Pharmacology Biochemistry and Behavior, Vol. 2, pp. 215–221. ANKHO International Inc., 1974. Printed in the U.S.A.

Morphine Analgesia, Two-Way Avoidance, and Consummatory Behavior Following Lesions in the Midbrain Raphe Nuclei of the Rat[1]

STANLEY A. LORENS

Institute of Physiology, University of Bergen, Aarestadveien 19, N-5000 Bergen, Norway

AND

LIBBY M. YUNGER

Department of Psychology, University of Iowa

(Received 4 November 1973)

LORENS, S. A. AND L. M. YUNGER. *Morphine analgesia, two-way avoidance, and consummatory behavior following lesions in the midbrain raphe nuclei of the rat.* PHARMAC. BIOCHEM. BEHAV. 2(2) 215–221, 1974. – Rats with lesions in the median raphe nucleus (MR group) or in both the dorsal and median raphe nuclei (R group) were compared with operated control animals on the following measures: telencephalic serotonin (5-HT) concentration; daily water consumption; acquisition of a two-way conditioned avoidance response; and morphine analgesia. Both lesions produced significant reductions in telencephalic 5-HT, reaching 33% in the MR group and 57% in the R group. Transient increases in water intake were observed in both groups, being more prolonged and of greater magnitude in the R group. On the other hand, facilitated shuttlebox avoidance learning was observed only in the R group. Neither lesion affected pain sensitivity or morphine (3–9 mg/kg) analgesia as measured by the hot-plate technique. Therefore, while the midbrain raphe appears to be involved in the regulation of water intake and some behavioral responses to painful stimuli, lesions in these nuclei and reduction of telencephalic 5-HT are not sufficient to block morphine analgesia.

Conditioned avoidance Morphine analgesia Raphe nuclei Serotonin Water-food consumption

REDUCTION in central 5-hydroxytryptamine (5-HT) concentration following either lateral hypothalamic medial forebrain bundle (MFB) lesions or parachlorophenylalanine (pCPA) administration [10, 12, 24] has been observed to increase sensitivity to painful stimuli as measured by the flinch-jump technique. In addition, hypersensitivity to noxious stimuli subsequent to 5-HT reduction has been suggested to underly the facilitated acquisition of an avoidance response (CAR) following pCPA in the rat [24] and midbrain raphe lesions in both the rat [16] and the cat [14].

Both pCPA treatment [25] and raphe lesions [21,22] have been reported to counteract the analgesic effect of morphine. These observations, however, have not received support from work in other laboratories. Harvey and Yunger [10] found that neither pCPA nor MFB lesions altered the analgesic potency of morphine as measured by the flinch-jump method. Bläsig *et al.* [1] and Reinhold *et al.* [20] have reported that neither raphe lesions, pCPA, nor intraventricular 5,6-dihydroxytryptamine affected morphine analgesia as measured by vocalization elicited by electrical stimulation of the tail. Buxbaum *et al.* [3] found that neither midbrain raphe lesions nor pCPA affected the antinociceptive effect of morphine as measured by the hot-plate and tail-flick procedures.

Since there were several differences in the procedure utilized in these studies (for example, lesion locus and size; sex of animals used; and, analgesia test employed) we attempted to replicate the studies of Samanin and his co-

[1]The major portion of this work was conducted while the first author was a member of the Department of Psychiatry, University of Iowa, and was supported by a grant from the State of Iowa Mental Health Fund. L. M. Y. was the recipient of predoctoral fellowship MH-10641-06. The authors gratefully acknowledge the technical assistance of R. Nurnberg, C. Easter, and A. Larsen. The results of this study were presented to the Third Scandinavian Meeting on Physiological Psychology and Psychophysiology, Copenhagen, May, 1973.

workers [21,22]. In addition, two of the behavioral effects of raphe lesions (elevated water intake and facilitated two-way CAR learning [4, 14, 16]) were determined as measures of lesion adequacy other than histological analysis and reductions in telencephalic 5-HT concentration.

METHOD

Animals

Eighty-two adult female rats (Sprague-Dawley) weighing 238 ± 2 g at the time of surgery were used. The animals were housed individually in Acme metabolism cages in a temperature- (72 ± 2°F), humidity- (37–54%), and illumination- (12 hr light, 12 hr dark cycle) controlled room. Ground rat chow and tap water were available ad lib.

Surgery and Histology

Electrolytic lesions were produced under ether anaesthesia by passing 2 mA d.c. for 20 sec through an intracranial cathode and a rectal anode. The cathode was a 0.25 mm diameter tungsten wire insulated with Epoxylite except for 0.5 mm of its tip. The lesion co-ordinates were: midline (that is, 0.0 lateral), 6.9 mm caudal to bregma, and 6.5 mm (for the dorsal nucleus) and 8.5 mm (for the median nucleus) below the surface of the skull. A Kopf stereotactic instrument was employed with the incisor bar set 3.2 mm above the interaural plane. In order to avoid damage to the mid-sagittal sinus the electrode was positioned 1 mm lateral to the midline, inserted 2 mm into the brain, returned to the midline, then lowered to the appropriate ventral co-ordinate. Lesions were produced in the median raphe (MR group) nucleus (n = 27) or in both the dorsal and median raphe (R group) nuclei (n = 27). Control animals (n = 28) underwent the same surgical procedure except that an electrode was not lowered into the brain. All animals received 75,000 units of penicillin G intramuscularly immediately post-operatively.

At the completion of the experiment the brainstems from 8 rats with median raphe lesions and 6 with dorsal plus median raphe lesions were processed for histological analysis. The brainstems were placed in 10% Formalin for at least 3 weeks, embedded in paraffin, then cut at 16 μ. Every tenth section was saved and stained by the thionin technique. The material thus obtained was examined microscopically and photographed.

Serotonin (5-HT) and Norepinephrine (NE) Determinations

The telencephalic concentrations of 5-HT and NE were measured spectrophotofluorometrically in the same manner as previously described [15]. In brief, 5-HT levels were analyzed by the method of Bogdanski *et al.* [2] and NE content by the Crout *et al.* [5] technique.

Apparatus

Analgesia was determined by the hot-plate method as previously detailed [27]. In short, the rat was placed on a copper surfaced (55–55.5°C) cylinder containing boiling acetone and its paw lick latency measured. If the rat did not respond within 30 sec, it was removed and returned to its home cage. CAR training was conducted in a shuttlebox as previously described [15].

Procedure

Following an adaptation period of 1 week, the 24 hr food and water intakes of almost all rats were measured for the 5 day period immediately preceding surgery and for the first 15 days postoperatively. Body weights were obtained every fifth day.

On the twentieth post-operative day the lesion and operated control groups were subdivided randomly into 4 subgroups. Each subgroup received either saline (1 ml/kg) or morphine sulfate (3, 6, or 9 mg/kg, calculated as the base) subcutaneously. Paw lick latencies were obtained 30 min before and 30, 60, and 90 min after injection.

On the thirtieth post-operative day 27 randomly selected rats (9 from each lesion group and 9 from the operated control group) underwent CAR training. The procedure followed was identical to that already described [15]. In brief, the animals received a maximum of 120 conditioning trials (10 trials/days) or were run until criterion (18 avoidance responses in last 20 trials) was reached. On Day 1 the first conditioning trial commenced 5 min after the rat entered the apparatus. On subsequent days the first trial began 1 min after the rat was placed in the shuttlebox. The CS was the sound from a conventional door buzzer, while the UCS was a 0.5 sec constant current (0.5 mA) shock. The CS-UCS interval was 5 sec, the CS being terminated upon the performance of either an escape or avoidance response. The animals were shocked only when motionless or moving in a direction away from the barrier. Inter-trial barrier crossings were recorded but not punished.

Twenty-six other rats (10 controls and 8 from each lesion group) were decapitated and their brains rapidly removed. The telencephali then were dissected free and analyzed for 5-HT and NE. The brainstems of the lesion animals were placed in Formalin for histological analysis.

The remaining 29 rats were sacrificed without further testing.

RESULTS

Histological and Neurochemical Analyses

The lesions destroyed the caudal 30–80% of the median raphe nucleus. The damage extended caudally into the pontine raphe nucleus but completely spared the pontine tegmental reticular nucleus. Unilateral involvement of the tectospinal tract, medial longitudinal fasciculus, and ventral tegmental nucleus of Gudden was observed in all MR lesion animals (Fig. 1).

In the R lesion group, damage to the median raphe nucleus was as described above. The lesions in the dorsal nucleus were confined to its caudal 25–75%. These lesions also extended into both the superior cerebellar peduncle and dorsal tegmental nuclei of Gudden (bilaterally in one rat). A representative lesion is shown in Fig. 1.

Lesions restricted to the median raphe nucleus produced a significant 33% reduction in telencephalic 5-HT without affecting NE concentration (Table 1). The lesions involving both the median and dorsal nuclei produced a significant 57% fall in telencephalic 5-HT which was accompanied by a significant 16% decrease in NE. The 5-HT content of the R group was significantly lower than that of the MR group, whereas the difference in NE content of the two lesion groups was not significant (Table 1).

As the lesions did not involve either the dorsal or ventral ascending NE pathways as defined by Ungerstedt [26], the 16% NE fall in the R group (Table 1) is probably not due to damage of these fiber systems. The lesions, furthermore, did not invade any of the midbrain dopamine cell groups (A8–10) [26].

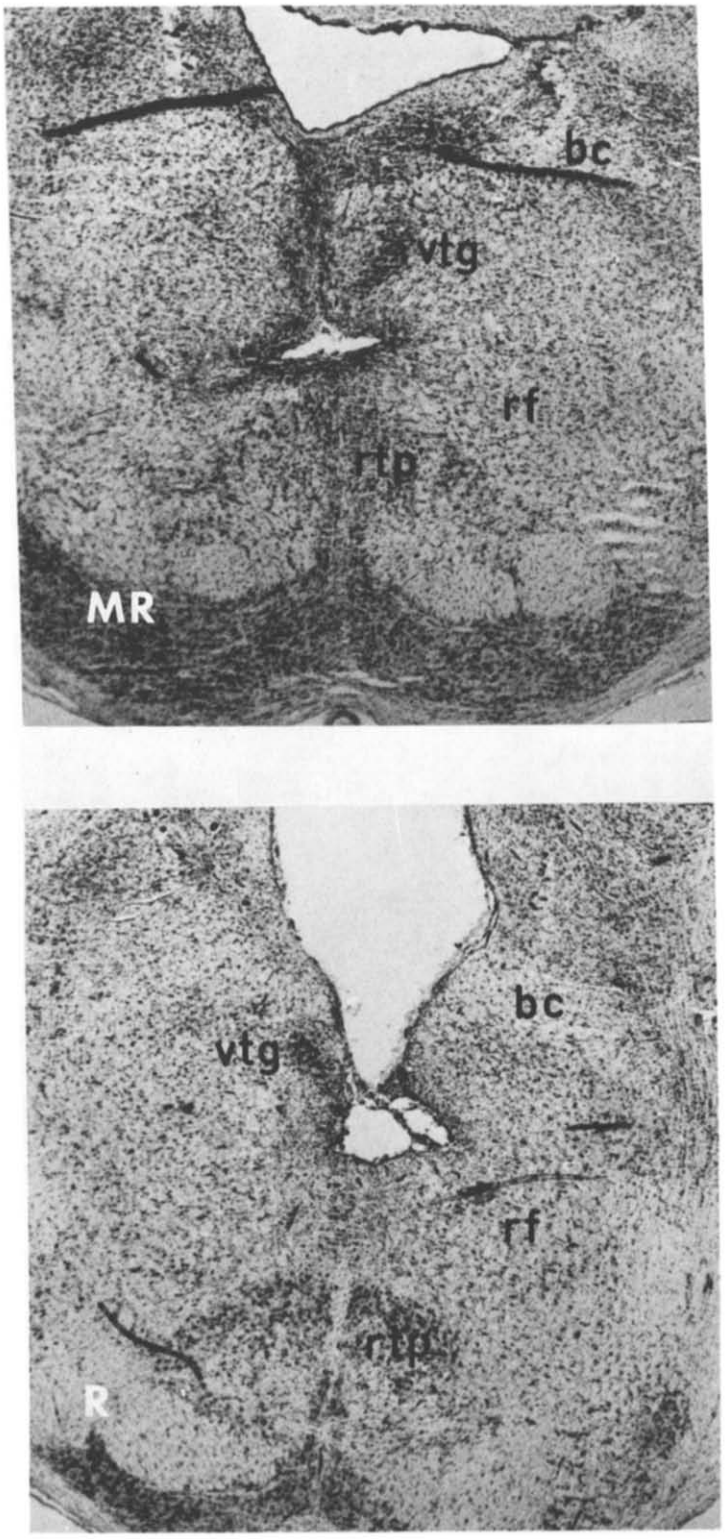

FIG. 1. Photomicrographs (X23) of representative lesions (at the level of the *nucleus reticularis tegmenti pontis*) in the median raphe nucleus alone (MR) and in both the dorsal and median raphe nuclei (R). The MR and R lesions depicted produced a 27% and a 50% reduction, respectively, in telencephalic 5-HT content. Abbreviations: bc: brachium conjunctivum (superior cerebellar peduncle); rf: reticular formation; rtp: pontine tegmental reticular nucleus; vtg: ventral tegmental nucleus of Gudden.

TABLE 1

MEAN (± SEM) TELENCEPHALIC CONCENTRATION (μg/g) OF 5-HYDROXYTRYPTAMINE (5-HT) AND NOREPINEPHRINE (NE)

Group	n	NE	%	p*	5-HT	%	p*
Control	10	0.31 ± 0.01	–	–	0.54 ± 0.01	–	–
Median Raphe	8	0.29 ± 0.01	− 6	NS	0.36 ± 0.03	−33	<0.02
Median-Dorsal Raphe	8	0.26 ± 0.01	−16	<0.02 (NS)	0.23 ± 0.05	−57	<0.002 (<0.038)

*Determined by Mann-Whitney U test, two-tailed; *p's* in parentheses refer to differences between lesion groups.

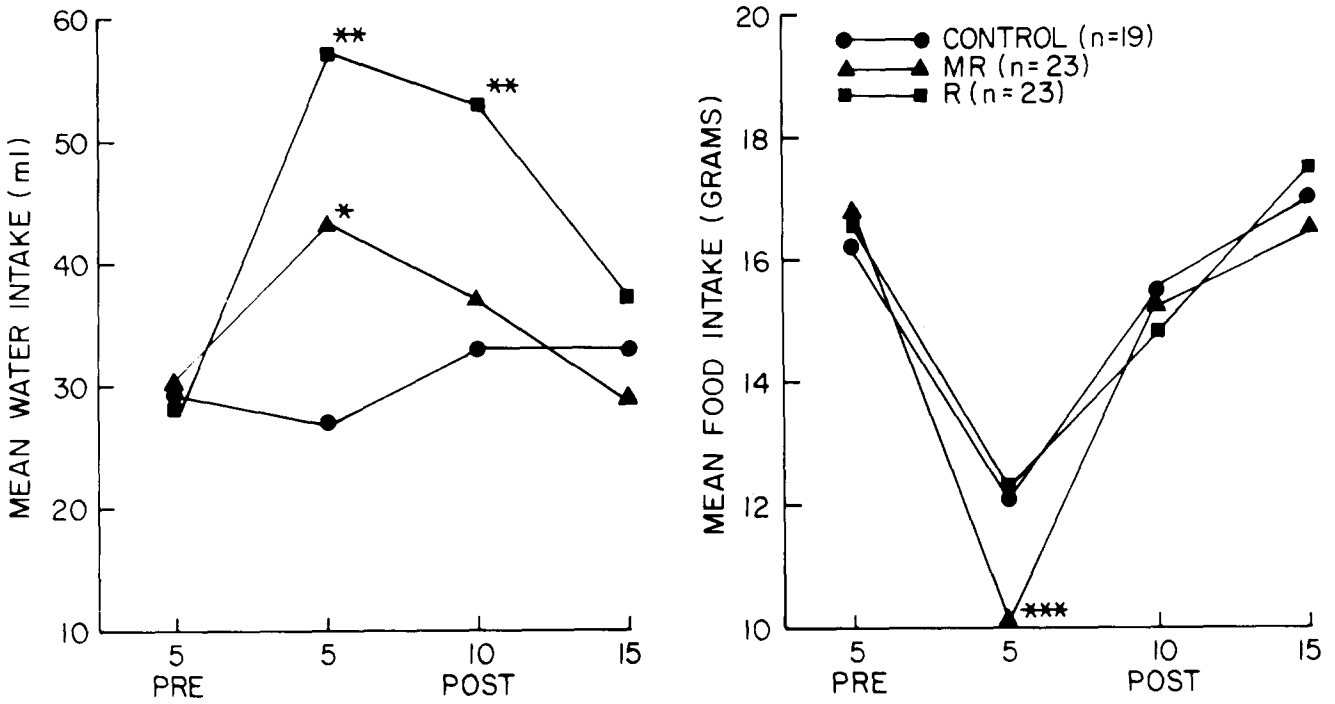

FIG. 2. Mean 5 day pre- and post operative food and water consumption: * = significantly higher than control group. ** = significantly higher than both the control and MR groups. *** = significantly less than the other two groups. MR = group with lesions in the median raphe nucleus alone. R = group with both dorsal and median raphe nucleus lesions.

Food and Water Consumption (Fig. 2)

Food intake was significantly reduced in all three groups, but only during the first 5 post-operative days. The MR group, furthermore, showed the largest reduction. Water intake, in contrast, was significantly increased in both lesion groups. The increase in the R group was of greater magnitude and more prolonged than that in the MR group.

Morphine Analgesia (Table 2)

The animals all appeared healthy post-operatively, no gross neurological or behavioral abnormalities being observed. On the day of analgesia testing the groups showed no differences in body weight (controls, M ± s.e.m. = 269 ± 3 g; MR group, 274 ± 4 g; and, R group, 266 ± 4 g). The paw lick latencies of the three groups did not differ at the time of the pre-drug test (controls, M ± s.e.m. = 8.6 ± 1.0 sec; MR group, 7.3 ± 0.5 sec; and, R group, 9.8 ± 1.0 sec).

In comparison to the control animals, the midbrain raphe lesions did not affect morphine analgesia. However, an analysis of variance (three-factor mixed design: repeated measures on one factor) did reveal a significant lesion effect ($F(2, 70) = 3.67$, $p<0.05$). Individual comparisons indicated that the only group differences for a given dose

TABLE 2

EFFECT OF MORPHINE ON PAW-LICK LATENCY (SEC) IN LESION AND CONTROL RATS

Group/Dose	N	Time (min) Before and After Injection			
		Pre 30	30	Post 60	90
Control					
Saline	7	10.1 ± 1.3	7.0 ± 0.7	6.6 ± 0.7	7.1 ± 1.3
3.0	7	8.2 ± 0.8	7.6 ± 1.4	5.9 ± 1.3	5.9 ± 0.7
6.0	7	8.4 ± 1.2	22.2 ± 3.7	16.4 ± 4.0	12.2 ± 4.6
9.0	7	7.8 ± 0.5	30.0 ± 0.0	27.9 ± 2.1	22.7 ± 3.7
Median Raphe					
Saline	7	6.9 ± 0.6	7.0 ± 1.2	5.8 ± 0.7	6.0 ± 1.4
3.0	6	6.6 ± 0.9	6.5 ± 1.7	4.1 ± 0.4	3.7 ± 0.6
6.0	7	8.4 ± 1.5	23.9 ± 4.0	23.2 ± 3.5	15.4 ± 4.3
9.0	7	7.2 ± 1.0	28.8 ± 1.2	25.5 ± 2.3	22.7 ± 3.8
Median-Dorsal Raphe					
Saline	7	9.0 ± 1.6	8.4 ± 1.5	7.7 ± 1.0	6.5 ± 1.0
3.0	6	11.0 ± 3.7	12.6 ± 1.3	12.7 ± 2.3	8.7 ± 1.6
6.0	7	9.7 ± 1.7	25.0 ± 2.5	24.8 ± 3.3	17.5 ± 4.5
9.0	7	9.6 ± 1.6	30.0 ± 0.0	29.1 ± 0.9	27.0 ± 2.8

TABLE 3

PERFORMANCE DURING TWO-WAY AVOIDANCE CONDITIONING

Group	N	Trials to Criterion		Errors to Criterion		Crossings First 5 Min		Total Intertrial Crossings	
		M ± SEM	p*	M ± SEM	p	M ± SEM	p	M ± SEM	p
Control	9	111 ± 6	–	100 ± 10	–	2 ± 1	–	7 ± 2	–
Median Raphe	9	93 ± 13	NS	74 ± 15	NS	3 ± 1	NS	35 ± 10	<0.002
Median-Dorsal Raphe	9	67 ± 11	= 0.002 (NS)	38 ± 10	<0.002 (NS)	6 ± 1	<0.002 (<0.02)	66 ± 28	<0.002 (NS)

*Determined by Mann-Whitney U test, two-tailed; p's in parentheses refer to differences between lesion groups.

were between the MR and R groups 60 and 90 min after 3.0 mg/kg morphine (Table 2). In both cases, the paw lick latency of the MR group was significantly shorter than that of the R group ($U = 2$, $n_1/n_2 = 6/6$, $p<0.008$).

As expected, the degree of morphine analgesia was a function of the dose ($F(3, 70) = 54.84$, $p<0.001$), time after injection ($F(3, 210) = 54.84$, $p<0.001$), and of the time × dose interaction ($F(9, 210) = 28.59$, $p<0.001$). The dose × lesion, time × lesion, and time × dose × lesion interactions were not significant.

Conditioned Avoidance Behavior (Table 3)

Only 3 of the MR and 2 of the control rats learned the avoidance task within the alloted 120 trials. In contrast, 7 of the R lesion rats reached criterion within 120 trials. Thus, a significantly greater number of R lesion than control animals acquired the CAR ($p = 0.05$, Fisher Exact Test). If a score of 120 was given animals not learning within 120 trials, the R lesion group acquired the CAR in significantly fewer trials than the control group. Analysis of the number of errors committed showed the same result.

The R lesion group, however, did not differ significantly from the MR group on any of these measures of CAR performance.

The R lesion group made a significantly larger number of barrier crossings during their first 5 min in the shuttlebox than either the MR lesion or control groups. In addition, both lesion groups showed a significantly larger number of inter-trial crossings during the course of conditioning.

DISCUSSION

The results of this study are in agreement with those which have previously reported transient elevations in water intake [4,16] and facilitated two-way CAR acquisition [16] following simultaneous ablation of both the dorsal and median raphe nuclei of the rat. Lesions confined to the median raphe nucleus alone, however, failed to affect shuttlebox CAR performance, and had a lesser influence on water consumption. On the other hand, we recently have shown that median raphe lesions alone, as well as dorsal plus median raphe lesions, retard the acquisition, retention, and forced extinction of a one-way CAR (Srebro and Lorens, manuscript in preparation). Thus the effects of midbrain raphe lesions on avoidance learning appear to be dependent on the task employed, the locus and extent of the damage, and on the degree of the associated reduction in telencephalic 5-HT.

The transient elevation in water intake following midbrain raphe lesions could be related to a corresponding reduction in forebrain acetylcholine (ACH) level. A correlation between increased water consumption and reduced brain content of ACH has been observed following septal lesions [18,23]. This relationship, however, has been questioned recently [17]. Nevertheless, Lewis and Shute [11] have reported that ascending ACH fibers originate in the midbrain raphe nuclei, and Pepeu (unpublished data, 1973) has found that midbrain raphe lesions produce a fall in brain ACH concentration.

The primary intent of the present study was to replicate the results of Samanin and co-workers [21,22]. Clearly, we failed in this endeavor, although we used the same sex (female) and strain (Sprague-Dawley) of rats with approximately the same body weights; the same analgesia test (hot-plate method); approximately the same doses of morphine sulfate; and, seemingly the same lesions. The only obvious difference in the two studies is that Samanin *et al.* [22] ran their analgesia tests 7 days post-operatively whereas ours were conducted on the twentieth post-operative day.

There are, however, two other possible explanations for the discrepant results. First, Samanin *et al.* [22] reported a 74% reduction in forebrain 5-HT following their median raphe lesion as measured one week post-operatively. We have never obtained such a dramatic decrease in either forebrain or telencephalic 5-HT concentration subsequent to a median raphe lesion alone. On the other hand, we have consistently employed much longer survival times. Nevertheless, it is quite surprizing that a 74% decrease in forebrain 5-HT after a median raphe lesion alone can be discerned only 7 days post-operatively [22] when reductions in brain 5-HT content following MFB lesions are seemingly not complete until the twelfth post-operative day [8,19], and especially since forebrain 5-HT would appear to depend not only on the B8 (median raphe nucleus) but the B7 (dorsal raphe nucleus) and B9 cell groups as well [6,7]. It thus would appear that the median raphe lesions of the Samanin group are quite large and produce a greater reduction in brain 5-HT than either our R or MR lesions, or those of Bläsig *et al.* [1]. It should be pointed out, however, that we [13,16], as well as Buxbaum *et al.* [3], have observed 75–85% falls in telencephalic and forebrain 5-HT concentration, but only after simultaneous destruction of areas B7 and B8.

The second possible source of difference is the puzzling footnote (a) in Table 1 of Samanin *et al.* [22] in which it is stated that animals "not responding within 45 sec" are not represented. It is thus possible that not all control and lesion animals injected with morphine were included in their final analysis. Thus, at best, the Samanin *et al.* [22] results appear to be due to the acute effects (like the transient elevation in water intake observed in the present as well as previous studies [4,16] of raphe lesions and possibly only in a select group of animals. In this respect, it is unfortunate that we did not conduct our analgesia tests on the seventh post-operative day, as did Samanin *et al.* [22]. It should be noted, however, that both Bläsig *et al.* [1] and Buxbaum *et al.* [3] tested their raphe lesion rats 8–15 days post-operatively and did not find any effect on morphine analgesia. Therefore, it would appear that reductions in brain 5-HT following lesions in the midbrain raphe nuclei do not influence the antinocicaptive action of morphine.

REFERENCES

1. Bläsig, J., K. Reinhold and A. Herz. Effect of 6-hydroxydopamine, 5,6-dihydroxytryptamine and raphe lesions on the antinociceptive actions of morphine in rats. *Psychopharmacologia* **31:** 111–119, 1973.
2. Bogdanski, D. F., A. Pletscher, B. B. Brodie and S. Udenfriend. Identification and assay of serotonin in brain. *J. Pharmac. exp. Ther.* **117:** 82–88, 1956.
3. Buxbaum, D. M., G. G. Yarbrough and M. E. Carter. Biogenic amines and narcotic effects. I. Modification of morphine-induced analgesia and motor activity after alteration of cerebral amine levels. *J. Pharmac. exp. Ther.* **185:** 317–327, 1973.
4. Coscina, D. V., L. D. Grant, S. Balagura and S. P. Grossman. Hyperdipsia after serotonin-depleting midbrain lesions. *Nature New Biology* **235:** 63–64, 1972.
5. Crout, J. R., C. R. Creveling and S. Udenfriend. Norepinephrine metabolism in rat brain and heart. *J. Pharmac. exp. Ther.* **132:** 269–277, 1961.
6. Dahlstrøm, A. and K. Fuxe. Evidence for the existence of monoamine-containing neurons in the central nervous system. *Acta physiol. scand.* **62:** Suppl. 232, 1964.
7. Dahlstrøm, A., J. Haggendal and C. Atack. Localization and transport of serotonin. In: *Serotonin and Behavior,* edited by Barchas, J. and E. Usdin, New York: Academic Press, pp. 87–96, 1973.
8. Harvey, J. A., A. Heller and R. Y. Moore. The effect of unilateral and bilateral medial forebrain bundle lesions on brain serotonin. *J. Pharmac. exp. Ther.* **140:** 103–110, 1963.
9. 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.
10. Harvey, J. A. and L. M. Yunger. Relationship between telencephalic content of serotonin and pain sensitivity. In: *Serotonin and Behavior,* edited by Barchas, J. and E. Usdin. New York: Academic Press, pp. 179–189, 1973.

11. Lewis, P. R. and C. C. D. Shute. The cholinergic limbic system: Projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and suptr-optic crest. *Brain* **90:** 521–540, 1967.
12. Lints, C. E. and J. A. Harvey. Altered sensitivity to footshock and decreased brain content of serotonin following brain lesions in the rat. *J. comp. physiol. Psychol.* **67:** 23–31, 1969.
13. Lorens, S. A. Effect of lesions in the raphe system on self-stimulation in the rat. *Physiol. Behav.* **7:** 815–818, 1971.
14. Lorens, S. A. Raphe lesions in cats: Forebrain serotonin and avoidance behavior. *Pharmac. Biochem. Behav.* **1:** 487–490, 1973.
15. Lorens, S. A., J. P. Sorensen and J. A. Harvey. Lesions in the nuclei accumbens septi of the rat: Behavioral and neurochemical effects. *J. comp. physiol. Psychol.* **75:** 284–290, 1970.
16. Lorens, S. A., J. P. Sorensen and L. M. Yunger. Behavioral and neurochemical effects of lesions in the raphe system of the rat. *J. comp. physiol. Psychol.* **77:** 48–52, 1971.
17. Pepeu, G., A. Mulas and M. L. Mulas. Changes in the acetylcholine content in the rat brain after lesions of the septum, fimbria and hippocampus. *Brain Res.* **57:** 153–164, 1973.
18. Pepeu, G., A. Mulas, A. Ruffi and P. Sotgiu. Brain acetylcholine levels in rats with septal lesions. *Life Sci.* **10:** 181–184, 1971.
19. Moore, R. Y. and A. Heller. Monoamine levels in rat brain following lateral hypothalamic lesion. *J. Pharmac. exp. Ther.* **156:** 12–22, 1967.
20. Reinhold, K., J. Bläsig and A. Herz. Changes in brain concentration of biogenic amines and the antinociceptive of morphine in rats. *Naunyn-Schmiedegergs Arch. exp. path. Pharmak.* **278:** 69–80, 1973.
21. Samanin, 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.
22. Samanin, R., W. Gumulka and L. Valzelli. Reduced effect of morphine in midbrain lesioned rats. *Eur. J. Pharmac.* **10:** 339–343, 1970.
23. Sorensen, J. P. and J. A. Harvey. Decreased brain acetylcholine after septal lesions in rats: Correlation with thirst. *Physiol. Behav.* **6:** 723–725, 1971.
24. Tenen, S. A. The effects of *p*-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behavior in the rat. *Psychopharmacologia* **10:** 204–219, 1967.
25. Tenen, S. S. Antagonism of the analgesic effect of morphine and other drugs by *p*-chlorophenylalanine, a serotonin depletor. *Psychopharmacologia* **12:** 278–285, 1968.
26. Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiol. scand.* Suppl. 367, 1–48, 1971.
27. Yunger, L. M., J. A. Harvey and S. A. Lorens. Dissociation of the analgesic and rewarding effects of brain stimulation in the rat. *Physiol. Behav.* **10:** 909–913, 1973.