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Exploratory Behaviour and Aversive Thresholds Following Intra-Amygdaloid Application of Opiates in Rats

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RODGERS, R. J. AND S. E. FILE. *Exploratory behaviour and aversive thresholds following intra-amygdaloid application of opiates in rats.* PHARMAC. BIOCHEM. BEHAV. 11(5) 505–511, 1979.—Rats were bilaterally implanted with guide cannulae aimed at the central or medial nucleus of the amygdala. Microinjections of morphine (10μg) at both sites significantly elevated the threshold of response in the flinch-jump test: but only at medial sites did naloxone (1μg) antagonise this effect. However, in the hole-board test, an opposite pattern of results emerged. Morphine injections into the central nucleus produced naloxone-reversible reductions in both exploration and activity whilst, in the medial nucleus, the morphine-induced decrease in exploration was not reversed by naloxone. It is concluded that (1) the presence or absence of naloxone-sensitive opiate receptors cannot always be deduced on the basis of a single behavioural test and (2) within the amygdaloid complex, two distinct naloxone-sensitive opiate systems appear to be involved in the modulation of behavioural responses to different forms of stimulation.

Morphine Naloxone Aversive thresholds Exploration Motor activity Central amygdala Medial amygdala

WITHIN the past decade, considerable advances have been made in our understanding of the central sites at which the opiates exert their potent behavioural effects ([35]-for review). Specific opiate binding sites and natural ligands (enkephalins/endorphins) have been located at levels from the spinal cord through the midbrain to various forebrain structures [2, 3, 4, 21, 26, 31, 33] . Strong analgesic responses have been produced by microinjection of morphine in spinal cord [34] and midbrain [18, 27, 28, 36] areas. Consequently, it has been argued that the analgesic effectiveness of morphine relates to its ability to mimic endogenous ligands at these sites. Such suggestions are supported by the findings that both brain-stimulation analgesia [1, 16] and acupuncture analgesia [18] are blocked by opiate antagonists.

However, it is perhaps misleading to consider this endogenous system as functioning only in relation to pain mechanisms. Indeed, a general role for these peptides in the mediation of stress responding has recently been proposed [29]. More specifically, several studies have argued for the involvement of the endogenous opiate system in exploration [12], sexual behaviour [6, 20], social affect [14, 22] and mental illness [30]. In this context, it should be noted that very high densities of opiate receptors and ligands are found in regions outside classical pain pathways. One such area is the amygdaloid complex [4], a subcortical structure more traditionally associated with limbic regulation of affective behaviour.

Affective responses to aversive stimulation are strongly inhibited by lesions of the medial amygdala [15], an effect that has been equated with the action of narcotic analgesics [7]. Recent results from our own laboratory support these findings. Microinjection of morphine into this limbic area, whilst ineffective in altering non-emotive spinal reflexes to pain, produced naloxone-reversible elevations in the more emotive jump response to electric shock [24, 25]. This profile suggested that a possible function of the amygdaloid opiate system may be to decrease affective responding in stressful situations.

To investigate further the possible behavioural significance of the amygdaloid opiate system(s), the current study examined the effects of intra-amygdaloid opiate injections on two behavioural tests: responses to a novel environment (hole-board test) and reactivity to electric shock (flinch-jump test). Two injection sites within the amygdaloid complex (central and medial nuclei) were chosen on the basis of differential opiate receptor and peptide distribution [4, 26, 31, 32].

METHOD

Animals and Surgery

One hundred and forty-eight male hooded rats (Olac Ltd, Bicester), weighing 300–350 g, were anaesthetised with Equithesin (4.0 ml/kg) and bilaterally implanted with stainless-steel guide cannulae (0.6 mm o.d.) aimed at sites 2 mm dorsal to the central (A/P: + 6.0, L: ± 3.9, V: 8.5) or medial (A/P: + 5.2, L: ± 3.5, V: 9.5) nucleus of the amygdala. Stereotaxic coordinates were based on calculations derived from the atlas of Pellegrino and Cushman [23] and level-head surgical procedure was adopted. Guide cannulae were kept free by flush-fitting stylets (0.3 mm o.d.) which, during injection, were replaced by injection units extending 2 mm beyond the guide tips. The rats were housed in groups of six for two weeks following surgery and then individually for one week prior to behavioural testing. They were maintained in a room with a constant light-dark cycle (lights on: 0600 hr/lights off: 1700 hr) and with food and water available ad libitum.

Apparatus

Hole-board. This consisted of a wooden box (65 x 65 x 45 cm) with four equally-spaced holes in the floor. Infra-red photocells placed just under the holes provided automated measures of exploration (number of head-dips; times spent head-dipping). This method of measuring exploration has been validated by File and Wardill [11]. Similar cells in the walls of the box provided an automated measure of motor activity.

Aversive thresholds. Reactivity to electric footshock was determined in a small test chamber(18 x 15 x 13 cm). Scrambled electric shock, from a Grason-Stadler shock source (Model 700) was supplied through the grid bars in the floor of the chamber.

Injection Technique and Drugs

Whilst hand-held, each animal received bilateral microinjections from two micrometer-driven Hamilton microsyringes (10 μl; Model 701N). All injections were made in a volume of 0.5 μl over a period of 40s and were administered immediately prior to behavioural testing.

Morphine sulphate (May and Baker Ltd) was used in a concentration of 10μg/0.5 μl and naloxone hydrochloride (Endo Lab. Inc.) in a concentration of 1 μg/0.5 μl. Both drugs were dissolved in sterile water which alone served for control injections. Sterile water was used in preference to saline as we have previously found behavioural change with amygdaloid saline injections (unpublished observations). Such changes were not apparent when sterile water was used. A further solution, containing both morphine and naloxone (10 μg + 1 μg, respec.) was also used.

Procedure

Within each placement site (central or medial amygdala) rats were randomly allocated to the hole-board or aversive threshold tests and then randomly assigned to vehicle (sterile water), morphine sulphate (10 μg), naloxone hydrochloride (1 μg) or morpine plus naloxone (10 μg + 1 μg) groups.

The rats tested in the holeboard received a single 10 min trial and were tested in a randomised order between 0800 and 1200 hr. After each trial, the rat was removed and the box thoroughly cleansed and dried. Aversive thresholds were measured using a modification of the flinch-jump technique [8, 24]. Rats were individually placed in the test chamber and received six series of eight electric shocks (0.5 sec duration at 10 sec intervals). Shocks were administered in alternating ascending and descending series with intensities ranging between 0.1—0.8 mA. For each series, the jump threshold (the intensity at which the animals hind feet left the grid bars) was determined. This procedure was carried out both before and after injection and comparisons made between baseline (preinjection) and response (postinjection) values.

Prior to sacrifice, animals received bilateral microinjections of trypan blue (0.5 μl) in order to aid localisation of injection sites. They were then overdosed with sodium pentobarbitone, intracardially perfused with formal saline and their brains retained for histological examination. Sections were cut on a freeze microtome and injection sites plotted on line drawings based on the atlas of Pellegrino and Cushman [23].

Statistics

The data from the holeboard were analyzed by a two-factor (morphine and naloxone) analysis of variance for each placement site. The results from the flinch-jump test were analyzed in two stages: first, the baseline thresholds across groups were compared using a one-factor analysis of variance; secondly, comparisons between baseline and post injection thresholds were made within each treatment group using two-tailed related *t*-tests.

RESULTS

Histology

Histological examination revealed that, in most cases, injection sites were accurately positioned within the desired amygdaloid nucleus. However, in several animals, cannulae were asymmetrically positioned (5 central animals/2 medial animals) and/or injections had considerably diffused to adjacent amygdaloid or caudate-putamen tissue (2 central animals/2 medial animals). The data from these animals were discarded from the statistical analyses. Figure 1 illustrates the area covered by injections within each placement group.

Holeboard Test

Morphine injections into the medial amygdala significantly reduced the mean time spent head-dipping, $F(1,38)=5.9$, $p< 0.02$, but this effect was not reversed by naloxone (see Fig. 2). At this site, morphine did not significantly alter either the number of head-dips or the motor activity score. Naloxone alone had no significant effect on any measure.

In the central amygdala, morphine produced naloxone-reversible decreases in the number of head-dips made, $F(1,42)=6.1$, $p<0.02$, and in motor activity, $F(1,42)=6.1$, $p<0.02$; naloxone alone was without effect on these measures (see Fig. 3). Both morphine and naloxone when given alone reduced the mean time spent head-dipping, but the rats injected with a combination of these drugs did not differ significantly from the controls, thus giving a singificant morphine x naloxone interaction effect, $F(1,42)=15.1$, $p<0.001$. See Fig. 3.

Flinch-Jump Test

There were no significant differences between groups on

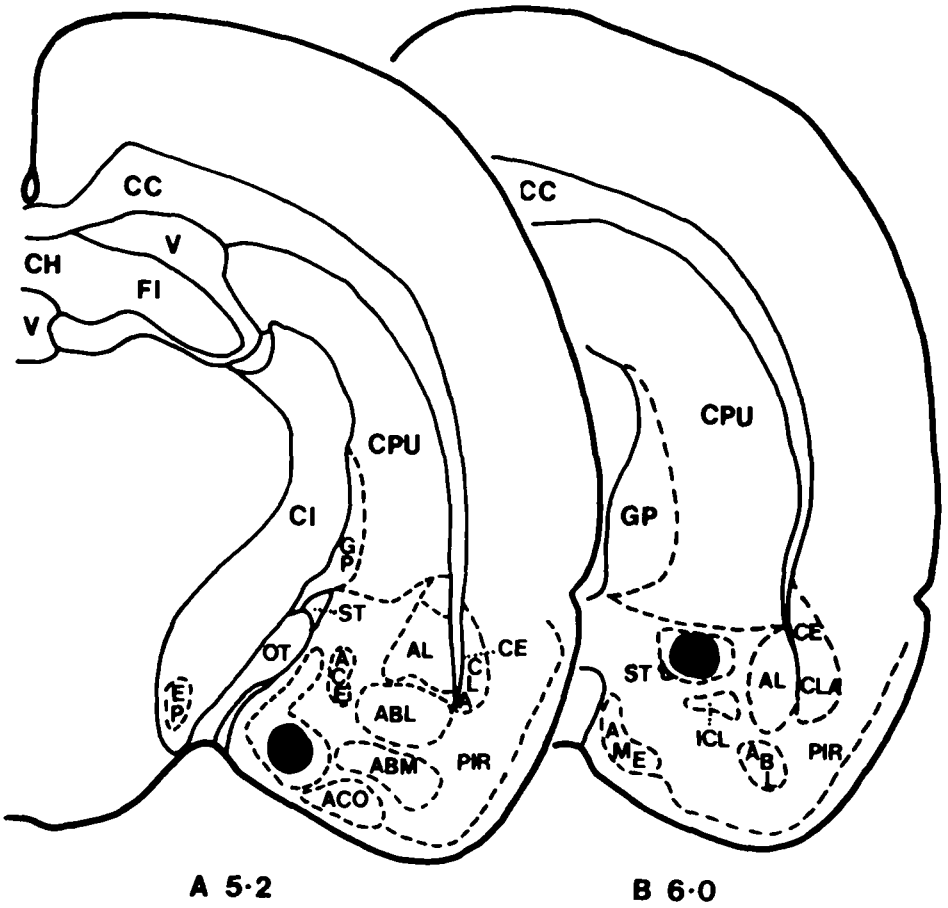

Fig. 1. Schematic summary of injection sites in (A) medial amygdala and (B) central amygdala. Line drawings adapted from the atlas of Pellegrino and Cushman [23].

baseline (i.e. preinjection) jump thresholds. In the medial amygdala, morphine significantly elevated the jump threshold, $t(7)=6.3$, $p<0.001$, an effect reversed by naloxone (see Fig. 4). No significant changes in thresholds were found after naloxone or vehicle injections into this area.

Morphine injections into the central amygdala significantly elevated the jump threshold, $t(6)=4.0$, $p<0.01$, but this effect was not antagonised by naloxone:—the group receiving the combination of morphine and naloxone also exhibited elevated thresholds, $t(5)=6.2$, $p<0.01$. Injections of vehicle or of naloxone alone were without effect in the central nucleus. See Fig. 5.

DISCUSSION

From the results presented in this paper, it can be seen that the presence or absence of naloxone-sensitive opiate receptors cannot always be deduced on the basis of a single behavioural test. The elevation of aversive thresholds produced by morphine injections into the central amygdala was not naloxone-reversible; whereas the morphine-induced reductions in motor activity and exploration were reversed by naloxone—even in the one case (time spent head-dipping) when injections of naloxone alone had produced similar effects to those seen after morphine. In contrast, morphine injections into the medial amygdala resulted in naloxone-reversible increases in aversive thresholds but non-naloxone reversible changes in exploration. Had the current study used only one behavioural test, it might have been concluded erroneously that naloxone-sensitive opiate receptors did not exist at one or other injection site. It would thus seem imperative to use more than one behavioural test when assessing the effects of intracranially-administered opiates.

Since some of the behavioural effects of morphine at each site were not antagonised by naloxone it would seem logical, it not tautological, to argue that these effects were mediated via morphine interaction with naloxone-insensitive opiate receptors. Indeed similar suggestions have recently been made concerning responses observed after morphine injections into other brain areas such as the periaqueductal gray matter [17] and midbrain reticular formation [13]. However, alternative explanations for the non-naloxone reversible effects may exist and since we have not determined whether these effects are stereospecific or whether they are produced by other pure agonists, further studies are required before this particular issue can be resolved.

The present results are both consistent and at variance with our previous findings. Naloxone-reversible elevations in aversive thresholds following morphine injections into the medial aspect of the amydgala confirm our earlier work but the present observation that morphine injections into the central nucleus produce elevated thresholds (although non-

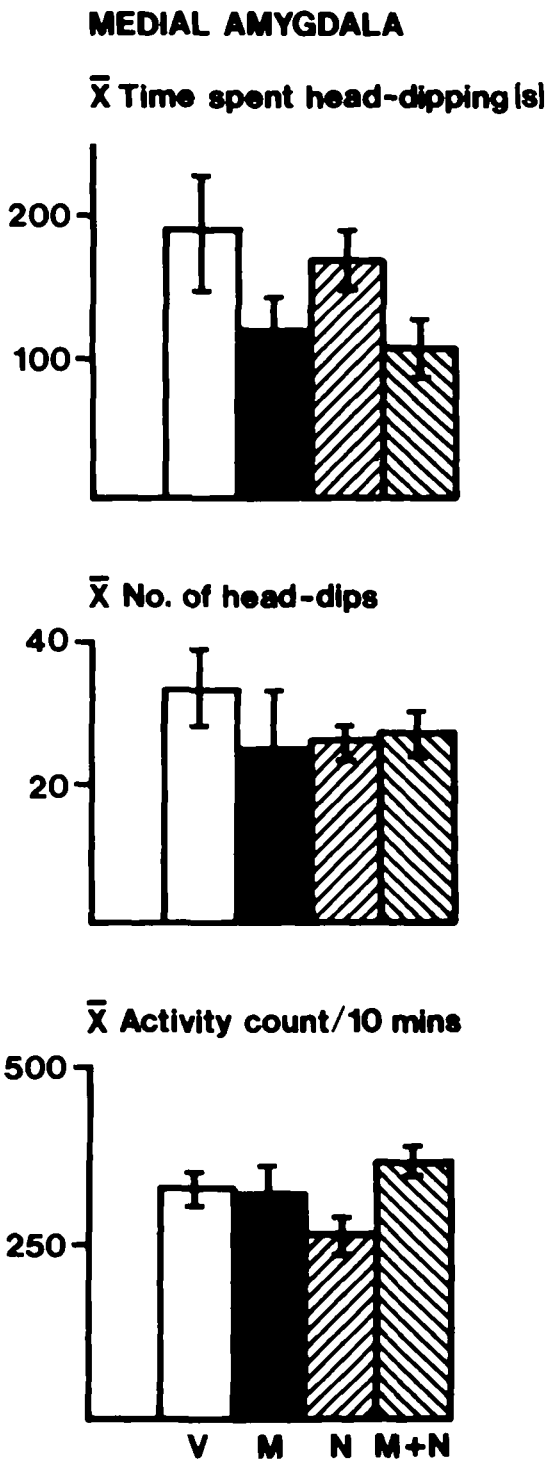

Fig. 2. Effects on holeboard measures (X ± SEM) of microinjection of vehicle (V), morphine (M), naloxone (N) or morphine plus naloxone (M+N) into the medial amygdaloid nucleus.

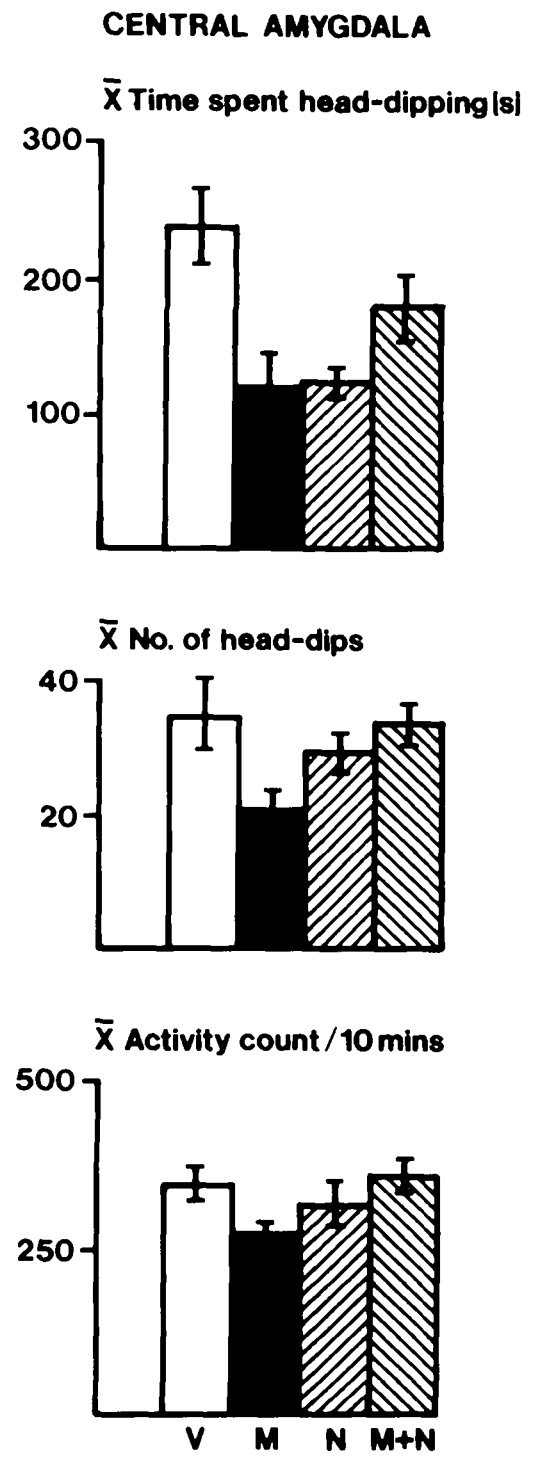

FIG. 3. Effects on holeboard measures (X̄ ± SEM) of microinjection of vehicle (V), morphine (M), naloxone (N) or morphine plus naloxone (M+N) into the central amygdaloid nucleus.

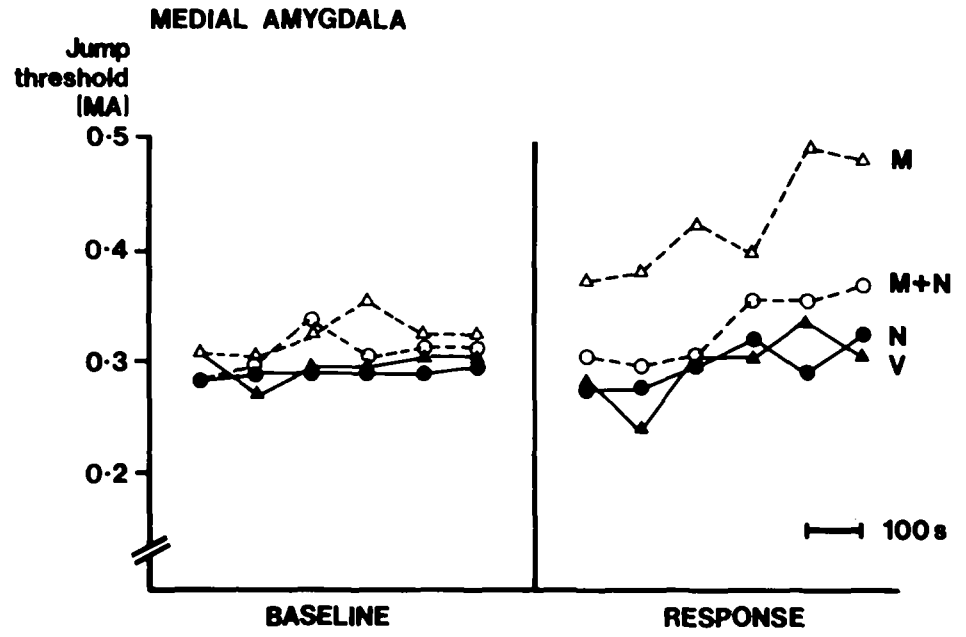

FIG. 4. Effects on jump thresholds (X) of micorinjection of vehicle (V), morphine (M), naloxone (N) or morphine plus naloxone (M·N) into the medial amygdaloid nucleus. Baseline refers to preinjection threshold and response to postinjection thresholds. Each data point represents the mean jump threshold for each shock series. See text for details.

naloxone reversible) contrasts with previous negative findings at this site [25]. The most likely interpretation of this inconsistency relates to localisation of injections within the central amygdaloid nucleus. Whereas in the present study injections were localised within the body of this nucleus, the ineffective sites in the earlier investigation were located more laterally.

The decrease in both exploration and motor activity following central amygdaloid injections of morphine could be interpreted as a general sedative effect of this treatment. However, in a parallel study [10], morphine injections into the central amygdala did not depress social interaction between pairs of male rats and neither was locomotor activity of the pairs decreased. In fact, when the test arena was un-

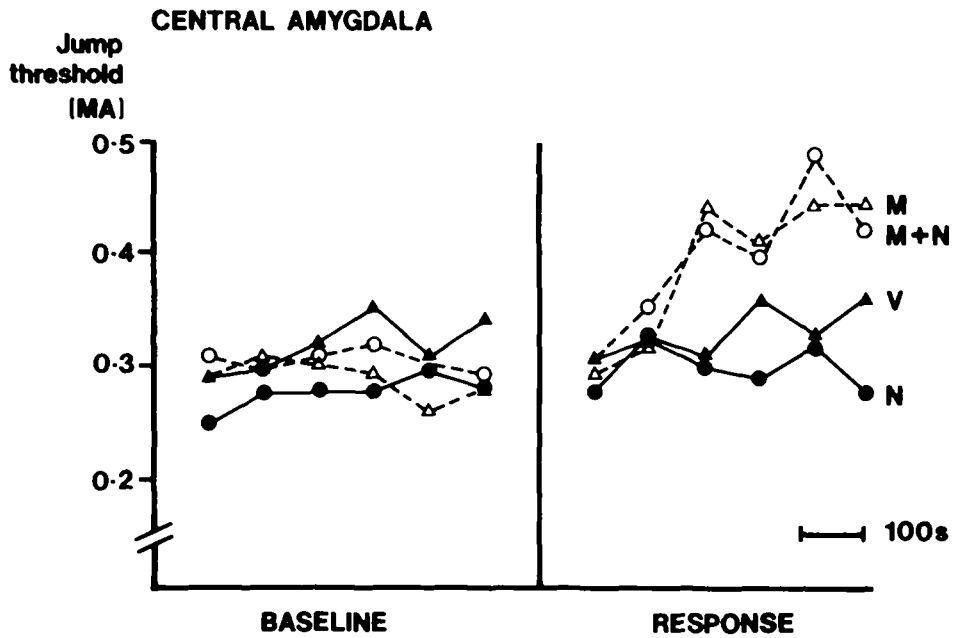

FIG. 5. Effects on jump thresholds ($\bar{X}$) of microinjection of vehicle (V), morphine (M), naloxone (N) or morphine plus naloxone (M·N) into the central amygdaloid necleus. See legend for Figure 4 for details.

familiar to the rats, morphine injections into the central nucleus significantly increased both social interaction and locomotor activity. The presence of another rat can modify a drug's effect on behaviour. For example, chlorpromazine reduces exploration in the holeboard when rats are tested singly, but does not reduce exploration when the rats are tested in pairs [9]. Thus, whilst the direction of the effect of morphine in the central amygdala may depend upon the social situation, this treatment does seem particularly to modify behaviour in novel situations.

Naloxone-sensitive opiate systems in different amygdaloid nuclei seem to be involved in modulating behavioural responses to different kinds of stimulation. It would appear that the central nucleus may be involved in mediating responses to a novel environment whilst the medial nucleus is more concerned with an organism's reactivity to painful stimulation. These different patterns may relate to the differential involvement of enkephalinergic and endorphinergic systems in behavioural regulation. For example, it is known that enkephalins and endorphins exist within totally separate neuronal systems in the brain [32]. More specifically, the medial amygdala receives a β-endorphinergic input from the basal hypothalamus [5] whilst the central nucleus contains a dense network of enkephalinergic interneurons [26]. Thus although both nuclei possess opiate receptors, the natural ligand in each case may differ.

Finally, our results emphasize that the amygdala is a subcortical complex of nuclei, each of which may be involved in different aspects of behavioural regulation. Thus it would no longer appear appropriate, as many still do, to view this limbic area as a functionally homogeneous structure.

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REFERENCES

1. Akil H., D. J. Mayer and J. C. Liebeskind. Antagonism of stimulation-produced analgesia by naloxone, a potent narcotic antagonist. *Science* **191:** 961–962, 1976.
2. Atweh S. F. and M. J. Kuhar. Autoradiographic localisation of opiate receptors in rat brain. I. Spinal cord and lower medulla. *Brain Res.* **124:** 53–67, 1977.
3. Atweh, S. F. and M. J. Kuhar. Autoradiographic localisation of opiate receptors in rat brain. II. The Brain Stem. *Brain Res.* **129:** 1–12, 1977.
4. Atweh, S. F. and M. J. Kuhar. Autoradiographic localisation of opiate receptors in rat brain. III. The telencephalon. *Brain Res.* **134:** 393–405, 1977.
5. Barchas, J. D., H. Akil, G. R. Elliott, R. B. Holman and S. J. Watson. Behavioural neurochemistry: neuroregulators and behavioural states. *Science* **200:** 964–973, 1978.
6. Bertolini, A., S. Genedani and M. Castelli. Behavioural effects of naloxone in rats. *Experientia* **34:** 771–772, 1978.
7. Charpentier, J. Analysis and measurement of pain in animals. In: *Pain* edited by A. Soulairac, J. Cahn and J. Charpentier. New York. Academic Press, 1968, pp. 171–200.
8. Evans, W. O. A new technique for the investigation of some analgesic drugs on a reflexive behaviour in the rat. *Psychopharmacologia* **2:** 318–325, 1969.
9. File, S. E. and J. H. Pope. The action of chlorpromazine on exploration in pairs of rats. *Psychopharmacologia* **37:** 249–254, 1974.
10. File, S. E. and R. J. Rodgers. Partial anxiolytic action of morphine sulphate following microinjection into the central nucleus of the amygdala in rats. *Pharmac. Biochem. Behav.* **11:** 313–318, 1979.
11. File, S. E. and A. G. Wardill. Validity of head-dipping as a measure of exploration in a modified holeboard. *Psychopharmacologia* **44:** 53–59, 1975.
12. Grevert, P. and A. Goldstein. Some effects of naloxone on behaviour in the mouse. *Psychopharmacology* **53:** 111–113, 1977.
13. Haigler, H. J. and D. D. Spring. A comparison of the analgesic and behavioural effects of (D-Ala^2) met-enkephalinamide and morphine in the mesencephalic reticular formation of rats. *Life Sci.* **23:** 1229–1230, 1978.
14. Herman, B. H. and J. Panksepp. Effects of morphine and naloxone on separation distress and approach attachment: evidence for opiate mediation of social affect. *Pharmac. Biochem. Behav.* **9:** 213–220, 1978.
15. Hoffmeister, F. Effects of psychotropic drugs on pain. In: *Pain* edited by A. Soulairac, J. Cahn and J. Charpentier. New York. Academic Press, 1968, pp. 309–319.
16. Hosobuchi, Y., J. E. Adams and R. Linchitz. Pain relief by electrical stimulation of the central gray matter in humans and its reversal by naloxone. *Science* **197:** 183–186, 1977.
17. Jacquet, Y. F. Opiate effects after adrenocorticotropin or β-endorphin in the periaqueductal gray matter of rats. *Science* **197:** 183–186, 1977.
18. Jacquet, Y. F. and A. Lajtha. Morphine action at central nervous system sites in the rat: analgesia or hyperalgesia depending on site and dose. *Science* **182:** 490–492, 1973.
19. Mayer, D. J., D. D. Price, J. Barber and A Rafii. Acupuncture analgesia: Evidence for activation of a pain inhibitory system as a mechanism of action. In: *Advances in Pain Research and Therapy* edited by J. J. Bonica and D. Albe-Fessard. New York: Raven Press, 1976, pp. 751–754.
20. Meyerson, B. J. and L. Terenius. β-endorphin and male sexual behaviour. *Eur. J. Pharmac.* **42:** 191–192, 1977.
21. Neale, J. H., J. L. Barker, G. H. Uhl and S. H. Synder. Enkephalin-containing neurons visualized in spinal cord cell cultures. *Science* **201:** 467–469, 1978.
22. Panksepp, J., B. Herman, R. Conner, P. Bishop and J. P. Scott. The biology of social attachments: opiates alleviate separation distress. *Biol. Psychiat.* **13:** 607–618, 1978.
23. Pellegrino, L. J. and A. J. Cushman. *A Sterotaxic Atlas of the Rat Brain*. New York: Appleton-Century-Crofts, 1967.
24. Rodgers, R. J. Elevation of aversive threshold in rats by intraamygdaloid injection of morphine sulphate. *Pharmac. Biochem. Behav.* **6:** 385–390, 1977.
25. Rodgers, R. J. Influence of intra-amygdaloid opiate injection on shock thresholds, tail-flick latencies and open field behaviour in rats. *Brain Res.* **153:** 211–216, 1978.
26. Sar, M., W. E. Stumpf, R. J. Miller, K. J. Chang and P. Cuatrecasas. Immunohistochemical localisation of enkephalin in rat brain and spinal cord. *J. comp. Neurol.* **182:** 17–38, 1978.
27. Sharpe, L. G., J. E. Garnett and T. J. Cicero. Analgesia and hyperreactivity produced by intracranial microinjections of morphine into the periaqueductal gray matter of the rat. *Behav. Biol.* **11:** 303–313, 1974.
28. Takagi, H., M. Satoh, A. Akaike and T. Shibata. The nucleus reticularis gigantocellularis of the medulla oblongata is a highly sensitive site in the production of morphine analgesia in the rat. *Eur. J. Pharmac.* **3:** 91–92, 1977.
29. Torda, C. Effects of recurrent postnatal pain-related stressful events on opiate receptor-endorgenous ligand system. *Psychoneuroendocrinol.* **3:** 85–91, 1978.
30. Verebey, K., J. Volavka and D. Clouet. Endorphins in psychiatry. *Arch. Gen. Psychiat.* **35:** 877–888, 1978.

31. Watson, S. J., H. Akil, S. Sullivan and J. D. Barchas. Immunocytochemical localisation of methionine enkephalin: preliminary observations. *Life Sci.* **21:** 733–738, 1977.
32. Watson, S. J., H. Akil, C. W. Richard and J. D. Barchas. Evidence for two separate opiate peptide neuronal systems. *Nature* **275:** 226–228, 1978.
33. Watson, S. J., J. D. Barchas and C. H. Li. β-lipotropin: localisation of cells and axons in rat brain by immunocytochemistry. *Proc. natl. Acad. Sci.* **74:** 5155–5158, 1977.
34. Yaksh, T. L. and T. A. Rudy. Analgesia mediated by a direct spinal action of narcotics. *Science* **192:** 1357–1358, 1976.
35. Yaksh, T. L. and T. A. Rudy. Narcotic analgesia: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. *Pain* **4:** 299–359, 1978.
36. Yaksh, T. L., J. C. Yeung and T. 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.