Effect of olfactory bulbectomy and enucleation on behavior of the mouse[1]

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Bilateral olfactory bulbectomy and enucleation were performed on two groups of mice. After recovery, these mice and their controls were studied and compared in reference to their social behavior, avoidance conditioning, and activity. The mice were then sacrificed and the levels of norepinephrine, serotonin, DOPA, dopamine, and epinephrine were measured on pooled whole brains. Although reactivity and certain behaviors of the mice were radically different, the amine levels were unchanged. The significance of this finding is discussed.

The purpose of this paper is to present the results of two experiments in which both behavioral and biochemical effects of central nervous system (CNS) ablation were determined in the laboratory mouse. The experiments were designed not only to show the change of activities but also to test whether a correlation exists between the behavioral and biochemical results, and furthermore, to test the effect on personality of sensory deficit (through ablation) in animals. Ablation studies have shown that removal of certain parts of the peripheral afferent nervous systems (bilateral glossopharyngealectomy) modifies the behavior of such animals (Scudder & Richardson, 1969) uniquely.

In rodents, ablation studies have largely been confined to rats. Heimer & Larsson (1967), Whitten (1959), and Bruce (1963) produced evidence of the importance of olfactory stimuli in the male rat's mating behavior. Wenzel & Jeffrey (1966) showed that mice that were almost completely sympathectomized during the first week of life by injection of nerve growth factor antiserum were significantly less reactive than the control group. This demonstrated that the sympathetic nervous system (afferent and efferent aspects) influences behavior at least in aversive situations.

It has been the contention recently that the biogenic amines, dopa, dopamine, norepinephrine, and epinephrine, and also serotonin, play a major role in the modulation of behavior of mammals. The catecholamines have been implicated because of their involvement with the sympathetic nervous system.

Examples of workers in this field are Bertler & Rosengren (1966), who studied the behavioral effects of dopa, and Carlsson, Jonasson, & Rosengren (1963), who correlated the time between the effects of reserpine on behavior and on amine level. Everett (1961) showed a relationship between reactivity to dopamine levels. Some electrophysiological and biochemical correlates between amines, stereotypy, and convulsive latencies have been reported from this laboratory (Scudder, Karczmar, Everett, Gibson, & Rifkin, 1966). It seems reasonable to assume that changes in the afferent input into the brain from discrete sensory ablations should evoke changes in behavior with accompanying changes in brain neurohumors.

EXPERIMENTAL DESIGN, ANIMALS, AND EQUIPMENT

There were two types of experiments with control replications for each. The two types are as follows: (1) chronic enucleation and (2) olfactory bulbectomy.

Chronic Enucleation

The mice used in this experiment were all male 40- to 45-day-old CF-I albino laboratory mice. The animals were enucleated 3 days after birth and maintained with their natural mothers until 20 days old, as were the unoperated controls. All animals were weaned and caged with a littermate on the 21st day. For this part of the experiment, 120 animals were used.

Olfactory Bulbectomy

The animals in this group were also CF-I 40- to 45-day-old male albino laboratory mice. A bilateral olfactory bulbectomy was performed on these animals. Before the operation, the animals were anesthetized with pentobarbital sodium. Pontocaine was applied subcutaneously, and an incision was made medially from the level of the ears to 1/8 in. from the tip of the nose. The skin was pulled to one side, exposing the skull. Two 1/8-in. holes were drilled bilaterally in the skull, exposing the olfactory bulbs. The dura mater was incised, and the bulbs were removed by means of suction through a micropipette. The hole in the cranium was then sealed with bone wax to prevent bleeding and infection. Antiseptic powder was applied to the wound before it was closed with a microwound clip. The animals were then placed in a clean cage warmed with a conventional lamp until recovery from the anesthesia. They were subsequently placed in their respective cages (two to a cage) for a recovery period of 21 days, after which they were tested for the behavioral and biochemical effects and then sacrificed, and the olfactory bulbectomy was confirmed macroscopically. For this part of the experiment, 120 animals were used.

EQUIPMENT

Three behavioral tests were used in this study: the "Mouse City" setup (Karczmar & Scudder, 1966; Scudder & Richardson, 1969), the "climbing screen" for measurement of rates of avoidance conditioning (Scudder, Avery, & Karczmar, 1965; Scudder & Richardson, 1969), and conventional photoactometers.

The "Mouse City" setup and the "climbing screen" have been described in detail in the above references. The exact technique was applied to these mice. The "Mouse City" quantitates the following behaviors: exploration, digging, stereotypic behavior, sleeping, freezing, contactual behavior, injection, sexual behavior, grooming behavior, carrying things, and aggression.

The climbing screen measures avoidance conditioning in terms of the ability of the average mouse to leave a base chamber and climb a ramp to avoid shock.

The photoactometers used in this study consisted of six conventional chambers 18-in. in diam. The chambers are semidark with a grid floor. The animals are without food and water for this experiment. The activity of each group of six mice is recorded every minute for the first 20 min, after which time the activity is recorded every 5 min for 1 h.

After the behavioral tests were measured, the animals were sacrificed and biochemical data was obtained; at this time, the ablation was confirmed.

The measurement of biogenic amines (dopa, dopamine, norepinephrine, and epinephrine) and serotonin was determined with an Aminco Bowman spectro-photoflurometer, according to the technique of Weigand & Perry (1961). The amine measurements were made on pooled lots of four brains.

RESULTS

Chronic Enucleation

Mouse City. The amount of a particular activity is represented as the average number of observations of the activity displayed per

Table 1
Photoactometer Data

	Mean Counts/Minute
OBE Control	50.00
OBE	86.00
Blind Control	46.00
Blind	41.00

SE = 1.05
SD = 7.21

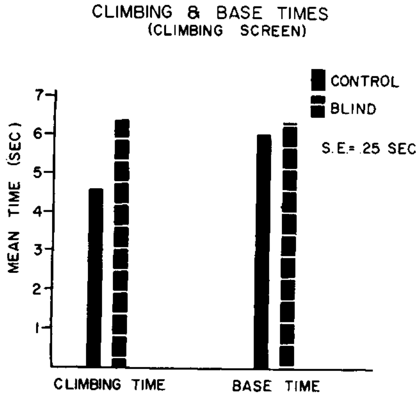

Fig. 1. Effects of enucleation on climbing and base times.

"city" study per mouse. A total of 40 city studies comprised this section of the experiment. There were 20 experimental and 20 control cities. Each city used 12 mice and was run in duplicate. The results obtained from the blind and the control animals were similar for all the traits studied, including aggression. The blinded animals, however, displayed a unique gesture, which was a slight "head bobbing," as if smelling or testing something in the air. This was not seen in the control animals.

Climbing screen. The data obtained from the climbing screen (Fig. 1) show that the enucleated and the control animals are not significantly different in their base times and are significantly different in their climbing times. The blind mice climb more slowly.

Photoactometers. The data for the photoactometer is presented in Table 1. There was a significant decrease in activity of the blind animals as compared to the controls.

Biogenic amines. There was no difference found in the levels of biogenic amines between the blind and control animals (Table 2).

Olfactory Bulbectomy

Mouse City. The results obtained for the "Mouse City" are summarized in Fig. 2. As in the previous section, 40 cities were conducted, 10 control with replicates and 10 experimental with replicates. Unlike the blind mice, the olfactory bulbectomized animals showed a pronounced decrease in exploration, sleeping, contactual behavior, and all forms of grooming behavior, as compared to the controls. Freezing behavior increased and stereotypic behavior remained constant. These animals were more reactive when approached by another animal. A "confrontation" between animals resulted in a "popcorn" or "dominoes" effect. That is, one animal would jump and hit another, who in turn would hit another. This lasted until the animals would go to their own cages, away from the other animals. However, if undisturbed, they were calm, showing slight motor activity. No aggression was seen in the treated mice.

Climbing screen. The data obtained from the "climbing screen" for the olfactory bulbectomized animals are presented in Fig. 3. The olfactory bulbectomized animals left the base chambers more quickly than their controls but resembled the controls in their climbing time.

Photoactometers. The photo activity data is presented in Table 1. The activity of the olfactory bulbectomized animals recorded in the photoactometers was significantly higher than that of their controls. This increase in activity was most probably a result of the previously mentioned "popcorn" effect. There was no significant difference in levels of biogenic amines between the operated animals and the controls (Table 2).

Table 2
Biogenic Amine Levels (/gm Wet Brain)

	D	DA	N	E	S
OBE Control	.109	.359	.478	.158	.277
OBE	110	.361	.466	.190	.288
Blind Control	.108	.360	.470	.180	.265
Blind	.115	.350	.480	.176	.268

DISCUSSION

These two different ablations of primary sensory centers seem to have caused two distinct behavioral syndromes in the mice. These two syndromes may be manifest only when the animal is placed in an aversive or threatening situation. The two treated groups reacted differently when presented with similar situations and changes in the environment.

The olfactory bulbectomized animals were highly reactive when approached by another animal, whereas the enucleated animals showed none of this activity. The base times of the olfactory bulbectomized animals were decreased as compared to the base times of the enucleated animals with respect to their controls, another indication of increased reactivity. The base times of the enucleated animals were slightly increased. Both of these situations, confrontation and the shock of the climbing screen, are alerting or arousal situations and produced motor reactions in the olfactory bulbectomized animals, whereas the same situations minimized the motor responses of the blind animals. The olfactory bulbectomized animals compared closely to the controls, but the enucleated animals showed a decrease in climbing times, again representing a decrease in reactivity under stress. This reactivity under stress is also manifest in the exploratory and aggression behaviors. The exploratory behavior of the olfactory bulbectomized animals in the city studies decreased. This decrease in exploration demonstrates that the animals were highly reactive to the environment, because when the animals began to explore, they would confront another animal and begin a "popcorn" effect which, due to the physical shape of the "mouse city" apparatus, enables the mice to go into different areas of the city to "get away from" the other animals. There is no such escape possible in the photoactometers, however, and the "popcorn" effect was allowed to perpetuate itself (thus increasing activity). The decrease in exploration accompanied an increase in freezing behavior for these bulbectomized animals that would freeze in the small chambers immediately after the "popcorn" effect ended. The absence of aggressive behavior in the olfactory bulbectomized animals possibly resulted from the decreasing number of encounters which, in turn, resulted from the decrease in exploration (Karczmar & Scudder, 1968).

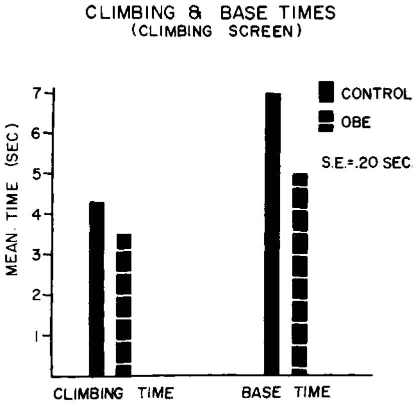

Fig. 2. Effects of olfactory bulbectomy on behavior. Control exploration = SC ± 1.30 checks/run/mouse; experimental exploration = SD ± 1.20 checks/run/mouse.

Subjective evaluation of the behaviors of both olfactory bulbectomized and enucleate animals were similar when the animals were solitary, but differences were immediately apparent in either a social or a stressful situation.

These then represent a definite "personality" change as a result of removal of neuronal tissue. This change in "emotion" or "mood" or "personality" has been related by some workers to changes in patterns of brain circuitry (Gellhorn, 1961; Stanley-Jones, 1965). This change in circuitry, according to many authors, depends upon, or at least is accompanied by, changes in the levels of the biogenic amines.

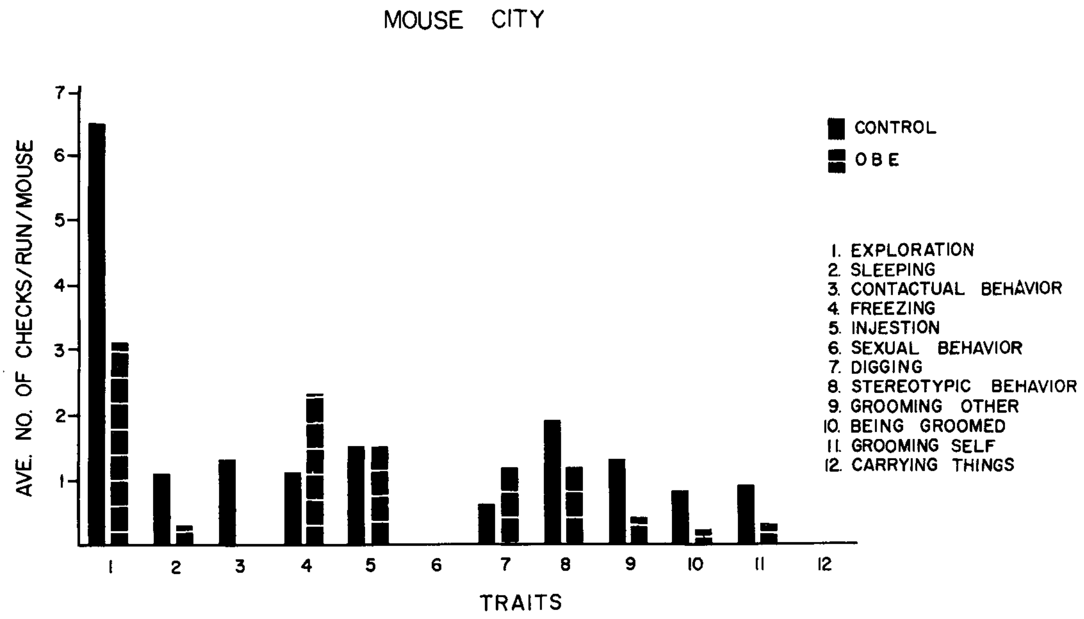

Fig. 3. Effects of olfactory bulbectomy on climbing and base times.

Our study shows a change in personality or mood without fluctuations in the total brain amine levels. This supports the contention that the behavior of animals may not be strongly dependent upon these biochemical substances as modulators of behavior in the central nervous system (Karczmar & Scudder, 1966, 1967; Richardson, Scudder, & Karczmar, 1969). The circuitry or system of synapses in the central nervous system responsible for behavior may depend upon the quantity and quality of sensory input, learned conditioned adaptations, and transmission nets (releasing mechanisms?) involving substances not measured in these experiments.

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NOTE

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