Ron Baxter
Zoology Senior Thesis

BEHAVIORAL COMPENSATION IN 6-HYDROXYDOPAMINE LESIONED PARKINSON RAT MODELS USING ADRENAL-MEDULLARY TISSUE AUTOGRAFTS.

Completed under the direction of
Dr. Darrell J. Graff
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"Behavioral Compensation in 6-Hydroxydopamine Lesioned Parkinson Rat Models Using Adrenal-Medullary Tissue Autografts."

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The thesis is approved and acceptable in quality and form.

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ABSTRACT

The study investigates the possibility and theoretical implications of reinnervation of a dopamine-deficient substantia nigra in a chemically-lesioned Sprague-Dawley rat using adrenal tissue autografts. The chemical 6-hydroxydopamine is used to chemically lesion the nigro-striatal pathway in the rat brain. The results suggest that when pieces of chromaffin tissue from the adult rat adrenal medulla are transplanted into the dopamine-denervated rat brain, behavioral compensation occurs. A summary of selected literature discussing nervous tissue reinnervation is included.

INTRODUCTION

Parkinson's disease afflicts more than a million Americans, usually beginning after age 50 and, most often, develops to a stage characterized by total incapacitation due to tremors, muscle rigidity, speech impairment and extreme difficulty initiating movement (Stromberg et al., 1984; Jorg and Janssen, 1987). The causative agent(s) may be viruses or environmental toxins, but the symptomatology stems from selective destruction of brain cells that produce dopamine, a central nervous system neurotransmitter (Stromberg et al., 1984). Neurons in the substantia nigra (Fig. 1), are the primary targets of the destructive agents (Neely and Jones, 1917; Brissaud, 1895; Greenfield, 1958).

The substantia nigra plays a crucial regulatory role in the control of muscle movements (Kish et al., 1988; Backlund et al., 1985). It does so through action on a second, much larger, brain area, the striatum, which is believed to govern initiation of voluntary movement. The nigral cell axons release dopamine in the striatum, where it is excitatory.

As the substantia nigra degenerates in Parkinson's disease, the striatum is gradually deprived of dopamine, stopping its activity. The drug L-dopa and analogs (bromocriptine) used to treat Parkinson's disease temporarily compensate for the decreased nigral function because they produce dopaminergic effects in the brain (Stromberg, et al., 1984). Unfortunately, L-dopa has severe side effects which may include disruptions of cardiac rhythm, nausea, headaches, dizziness, and depression. In addition, its effectiveness is lost after several years. Hence, it cannot alter the course of substantia nigra degeneration (Diamond et al., 1976; Hunter et al., 1973).

The symptoms of Parkinson's disease can be mimicked by artificial means in the laboratory upon disruption of the nigro-striatal pathway. Lesions of the nigro-striatal neurons with 6-hydroxydopamine (6-OHDA) were first demonstrated in 1968 (Ungerstedt, 1968) and have become a very popular model for Parkinson's disease. Histological examination of brain tissue from lesioned animals shows that intracerebrally injected 6-OHDA is able to induce degeneration of dopamine cell bodies, axons and terminals in the vicinity of the site of injection (Ungerstedt,

1968). It seems most plausible that 6-OHDA is taken up selectively by sympathetic neurons in a manner similar to that used for dopamine, the physiological transmitter. It remains to be elucidated if 6-OHDA is also stored in synaptic vesicles within the axon terminal. Once localized in the terminals, 6-OHDA and/or its potential metabolites provoke neuronal degeneration by a mechanism which remains obscure; 6-OHDA is a strong reducing agent, undergoing rapid oxidation in neutral aqueous solution. This property might be responsible in part for its effects (Tranzer and Thoenen, 1968).

Rats with large unilateral chemical lesions of the nigro-striatal dopamine system are known to turn or rotate after treatment with various drugs that interfere with monoamine transmission (Ungerstedt and Arbuthnott, 1970). This behavior was reinvestigated after 6-OHDA lesions of the nigro-striatal dopamine system. A method of quantifying the behavior in a specially designed "rotometer" has been developed (Ungerstedt and Arbuthnott, 1970). The rotational behavior is highly reproducible; its severity seems to parallel the degree of dopamine receptor stimulation (Ungerstedt, 1971).

The objectives of this study were 1) induce Parkinson-like symptoms in rats using 6-hydroxydopamine (6-OHDA) and 2) reinnervate the lesioned pathway by implanting adrenal-medullary tissue into the host brain. These animals were then examined for behavioral evidence demonstrating compensation by dopamine producing adrenal-medullary cells.

There are several reasons for choosing adrenal-medullary tissue in attempting to counteract the symptoms of experimental Parkinsonism. First, several studies (Stromberg et al., 1984; Freed et al., 1983; Lishajko, 1976; Sider and Carlsson, 1972) have shown that the normal adrenal medulla produces dopamine as an intermediary in the synthesis of adrenaline (Fig. 3). Second, chromaffin cells have been shown to be converted from an endocrine phenotype toward an adrenergic neuronal phenotype in the absence of glucocorticoids and in the presence of nerve growth factor (Olsen, 1970; Pohorecky and Wurtman 1971; Tischler and Green, 1980). Adrenal chromaffin cells, normally rounded in shape, become angular and develop processes when grown as grafts in the anterior eye chamber or when grown in culture in the absence of corticosteroids (Backlund, 1985; Stromberg et al., 1984; Olsen, 1970). Finally, such intraocular chromaffin grafts are able to effectively innervate brain tissue grafts of cortex cerebri or hippocampal formation as demonstrated by histochemical studies (Stromberg, 1984).

METHODS AND RESULTS

Control Animals

Five rats (Sprague-Dawley, 350-400 g.) were injected (IP) with 5 mg/kg of the dopamine-releasing agent, methamphetamine dissolved in 5 mg/ml isotonic saline (Dunnett et al., 1981) to test for unilateral dopamine deficiency within the nigro-striatal

tissue (Kelly, 1975). Such injections induce intense rotational behavior in the direction of the dopamine deficiency (Ungerstedt, 1971; Ungerstedt and Arbuthnott, 1970). There were no abnormal rotational behaviors observed in any of the rats.

Constructing the Parkinson Model

Animals were anesthetized by intraperitoneal injection with sodium pentobarbital (Nembutal;Sigma) at a dosage of 40 mg/kg. In order to minimize the possibility of infection and to facilitate surgery, the top of the animal's head from between the eyes to well behind the ears was shaved. The animals were then mounted in a stereotaxic frame (Fig. 2).

Longitudinal midline incisions were made in the skin on top of the head exposing the skull (Fig. 1a). The incisions were about 2.0 cm long, permitting both bone suture junctions, bregma and lambda, to be seen in the wound (Fig. 1a). The periosteal connective tissue adhering to the bone was scraped away with the blunt edge on the back of a scalpel blade; this procedure minimizes bleeding because the capillaries are crushed rather than cut smoothly. Injection coordinates with respect to bregma were: anterior, +0.9 mm; lateral, +2.8 mm; and vertical, -5.0 mm (Fig. 1b,c), with the incisor bar of the apparatus set 2.3 mm below the intra-aural line.

All animals were given unilateral right nigro-striatal chemical lesions by injection of eight ug 6-OHDA HCL (free base weight) dissolved in 4 ul ascorbate solution (0.2mg ascorbic acid

per 1 ml 0.9% saline) and injected stereotaxically over 4 min. After surgery, the rats were dosed with Terramycin (tetracycline) intra-muscularly and sulfathiozole topically. The bone was then sealed with wax and the skin was sutured.

Testing the Model

The initial methamphetamine test was conducted 12 days after the 6-OHDA injection. Rats were injected (IP) with 5 mg/kg methamphetamine (dissolved in 5 mg/ml isotonic saline; pH 5.5) and observed for rotational behavior. After several minutes some stretching and cleaning behavior was observed. When the rats were placed in an open field they began turning in tight circles. Rotation was measured by tallying the number of turns ipsilateral with respect to the lesion. All animals achieved a consistently high percentage of ipsilateral turns and were used in subsequent testing or underwent transplantation surgery.

Transplants

Approximately 2 months after the 6-OHDA denervations, each animal was anesthetized with Nembutal and placed in a stereotaxic frame (Fig. 2). Adrenalectomies were then performed on several non-lesioned rats to obtain adrenal glands. Adrenal capsules were opened and the adrenal cortex cut away from the medulla. In order to ensure as complete a removal of the cortical tissue as possible, 10-20% of the outermost medullary tissue also was removed.

Using a small dental drill, a hole was made in the skull of the lesioned animals at the same coordinate site used for the chemical lesion. All adrenal-medullary implants were made into the head of the caudate nucleus (Fig. 1b,c) at the coordinate site, (A: +0.9 mm; L: +2.8 mm; V: -5.0 mm;), using a 10 ul glass capillary attached to a 25 ul Hamilton syringe.

Results

All rats survived the surgical procedures without any noticeable changes in behavior. A second methamphetamine-induced rotation test was carried out as described above. Compensation was observed in all three transplanted animals; the rats no longer turned ipsilaterally in tight circles. A video was produced illustrating the surgical procedures and the behavioral responses due to the injection of methamphetamines and is currently on file at the Weber State College Zoology Dept.

DISCUSSION

Previous workers have shown that grafts of human fetal substantia nigra to the vicinity of, or into, the striatum counteract the effects of 6-OHDA-induced degeneration of the nigro-striatal system (Brundin et al., 1986; Stromberg et al., 1986; Brundin and Bjorklund, 1987). Thus, the idea of substituting a missing transmitter in brain tissue by intracranial grafting of cells that synthesize the missing compound

has proven experimentally feasible.

Studies of intraventricular chromaffin tissue grafts demonstrated that such grafts survived, synthesized catecholamines, and counteracted the methamphetamine-induced rotational behavior in 6-OHDA-denervated rats to approximately the same extent as substantia nigra grafts (Olsen, 1970). The results of this study are in agreement with Olsen's work. Freed et al. (1983) found that, intraventricularly, the chromaffin cells formed very few nerve fibers. They concluded that the restorative effect on the rotational behavior caused by the grafts was probably due mainly to diffusion of catecholamines into the host striatum from the ventricle. Intracranially, there was an absence of glucocorticoids since the grafts were devoid of adrenal cortex. Therefore, the very low number of fibers formed by the intracranial chromaffin grafts is probably best explained by an absence of nerve growth factor in the brain. If needed, improved fiber formation by the chronic chromaffin grafts might be achieved by local addition of nerve growth factor.

A recent study examined the pattern and degree of dopamine loss in the striatum obtained at autopsy from eight patients with idiopathic Parkinson's disease (Kish et al., 1988). In the putamen, there was a nearly complete depletion of dopamine in all subdivisions, with the greatest reduction occurring in the caudal portions (less than 1 percent of the dopamine remaining). In the caudate nucleus (Fig. 1b,c), the only subdivision with severe dopamine reduction was the most dorsal and rostral part; other

subdivisions still had substantial levels. It was proposed that the motor deficits that are a constant and characteristic feature of idiopathic Parkinson's disease are for the most part a consequence of dopamine loss in the putamen, and that the dopamine-related caudate deficits are, if present, less marked or restricted to discrete functions only. Therefore, the putamen, particularly its caudal portions, may be the most appropriate site for intrastriatal application of dopamine-producing autografts in patients with idiopathic Parkinson's disease.

Occasionally, in the breeding colony at Weber State College we find rats which exhibit abnormal motor behavior. They appear to have very poor equilibrium and usually hold their heads to the side. If these rats are picked up by the tail they will twist in the air in tight circles. We tested the hypothesis that this behavior results from dopamine deficiency in the nigro-striatal pathway by administering a 0.5 ml dose of methamphetamine (IP). The methamphetamine produced CNS stimulation, but had no effect on rotational behavior.

We also tested varying doses of methamphetamine. After a 0.5 ml injection of methamphetamine solution (IP) one rat did not respond with the usual behavior. A second injection of 0.5 ml caused the rotational behavior characteristic of dopamine deficiency. The rat became extremely hypersensitive to light and sound and started behaving abnormally within five minutes. The rat died that night unobserved.

It has been a year now since researchers from Mexico

reported dramatic improvement in two Parkinson's disease patients who had received transplants of adrenal tissue into their brains (Lewin, 1988). Here, it appeared was a real breakthrough in Parkinson's disease therapy; the first since the introduction of L-dopa treatment in the late 1960's. Currently, though, there is a disparity between what we see in the American patients and what we have been told about the Mexican patients (Lewin, 1988). Part of the problem is that apart from the initial report a year ago, the Mexican team has published few details of its results. However, their study of 30 patients is currently being prepared. The scientific world is awaiting this report.

In addition, it was reported recently that many patients appear to experience a series of unusual and unexpected behavioral changes that begin immediately after surgery and may persist for several months (Lewin, 1988). First, patients had a significantly reduced need for analgesics immediately following surgery. Second, about three days after surgery, patients' sleep patterns changed for three or four days. Third, delusions of various sorts occurred, ranging from simple to flamboyant. Fourth, some patients experienced personality change, including disinhibition and mania, sometimes shifting from a reserved, conservative personality to being exuberantly outgoing. And fifth, several patients experienced visual and auditory hallucinations (Lewin, 1988).

A year after the first rush of enthusiasm, the prospects for

repairing some diseases of the brain in humans are perhaps dimmer in the short term but could certainly be brighter in the long term. It is an exciting era for neurological research. We may be on the threshold of some very exciting breakthroughs in the treatment of Parkinson's disease and other neurological disorders by way of tissue transplants.

CONCLUSION

The research reported here shows that when pieces of chromaffin tissue from the adrenal medulla of an adult rat are transplanted into the dopamine-denervated rat brain, behavioral compensation for the effects of the dopamine deficient nigro-striatal pathway occurs. Even though the sample size is small, the behavioral compensation is significantly and consistently demonstrable.

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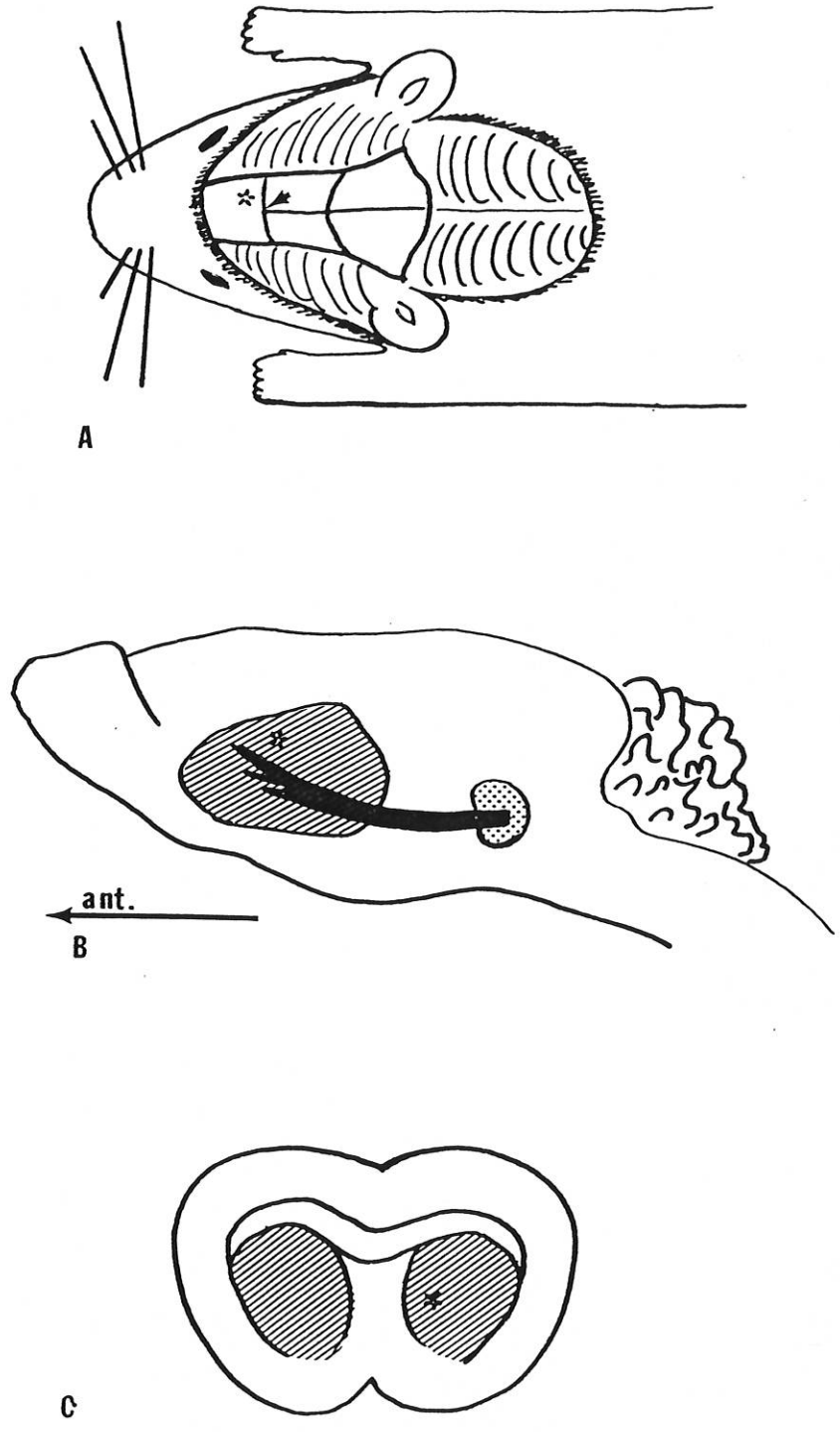

Figure 1: Crosshatching indicates caudate nucleus; stippling indicates substantia nigra; nigro-striatal pathway is in black; asterisk indicates lesion site; arrowhead indicates the landmark bregma. a) Dorsal view of exposed rat skull. b) Longitudinal midline section of rat brain. c) Cross-section of rat brain through the caudate nucleus.

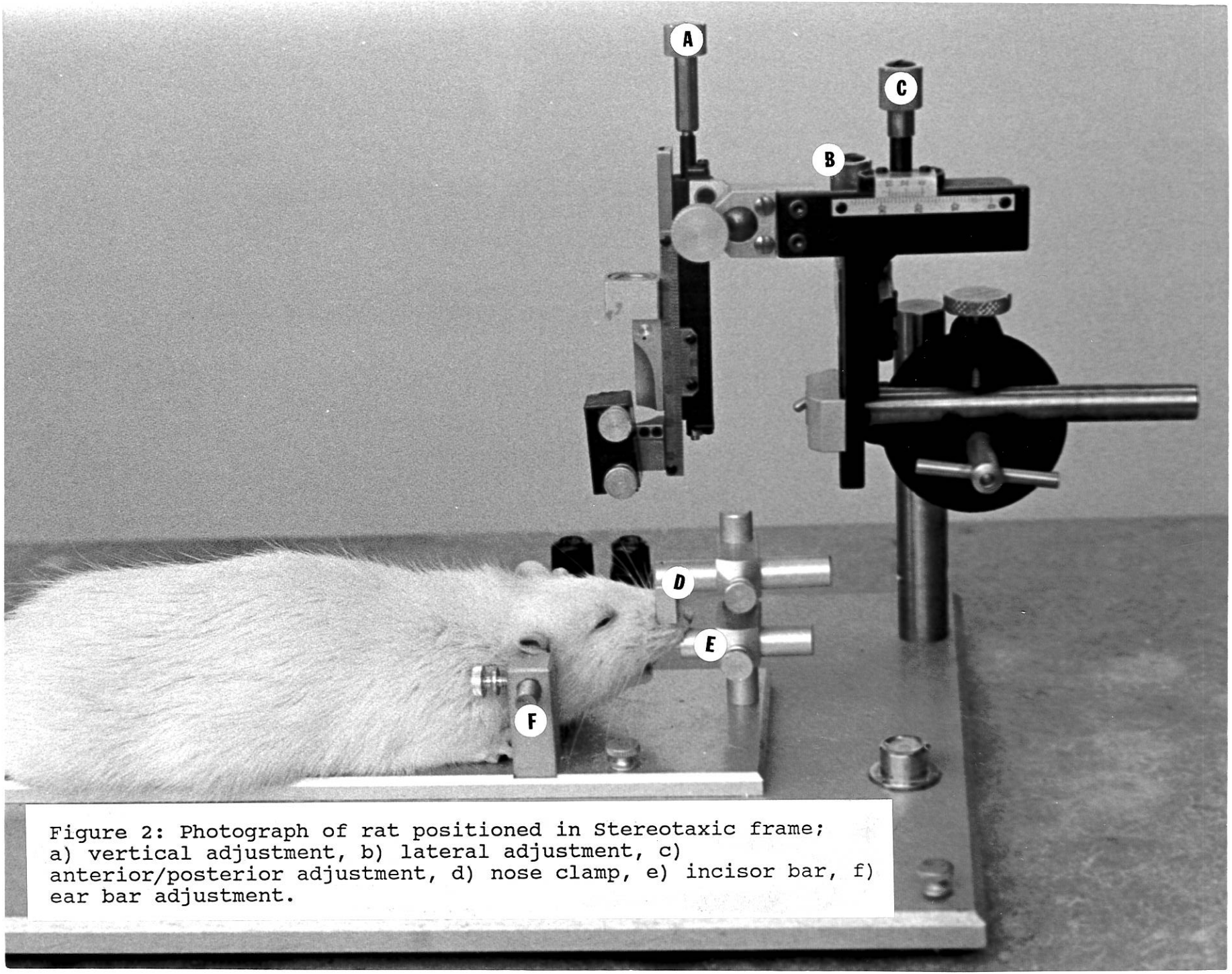

Figure 2: Photograph of rat positioned in Stereotaxic frame; a) vertical adjustment, b) lateral adjustment, c) anterior/posterior adjustment, d) nose clamp, e) incisor bar, f) ear bar adjustment.

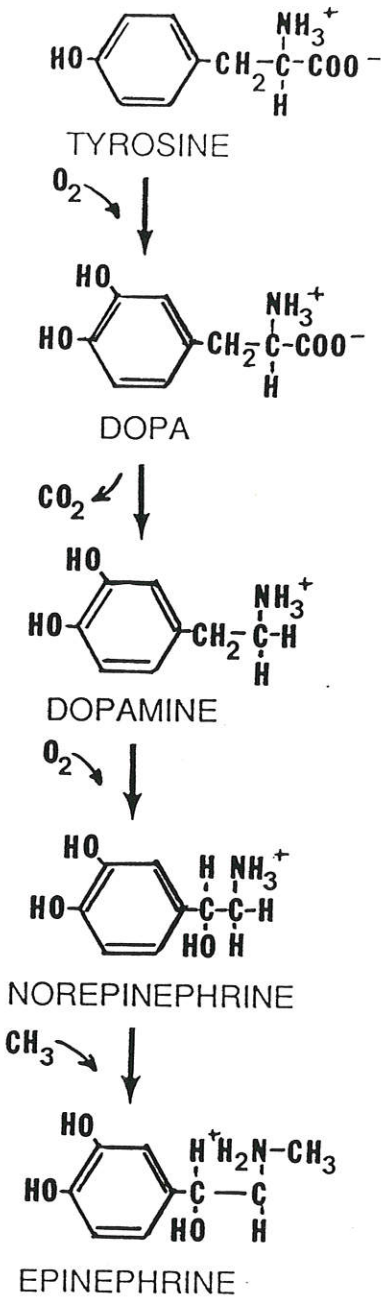

Figure 3: Catecholamine biosynthetic pathway.