 1975.
2. Balagura, S. and T. Ralph. The analgesic effect of electrical stimulation of the diencephalon and mesencephalon. *Brain Res.* **60:** 369–379, 1973.
3. Beecher, H. K. *Measurement of Subjective Responses. Qualitative Effect of Drugs.* New York: Oxford University Press, 1959.
4. Costall, B. and R. J. Naylor. The behavioural effects of dopamine applied intracerebrally to areas of the mesolimbic system. *Eup. J. Pharmac.* **32:** 87–92, 1975.
5. Costall, B., D. H. Fortune and R. J. Naylor. Biphasic changes in motor behaviour following morphine injection into the nucleus accumbens. *Br. J. Pharmac.* **57:** 423, 1976.
6. Cox, V. C. and E. S. Valenstein. Attenuation of aversive properties of peripheral shock by hypothalamic stimulation. *Science* **149:** 323–325, 1965.
7. Dahlstrom, A. and K. Fuxe. Evidence from the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain Stem neurons. *Acta Physiol. scand.* **62:** Suppl. 232: 1–55, 1965.
8. Delgado, J. M. R., H. F. Rosvold and E. Looney. Evoking conditioned fear by electrical stimulation of subcortical structures in the monkey brain. *J. comp. physiol. Psychol.* **49:** 373–380, 1956.
9. Eleftheriou, B. E. *The Neurobiology of the Amygdala.* New York: Plenum Press, 1972.
10. Evans, W. O. A new technique for the investigation of some analgesic drugs on a reflexive behaviour in the rat. *Psychopharmacologia* **2:** 318–325, 1961.
11. Haigler, H. J. Morphine: Ability to block neuronal activity evoked by a nociceptive stimulus. *Life Sci.* **19:** 841–858, 1976.
12. Hayward, J. N. Effects on drugs of abuse on motivated behaviour and magnocellular neuroendocrine cells. In: *Narcotics and the Hypothalamus,* edited by E. Zimmerman and R. George. New York: Raven Press, 1974, pp. 83–93.
13. Jacquet, Y. F. and A. Lajtha. Morphine action at central nervous system sites in the rat. Analgesia or hyperalgesia depending upon site and dose. *Science* **182:** 490–492, 1973.
14. Jacquet, Y. F. and A. Lajtha. The periaqueductal gray: Site of morphine analgesia and tolerance as shown by 2-way cross tolerance between systemic and intra-cerebral injections. *Brain Res.* **103:** 501–513, 1976.
15. Jaffe, J. H. Narcotic analgesics. In: *The Pharmacological Basis of Therapeutics,* edited by L. S. Goodman and A. Gilman. London: Collier-MacMillan, 1970, pp. 237–275.
16. Kaada, B. Brain mechanisms related to aggressive behaviour. In: *Aggression and Defence: Neural Mechanisms and Social Patterns,* Vol. 5, edited by C. D. Clemente and D. B. Lindsley. Brain Function. Los Angeles: University of California Press, 1967, pp. 95–135.
17. Konig, J. F. R. and R. A. Klippel. *The Rat Brain. A Stereotaxic Atlas.* Baltimore: Williams and Wilkins, 1963.
18. Kuhar, M. J., C. B. Pert and S. H. Synder. Regional distribution of opiate receptor binding in monkey and human brain. *Nature* **245:** 447–450, 1973.
19. Liebeskind, J. C., G. Guilbaud, J.-M. Besson and J. C. Oliveras. Analgesia from electrical stimulation of the periaquaductal gray matter in the cat: behavioural observations and inhibitory effects on spinal cord interneurons. *Brain Res.* **50:** 441–446, 1973.
20. Mayer, D. J. Pain inhibition by electrical brain stimulation: comparison to morphine. In: *Opiate Receptor Mechanisms,* edited by S. H. Synder and S. Matthyse. New York: MIT Press, 1975.
21. Mayer, D. J. and J. C. Liebeskind. Pain reduction by focal electrical stimulation of the brain: an anatomical and behavioural analysis. *Brain Res.* **68:** 73–93, 1974.
22. Mayer, D. J., T. L. Wolfe, H. Akil, B. Carder and J. C. Liebeskind. Analgesia from electrical stimulation in the brainstem of the rat. *Science* **174:** 1351–1354, 1971.
23. Melzack, R. and K. L. Casey. Sensory, motivational and central control determinants of pain: A new conceptual model. In: *The Skin Senses,* edited by D. Kenshalo. Springfield: Thomas, 1968, pp. 423–443.
24. Pert, A. and T. L. Yaksh. Sites of morphine induced analgesia in the primate brain: relation to pain pathways. *Brain Res.* **80:** 135–140, 1974.
25. Pert, C. B., D. Aposhian and S. H. Synder. Phylogenetic distribution of opiate receptor binding. *Brain Res.* **75:** 356–361, 1974.
26. Price, M. T. C. and H. C. Fibiger. Ascending catecholamine systems and morphine analgesia. *Brain Res.* **99:** 189–193, 1975.
27. Proudfit, H. K. and E. G. Anderson. Morphine analgesia: blockade by raphe magnus lesions. *Brain Res.* **98:** 612–618, 1975.

28. Reynolds, D. V. Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science* **164:** 444–445, 1969.
29. Rodgers, R. J., J. M. Semple, S. J. Cooper and K. Brown. Shock induced aggression and pain sensitivity in the rat: Catecholamine involvement in the cortico-medial amygdala. *Aggressive Behaviour* **2:** In press, 1976.
30. Ruda, M. A. An autoradiographic study of the efferent connections of the midbrain central gray in the cat. *Anat. Rec.* **181:** 468, 1975.
31. Saminin, R. and S. Bernasconi. Effects of intraventricularly injected 6-OH dopamine or midbrain raphe lesions on morphine analgesia in rats. *Psychopharmacologia* **25:** 175–182, 1972.
32. Simantov, R., M. J. Kuhar, G. W. Pasternak and S. H. Synder. The regional distribution of a morphine-like factor enkephalin in monkey brain. *Brain Res.* **106:** 189–197, 1976.
33. Sharpe, L. G., J. E. Garnett and T. J. Cicero. Analgesia & hyperreactivity produced by intracranial microinjections of morphine into the periaqueductal gray matter of the rat. *Behav. Biol.* **11:** 303–313, 1974.
34. Sparkes, C. G. and P. S. J. Spencer. Antinociceptive activity of morphine after injection of biogenic amines into the cerebral ventricles of the conscious rat. *Br. J. Pharmac.* **42:** 230–241, 1971.
35. Synder, S. H. Opiate receptor function in normal and drug altered brain. *Nature* **257:** 185–189, 1975.
36. Teitelbaum, H., J. Blosser and G. Catravas. Bilateral electroencephalographic response and unilateral tolerance to unilateral morphine injections. *Nature* **260:** 158–159, 1976.
37. Tilson, H. A., R. H. Rech and S. Stolman. Hyperalgesia during withdrawal as a means of measuring the degree of dependence in morphine dependent rats. *Psychopharmacologia* **28:** 287–300, 1973.
38. Yaksh, T. L., J. C. Yeung and R. A. Rudy. Systematic examination in the rat of brain sites sensitive to the direct application of morphine: Observation of differential effects within the periaqueductal gray. *Brain Res.* **114:** 83–103, 1976.