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Serotonergic Reduction of Dorsal Central Gray Area Stimulation-Produced Aversion

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KISER, R. S., D. C. GERMAN AND R. M. LEBOVITZ. *Serotonergic reduction of dorsal central gray area stimulation-produced aversion.* PHARMAC. BIOCHEM. BEHAV. 9(1) 27–31, 1978.—Stimulating electrodes were implanted into the dorsal central gray area (DCG) of rats. The animals were trained to bar press to decrement the aversive DCG stimulation current. Rats treated with 5-hydroxytryptophan (5-HTP), 75 mg/kg or 150 mg/kg, showed a dose-dependent reduction in decremental bar pressing. In a second study, animals received either chlorimipramine, 15 mg/kg, protriptyline, 15 mg/kg, or 5-HTP, 150 mg/kg. Chlorimipramine, a strong blocker of serotonin reuptake, and 5-HTP produced significant reductions in decremental bar pressing. Protriptyline, a weak serotonin reuptake blocker, produced no significant effect. These results suggest that serotonin reduces aversive neural mechanisms associated with the dorsal central gray area.

Aversion Serotonin Pain mechanisms Stimulation-produced analgesia Tricyclic antidepressants

A NUMBER of studies have suggested that the central gray area is involved in central pathways for pain integration and relay. The dorsolateral part of the central gray area receives collaterals from paleospinothalamic pain pathways [36]; and electrical stimulation of the dorsal central gray area (DCG) produces aversive behavior which has been described as either "fearlike" [26,40] or "painlike" [53].

We have observed that DCG stimulation-produced aversion in rats is apparently affected by manipulations of brain serotonin (5-HT) function. The serotonin-depleting drug, para-chlorophenylalanine (PCPA), increases decremental bar pressing to escape DCG stimulation [29,30], and these findings suggest that 5-HT decreases the aversion associated with DCG stimulation.

Such findings are consistent with other studies which have also found a role for 5-HT in reducing pain responsivity. Lowered pain thresholds have been reported in rats following forebrain 5-HT depletion produced either by lesions which destroy ascending 5-HT axons or by administration of PCPA [22, 32, 55, 64, 65], and these lowered pain thresholds have been reversed by the administration of the serotonin precursor 5-hydroxytryptophan (5-HTP) [23,55]. Serotonin has been implicated in the antinociceptive actions of opiates. Morphine analgesia has been enhanced by increasing brain 5-HT levels by either systemically administered 5-HTP [13,46] or intraventricularly administered 5-HT [6,50]. Morphine analgesia has been reduced by 5-HT depletion induced by a variety of drugs [14, 18, 49, 54, 56, 59] and by lesions of 5-HT nuclei and axons [17, 42, 43, 47, 48, 62]. Potent analgesia results from intracerebral morphine injections into the area around the serotonergic dorsal raphe nucleus (DRN) [63], and the antinociceptive effects of morphine microinjected into this area are blocked by the 5-HT antagonists, methysergide and cinanserin [61].

To further test the hypothesis that 5-HT reduces the aversion of DCG stimulation, in the present study we have administered drugs which enhance 5-HT function. The drugs used were 5-HTP, and the two tricyclic antidepressant drugs, chlorimipramine and protriptyline. 5-HTP facilitates 5-HT function by increasing total brain 5-HT levels [19], and chlorimipramine is a potent blocker of 5-HT reuptake [7, 8, 35, 51]. Protriptyline only weakly blocks 5-HT reuptake [8, 16, 51].

METHOD

Male albino rats weighing approximately 250 g at the time of surgery were used in these studies. The details of the electrode implantation surgery have been described elsewhere [29]. The animals were stereotaxically implanted with a bipolar electrode whose tip was aimed at the DCG (Coordinates: AP=−0.25 mm, L=+0.7 mm, V=−0.1 mm) [52]. The electrode was insulated except for the two 0.2 mm dia. cross-sections at the tip. After surgery and throughout the course of the experiments, all animals were individually housed with unlimited access to food and water.

One week after surgery, the animals were screened for aversive stimulation effects. The animals were placed into a behavioral box enclosed in a sound-proof isolation chamber (BRS Foringer). The stimulation current consisted of trains of capacity-coupled negative square wave pulses (5 trains/sec, train duration=100 msec, stimulation pulse duration=0.5 msec, stimulation frequency=60 Hz). Animals were shaped to escape brain stimulation by bar pressing. Those animals which demonstrated stable escape bar press-

ing at stimulation currents below 500 μA (peak pulse) were next trained in a decremental bar-pressing paradigm. In this paradigm the animals were presented with 120-sec stimulation periods alternating with 60-sec rest periods in which no stimulation occurred. At the beginning of each stimulation period, the stimulation current was initiated at a level which was pre-selected by the experimenter. During the stimulation periods each bar press decremented the stimulation current by 5% of its initial level. The 5% decrementation occurred up to a total of nine bar presses. The tenth bar press terminated the stimulation and started a rest period. Should ten bar presses not occur in a stimulation period, the following rest period was automatically started at the end of 120 sec of stimulation. A stimulation current level was chosen for each animal such that its average baseline performance was to reduce the stimulation current level by approximately 20–35 percent (i.e., 4–7 bar presses). In each experiment the baseline level of decremental bar pressing consisted of the average number of decremental bar presses in ten consecutive stimulation periods. Each animal then received an intraperitoneal injection of the drug in question and was immediately returned to the behavioral box and run at the same stimulation parameters for 40 stimulation periods (2 hr) and again for ten stimulation periods at 24 hr postinjection.

In the first study, each animal received an injection of either 5-HTP (75 mg/kg), 5-HTP (150 mg/kg), or normal saline, chosen in random order. The 5-HTP (L-5-Hydroxytryptophan methyl ester HCl- Calbiochem) was dissolved in 0.6 ml of normal saline and administered intraperitoneally. In following weeks, each animal eventually received all of the above drug injections in experiments separated by at least one week. Separate animals were used in the second study. In this experiment, the animals received intraperitoneal injections of either chlorimipramine (Anafranil; Ciba-Geigy) (15 mg/kg), protriptyline (Vivactyl; Merck, Sharp & Dohme) (15 mg/kg), 5-HTP (150 mg/kg), or normal saline. As in the first study, the order of drug injections was random, and all animals received all drugs in experiments at least one week apart. After the completion of the studies, the animals were sacrificed with an overdose of sodium pentobarbital and perfused transcardially with neutral buffered Formalin. The locations of the stimulating electrode tips were determined from 50 micron thick frozen brain sections stained with cresyl violet.

RESULTS

Histological examination showed that the stimulating electrode tips were located in the caudal portion of the dorsal central gray area and the immediately adjacent tegmentum. A typical stimulation site is shown in Fig. 1. Stimulation at these sites produced general agitation with frantic rearing and running, as if the animal were searching the behavioral box for exits. Occasionally urination, defecation and/or piloerection were seen. During initial animal screening, these behaviors appeared to be produced in direct proportion to the stimulation current intensity. As the animals reduced the current by bar pressing, their frantic, escape-seeking behavior progressively diminished to a point at which they could sit undisturbed in the behavioral box.

In the first study, 5-HTP reduced decremental bar pressing in a dose-dependent manner (Fig. 2). A two-factor analysis of variance was conducted on the 5-HTP data, and significant F values were found for the drug treatment effect ($p=0.002$) and the trials effect ($p<0.001$). A subsequent Student Newman-Keuls analysis revealed that 5-HTP at both 75 and 150 mg/kg produced a significant reduction in bar pressing compared to normal saline ($p<0.01$ in each case), and the reduction at 150 mg/kg was significantly greater than that produced by 75 mg/kg ($p<0.05$). One hr after receiving either dose of 5-HTP, the level of motor agitation produced at stimulation onset was greatly attenuated. There were no signs of sedation or motor impairment after either dose of 5-HTP, and the animals remained alert and normally active throughout the course of the experiment.

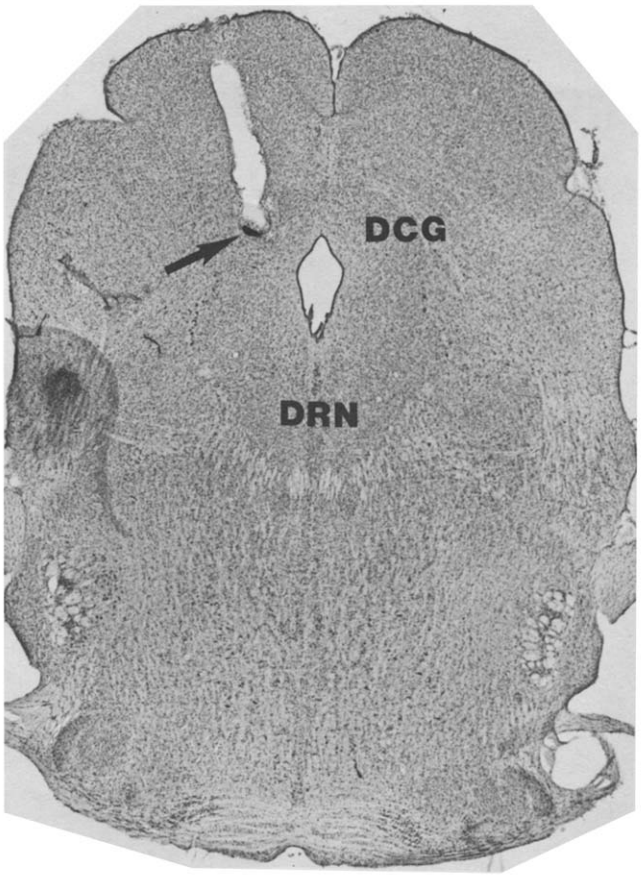

FIG. 1. Histological section showing a stimulating electrode tip (arrow) lying in the dorsal central gray area. Cresyl violet stain. Legend—DCG: dorsal central gray, DRN: dorsal raphe nucleus.

The results of the second study are shown in Fig. 3. A two-factor analysis of variance was conducted on the data of this study. Significant F values were found for the effects of both drug treatment ($p=0.008$) and trials ($p=0.002$). A subsequent Student Newman-Keuls analysis showed that the reduction in bar pressing after chlorimipramine and 5-HTP each differed significantly from normal saline ($p<0.01$) and from protriptyline ($p<0.01$), but the effects of chlorimipramine and 5-HTP did not differ significantly from each other ($p>0.05$). There was no significant difference between the effects of protriptyline and normal saline ($p>0.05$). By 60 min after receiving either chlorimipramine or 5-HTP, the animals' general motor agitation during stimulation periods was substantially less than at baseline. At all times the animals receiving the experimental drugs appeared alert, with

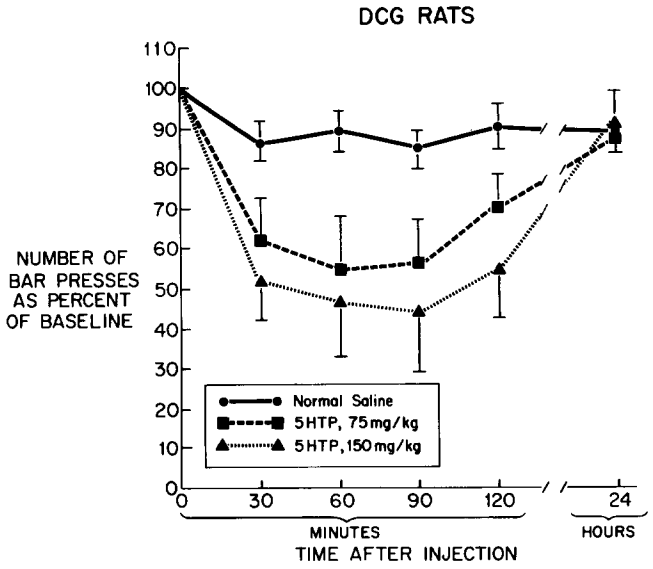

FIG. 2. Effects of 5-hydroxytryptophan on decremental bar-pressing performance (N=8). Each point represents the mean (± SEM) for 10 stimulation periods in all rats. Number of bar presses is expressed as a percentage of baseline.

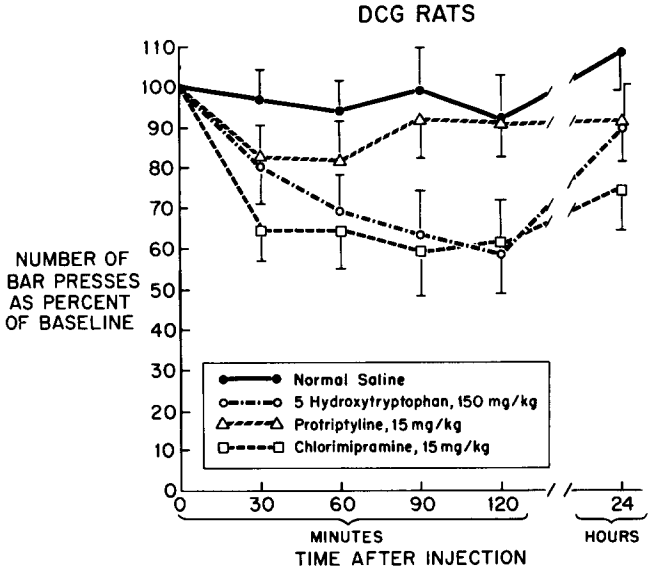

FIG. 3. Effects of tricyclic antidepressant drugs and 5-hydroxytryptophan on decremental bar pressing performance (N=15). The data are shown in the same manner as described for Fig. 2.

normal motor function, and in this respect were indistinguishable from saline-injected controls.

DISCUSSION

Several laboratories have reported that DCG stimulation is aversive [1, 11, 12, 26, 39, 40, 45, 53, 58]. The aversive nature of DCG stimulation may derive from the fact that this area receives collaterals from paleospinothalamic pain pathways [36] which are concerned with protopathic, or affective pain sensation [4,9]. The central gray area in turn has afferent and efferent connections with numerous limbic, hypothalamic, and thalamic sites [5, 21, 38]. This nodal position of the central gray area between paleospinothalamic pain pathways and forebrain limbic, hypothalamic, and thalamic structures has led to the idea that the central gray area has a role in the integration of protopathic pain stimuli with "painful" affects such as distress, suffering, and fear [24,37].

The results of the present studies support the hypothesis that 5-HT mechanisms reduce the aversion associated with DCG stimulation in rats. 5-Hydroxytryptophan, at doses of 75 mg/kg and 150 mg/kg, is reported to increase brain 5-HT concentration by 125 and 200%, respectively [19]. The time course of these 5-HT elevations corresponds closely to the time course of the reduction in decremental bar pressing which was seen in this study. The doses of chlorimipramine and protriptyline used in the second study produce respective 5-HT reuptake inhibitions of approximately 60% and 15% [8]. These relative 5-HT reuptake inhibitions correspond well with the relative decreases in decremental bar pressing produced by the two drugs.

The mechanism by which 5-HT could inhibit DCG stimulation-produced aversion is unknown. One possibility is that the serotonergic dorsal raphe nucleus is involved. The DRN lies in the ventrocaudal portion of the central gray area [15], and several studies have found that stimulation in the region of the DRN produces profound analgesia to noxious peripheral stimuli [2, 3, 31, 33, 34, 41]. Serotonin appears to be important for the production of this analgesia, since PCPA [2,3] and LSD [25] block analgesia produced by stimulation in this region, and the PCPA-blocked analgesia is restored by 5-HTP [2]. Although no direct connections between the DCG and the DRN have been described, several limbic and thalamic structures receive overlapping projections from both the DCG [5,21] and the DRN [10, 20, 57]. These sites of overlapping projections could represent important loci for the regulation of aversive neural mechanisms by 5-HT.

Although our data are consistent with the hypothesis that 5-HT reduces aversion associated with DCG stimulation, it should be noted that 5-HT has been implicated in a number of behaviors other than those associated with aversion [60]. Because of this possible broad role for 5-HT, it is not certain that an enhancement of serotonin function by the drugs in our study directly and specifically reduced aversive neural mechanisms. Our results are consistent, however, with the broad range of other studies described earlier [2, 3, 6, 13, 14, 17, 18, 22, 23, 25, 29–31, 33, 34, 41, 43, 46–50, 54–56, 59, 61–65] which have utilized a variety of experimental paradigms and supported the hypothesis that 5-HT reduces aversion.

Tricyclic antidepressant drugs of the type used in this study are widely used in clinical psychiatry for the treatment of depressive neuroses. Those tricyclic antidepressants such as chlorimipramine, which have tertiary amines in their side chains, are more potent in blocking 5-HT reuptake than are tricyclic drugs such as protriptyline, which have secondary amine side chains [8,16]. The tertiary amine tricyclic antidepressant drugs are also more effective than are the secondary amine drugs in treating depressions which include prominent symptoms of anxiety, tension, distress, and suffering [28,44]. These dysphoric mental symptoms are similar to the affective symptoms of protopathic pain states [27], and

the neurobiological basis of these painful mental sensations possibly involves the DCG and its proposed role in the integration of protopathic pain stimuli with affective processes of the limbic system. The reduction of these symptoms by tertiary amine tricyclic antidepressant drugs could involve an enhanced serotonergic inhibition of aversive neural mechanisms associated with the DCG.

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REFERENCES

1. Adams, D. B. The activity of single cells in the midbrain and hypothalamus of the cat during affective defense behavior. *Archs ital. biol.* **106:** 243–269, 1968.
2. Akil, H. and J. C. Liebeskind. Monoaminergic mechanisms of stimulation-produced analgesia. *Brain Res.* **94:** 279–296, 1975.
3. Akil, H. and D. J. Mayer. Antagonism of stimulation-produced analgesia by p-CPA, a serotonin synthesis inhibitor. *Brain Res.* **44:** 692–697, 1972.
4. Bowsher, D. Termination of the central pain pathway in man: The conscious appreciation of pain. *Brain* **80:** 606–624, 1957.
5. Browder, S., D. C. German, M. Mendershausen, R. T. Brown, R. S. Kiser and G. A. Milhailoff. Autoradiographic tracing of dorsal central gray projections in the rat. *Neurosci. Abst.* Vol. **III:** Abstract No. 1215, 1977.
6. Calcutt, C. R., S. L. Handley, C. G. Sparkes and P. S. J. Spencer. Roles of noradrenaline and 5-hydroxytryptamine in the antinociceptive effects of morphine. In: *Agonist and Antagonist Actions of Narcotic Analgesic Drugs*, edited by H. W. Kosterlitz, H. O. J. Collier and J. E. Villareal. Baltimore: University Park Press, 1971, pp. 176–191.
7. Carlsson, A. Structural specificity for inhibition of [^{14}C]-5-hydroxytryptamine uptake by cerebral slices. *J. Pharm. Pharmac.* **22:** 729–732, 1970.
8. Carlsson, A., H. Corrodi, K. Fuxe and T. Hökfelt. Effect of antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-methyl-α-ethyl-meta-tyramine. *Eur. J. Pharmac.* **5:** 357–366, 1969.
9. Casey, K. L. and R. Melzack. Neural mechanisms of pain: A conceptual model. In: *New Concepts in Pain and Its Clinical Management*, edited by E. L. Way. Philadelphia: Davis, 1967, pp. 13–31.
10. Conrad, L. C. A., C. M. Leonard and D. W. Pfaff. Connections of the median and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study. *J. comp. Neurol.* **156:** 179–206, 1974.
11. Delgado, J. M. Cerebral structures involved in transmission and elaboration of noxious stimulation. *J. Neurophysiol.* **18:** 261–275, 1955.
12. De Molina, A. and R. W. Hunsperger. Central representation of affective reactions in forebrain and brain stem: electrical stimulation of amygdala, stria terminalis, and adjacent structures. *J. Physiol. (Lond.)* **145:** 251–265, 1959.
13. Dewey, W. L., L. S. Harris, J. F. Howes and J. A. Nuite. The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. *J. Pharmac. exp. Ther.* **175:** 435–442, 1970.
14. Diaz, J., G. Ellison and D. Masuoka. Opposed behavioral syndromes in rats with partial and more complete central serotonergic lesions made with 5,6-dihydroxytryptamine. *Psychopharmacologia* **37:** 67–79, 1974.
15. Fuxe, K., T. Hökfelt and U. Ungerstedt. Morphological and functional aspects of central monoamine neurons. *Int. Rev. Neurobiol.* **13:** 93–126, 1970.
16. Fuxe, K. and U. Ungerstedt. Histochemical studies in the effect of (+)-amphetamine, drugs of the imipramine group and tryptamine on central catecholamine and 5-hydroxytryptamine neurons after intraventricular injection of catecholamines and 5-hydroxytryptamine. *Eur. J. Pharmac.* **4:** 135–144, 1968.
17. Garau, L., M. L. Mulas and G. Pepeu. The influence of raphe lesions on the effects of morphine on nociception and cortical ACh output. *Neuropharmacology* **14:** 259–263, 1975.
18. Gorlitz, B. and H. Frey. Central monoamines and antinociceptive drug action. *Eur. J. Pharmac.* **20:** 171–180, 1972.
19. Green, H. and J. L. Sawyer. Biochemical-pharmacological studies with 5-hydroxytryptophan, precursor of serotonin. *Prog. Brain Res.* **8:** 150–187, 1964.
20. Halaris, A. E., B. E. Jones and R. Y. Moore. Axonal transport in serotonin neurons of the midbrain raphe. *Brain Res.* **107:** 555–574, 1976.
21. Hamilton, B. L. Projections of the nuclei of the periaqueductal gray matter in the cat. *J. comp. Neurol.* **152:** 45–58, 1973.
22. Harvey, J. A. and C. E. Lints. Lesions in the medial forebrain bundle: delayed effects on sensitivity to electric shock. *Science* **148:** 250–252, 1965.
23. Harvey, J. A. and C. E. Lints. Lesions in the medial forebrain bundle: relationship between pain sensitivity and telencephalic content of serotonin. *J. comp. physiol. Psychol.* **74;** 28–36, 1971.
24. Hassler, R. Die zentralen Systeme des Schmerzes. *Acta Neurochirurgica* **8:** 353–423, 1960.
25. Hayes, R. L., P. G. Newlon, J. A. Rosecrans and D. J. Mayer. Reduction of stimulation-produced analgesia by lysergic acid diethylamide, a depressor of serotonergic neural activity. *Brain Res.* **122:** 367–372, 1977.
26. Hunsperger, R. W. Role of substantia grisea centralis mesencephali in electrically-induced rage reactions. In: *Progress in Neurobiology*, edited by J. Ariens-Kapper. Amsterdam: Elsevier, 1956, pp. 289–292.
27. Jaffe, J. H. and W. R. Martin. Narcotic analgesics and antagonists. In: *The Pharmacological Basis of Therapeutics*, edited by L. S. Goodman and A. Gilman. New York: MacMillan, 1975, pp. 245–283.
28. Kieholz, P. Die behandlung endogener depressionen mit psychopharmaka. *Dt. Med. Wschr.* **93:** 701, 1968.
29. Kiser, R. S. and R. M. Lebovitz. Monoaminergic mechanisms in aversive brain stimulation. *Physiol. Behav.* **15:** 47–53, 1975.
30. Kiser, R. S., R. M. Lebovitz and D. C. German. Anatomic and pharmacologic differences between two types of aversive midbrain stimulation. *Brain Res.*, in press, 1978.
31. Liebeskind, J. C., G. Guilbaud, J. Besson and J. Oliveras. Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioral observations and inhibitory effects on spinal cord interneurons. *Brain Res.* **50:** 441–446, 1973.
32. Lints, C. E. and J. A. Harvey. Drug induced reversal of brain damage in the rat. *Physiol. Behav.* **4:** 29–31, 1969.
33. Mayer, D. J. and J. C. Liebeskind. Pain reduction by focal electrical stimulation of the brain: an anatomical and behavioral analysis. *Brain Res.* **68:** 73–93, 1974.
34. Mayer, D. J., T. L. Wolfle, H. Akil, B. Carder and J. C. Liebeskind. Analgesia from electrical stimulation in the brainstem of the rat. *Science* **174:** 1351–1354, 1971.

35. Meek, J. and B. Werdinius. Hydroxytryptamine turnover decreased by the antidepressant drug, chlorimipramine. *J. Pharm. Pharmac.* **22:** 141–143, 1970.
36. Mehler, W. R. Some observations on secondary ascending afferent systems in the central nervous system. In: *Henry Ford Hospital International Symposium on Pain*, edited by R. S. Knighton and P. R. Dumke, 1966, pp. 11–32.
37. Mehler, W. R., M. E. Feferman and W. J. H. Nauta. Ascending axon degeneration following anterolateral cordotomy. An experimental study in the monkey. *Brain* **83:** 718–752, 1960.
38. Nauta, W. J. H. Hippocampal projections and related neural pathways to the mid-brain in the cat. *Brain* **81:** 319–341, 1958.
39. Olds, J. Approach-avoidance dissociations in rat brain. *Am. J. Physiol.* **199:** 965–968, 1960.
40. Olds, M. E. and J. Olds. Approach-avoidance analysis of rat diencephalon. *J. comp. Neurol.* **120:** 259–283, 1963.
41. Oliveras, J. L., J. M. Besson, G. Guilbaud and J. C. Liebeskind. Behavioral and electrophysiological evidence of pain inhibition from midbrain stimulation in the cat. *Expl Brain Res.* **20:** 32–44, 1974.
42. Price, D. D., R. L. Hayes, G. J. Bennett, G. L. Wilcox and D. J. Mayer. Effects of dorsolateral spinal cord lesions on narcotic and non-narcotic analgesia in the rat. *Proc. Soc. Neurosci.* **2:** 947, 1976.
43. Proudfit, H. K. and E. G. Anderson. Morphine analgesia: blockade by raphe magnus lesions. *Brain Res.* **98:** 612–618, 1975.
44. Rossi, G. V. Pharmacology of tricyclic antidepressants. A review. *Am. J. Pharmac.* **148:** 37–45, 1976.
45. Routtenberg, A. Hippocampal activity and brainstem reward-aversion loci. *J. comp. physiol. Psychol.* **72:** 161–170, 1970.
46. Saarnivaara, L. Effect of 5-hydroxytryptamine on morphine analgesia in rabbits. *Ann. Med exp. Biol. Fenn.* **47:** 113, 1969.
47. Samanin, R., D. Ghezzi, C. Mauron and L. Valzelli. Effect of midbrain raphe lesion on the antinociceptive action of morphine and other analgesics in rats. *Psychopharmacologia* **33:** 365–368, 1973.
48. Samanin, R., W. Gumulka and L. Valzelli. Reduced effect of morphine in midbrain raphe lesioned rats. *Eur. J. Pharmac.* **10:** 339–343, 1970.
49. Schneider, J. A. Reserpine antagonism of morphine analgesia in mice. *Proc. Soc. exp. Biol. Med.* **87:** 614–615, 1954.
50. Sewell, R. D. E. and P. S. J. Spencer. Modification of the antinociceptive activity of narcotic agonists and antagonists by intraventricular injection of biogenic amines in mice. *Brit. J. Pharmac.* **51:** 140P–141P, 1974.
51. Sheard, M. H., A. Zolovick and G. K. Aghajanian. Raphe neurons: Effect of tricyclic antidepressant drugs. *Brain Res.* **43:** 690–694, 1972.
52. Skinner, J. E. *Neuroscience: A Laboratory Manual.* Philadelphia: Saunders, 1971.
53. Spiegel, E. A., M. Kletzkin and E. G. Szekely. Pain reactions upon stimulation of the tectum mesencephali. *J. Neuropath. exp. Neurol.* **13:** 212–220, 1954.
54. Takagi, H., T. Takashima and K. Kimura. Antagonism of the analgesic effect of morphine in mice by tetrabenazine and reserpine. *Arch. int. Pharmacodyn.* **149:** 484–492, 1964.
55. Tenen, S. W. The effects of PCPA, a serotonin depletor, on avoidance acquisition, pain sensitivity, and related behavior in the rat. *Psychopharmacologia* **10:** 204–219, 1967.
56. Tenen, S. W. Antagonism of the analgesic effect of morphine and other drugs by p-chlorophenylalanine, a serotonin depletor. *Psychopharmacologia* **12:** 278–285, 1968.
57. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand.* **367:** 1–48, 1971.
58. Valenstein, E. S. Independence of approach and escape reactions to electrical stimulation of the brain. *J. comp. physiol. Psychol.* **60:** 20–30, 1965.
59. Vogt, M. The effect of lowering the 5-hydroxytryptamine content of the rat spinal cord on analgesia produced by morphine. *J. Physiol., Lond.* **236:** 483–498, 1974.
60. Weissman, A. Behavioral pharmacology of p-chlorophenylalanine (PCPA). In: *Serotonin and Behavior*, edited by J. Barchas and E. Usdin. New York: Academic Press, 1973, pp. 235–248.
61. Yaksh, T. L., J. C. Duchateau and T. A. Rudy. Antagonism by methysergide and cinanserin of the antinociceptive action of morphine administered into the periaqueductal gray. *Brain Res.* **104:** 367–372, 1976.
62. Yaksh, T. L., R. L. Plant and T. A. Rudy. Studies on the antagonism by raphe lesions of the antinociceptive action of systemic morphine. *Eur. J. Pharmac.* **41:** 399–408, 1977.
63. 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.
64. Yunger, L. M. and J. A. Harvey. Effects of lesions in the medial forebrain bundle on three measures of pain sensitivity and noise-elicited startle. *J. comp. physiol. Psychol.* **83:** 173–183, 1973.
65. Yunger, L. M. and J. A. Harvey. Behavioral effects of L–5–hydroxytryptophan after destruction of ascending serotonergic pathways in the rat: the role of catecholaminergic neurons. *J. Pharmac. exp. Ther.* **196:** 307–315, 1976.