Pharmacology Biochemistry & Behavior, Vol. 7, pp. 205–210.

5,7-Dihydroxytryptamine Lesions of the Ascending 5-Hydroxytryptamine Pathways: Habituation, Motor Activity and Agonistic Behavior[1]

KJELL HOLE, GEORG ESPOLIN JOHNSON AND ODD-GEIR BERGE

Institute of Physiology, University of Bergen, Norway

(Received 19 January 1977)

HOLE, K., G. E. JOHNSON AND O.-G. BERGE. *5,7-Dihydroxytryptamine lesions of the ascending 5-hydroxytryptamine pathways: habituation, motor activity and agonistic behavior.* PHARMAC. BIOCHEM. BEHAV. 7(3) 205–210, 1977. – Rats were injected stereotactically in mesencephalon with 5,7-dihydroxytryptamine (5,7–DHT) in the medial 5-hydroxytryptamine (5–HT) pathway (n = 8) and in the medial plus the lateral 5–HT pathways (n = 7) or injected with vehicle (n = 8), or sham-operated (n = 8). The 5,7–DHT lesions reduced the *in vitro* ^{3}H-5-HT uptake in the hypothalamus and the cortex cerebri to 27–51% of control values, ^{3}H-noradrenaline uptake was not significantly changed. 5,7–DHT lesions of the medial, and of the medial plus the lateral, 5–HT pathways induced mouse killing behavior and increased number of boxing positions in the shock elicited fighting test. Both lesions also reduced the rate of habituation to touch, but only the lesion of the medial plus the lateral 5–HT pathway significantly reduced the rate of habituation to acoustic stimulation. Activity in the home cage was not significantly changed by the lesions. It was concluded that selective chemical lesions of the ascending 5–HT pathways result in prolonged habituation of the orienting response and increase in particular components of agonistic behavior. The increase in locomotor activity observed after electrolytic lesions of nucleus raphe medianus seems not to be due only to lesion of the 5–HT neurons ascending from this nucleus.

5–Hydroxytryptamine 5,7–Dihydroxytryptamine Habituation of orienting Agonistic behavior Motor activity

IT HAS been suggested that the 5-hydroxytryptamine (5–HT) neurons originating in the midbrain raphe nuclei are important in central control of pain sensitivity [12], motor activity [9, 15, 18, 24], habituation or sensitization to sensory stimulation [3, 4, 5], and aggressive behavior [6, 17, 22]. In the majority of previous studies the function of the 5–HT neurons was altered by electrolytic lesions, parachlorophenylalanine (PCPA) treatment, or other pharmacological manipulations. These methods affect other types of neurons as well as the 5–HT neurons. Recently it was observed that when the 5–HT neurons are lesioned selectively with, 5,7-dihydroxytryptamine (5,7–DHT) injected stereotactically into the 5–HT pathways, open field locomotor activity is reduced and pain sensitivity is unchanged [15,16], in contrast to the increase in locomotor activity [24] and increase in pain sensitivity [12] observed after electrolytic lesions. Possibly some of the behavioral changes observed after electrolytic lesions of the midbrain raphe nuclei or the ascending 5–HT pathways are due to damage of other neurons than the 5–HT neurons. The behavioral effects of selective chemical lesions of the 5–HT pathways therefore should be further investigated.

In the present study the effect of 5,7–DHT lesions of the ascending 5–HT pathways on habituation of the orienting response, agonistic behavior, and motor activity in two different stimulus situations in the home cage, were studied.

METHOD

Animals

Thirty-one male albino rats (Möll-Wistar) weighing 240–280 g at time of surgery were used. They were housed individually in conventional rat cages and had food and water available ad lib. Testing was performed during the light phase of a 12 hr light-dark cycle.

[1] The authors gratefully acknowledge Miss Nina Holmelid and Mrs. May-Elin Figueroa for their excellent technical assistance. This research was supported in part by Norwegian Research Council for Science and the Humanities and Andssvakesakens Forskningsfond.

Surgery

A Kopf stereotaxic instrument was used with the incisor bar set 2.4 mm above the interaural plane. Under KETALAR anesthesia (100–200 mg/kg), 5,7–DHT (5,7-dihydroxytryptamine creatine sulfate, Regis) dissolved in 0.9% NaCl containing ascorbic acid (0,1 mg/ml) was injected stereotactically with a 10 μl Hamilton syringe in the region of the ascending 5–HT bundles [15] in the mesencephalon.

The coordinates were for the medial 5–HT bundle (group Med, n = 8): posterior to bregma 5.6, lateral 0.0, ventral to the surface of cortex 7.5 mm; and for the combined lesions of medial and lateral bundles (group Med + Lat, n = 7): posterior 5.6, lateral ± 0.6, and ventral 7.0 mm. At each injection site 4 μg, 5,7–DHT (calculated as the base) in 4 μl was injected (1 μl/min). The needle was left *in situ* for 1 min after completion of each injection. Twenty min before the intracerebral injections protriptyline HCl (Merck, Sharp and Dohme) 25 mg/kg was injected IP [15]. Control rats were treated in the same manner as the 5,7–DHT rats, except that they were injected intracerebrally with the vehicle only (group Veh, n = 8), or the cannula was not lowered intracranially (group Sham, n = 8).

Histological and Biochemical Analyses

Forty-six days after surgery the animals were sacrificed by decapitation. The brainstems of the 5,7–DHT and vehicle injected rats were kept in 10% Formalin for one week. Frozen sections then were cut at 30 μm and every third section saved and stained by the eosin technique.

The in vitro uptake of ^{3}H-5-HT and ^{3}H-NA was studied in punched-out slices (diameter 3 or 2 mm and thickness about 0,6 mm) from the cortex cerebri and the hypothalamus. The incubation medium was a modified Krebs-Ringer bicarbonate buffer [11], pH 7.4, containing ascrobic acid 0.2 mg/ml, Pargyline 20 μg/ml, EDTA 46.5 mg/l, and saturated with 95% O_2 and 5% CO_2. The slices were preincubated in 2 ml of this buffer at + 37° C for 5 min in a metabolic shaker. Then ^{3}H-5-HT (21.4 Ci/mmol) or ^{3}H-NA (10.3 Ci/mmol, New England Nuclear) was added to the medium to give a final concentration of $2 \cdot 10^{-8}$ M, and the incubation continued for 10 min. After termination of the incubation, the slices were dissolved in SOLUENE 350 (Packard Instruments), and scintillation fluid (toluene/PERMABLEND III, Packard) was added. The samples were counted in a Nuclear-Chicago Mark I liquid scintillation spectrometer.

Mouse Killing Behavior

Nine days after surgery an adult albino mouse was introduced into the rat's home cage. The behavior of the rat was observed for 30 min, and biting attacks on the mouse were scored.

Competition Tests

Twelve days after surgery, after 36 hr of water deprivation, competition for a water bottle was tested. Following this test session a 5,7–DHT injected rat and a control rat lived together in the test cage for 48 hr. At the end of this period, after another 36 hr period of water deprivation, competition for water was again tested. Fifteen days after surgery a tube fighting test (attempting to force another rat out of a tube) was performed. There were no significant differences between groups in any of these competition tests, and they are not further reported.

Habituation to Acoustic Stimulation

Testing was performed on the 16th, 17th and 18th day postoperatively in a Grason-Stadler (Model E 3125–A 100) Skinner box (19 × 29 × 23 cm) contained within a sound attenuated chamber (Model E 3125AA–3). Background white noise was provided by the ventilation fan. The stimulus was a white noise (85 dB for 3 sec) delivered from a Grason Stadler Noise Generator Model 901B. The rat was placed in the apparatus 10 min before testing. The stimulus was presented with at least 5 sec intervals, and only when the rat was lying or sitting motionless.

The rat's response to the stimulus was rated in 5 grades as described previously [14]. The rat attained the habituation criterion when no response was observed to 3 successive stimuli.

Habituation to Touch

Testing was performed 17–18 days after surgery, in the home cage. The touch was applied with a stiff hair attached to a plastic rod, on a mark on the back of the rat, in the midline, 3 cm from the tail (eliminating visual stimuli).

Care was taken to apply the stimulus in a constant way each time, when the rat was not moving. The stimulus was terminated when the hair was visibly bent. Intervals between stimuli were at least 15 sec.

The rat's response to the touch was scored in 4 grades. Criterion for habituation was attained when the rat showed no visible response for 3 successive trials.

Activity in Home Cage

Observation of home cage activity was performed 20 days after surgery in the animal quarters. The floor of the home cage was divided by imaginary lines into 6 squares, 11 cm × 11 cm each. Number of squares entered with both forepaws, number of rearings, and number of rearings of duration 10 sec or more were counted. The rat was observed for two 5 min periods. During the first period the cage was in the rack. Then the cage was gently moved to a table in the same room, and the second observation period started immediately.

Shock Elicited Fighting

Testing was performed 20 days after surgery. The testing apparatus was the same as the one used for habituation to acoustic stimulation. Scrambled shocks (40 shocks, 0.8 mA, duration 0.5 sec with 2.5 sec intervals) were delivered to the grid floor from a Grason Stadler (Model E1004GS) shock generator. Two rats from the same experimental group were tested together and the occurrence of a boxing position [1] was scored for each shock. For statistical purposes all control rats (Sham and Veh) were treated as one group.

Statistical Analysis

When not stated otherwise statements of statistical significance are based on Students *t*-test, two-tailed, with the confidence limit set at the 5% level. The *p*-values refer to comparisons of the 5,7–DHT injected groups or the sham operated group with the vehicle group.

RESULTS

The animals appeared healthy, and there were no significant group differences in body weight throughout the experiment. Biochemical and behavioral data for the rats injected with vehicle in the midline (n = 4) and bilaterally (n = 4) were approximately the same, and all vehicle injected rats therefore were treated as one group in the data presentation. Group Veh was not significantly different from group Sham for any of the biochemical or behavioral data.

Histology

In the 5,7–DHT injected and vehicle injected rats visible damage to the brain tissue was observed only in the needle track and its immediate surroundings (not exceeding 200 μm in diameter in any rat). With the histological technique used, there was no detectable difference between 5,7–DHT injected and vehicle injected rats. Fluorescence histochemistry of brains with this type of 5,7–DHT lesions has been described in a previous paper [15].

In Vitro Uptake of ^{3}H–5–HT and ^{3}H–NA

Figure 1 shows that 5,7–DHT injections in the medial 5–HT bundle reduced ^{3}H–5–HT uptake to 51% of control values in the cortex cerebri, and to 44% in the hypothalamus, ^{3}H–NA uptake in the cortex was not changed. 5,7–DHT lesions of the medial plus lateral 5–HT bundles reduced ^{3}H–5–HT uptake to 42% of control values in the cortex and to 27% in the hypothalamus; a reduction in ^{3}H–NA uptake in the cortex to 75% of control in this group was not statistically significant. In a previous study [15], we observed that similar bilateral 5,7–DHT lesions produced some damage to the dorsal NA bundle in some of the rats. The behavioral data of the three rats of the Med + Lat group with the lowest cortical ^{3}H–NA uptake therefore was compared to the data of the rest of the group, but no clear differences were found.

Vehicle injections did not significantly affect ^{3}H–5–HT or ^{3}H–NA uptake.

Mouse Killing Behavior

None of the rats in the two control groups killed the mouse during the observation period. However, 4 of the 7 rats in group Med ($p<0.025$, Fisher-test, two tailed) and 6 of the 8 rats in group Med + Lat ($p<0.005$) killed the mouse.

Habituation to Acoustic Stimulation

The Med + Lat group required significantly more trials to reach the habituation criterion than the controls ($p<0.02$) and group Med ($p<0.05$). Group Med was not significantly different from the controls ($p>0.10$) (Fig. 2).

Habituation to Touch

Both 5,7–DHT injected groups required significantly more trials than the control groups to reach the habituation criterion ($p<0.01$) (Fig. 3).

Activity in Home Cage

When the cage was in the rack, the activity was very low in all groups (0 or 1 crossing for most rats), and there was no significant difference between the groups.

After the cage was moved to the table, locomotor activity was 25% lower in the group Med + Lat than in group Veh, however, this difference was not statistically significant. Number of rearings, and number of rearings of duration 10 sec or more, were not significantly changed by the 5,7–DHT injections.

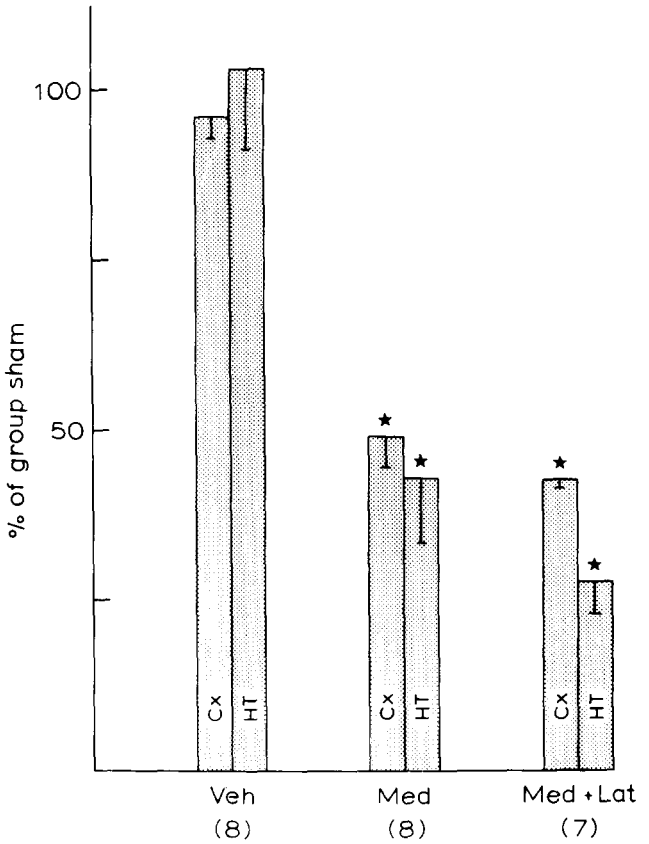

FIG. 1. In vitro ^{3}H–5–HT uptake (mean and SEM), given as percent of the mean uptake in group Sham. Number of rats in ( ). * = $p<0.001$ (compared to group Veh). CX = Cortex. HT = Hypothalamus.

Shock Elicited Fighting

Both in group Med and group Med + Lat the shocks elicited a significant higher number of boxing positions than in the controls ($p<0.05$) (Fig. 4).

DISCUSSION

The 5,7–DHT injections into the ascending 5-HT pathways resulted in extensive lesions of these pathways, as indicated by the reduced in vitro uptake of ^{3}H–5–HT in the terminal areas in the cortex and the hypothalamus. Both the histological examination of the injection site and the data for the ^{3}H–NA uptake in the cortex suggested that the lesions were specific for the 5-HT neurons. In a previous study [15], fluorescence histochemical analysis of brains with similar 5,7–DHT lesions indicated that the unspecific lesions produced is small compared to the lesion of the 5–HT pathways. The fluorescence histochemical

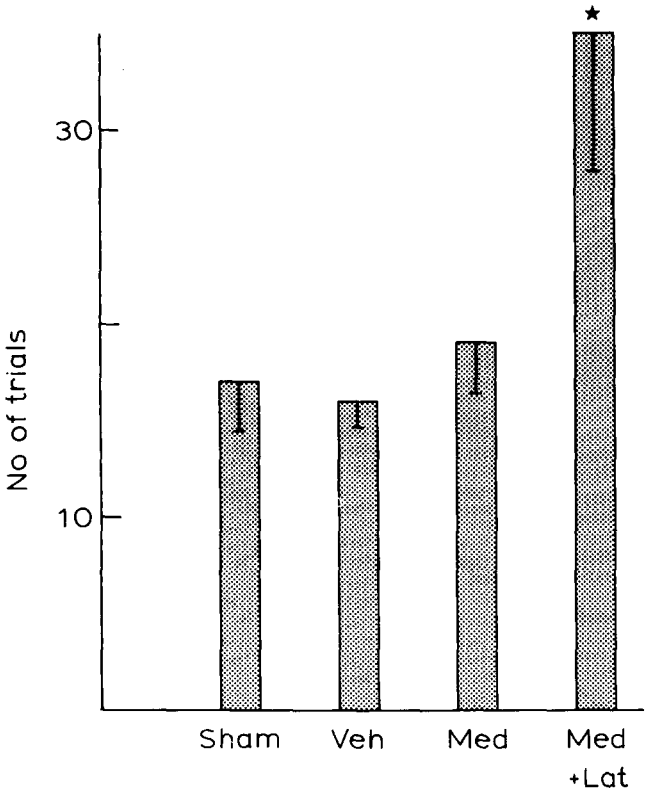

FIG. 2. Habituation to acoustic stimulation. Number of trials to criterion (mean and SEM . * = $p<0.02$.

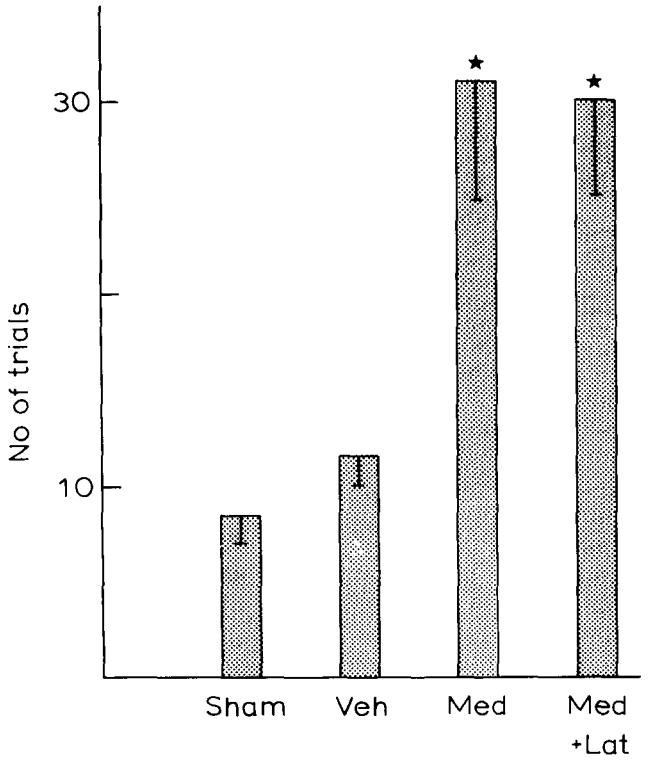

FIG. 3. Habituation to touch. Number of trials to criterion (mean and SEM). * = $p<0.01$.

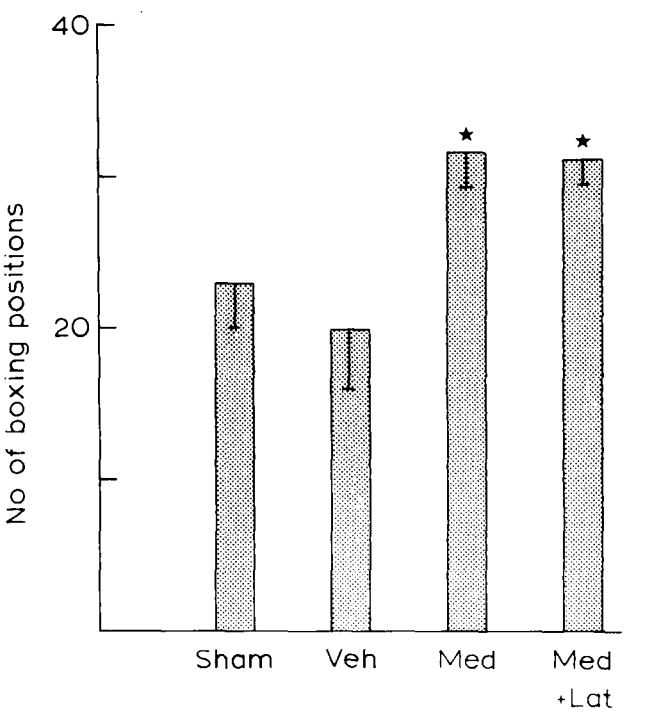

FIG. 4. Shock elicited fighting. Number of shocks (mean and SEM) that elicited a boxing position. Number of pairs of rats in ( ). Each pair received 40 shocks. * = $p<0.05$, compared to all control rats (group Sham + group Veh).

analysis also showed that the medial lesion mainly damaged the medial 5–HT pathway, while the medial plus lateral lesion affected both the medial and the lateral 5–HT pathways.

In this experiment, the medial lesion reduced the rate of habituation to touch, and the medial plus lateral lesion both to touch and to acoustic stimulation. This experiment therefore supports previous studies indicating that lesion of the ascending 5–HT neurons or reduced synthesis of 5–HT reduce the rate of habituation or increase sensitization [3, 4, 5]. Several other studies, however, have failed to observe changes in the habituation rate in rats with impaired function of the 5–HT neurons [8, 9, 25]. Methodological differences between the tests may underlie some of these differences. This is also suggested by the present results, since the group Med evidenced a reduced rate of habituation only with regard to the response to touch, but not to audtiory stimulation.

A general increase in reactivity may result in prolonged habituation. Furthermore, it should be pointed out that electrolytic lesions in the brainstem may produce a reduced rate of habituation [19]. The necrosis at the site of injection was very small both after 5,7–DHT and vehicle injections, and there was no clear difference between the two groups in the size of this unspecific lesion. However, cell counting of the lesion area was not performed. The possibility that there may exist small differences between the two groups in the damage to non-5–HT neurons therefore has not been excluded.

Lesions both of the medial and of the medial plus the lateral 5–HT bundles increased mouse killing behavior, and also increased number of times the electric shocks elicited a boxing position. These results are in accordance with

several previous studies. Both electrolytic lesions in the raphe nuclei [17,26], inhibition of 5–HT synthesis with PCPA [6, 22, 23], or with parachloro-N-methylamphetamine [13], and general lesion of 5–HT terminals with intracisternal injection of 5,7–DHT [2] produce similar behavioral changes. This experiment further supports the assumption that central 5–HT neurons are involved in muricide and shock elicited fighting behavior. Furthermore, the experiment indicates that lesion of the medial 5–HT bundle is sufficient to produce these behavioral changes. Everitt, Fuxe and Jonsson [7] observed biting attacks on males by female rats with 5,7–DHT lesions of the medial 5–HT bundle. Thus, in rats the ascending medial 5–HT pathway seems to be important in control of agonistic behavior both in males and females, while in mice it has been suggested that only female aggressive behavior is controlled by serotonergic mechanisms [21].

The shock elicited fighting model has been the most frequently used paradigm for aggressive behavior. Blanchard and Blanchard [1], however, have shown that the pain induced boxing position mainly is a defensive posture. This test therefore should be regarded mainly as a test for defensive behavior. In the mouse killing behavior there seems to be an important component of conspecific attack, in addition to predation and possible defensive components [1]. It is possible therefore that both conspecific attack and defensive behavior are increased by the lesion of the medial 5–HT pathway.

In a previous study it was shown that open field locomotor activity was reduced in rats with 5,7–DHT lesions of the medial plus lateral 5–HT bundles [15], in contrast to the increase in activity after electrolytic lesion of the median raphe nucleus [18,24]. One possible reason for a decreased open field locomotor activity could be an increase in reactivity or emotionality in the 5,7–DHT lesioned rats, particularly since the field was brightly illuminated [15]. In the present study the rats therefore were observed in the home cage (in a situation without any new stimuli), and in a situation with a rather weak stimulus (gently moving the home cage to another part of the room, without handling of the rat). Hyperactivity, as in the electrolytic raphe lesioned rats, was not observed in any of these situations. On the contrary, rats with combined lesions in the medial and the lateral 5–HT bundles tended to show a reduction in the locomotor activity also in this test, however, this reduction was not statistically significant.

In conclusion, prolonged habituation and increase in mouse killing behavior and in shock elicited defensive behavior can be observed not only after electrolytic lesions of the 5–HT neurons or pharmacological alterations of the function of these neurons, but also after selective chemical lesions of the 5–HT neurons. It seems likely therefore, that these behavioral changes are due to lesion of the ascending 5–HT pathways. Competitive and dominance behaviors in other tests were not changed, and lesion of the ascending 5–HT pathways does not seem to result in a general increase in agonistic behavior, but rather in particular components of this behavior. Furthermore, the study supports previous studies [15,20], concluding that the increased ambulatory activity observed in rats with electrolytic lesions in the median raphe nucleus is not only due to lesion of the 5–HT neurons ascending from this nucleus.

REFERENCES

1. Blanchard, R. J. and D. C. Blanchard. Aggressive behavior in the laboratory rat. *Behav. Biol.*, in press.
2. Breese, G. R. and B. R. Cooper. Behavioral and biochemical interactions of 5,7-dihydroxytryptamine with various drugs when administered intracisternally to adult and developing rats. *Brain Res.* **98:** 517–527, 1975.
3. Carlton, P. L. and C. Advokat. Attenuated habituation due to parachlorophenylalanine. *Pharmac. Biochem. Behav.* **1:** 657–663, 1973.
4. Conner, R. L., J. M. Stolk, J. D. Barchas and S. Levine. Parachlorophenylalanine and habituation to repetitive auditory startle stimuli in rats. *Physiol. Behav.* **5:** 1215–1219, 1970.
5. Davis, M. and M. H. Sheard. Habituation of the rat startle response: Effects of raphe lesions. *Physiol. Behav.* **12:** 425–431, 1974.
6. Eichelman, B. S., Jr. and N. B. Thoa. The aggressive monoamines. *Biol. Psychiat.* **6:** 143–164, 1973.
7. Everitt, B. J., K. Fuxe and G. Jonsson. The effects of 5,7-dihydroxytryptamine lesions of ascending 5-hydroxytryptamine pathways on the sexual and aggressive behaviour of female rats. *J. Pharmac.* **6:** 25–32, 1975.
8. File, S. E. Effects of parachlorophenylalanine and amphetamine on habituation of orienting. *Pharmac. Biochem. Behav.* **3:** 979–983, 1975.
9. Geyer, M. A., A. Puerto, D. B. Menkes, D. S. Segal and A. S. Mandell. Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. *Brain Res.* **106:** 257–270, 1976.
10. Grant, L. D., D. V. Coscina, S. P. Grossman and D. X. Freedman. Muricide after serotonin depleting lesions of midbrain raphe nuclei. *Pharmac. Biochem. Behav.* **1:** 77–80, 1973.
11. Hamberger, B. Reserpine-resistent uptake of catecholamines in isolated tissues of the rat. *Acta physiol. scand. Suppl* **295:** 1–64, 1967.
12. 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.
13. Hodge, G. K. and L. L. Butcher. 5-Hydroxytryptamine correlates of isolation-induced aggression in mice. *Eur. J. Pharmac.* **28:** 326–337, 1974.
14. Hole, K. Behavior and brain growth in rats treated with p-chlorophenylalanine in the first weeks of life. *Devl. Psychobiol.* **5:** 157–173, 1972.
15. Hole, K., K. Fuxe and G. Jonsson. Behavioral effect of 5,7-dihydroxytryptamine lesions of ascending 5-hydroxytryptamine pathways. *Brain Res.* **107:** 385–399, 1976.
16. Hole, K. and S. A. Lorens. Response to electric shock in rats: Effects of selective midbrain raphe lesions. *Pharmac. Biochem. Behav.* **3:** 95–102, 1975.
17. Jacobs, B. L. and A. Cohen. Differential behavioral effects of lesions of the median or dorsal raphe nuclei in rats: Open field and pain-elicited aggression. *J. comp. physiol. Psychol.* **90:** 102–108, 1976.
18. Jacobs, B. L., W. D. Wise and K. M. Taylor. Differential behavioral and neurochemical effects following lesions of the dorsal or median raphe nuclei in rats. *Brain Res.* **79:** 353–361, 1974.
19. Le Moal, M., L. Stinus and D. Galey. Radiofrequency lesions of the ventral mesencephalic tegmentum: neurological and behavioral considerations. *Expl Neurol.* **50:** 521–535, 1976.
20. Lorens, S. A., H. C. Guldberg, K. Hole, C. Köhler and B. Srebro. Activity, avoidance learning and regional 5-hydroxytryptamine following intrabrainstem 5,7-dihydroxytryptamine and electrolytic midbrain raphe lesions in the rat. *Brain Res.* **108:** 97–113, 1976.

21. Neckers, L. M., M. X. Zarrow, M. M. Myers and V. H. Denenberg. Influence of olfactory bulbectomy and the serotonergic system upon intermale aggression and maternal behavior in the mouse. *Pharmac. Biochem. Behav.* **3:** 545–550, 1975.
22. Sheard, M. M. and M. Davis. Shock elicited fighting in rats: Importance of intershock interval upon the effect of p-chlorophenylalanine (PCPA). *Brain Res.* **111:** 433–437, 1976.
23. Sheard, M. H. Brain serotonin depletion by p-chlorophenylalanine or lesions of raphe neurons in rats. *Physiol. Behav.* **10:** 809–811, 1973.
24. Srebro, B. and S. A. Lorens. Behavioral effects of selective midbrain raphe lesions in the rat. *Brain Res.* **89:** 303–325, 1975.
25. Swonger, A. K. and R. H. Rech. Serotonergic and cholinergic involvement in habituation of activity and spontaneous alteration of rats in a Y maze. *J. comp. physiol. Psychol.* **81:** 509–522, 1972.
26. Vergnes, M. and C. Penot. Aggression intraspécifique induite par chocs électriques et réactivite après lésion du raphé chez le rat.. Effets de la physostigmine. *Brain Res.* **104:** 107–119, 1976.