On the behavioral effects of bilateral glossopharyngeallectomy in mice¹

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Bilateral glossopharyngeallectomy was performed on male Mus musculus SC-1 mice; the operated, sham operated, and control animals were subsequently tested for avoidance conditioning rates and numerous other types of behavior (aggression, exploration, sexual activity, digging, stereotypy, grooming, etc.). There was a significant behavioral change only in reference to exploratory activity which was decreased in the glossopharyngeallectomized animals. This phenomenon is discussed in relation to theories of autonomic imbalance and neural drive.

D. Stanley-Jones has presented a hypothesis that cyclothymia is a disease of the emotions caused by an imbalance of the central autonomic systems which in turn is perceived as emotion in the conscious psyche (Stanley-Jones, 1965). The development of Stanley-Jones' ideas rests on a theory that sensory receptors outside of the nervous system drive the neural apparatus and that no neural impulse can arise de novo within the nervous system.

It is not the object of this paper to evaluate Dr. Stanley-Jones' theories on manic depressive psychosis but to give credit to him for providing the ground plan and hypothesis behind the present research. The suggestion is made in his paper that the "oscillations of true cyclothymia could be interrupted by a section of those branches of the parasympathetic that convey the output of the stretch-receptors in the aortic wall and the carotid sinus."

If the theory of neural dynamics is correct, some change in behavior accom-

panying changed afferent neural drive might occur even in normal Ss. For example, a decrease in the number of parasympathetic afferent impulses to the central nervous system might be expected to change the emotional balance of animals either by resetting the hypothalamus in favor of the sympathetic system or by causing more activity in the orbital cortex. The net behavioral result of glossopharyngeallectomy might be, according to the Stanley-Jones hypothesis, an increase in arousal, activity, and emotional tone. Glossopharyngeallectomy might be expected to increase the number of aggressive episodes between mice and to decrease inhibition of exploratory activity.

ANIMALS, METHODS, AND EXPERIMENTAL DESIGN

Seventy-two white male SC1 mice, 60 days old, and weighing on the average 24.5 g, were obtained from Scientific Small Animal Laboratory, and were divided into six groups of 12 mice each. They were then randomly isolated, two per cage, for a period of 4 weeks, and were housed in a constant-temperature chamber at 75 ± 5 deg F. Food and water were available ad lib. Groups 1 and 2 were used as untreated control groups. Groups 3 and 4 were used as sham-operated groups. Double glossopharyngeallectomies were performed on the mice of Groups 5 and 6.

The bilateral glossopharyngeallectomy was performed as follows: The mouse was anesthetized with Nembutal sodium, 60 mg/kg, i.p. The animal was shaved and placed on its back on an operating table. When the animal was sufficiently anesthetized, a 5/8-in. transverse incision was made in the throat with presterilized instruments. The submaxillary gland was lifted and held by a hemostat. A dull probe and sharp forceps were used in teasing muscles and fascia until the trachea could be seen. The trachea was then lifted slightly

laterally and, at the site of the carotid sinus, between the junction of the internal and external carotid arteries, the glossopharyngeal nerve was located. An A. C. Baker dissecting microscope was used to locate the nerve. The glossopharyngeal was lifted by means of a microhook just posterior to the lingual branch to the posterior part of the tongue and was then cut with a micro-scissors. A 3-mm section was excised from the nerve between the carotid and the lingual branch.

This complete operation was performed bilaterally on 24 mice (Groups 5 and 6). An identical procedure was carried out on the mice of Groups 3 and 4 (sham) with the exception that the glossopharyngeal nerve was merely identified, not cut.

Two 7-mm wound clips were used to close the incision. The mouse was then placed on its stomach in a clean cage; it was warmed postoperatively by a conventional 100-W lamp until it recovered from the anesthetic. After 2 h the animal was placed in the constant temperature chamber with its mate.

After the 4-week isolation period each group was run in the "Mouse City" experiment (Karczmars & Scudder, 1967). In this experimental design one pair of mice was placed in each of six small chambers, each of which connects to a central communal chamber by way of a tubular runway (see Fig. 1). The floors were covered with sawdust, and food and water was present in the main chamber. The experiment was run under red light (60 W), between 10 a.m. and 2 p.m.; the temperature was kept at 72 deg F.

Fifteen minutes after the mice were placed in the chambers, the tubular runways were opened and the mice were permitted to interact. The activities of each of the 12 mice were recorded at 5-min intervals. The activity of each mouse at observation was categorized as follows: contactual behavior, digging, stereotypic behavior, freezing, ingestion, being groomed, grooming self, grooming others, sleeping, and exploration. All cases of aggression were recorded with reference to the number of attacks. Each experiment lasted 100 min. Two experiments were run each day, one at 11 a.m. and one at 1 p.m. Each group was run on each of 3 consecutive days. After these initial control experiments were completed, a sham operation was performed on the mice of Groups 3 and 4 and a glossopharyngeallectomy was performed on Groups 5 and 6. The mice were isolated for 4 weeks during which the operated animals recovered. Thereafter,

Fig. 1. A diagram of the "Mouse City" in which the behavioral observations were made.

Fig. 2. A diagram of the automated avoidance conditioning climbing screen which was used to study learning.

all groups of mice were again run in the "Mouse City" under double blind conditions. Weights and body temperature of all animals were recorded before and after the experiment.

Three weeks after the "Mouse City" experiments all animals of each group, glossopharyngealctomized, sham, and control mice, were tested for learning in an automated avoidance conditioning climbing screen (Scudder, Avery, & Karczmar, 1965). Briefly (Fig. 2), this apparatus consisted of five base chambers (A 1-5) with shocking grid floors. Each chamber was connected to another by a 35-deg inclined tunnel (D 1-5), the floors of which were also electrified by means of four consecutive grids. The door (J) from each chamber opened 5 sec before the animal was shocked and the wave of shock was carried progressively in segments of the grid up to the next chamber at 10-sec intervals forcing the animal to climb. The mean time the animals spent in the base chambers (base times) and climbing (climbing times) were recorded by means of timers (B 1-5) and (C 1-5). Each animal was processed in the machine 10 successive times (Scudder, Avery, & Karczmar, 1969).

Immediately after testing for avoidance conditioning all the animals were sacrificed to confirm the separation of the glossopharyngeal nerve. No histological examination was made on the brains of the animals.

RESULTS

The glossopharyngeal nerves were separated completely in the operated and were fully intact in the sham animals. There were no indications of infections or other lesions.

The behavioral results for exploration in the "Mouse City" experiment are presented in Table 1. All behavioral traits, except exploration, remained unchanged in all groups of mice and do not appear in this table. The contactual behavior, for example, which is a good measure of body temperature, did not change for any group in relation to its control. All the mice exhibited an increase in contactual behavior; however, the increase was proportional in all groups, and thus must be considered an effect caused by aging. Aging effects appeared in freezing and grooming behavior as well. Fluctuations in digging, stereotypic behavior, and aggression were within the norm.

Exploration, on the other hand, was markedly decreased in the bilaterally glossopharyngealctomized mice. Generally, the animals of Groups 1, 2, 3, and 4 explored more postoperatively (when they were older) than they had during the initial runs. Exploration is a quality all normal mice show, and its pronounced decrease in the operated animals clearly sets Groups 5 and 6 apart from the others. The glossopharyngealctomized animals were not nervous, frightened, or timid; they were calm and quiet. Lack of exploration may be a reflection of placidity, sluggishness, or lack of emotional tone; it is not, however, the result of obesity in this case. All of the operated animals, both sham and real, were considerably overweight. The decreased exploration of the glossopharyngealctomized mice, was not due to their increased weight, since their average weight was almost equal to that of the sham operated animals.

The behavioral results for the "climbing screen" experiment showed conclusively that there was no significant change in avoidance conditioning in either the control, the sham, or the glossopharyngealctomized animals.

DISCUSSION

The operated mice were tested for learning in the "climbing screen" to find out if the operation had caused motor impairment or changes in performance due to the lack of cardiovascular reflexes. It was not expected that the differing personalities resulting from the operation would directly affect the rate of learning, but the climbing of the mice might have been altered if they were hypertensive or physically aberrant. The similarity of all the groups indicated that the change in behavior was a mild effect present in the unstressed animal and not particularly pathological. Large behavioral changes associated with central nervous system ablations were not expected

(Lennart & Larson, 1967; Schmaltz & Isaacson, 1967).

The importance of this research is twofold. Primarily, it indicates that perhaps there is some validity in the cybernetic, neural dynamic hypothesis of behavior. Changes in the quantity and location of input into the central nervous system have behavioral consequences of possible therapeutic and theoretical interest. Secondly, it should be pointed out that psychotropic drugs may modify the psyche indirectly by just such an imbalance of visceral receptors. This possible source of behavioral change has not received the attention it merits on the basis of these experiments.

It is not easy to relate these findings in normal mice to the possible therapeutic uses of bilateral glossopharyngealctomy in diseases of emotions in humans. Studies in mice suggest that sympathomimetic drugs do cause a decrease in exploratory behavior; but mice so treated are not calm in appearance as were the glossopharyngealctomized mice. Furthermore, drugged animals show, on occasion, an increase in stereotypy, which these operated mice did not show (Karczmar & Scudder, 1967). Drug effects, however, hit many cells which are perhaps not affected by glossopharyngealctomy. Autonomic balance in theory is subtly mixed (Gelhorn, 1961) to produce varied emotional states. It is not surprising, therefore, that the behavior of the operated animals was not as expected.

From a psychological point of view, one line of reasoning is particularly interesting. Curiosity has been studied in many animals (Glickman & Sroges, 1966). In humans there is no doubt that curiosity has a conscious emotional attribute which is expressed as a "quest for truth" or "search for knowledge." In mice curiosity and exploration are directly proportional and may well be a measure of the same quality (Scudder & Karczmar, in press). The decrease of exploration or curiosity by the glossopharyngealctomized mice possibly represents a real personality change, i.e., a sublimation of (parasympathetic) libido brought about by a decrease in neuronal

Table 1
The Relative Frequency of Occurrence of Exploration Exhibited by the Control, the Sham, and the Glossopharyngealctomized Mice.

Groups	Average number of checks/experiment/mouse					
	Control 1 Pre	2 Post	Sham 3 Pre	4 Post	Operative 5 Pre	6 Post
Exploration	4.00	6.00	4.10	5.50	4.50	3.80
SD	±1.61	±1.22	±1.11	±0.90	±1.58	±1.20

drive to parasympathetic centers of the visceral brain.

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NOTE

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Amphetamine depression of a multiple CRF-EXT operant schedule: A sex comparison¹

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Amphetamine, 0.5 mg/kg administered on five successive trials, significantly depressed the operant performance of both male and female rats maintained on a multiple CRF-EXT schedule. That the depression of behavior was due to the drug was indicated by the recovery of performance in both sexes on two subsequent distilled water injection trials. Some basic sex differences in reaction to the reinforcement schedule also were observed on all trials of the study.

The depressant effect of amphetamine on operant performance maintained by various reinforcement schedules is well established and has been reviewed elsewhere (Cole, 1967). However, some reversal effects of the drug have been noted with DRL schedules (Kelleher et al, 1961) and FR schedules with pre-time-out (Ferster et al, 1962).

In an operant task where 5-min periods of continuous reinforcement (CRF) and extinction (EXT) were successively alternated, the depressant effect of amphetamine in male albino rats has been found to hold for a 2.0 mg/kg dose (Cole, 1968). The present study was undertaken for the

purpose of making a sex comparison of the depressant action of the drug on operant behavior maintained by a similar multiple CRF-EXT schedule while employing a smaller dose. Special attention was focused on the generality of such a depressant effect or its dependency on sex.

SUBJECTS

Sixteen albino rats (eight males and eight females), purchased from West Jersey Biological Supply, served as Ss. At the beginning of training, they were approximately 90 days old with an average body weight of 250 g. The Ss were housed individually in a temperature-controlled laboratory and were allowed ad lib access to Purina chow and water in the home cages.

PROCEDURE

Initially, all Ss were trained in a standard commercial operant chamber (Gerbrands Co.) during 30-min CRF sessions under 48-h food deprivation. Training was continued until each S had established a baseline operant rate, following which time, they were assigned to either a distilled water (DW) or amphetamine (AMP) group with the restriction that half of the males and half of the females were assigned to each group. Statistical analysis of the groups indicated no initial differences in operant rate due to sex or assignment.

All Ss then were given seven 30-min trials of multiple CRF-EXT schedule with 5 days between trials. On Trials 1-5, the Ss were food deprived for 48 h immediately prior to testing and then injected intramuscularly with DW or 0.5 mg/kg d-amphetamine sulfate² 15 min before placement in the apparatus. In the case of the DW groups, the Ss were administered a controlled-volume injection adjusted to body weight. Trials 6 and 7 served as "recovery-from-drug" trials with all Ss receiving DW injections 15 min before testing. In all other respects, Trials 6 and 7 were identical to the five preceding trials.

On all seven trials, the 30-min test period was divided into 10 successively alternating 3-min periods of CRF and EXT. Half of the Ss under each of the four major experimental conditions (male-DW; male-0.5 AMP; female-DW; female-0.5 AMP) received the CRF, EXT, CRF, EXT, CRF, EXT, CRF, EXT, CRF, EXT sequence with the other half of the Ss receiving the EXT, CRF, EXT, CRF, EXT, CRF, EXT, CRF, EXT, CRF sequence. During the CRF periods, the S received a single 45-mg precision food pellet³ for each bar press. A cue light, directly above the food tray, remained on for the entire CRF period but was off during EXT periods.

RESULTS AND DISCUSSION

The mean number of total bar presses for the four groups on the five drug and two "recovery-from-drug" trials is presented in Fig. 1. An overall trend analysis of the data on Trials 1-5 yielded a significant drug effect ($F = 15.44$; $df = 1/12$; $p < .01$) and a significant difference between sexes ($F = 6.15$; $df = 1/12$; $p < .05$). Consistently higher average rates were observed in both female groups on Trials 1-5 when compared to their male counterparts (female-DW > male-DW; female-0.5 AMP > male-0.5 AMP). Analysis of Trials 6 and 7 indicated a complete recovery from drug effect ($F < 1.00$) on both sexes. Although not reaching statistical significance on the two "recovery-from-drug" trials, the average rates of the two female groups continued to be higher than those for the male groups.

In an attempt to ascertain possible differences in the depressant effect of the drug with sexes, further trend comparisons were made on Trials 1-5. Analysis indicated that the difference in trends of the female DW and 0.5 AMP groups was significant ($F = 7.53$; $df = 1/6$; $p < .05$) and that the difference in the performance trends of the male DW and 0.5 AMP groups also was significant ($F = 9.49$; $df = 1/6$; $p < .05$).

The results of the present study demonstrate the effectiveness of amphetamine (0.5-mg/kg dose) in depressing the general operant performance of both male and female rats maintained on a multiple