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Effects of Nicotine on the Exploratory Locomotion Patterns of Female Roman High- and Low-Avoidance Rats[1]

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BÄTTIG, K., P. DRISCOLL, J. SCHLATTER AND H. J. USTER. *Effects of nicotine on the exploratory locomotion patterns of female Roman high- and low-avoidance rats.* PHARMAC. BIOCHEM. BEHAV. 4(4) 435–439, 1976. – Utilizing an automated, Dashiell-type hexagonal maze, it was demonstrated that RHA rats: 1) were more active, 2) reversed direction more often, 3) entered radial (blind) alleys less often, and 4) displayed shorter latencies than did RLA rats. Direction reversals (U-turns) tended to increase from day to day with the RHA rats, whereas the opposite was true for the RLA rats. Nicotine injections (0.2 mg/kg) increased activity and the number of U-turns, shortened the latencies and lessened the likelihood of entering radial alleys for both strains. The RHA rats were more sensitive to nicotine than were the RLA rats in all of these measurements, which varied, depending upon alley length and structural complexity, among the maze configurations.

Nicotine Exploratory patterns RHA/RLA rats Day/night cycle

NICOTINE injections have been shown to exert stimulant and/or depressant effects on the spontaneous activity of rats, depending upon the time of day [4], the strain of rats being tested [11,15] and several other factors [6]. Even rats selected, but not bred, for different activity levels have shown a dissimilar susceptibility to the effects of nicotine on such behavior [18,19], and it has been further demonstrated that female rats were more sensitive to the stimulating effects of nicotine than were male rats [11,19]. However, little systematic research has been devoted to the question of to what extent sensory aspects and familiarity with a testing situation might influence the effects of nicotine on exploratory behavior. It was the purpose of this present study to consider this problem, as well as to simultaneously investigate the influence of genetic and circadian effects on this interaction.

Roman high-avoidance (RHA) and Roman low-avoidance (RLA) rats were used to evaluate the genetic factor in this experiment. Developed from the same Wistar stock, through repeated shuttlebox testing and selective breeding [3], these strains have continued to demonstrate divergent patterns of behavior in the shuttlebox over many generations [7,9]. Other inborn behavioral differences observed include open field ambulation [5,7] and rearing frequency [11], in both of which the RHA rats have been more active.

The experimental model chosen for the present study consists of serial exposures to maze configurations differing in alley length and structural complexity. The test apparatus used [1, 2, 23] permits a comprehensive analysis of the exploratory behavior of rats including activity levels and patterns, all modes of directional change under various choice situations, and the respective time sequences involved.

METHOD

Apparatus

Barriers were introduced into an automated Dashiell-type hexagonal maze [1,23], so as to produce 6 different maze configurations. The design and structure symbols for these are shown in Fig. 1. By means of 42 photocells and appropriate programming equipment, the location of the animal was recorded 5 times per sec on a continuously running punch tape. The continuous noise of the puncher, damped by a sound-attenuating box, served as background

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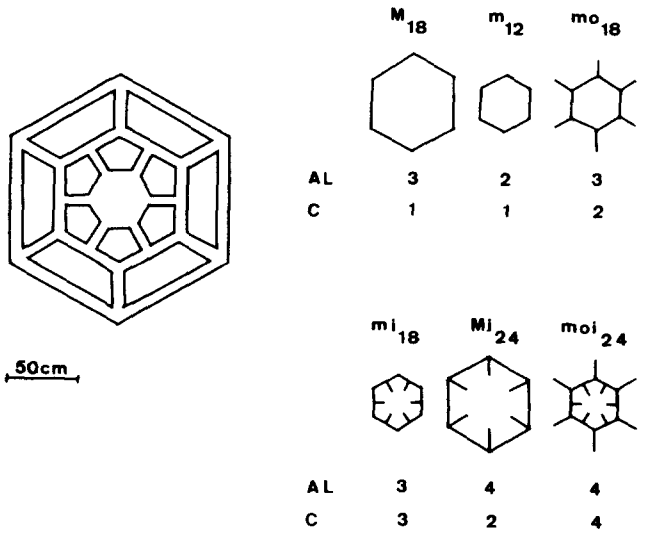

FIG. 1. The labyrinth and the plans for the 6 maze configurations. M = outer (longer) hexagonal alley, m = inner (shorter) hexagonal alley. o = outer radial alleys, i = inner radial alleys. 12, 18 and 24 = number of photocells (total surface area). AL = relative alley length, C = relative complexity.

noise. All alleys were 8 cm wide, the side-walls were 15 cm high, and the distance between neighboring photocells was 30 cm. The walls and floor were black and the maze was covered by a black plastic cloth.

Animals

The 24 female RHA and 24 female RLA rats used averaged, respectively, 208 and 194 g at the start of the experiments, at which time all were 15 weeks old (± 3 days). They were divided into 8 experimental groups of 6 animals each, following the split-litter technique, and housed 3 rats per cage, with free access to food and water. The animal quarters and test room were illuminated from 0600 to 1800 hr, and each rat was handled daily during the 5 days preceding the experiment.

Experimental Design

The experimental groups were brought into the test room 50 min before the beginning of their runs. A run consisted of 6 min in the appropriate maze configuration. Nine min intervals were allowed between the testing of 2 animals, for rearranging the barriers when necessary, and for cleaning the maze floor with a damp sponge. Each rat was exposed to each of the 6 configurations once on each of 6 consecutive days (or nights), in a latin-square sequence, with this procedure being repeated after 1 test-free day. Half of all groups were tested during the day (between 0830 and 1600 hr), and the other half during the night (between 2030 and 0400 hr). All animals were injected SC 35 min before testing, with half of them receiving physiological saline throughout the experiment and the other half receiving 0.2 mg/kg nicotine (nicotine H^+-tartrate, calculated as nicotine base). According to the 2 × 2 × 2 design of RHA or RLA strain (H,L), nicotine or saline treatment (N,S) and day or night testing (d,n), the groups have been designated as HNd, HSd, LNd, LSd, HNn, HSn, LNn and LSn. The following parameters were calculated by computer for each run: 1) activity as represented by the number of photocell activations per run; 2) the average decrease of activity per 30 sec within each run, calculated as the regression coefficient over the activity of the 12 successive time blocks of a run; 3) the number of direction reversals (U-turns) per run, defined as a repeated interruption of the same 3 photocells in a reversed sequence; 4) the frequency of leaving the hexagonal alley and entering a radial (blind) alley; and 5) the latencies between activation of neighboring photocells for each of 7 different types of choice situation.

Statistics

Separate analyses of variance were calculated for each of the variables and for each of the 8 groups. Comparisons for statistical significance within groups and variables were made by using the Duncan technique, and comparisons among groups were determined by the multiple t test.

Brain-amine Analysis

Vaginal smears were examined daily for 2 weeks immediately following testing, and the estrus cycles were determined for all 48 rats. On the next proestrus day (or night, depending upon when the rat had been tested), each animal was sacrificed by decapitation and the brain was immediately frozen in dry ice. One hr before decapitation, 1/3 of the rats, equally divided among the experimental groups, were injected IP with 100 mg/kg nialamide HCl, a monoamine oxidase inhibitor. The other 2/3 of the rats were injected IP with physiological saline. The brains were kept deep-frozen until examined for serotonin (5-HT), dopamine (DA) and noradrenaline (NA) content (and turnover), using a modified fluorometric-determination method [13,22].

RESULTS

The development of the average activity measurements during the 12 testing days for the 8 experimental groups is shown in the top half of Fig. 2. The values obtained for day and night groups differed only slightly with a tendency toward higher scores in the night than in the day measurements. An initial increase in activity from Day 1 to Day 2 was observed in all groups regardless of strain, treatment or day/night cycle. From then on, activity remained about the same in all saline treated groups, whereas it continued to increase in all nicotine treated groups, levelling off only toward the end of the 12 day observation period in the latter. Significant positive regression coefficients ($p<0.01$) of the average group activity over the 12 days were obtained for all nicotine treated but none of the saline treated groups. The differences between strains were clear cut for both the nicotine and saline conditions, with higher activity levels and more sensitivity to nicotine being consistently seen in the RHA groups. Significant ($p<0.05$) intergroup differences in activity, averaged over the 12 days, were found in the day groups with HNd> (HSd,LNd)>LSd, and in the night groups with HNn> (HSn,LNn)>LSn.

The bottom half of Fig. 2 shows the number of U-turns, the development of which over the 12 days roughly paralleled the development of activity. In the 2 saline treated RHA groups, however, the number of U-turns showed a tendency toward a gradual and continuous

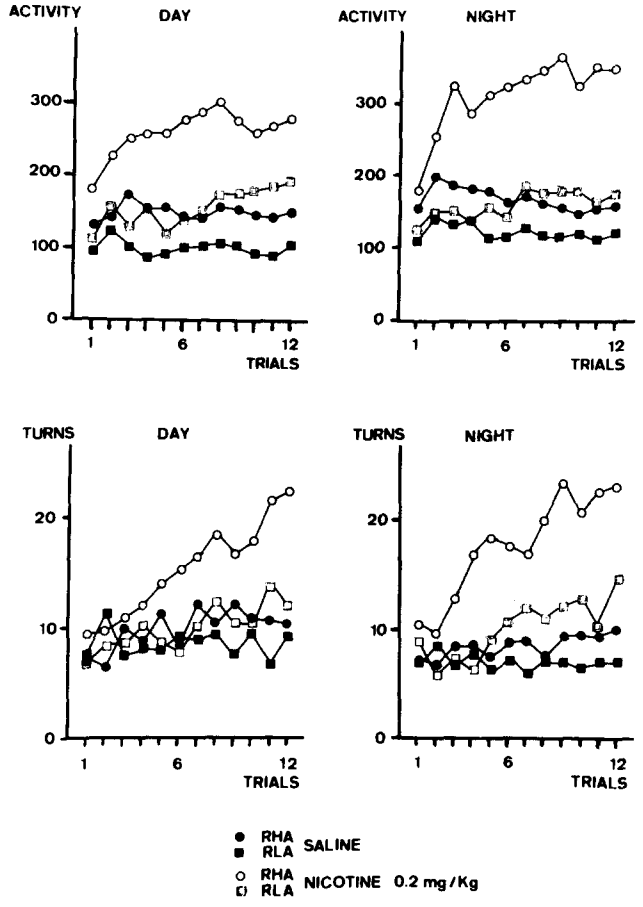

FIG. 2. Comparison of all groups with regard to activity and U-turns over the 12 testing days.

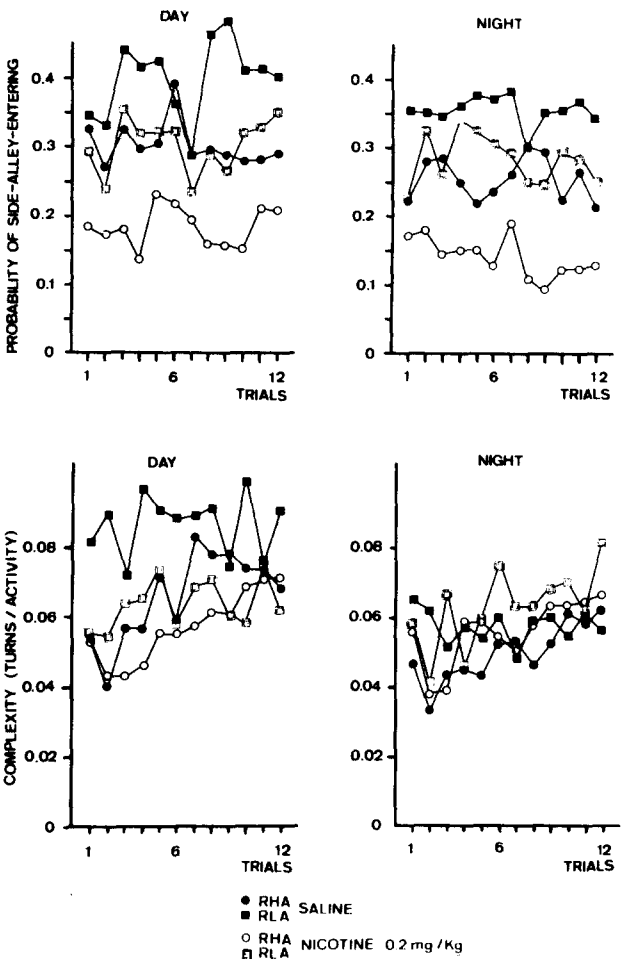

FIG. 3. Comparison of all groups with regard to entering radial alleys/choice situations and locomotion complexity over the 12 testing days.

increase, although the activity scores showed no such trend. Furthermore, the respective regression coefficients over the 12 days reached significant levels ($p<0.01$) in all nicotine treated groups, and in the 2 saline treated RHA groups. The increase for the nicotine treated groups was, once again, greater for the RHA than for the RLA rats. Statistical comparisons of the group values averaged over the 12 days showed, in the day groups, that HNd>(HSd,LNd,LSd) and that LNd>LSd. In the night groups it was found that HNn>LNn>HSn>LSn ($p<0.05$).

The top half of Fig. 3 shows the frequency of entering radial alleys relative to the number of choice situations. These curves show approximately the opposite intergoup order as that seen with activity and U-turns. The more active animals, therefore, were less likely to enter radial alleys. This tendency remained almost unchanged within groups over the 12 days. Statistically significant ($p<0.05$) intergroup differences were found in the day groups with LSd>(LNd,HSd)>HNd, and in the night groups with LSn>(LNn,HSn)>HNn.

The relative locomotion complexity, calculated as the number of U-turns per unit of activity, is shown in the bottom half of Fig. 3. This index of non-stereotypy was seen to increase in all groups with the exception of the saline treated RLA day group, where locomotion complexity was considerably higher than in all other groups to start with. Although the nature of this index did not permit statistical intergroup comparisons, several interesting trends were noted. The intergroup differences were generally more pronounced among the day than among the night groups, and within both samples the scores tended to be higher for the RLA than for the comparable RHA groups. In addition, the nicotine-treated day groups generally showed lower scores than the respective controls, whereas the opposite trend was seen for the comparable night groups.

The slope of intrasession activity decline, as represented by the regression coefficients over the twelve 30 sec blocks of each run, was also estimated. This measure, referred to as intrasession habituation, did not show any systematic changes over the 12 days. Statistically significant intergroup differences coincided with those found for activity alone.

A further analysis dealt with the question of strain, treatment and day/night cycle influence on the maze-specific aspects of locomotion. Three comparisons, among the 6 maze configurations, were possible within each of the 8 groups with respect to structural complexity, as 3 pairs of configurations were equal in alley length but different in complexity (configurations 1 and 3, 1 and 4, 5 and 6). Two comparisons were possible for different alley length but equal structural complexity (configurations 1 and 2, 3 and 5). Regardless of strain, treatment or day/night cycle, it was

seen that increased structural complexity decreased activity, with 22 out of 24 possible comparisons being significant. On the other hand, increased alley length increased activity, with all 16 comparisons being significant ($p<0.05$).

The last analysis was made to detect possible intramaze situational effects on locomotion. Average latencies for successive interruptions of photocells were calculated separately for the straight sections and the corner sections of the hexagonal alleys, as well as for reentering the hexagonal alleys upon leaving a radial alley. Regardless of strain, treatment or day/night cycle, these latencies were found to increase by a factor of about 1 : 2 from unbranched straight sections to branched straight sections and corners, and by a similar ratio for reentries into hexagonal alleys. Statistical intergroup comparisons of these situationally-different latencies emphasized, once again, the greater nicotine sensitivity of the RHA rats. Within the RHA groups, the treatment differences reached significant levels ($p<0.05$) in 25 out of 34 possible comparisons, but in only 2 comparisons within the RLA groups. Also, RHA-RLA strain differences reached significant levels in all 17 comparisons within the nicotine treated night groups.

Table 1 shows the results of the brain-amine examinations for the 48 rats used in this experiment. The 2 most significant findings were 1) that about 30% less total amines were found in all rats observed (and sacrificed) during the night than during the day, and 2) that the 5-HT synthesis rate appeared to be higher in the RHA rats than in the RLA rats, especially during the day.

TABLE 1

RESULTS OF THE BRAIN-AMINE ANALYSIS, IN $\mu g/g \pm SD$*

	NaCl		Nialamid	
	RHA	RLA	RHA	RLA
Day				
5-HT	0.67±0.01	0.74±0.13	0.84±0.06	0.72±0.03
DA	2.71±0.38	2.36±0.82	1.81±0.13	1.86±0.27
NA	0.20±0.03	0.22±0.06	0.21±0.04	0.21±0.03
Night				
5-HT	0.49±0.06	0.46±0.07	0.67±0.03	0.58±0.07
DA	2.03±0.38	1.52±0.09	2.02±0.45	1.78±0.22
NA	0.13±0.00	0.12±0.01	0.23±0.05	0.18±0.01

*Courtesy of Pharmacology Institute, Zurich University.

DISCUSSION

An earlier study, testing nontreated, male Wistar rats at night only, had shown that increases of structural complexity in this same labyrinth decreased the amount of locomotion, and vice-versa [23]. The present study has confirmed these findings, and has further demonstrated that strain, treatment and day/night cycle have no effect on this phenomenon per se. This result of the intermaze comparisons in both studies was further supported by intramaze comparisons regarding the latencies for passing straight-, corner- and branching-sections of the alleys. Stimulus density can, therefore, be considered one of the critical factors influencing maze exploration.

Another finding in the previous study, however, that locomotion complexity became less stereotyped with increasing experience, was confirmed in the present study with the RHA rats only. Locomotion complexity started at a low initial level in the RHA rats, subsequently increasing as a function of experience. This may have represented a tendency to shift behavior from stereotyped foward-running toward a more detailed inspection of the surroundings [8]. In the saline treated RLA rats, locomotion complexity was initially high, and subsequently failed to increase. Simultaneously, the amount of activity was considerably lower in these animals than in the RHA rats throughout the 12 days. This suggested that exploration strategy in this strain was determined, in comparison to the RHA rats, by different or additional factors. Such factors could not be related to differential effects of the maze configurations between the 2 strains, since the effects of maze geometry were basically the same in both. A clue to this difference might be found in the higher emotional level of the RLA rats, as suggested by open-field observations with the 2 strains [5,7]. Locomotion complexity may have been high in the RLA rats as a result of fear or anxiety which inhibited stereotyped forward-running. For example, if it could be determined that "hypertension-susceptible" rats were poorer 2-way avoiders, explored less and were less aggressive than "hypertension-resistant" rats [10], as the RLA rats have also been to date, it might then be possible that RLA rats were less capable of inhibiting fear-motivated responses, this having been the explanation given for the behavior of the "hypertension-susceptible" rats.

At least 2 other possible motivational approaches to the genetic differences seen in this present study merit mentioning. One would involve the role of curiosity at the onset of each exposure [12,14], a proposal which has been previously discussed in connection with an earlier maze study of this type [2]. According to that approach, the RHA rats would be reacting to the maze configurations with a higher degree of curiosity than the RLA rats and would thereby experience a stronger locomotor drive. Another approach would consider the strain differences in the light of habituation to a novel environment, and in relation to brain 5-HT metabolism. According to this proposal [17], a more functional 5-HT system would depress the ability of the animal to habituate, by depressing sensory input. The 5-HT function would, in that case, be positively correlated to activity [20]. In regard to the present study, the implications of this approach would be that the RHA rats, with their apparently higher 5-HT synthesis rate, would be considered to have a reduced sensory input, reduced ability to habituate and, as a result, to be more active than the RLA rats in a novel situation. Although a high NA activity (synthesis) rate is generally associated with low brain levels of NA, high brain levels of 5-HT are indicative of high 5-HT activity [21]. Thus the observation that nicotine stimulates both activity and the accumulation of 5-HT (especially in the diencephalon) of female rats [19], also provides a possible explanation for the observed effects of nicotine in our results. With respect to the circadian differences observed in 5-HT activity, and to the distinct intramaze locomotion latencies seen, it might also be remembered that the strain differences reached significant levels in all comparisons within the nicotine-night groups, as compared to only a few times within the saline-night and both day groups.

Nicotine injections produced, in both strains, increases in activity and in the number of U-turns, without influencing the differential effects of maze geometry. This

stimulating effect was considerably greater in the more active RHA rats. This finding was in agreement with an earlier study dealing with rearing frequency which made the additional observation that significant differences due to nicotine injections, on that parameter, could only be demonstrated with the female RHA rats [11]. It has been found, by measuring running-wheel behavior, that nicotine increased activity during the day, while decreasing this behavior during the night in rats [4]. In regard to the comparatively modest effects of the day/night cycle seen in this present study, it must be remembered that these 2 methods differ from each other in at least 2 important aspects, in that the observation periods here were very short in comparison to the running-wheel method, and that the animals were also presented with a new situation at each maze exposure. Circadian rhythm effects could still be detected in the present study, however. Locomotion complexity was observed to be considerably higher during the day than at night in the saline treated RLA rats. Furthermore, nicotine, which appeared to lower locomotion complexity in both strains during the day, failed to do so at night and even tended toward an opposite effect. No conclusive explanation for these effects can be offered without further experimentation, however, as both emotionality and accumulated experience (or familiarity) were likely to increase locomotion complexity.

Among the experience-related behavioral changes, the finding that intertreatment differences were negligible during the early days and pronounced toward the end of testing is of primary importance. This could explain, in part, why previous studies have occasionally failed to find stimulant effects of nicotine on spontaneous activity. The most probable reason for such results, previously put forth [16], may be a depressant or inhibiting component of nicotine action which, in contrast to its stimulant actions, would be subject to tolerance. Other possibilities, such as a differential mode of nicotine action at the various stages of familiarization with a given testing situation, are also being presently investigated.

REFERENCES

1. Bättig, K. Drug effects on exploration of a combined maze and open-field system by rats. *Ann. N. Y. Acad. Sci.* **159**: 880–897, 1969.
2. Bättig, K. and H. U. Wanner. Die spontane Lokomotion von Ratten in einer Kombination von Labyrinthgängen und Offenfeld. *Helv. physiol. Acta* **25**: 249–261, 1967.
3. Bignami, G. Selection for high rates and low rates of avoidance conditioning in the rat. *Anim. Behav.* **13**: 221–227, 1965.
4. Bovet-Nitti, F. and D. Bovet. Différente action de la nicotine en cours de la journée et de la nuit dans l'activite spontanée du rat. *C. R. Acad. Sci. (Paris)* **262**: 316–320, 1966.
5. Broadhurst, P. L. and G. Bignami. Correlative effects of psychogenic selection: a study of the Roman high and low avoidance strains of rats. *Behav. Res. Ther.* **2**: 273–280, 1965.
6. Driscoll, P. and K. Bättig. Cigarette smoke and behavior: some recent developments. *Rev. Envirn. Hlth.* **1**: 113–133, 1973.
7. Dütsch, H. R. and K. Bättig. Comparison of RHA and RLA rats in four different tests. *Experientia* **31**: 708, 1975.
8. Eisenberger, R. Explanation of rewards that do not reduce tissue needs. *Psychol. Bull.* **77**: 319–339, 1972.
9. Fleming, J. C. and P. L. Broadhurst. The effects of nicotine on two-way avoidance conditioning in bi-directionally selected strains of rats. *Psychopharmacologia* **42**: 147–152, 1975.
10. Friedman, R. and K. R. Weiss. Avoidance behavior of two strains of rats with opposite genetic susceptibility to hypertension. *Psychophysiol.* **11**: 222–223, 1974.
11. Garg, M. Variation in effects of nicotine in four strains of rats. *Psychopharmacologia* **14**: 432–438, 1969.
12. Glanzer, M. Curiosity, exploratory drive and stimulus satiation. *Psychol. Bull.* **60**: 257–268, 1958.
13. Maickel, R. P. and F. P. Miller. The fluorometric determination of indolealkylamines in brain and pineal gland. *Adv. Pharmacol.* **6A**: 71–77, 1968.
14. Montgomery, K. C. The role of the exploratory drive in learning. *J. comp. physiol. Psychol.* **47**: 60–64, 1954.
15. Morrison, C. F. and P. N. Lee. A comparison of the effects of nicotine and physostigmine on a measure of activity in the rat. *Psychopharmacologia* **13**: 210–221, 1968.
16. Morrison, C. F. and J. A. Stephenson. The occurrence of tolerance to a central depressant effect of nicotine. *Br. J. Pharmac.* **45**: 151–156, 1972.
17. Rosecrans, J. A. Differences in brain area 5-hydroxytryptamine turnover and rearing behavior in rats and mice of both sexes. *Eur. J. Pharmac.* **9**: 379–382, 1970.
18. Rosecrans, J. A. Effects of nicotine on behavioral arousal and brain 5-hydroxytryptamine function in female rats selected for differences in activity. *Eur. J. Pharmac.* **14**: 29–37, 1971.
19. Rosecrans, J. A. Effects of nicotine on brain area 5-hydroxytryptamine function in male and female rats separated for differences of activity. *Eur. J. Pharmac.* **16**: 123–127, 1971.
20. Rosecrans, J. A. and M. D. Schechter. Brain 5-hydroxytryptamine correlates of behavior in rats: strain and sex variability. *Physiol. Behav.* **8**: 503–510, 1972.
21. Scheving, L. E., W. H. Harrison, P. Gordon and J. E. Pauly. Daily fluctuation (circadian and ultradian) in biogenic amines of the rat brain. *Am. J. Physiol.* **214**: 166–173, 1968.
22. Schlumpf, M., W. Lichtensteiger, H. Langemann, P. G. Waser and F. Hefti. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. *Biochem. Pharmac.* **23**: 2437–2446, 1974.
23. Uster, H. J., K. Bättig and H. H. Nägeli. Effects of maze geometry and experience on exploratory behavior in the rat. *Anim. Learn. Behav.* **4**(1A): 84–88, 1976.