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Influences on Handedness of Local Application of Acetylcholine with Glutamic Acid to Cerebral Cortex of the Rat

Edward R. Suess

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SUESS

INFLUENCE ON HANDEDNESS OF APPLICATION OF
ACETYLCHOLINE TO CEREBRAL CORTEX of the RAT

NAME AND ADDRESS	DATE

INFLUENCE ON HANDEDNESS OF LOCAL APPLICATION OF ACETYLCHOLINE WITH GLUTAMIC ACID TO CEREBRAL CORTEX OF THE RAT

By

Edward R. Suess

A Thesis
In partial fulfillment of the
Requirements for the Degree of
Master of Science in Psychology

The University of New Mexico
1949

This thesis, directed and approved by the candidate's committee, has been accepted by the Graduate Committee of the University of New Mexico in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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Aug. 1, 1949
DATE

INFLUENCE OF HANDEDNESS OF LOCAL APPLICATION OF ACETYLCHOLINE WITH GLUTAMIC ACID TO CEREBRAL CORTEX OF THE RAT

BY

Edward R. Suess

Thesis committee

Drown Peterson
CHAIRMAN

Morton J. Keston

C. Clayton Hoff

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

CHAPTER I

THE PROBLEM AND DEFINITION OF TERMS USED

This study is a continuation of investigation begun by Peterson and continued by Peterson and Rigney.[1] In an unpublished report to the AAAS in Philadelphia in 1940, Peterson first outlined the nature of the investigation of this problem.[2]

Essentially, it is to seek the neural equivalent of practice. Since the behavior of an organism is dependent upon organic structures, and since behavior changes with practice or experience, it may be assumed that some kind of organic change takes place within the organism, presumably in the nervous system, when such changes are manifested in behavior. What the nature of this change is, remains

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[1] Geo. M. Peterson, and Joseph W. Rigney, "Influence on Handedness of Local Application of Acetylcholine with Other Chemicals to the Cerebral Cortex of the Rat," _Jour. of Comp. and Physiol. Psychol._, In Press.

[2] Geo. M. Peterson, "Acetylcholine as a Neurochemical Equivalent of Practice," Unpublished report to the AAAS, Philadelphia, December, 1940, p. 1.

a persistent problem for psychology. Peterson selected an organic chemical, acetylcholine, as a promising drug to work with in attacking this problem. If it could be shown to have a facilitating effect upon a behavior trait subject to practice effects, this might be a first step in establishing a relationship between these behavior changes due to practice and physiological changes within the organism. Since acetylcholine does bear a close relationship to synaptic nerve conduction and since a particular behavior trait has definitely been established and localized as to function in the brain with a fairly accessible region for neurological work, it appears wholly warranted and feasible to approach the problem in the manner presented.

Handedness in the albino rat, a form of behavior easily observed and measured, is the particular trait selected for study.[3] Methods and materials employed to determine handedness along with the frequency of occur-

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[3] Geo. M. Peterson, "Mechanisms of Handedness in the Rat," Comparative Psychology Monographs, Vol. 9, No. 6, Baltimore: The Johns Hopkins Press, April, 1934. Pp. 1-67.

rence of predominantly single-handed and ambidexterous rats have been reviewed by Peterson.[4] The rat, regardless of his original hand preference, can be taught the use of his non-preferred hand through application of forced practice. This is ordinarily accomplished by employing an off-set feeding dish which makes the use of the preferred inconvenient. Practice with the non-preferred hand may result in transfers to its use in the normal situation with the center food dish. Unpublished studies in the University of New Mexico laboratory indicate that anywhere from one hundred to one thousand reaches in this situation may cause a shift in handedness to the use of the non-preferred hand. The degree of shift when placed in the normal feeding situation may be anywhere from an occasional use of the non-preferred hand to a complete shift.[5] Peterson attempted

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[4] *Loc. cit.*

[5] Peterson, *op. cit.*, "Acetylcholine as a Neurochemical Equivalent of Practice," Table I.

to induce a similar shift in handedness in the rat by applying acetylcholine to that portion of the cerebral cortex which controls the use of this non-preferred hand. His design was planned to effect these changes and to observe any significant shifts through this drug application.

Preliminary work with acetylcholine gave no consistently positive results; however, some interesting and significant observations were made as to the effects of the application of this drug. One hundred and fifty rats were utilized. One hundred and eight were single-handed and forty-two were ambidexterous. Only two single-handed rats and fifteen ambidexters were influenced in a positive direction; however, some of the animals exhibited clonic movements of the contralateral forelimb a few minutes after acetylcholine was applied to the cortex.[6] It can at least be assumed that the drug did reach the motor neurons of

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[6] Geo. M. Peterson, "Changes in Handedness in the Rat from the Local Application of Acetylcholine to the Cerebral Cortex," Jour. of Comp. and Physiol. Psychol., In press.

that portion of the cortex responsible for the innervation of that particular limb.

Later work in this direction made use of other chemicals with acetylcholine.[7] Those selected were inhibitors of cholinesterase, buffers, solvents natural to nerve tissue, and chemicals with parallel effects. One of the chemicals tested in combination with acetylcholine was glutamic acid. The natural glutamic acid was applied to three rats. A positive shift resulted in two instances, and no shift in one. Various combinations of other chemicals were also tried, none of which gave consistently positive results. It was found, however, that smaller concentrations of acetylcholine could be used to produce shifts than was previously used. One half of one percent to three percent solutions of acetylcholine were employed instead of the former five to ten percent concentrations.[8]

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[7] Peterson and Rigney, op. cit.

[8] Ibid.

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[7] Peterson and Rigney, *op. cit.*

[8] *Ibid.*

I. THE PROBLEM

Statement of the problem. This study was undertaken to determine the effects on handedness in the rat upon application of glutamic acid and acetylcholine to the cortex.

Importance of the study. Since acetylcholine is a natural constituent of nerve tissue, it is more than feasible to explore the effects of this chemical. Its role of pre-and post-synaptic conduction and also the assumption of a similar role at synaptic surfaces has already been indicated.[9] The literature is replete with the possibilities and potentialities of acetylcholine and its relationship and bearing to the nerve impulse. Recent investigations have pointed to glutamic acid as a chemical worth considering to aid in the facilitation of greater effects of acetylcholine.

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[9] T. H. Bullock, H. Grundfest, D. Nachmansohn, M. A. Rothenberg, "Generality of the Role of Acetylcholine in Nerve and Muscle Conduction," Jour. Neurophysiol., Vol. X, No. 1, January 1947, (Springfield: Charles C. Thomas), pp. 11-22.

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II. DEFINITION OF TERMS USED

Acetylcholine. This ester is a methylated quartternary ammonium base formed from choline and acetic acid. It is hygroscopic, soluble in alcohol and water; it is easily decomposed by heat or alkali.[10] Acetylcholine, liberated between many conducting tissues throughout the body, is synthesized by an enzyme, choline acetylase.[11] It is a very unstable ester in living tissue, being split by another enzyme, choline esterase, in a matter of milliseconds at the site of action during transmission of nerve impulses.[12] Acetylcholine is formed in brain slices under aerobic conditions if glucose or pyruvate is present.[13]

Glutamic acid. Glutamic acid is a monoamino dicarboxylic acid, and is one of the amino acids present in

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[10] Goodman and Gilman, The Pharmacological Basis of Therapeutics, (New York: The MacMillan Company, 1941).

[11] David Nachmansohn, "Role of Acetylcholine in Nerve Activity," Vitamins and Hormones, Vol. III, (New York: The Academic Press, 1945), p. 365.

[12] Ibid., p. 341.

[13] Ibid., p. 365.

II. DEFINITIONS OF [illegible]

Acetylcholine. This [illegible] is a quat-ernary ammonium base formed [illegible] and acetic acid.[10] It is hygroscopic, soluble in [illegible] and [illegible], easily decomposed by heat [illegible] liberated between [illegible] body, is synthesized by an enzyme, choline acetylase.[11] It is a very unstable [illegible] by another enzyme, cholinesterase, in a matter of milli-seconds at the site of action in the transmission of nerve impulses.[12] Acetylcholine is synthesized [illegible] aerobic conditions if glucose or [illegible] is present.[13]

Glutamic acid. Glutamic acid is [illegible] carboxylic acid, and is one of the amino acids [illegible]

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[10] Goodman and Gilman, The Pharmacological Basis of Therapeutics, (New York: The [illegible])

[11] David Nachmansohn, "[illegible] Nerve Activity," Vitamins and Hormones, Vol. III, (New York: The Academic Press, [illegible]

[12] Ibid., p. [illegible].

[13] Ibid., p. [illegible].

the body. The vast majority of amino acid molecules are derived from plant proteins where they are produced from their inorganic constituents by the action of photosynthesis.[14]

Glutamic acid is officially classified as a "non essential" amino acid; however, it is the only amino acid which is metabolized by brain tissue.[15] It has important therapeutic properties in that the mild form of epilepsy known as <u>petit mal</u> is now treated with it.[16] This acid is easily synthesized by the body.[17]

Glutamic acid is positively correlated with mental behavior. Statistically significant results were obtained for an increase in learning ability of rats to run a maze

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[14] R. A. Gortner, Outlines of Biochemistry, (New York: John Wiley and Sons, Inc., 1929), p. 423.

[15] B. J. Harrow, Textbook of Biochemistry, (Philadelphia: W. B. Saunders Company, 1947), p. 537.

[16] J. C. Price, H. Waelsch, and T. J. Putnam, "dl-Glutamic Acid Hydrochloride in Treatment of Petit Mal and Psychomotor Seizures," Jour. of Amer. Med. Assoc., Vol. 122, No. 17, (Chicago, 1943), p. 1153.

[17] Harrow, op. cit., p. 537.

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[17] Harrow, _op. cit._, p. 537.

when the l(+) form of glutamic acid was administered.[18] Glutamic acid contains a property not common to amino acids in general, since it cannot be replaced by glycine.[19]

Amino acids may act as buffers since they are amphoteric and can form fairly stable salts with either acids or bases. They may act both as acids and weak bases when in solution. Specifically, glutamic acid, existing in proteins in the form of an amide, is a stronger acid than base since it possesses the properties of two carboxyl groups.[20]

The optically active glutamic acid forms both d and l acids. Upon alkaline hydrolization, this active amino acid becomes a racemic amino acid in that one of the unions of the double bond within glutamic acid

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[18] F. T. Zimmerman, and S. Ross, "Effect of Glutamic Acid and Other Amino Acids on Maze Learning in the White Rat," Arch. of Neurol. and Psychiat., Vol. 51, (Chicago, 1944), pp. 446-51.

[19] Harrow, op. cit., p. 537.

[20] R. J. Williams, An Introduction to Biochemistry, (New York: D. Van Nostrand Company, Inc., 1931), p. 93.

may later become attached to hydrogen; therefore, rather than an active amino acid, we find equal amounts of d and l acids.[21] The symbols d and l indicate both the configuration and the direction of rotation. Thus, d(+) indicates d-configuration and dextrorotation. Various factors actually influence and determine the direction of rotation and subsequently the classification of amino acids. The d forms are usually represented in the literature as the natural forms. Glutamic acid pre-formed in the protein molecule has the same configuration around the alpha-carbon atom, namely, the l-form.[22]

Handedness and Ambidexterity. When placed in a feeding situation where they must reach with their forepaws for food, rats exhibit a preference for one hand or the other.[23] About ninety percent of all rats will either be completely left handed or completely right handed. The

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[21] Gortner, *op. cit.*, p. 406.

[22] P. H. Mitchell, *A Textbook of Biochemistry*, (New York: McGraw-Hill Book Co., Inc., 1946), pp. 95-96.

[23] Peterson, *op. cit.*, "Mechanisms of Handedness in the Rat," p. 1.

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[21] Cortner, op. cit., [illegible]

[22] [illegible] York: McGraw-Hill [illegible]

[23] Peterson, op. cit., [illegible] the Rat," p. [illegible]

remaining ten percent are more or less ambidexterous, although most of these continue to show a preference for one hand. Rats with greater degrees of ambidexterity presumably could be most easily influenced by drug application; therefore, these rats were more carefully sought for our study.

Practice. This is the repeated performance of the white rat in reaching for food, grasping it, and then conveying it to the mouth, as practiced in the forced reaching situation. Continued practice or use of the non-preferred hand brings about an inclination of the rat towards the use of this hand.

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CHAPTER II

REVIEW OF THE LITERATURE

The literature dealing with acetylcholine and its role as mediator of nerve impulses is voluminous. This work has been reviewed by Rigney.[1] What will be presented here is a brief discussion of its relationship to glutamic acid.

I. ACETYLCHOLINE

This ester is found within the parasympathetic nervous system as well as the central nervous system. Due to its presence at synapses and myoneural junctions at the time of nerve stimulation, it has received a great deal of attention in recent years. At least one investigator, Nachmansohn, regards it as the propagator as well as transmitter of the nerve impulse. His three lines of investigation center about the facts that cholinesterase

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[1] Joseph W. Rigney, "Influence on Handedness of Local Application of Acetylcholine with DFP and Glycine to Cerebral Cortex of the Rat," (Unpublished Master's thesis, The University of New Mexico, Albuquerque, 1948).

is present in high concentrations at the nerve during the time of neural activity; there is a definite localization of cholinesterase at the neuronal surface; and a close parallel exists between the action potential and the activity of this enzyme, as has been evidenced in the electric organs of certain fish.[2] Cholinesterase is also used as an index to measure, indirectly, the amount of acetylcholine present at the particular locus of nerve activity or transmission.[3] Other investigations with acetylcholine include Elliot, working within the autonomic nervous system;[4] the extensive work of Miller and his co-workers;[5] Loewi's experiments with frog hearts and the study of transmission

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[2] Nachmansohn, op. cit., p. 341.

[3] Ibid., p. 342.

[4] T. R. Elliot, "On the Action of Adrenalin," (J. Physiol., 31:XX), cited by Cannon and Rosenbleuth, Autonomic Neuro-Effector Systems, Experimental Biology Monographs, (New York: The MacMillan Co., 1937), p. 21.

[5] F. R. Miller, G. W. Stravraky, and G. A. Woonton, "Effects of Eserine, Acetylcholine and Atropine on the Electroencelpalocorticogram," Jour. Neurophysiol., Vol. III, No. 2, March, 1940, (Springfield: Charles C. Thomas), pp. 131-38.

at parasympathetic nerve endings;[6] and Kibjakow and Dale's contributions to the theory of chemical transmission in the central nervous system.[7] A recent review by Feldberg deals with acetylcholine and the problem faced in reconciling the chemical theory and the objections against such a theory.[8] One of the most severe critics of the chemical mediation theory is Eccles, who lists several criticisms in one of his earlier reports.[9] Work by Nachmansohn and his associates on the pharmocological effects of acetylcholine,[10] and the studies of Sjostrand,[11] deal further with the prop-

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[6] Otto Loewi, Harvey Lectures, 28 (1932-33), 218 (1934), cited by Nachmansohn, op. cit., p. 338.

[7] Nachmansohn, op. cit., p. 339.

[8] W. Feldberg, "Present Views on the Mode of Action of Acetylcholine in the Central Nervous System," Physiol. Rev., Vol. 25, (Baltimore, Maryland, 1945), p. 596; also Nachmansohn, op. cit., p. 339.

[9] J. C. Eccles, "Synaptic and Neuro-muscular Transmission," Physiol. Rev., Vol. 17, (Baltimore, 1937), pp. 538, ff.

[10] M. A. Rothenberg, D. B. Sprinson, and D. Nachmansohn, "Site of Action of Acetylcholine," Journal of Neurophysiology, Vol. XI, No. 2, March, 1948.

[11] T. Sjostrand, Jour. Physiol., 90:41P., 1937, cited by Feldberg, op. cit., p. 598.

erties and effects of this chemical. A study of the synaptic region of the neuron is taken up by Gasser in his report.[12] Erlanger discusses the fundamental differences between nerve fiber and synapse conduction.[13]

It might be noted that due to recent discoveries by Nachmansohn, cited at the beginning of this chapter, a modified theory of the activity of acetylcholine in the nervous system has been introduced. He has replaced the oldger extracellular theory with an intracellular theory. This indicates a similar role for acetylcholine in conduction along fibers and across synapses.[14]

II. GLUTAMIC ACID

With the recent discovery through clinical observations that glutamic acid has a fovorable effect on pat-

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[12] H. S. Gasser, "Axons as Samples of Nervous Tissue," Synapse Symposium, Journal of Neurophysiology, Springfield, Illinois: Charles C. Thomas, September, 1939, Vol. 11, No. 5, pp. 361-69.

[13] J. Erlanger, "Initiation of Impulses in Axons," Synapse symposium, Journal of Neurophysiology, Vol. 11, No. 5, Sept. 1939, (Springfield: Charles C. Thomas), pp. 370-78.

[14] Nachmansohn, op. cit., p. 360.

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[13] J. Erlanger, "Interrelations of Axons and Synapses," Synapse symposium, Journal of Neurophysiology, Vol. II, No. 5, Sept. 1939, (Springfield, Ill.: Charles C. Thomas), pp. 370-78.

[14] Nachmansohn, op. cit., p. 30.

ients suffering from petit mal, the mild form of epilepsy, further work has been taken up to test its effects on nerve activity and its relationship to acetylcholine.[15] Nachmansohn and his associates found that l(+) glutamic acid in 2 X 10^{-2}M concentrations increases the rate of formation of acetylcholine by about four to five times.[16] The d(-) glutamic has a small effect. Of all other amino acids tested thus far, glutamic acid rates second only to cysteine in having the strongest effect on acetylcholine formation.[17] As has been earlier mentioned in this report, Peterson used the natural glutamic acid in establishing two positive shifts in handedness upon application with acetylcholine to the cerebral cortex of three animals.[18]

It is view of the above facts that this study was run systematically to determine any possible effects of this amino acid with acetylcholine.

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[15] J. C. Price, H. Waelsch, and T. J. Putnam, loc. cit.

[16] D. Nachmansohn, H. M. John, and H. Waelsch, "Effect of Glutamic Acid on the Formation of Acetylcholine," Journal of Biological Chemistry, Vol. 150, No. 2, October, 1943. Pp. 485-86.

[17] Nachmansohn, op. cit., p. 367.

[18] Peterson, op. cit., "Changes in Handedness in the Rat from the Local Application of Acetylcholine to the Cortex."

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[15] J. G. [illegible]

[16] D. [illegible] of Glutamic [illegible] of Biological Chemistry, Vol. [illegible] Pp. [illegible]

[17] Nachmansohn, [illegible]

[18] Peterson, [illegible] Rat from the [illegible]

III. HANDEDNESS AS A CRITERION OF CEREBRAL FUNCTIONING

Handedness was chosen as a suitable trait to be observed and measured in testing the influence of drug application since it has been localized in the brain.

Locus on cerebral cortex which controls handedness of the white rat. Cerebral dominance of handedness and the effects of destruction in particular areas of the cortex of the rat has been the object of several studies.[19,20] In later studies, the locus controlling handedness was more clearly specified. It was found that a small region in the contralateral frontal lobe of the cerebral cortex is essential for the control of handedness.[21]

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[19] Geo. M. Peterson, "A Preliminary Report on Right and Left Handedness in the Rat," Jour. of Comp. Psychol., Vol. XII, (Baltimore, Md., 1931), p. 248.

[20] Peterson, op. cit., "Mechanisms of Handedness in the Rat," pp. 1-67.

[21] Geo. M. Peterson, and La Charles Fracarol, "The Relative Influence of the Locus and Mass of Destruction Upon the Control of Handedness by the Cerebral Cortex," Jour. of Comp. Neurology, Vol. 68, No. 2, (Philadelphia: The Wistar Institute Press), Feb., 1938, p. 189.

III. [illegible]

[illegible]

Handedness [illegible]

observed and measured [illegible]

application since it [illegible].

Locus on cerebral cortex [illegible]

of the white rat. Cerebral [illegible]

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[19] Geo. W. Peterson, [illegible] and Left Handedness in the Rat, [illegible] Vol. XII, (Baltimore, [illegible]).

[20] Peterson, op. cit., [illegible] in the Rat," pp. [illegible].

[21] Geo. W. Peterson, [illegible] Relative Influence of [illegible] Upon the Control of Handedness [illegible] Jour. of Comp. Psychology, Vol. [illegible] The Wistar Institute Press, [illegible].

Reliability of ambidexterity. The first fifty reaches during an observation period were found to be a good indication as to the variation expected from day to day.[22] Statistical analysis confirmed the high reliability of these observations in a recent study.[23]

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[22] Ibid., p. 176.

[23] Rigney, op. cit.

CHAPTER III

METHODS USED AND THE EXPERIMENTAL DESIGN

The procedure followed in this study is similar to that used by Rigney.[1] A factorial design was laid out according to Fisher.[2] Since d and l glutamic acid were both available, an additional measurable influence was possible regarding application to the left and right hemisphere.

Observation of Handedness. Rats were first observed and selected for varying degrees of ambidexterity. Since there were not a sufficient number of natural ambidexters available to complete the experimental design, it was necessary to place ten single-handed rats into a forced reaching situation whereby they eventually learned to make use of their non-preferred hand as well as the normally preferred hand. These artificial ambidexters were treated

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[1] Rigney, op. cit.

[2] Ronald A. Fisher, The Design of Experiments, (London: Oliver and Boyd, 1947).

in the same manner as the normal ambidexters.

After selection, each rat was observed for three days prior to the operation day. These observations included two sets of fifty reaches for each day. On the fourth day, operation day, a set of fifty reaches was taken and the rat then immediately put through the operational procedures. After the operation, the rat was again placed into the feeding situation to be observed for fifty or more post-operational reaches. Fifty to one hundred reaches were also observed from three to five days after the operation.

Operational procedure. After the first fifty reaches on operation day, the animal was immediately anesthesized by ether. The hair was clipped from the top of the head where an incision was to be make. The skull was exposed and trephined at the frontal lobe. The drug or drug combinations were applied directly to the cerebral cortex by a small ball of cotton saturated with these solutions. The incision was again closed with wound clips and the rat placed once more into the feeding cage

In the same manner [illegible]

After selection, [illegible] days prior to the [illegible] cluded two sets of fifty [illegible] fourth day, operation day, [illegible] taken and the rat then [illegible] ational procedures. [illegible] again placed into the [illegible] for fifty or more post-[illegible] one hundred reaches [illegible] days after the operation.

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from which he had previously been taken.

With a few rats, drugs were re-applied a few days after the initial operation. The procedure followed was much the same as that mentioned above.

Factorial design and drug combinations. A Fisher factorial design on analysis of variance technique made use of combinations of twelve rats or any multiple thereof. This design had one replication, thereby utilizing twenty-four animals. The limitation in number was due to the non-availability of ambidexters. The drug combinations used were assigned the following letters: Acetylcholine, A; l-glutamic acid, B; d-glutamic acid, B'. Operation and application to left and right hemisphere were assigned the letters C and c respectively. Total possible combinations are shown in table I. Samll letters designated the absence of the drug in the various combinations.

The chemicals used were of approximately the following concentrations: Acetylcholine 3 to 5 percent, glutamic acid .1 M.

TABLE I

RATS, COMBINATIONS, AND DRUGS

Rats		Combinations	Drugs
1M	13M	ABC	Acetylcholine, l-glutamic acid left hemisphere
2M	14M	AB'C	Acetylcholine, d-glutamic acid left hemisphere
3M	15M	ABc	Acetylcholine, l-glutamic acid right hemisphere
4M	16F	AB'c	Acetylcholine, d-glutamic acid right hemisphere
5M	17M	AbC	Acetylcholine, left hemisphere
6M	18F	Abc	Acetylcholine, right hemisphere
7M	19M	aBC	l-glutamic acid, left hemisph.
8M	20F	aB'C	d-glutamic acid, left hemisph.
9M	21F	aBc	l-glutamic acid, right hemisph.
10M	22M	aB'c	d-glutamic acid, right hemisph.
11F	23M	abC	control rats, left hemisphere
12F	24M	abc	control rats, right hemisphere

CHAPTER IV

RESULTS, TABLES, AND ANALYSIS

The results to be discussed include the reliability of ambidexterity in the rat and the possible influence of orders; and the variances from the factorial design including the effects of acetylcholine and glutamic acid.

I. INFLUENCE OF ORDERS ON AMBIDEXTERITY

An analysis of variance was done on pre-operational data of twenty-four rats used in this study to determine the influence of orders on handedness. Results are shown in table II. The total contribution of orders to the total variance was non-significant. These results are in accord with those found by Rigney.[1] Since orders were judged to be two samples of the same distribution, it was justifiable to determine the contribution of drugs accounting for any differences between operation day orders.

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[1] Rigney, *op. cit.*

II. THE DRUGS AND THEIR EFFECTS

Acetylcholine. The variance contributed by acetylcholine was only .04, which is considerably less than experimental error. Since this is the lesser mean square, it was used in the denominator to determine the F ratio. This ratio is significant at the five percent level, indicating, if this level of significance is accepted, that there is greater similarity between acetylcholine rats and non-acetylcholine rats than would be expected by chance. Since there is nothing in the experimental design to warrant such a result, we can only conclude that the one time in about twenty to be expected, has occurred here.

Glutamic acid. The variance contributed by glutamic acid was 9.30. This was significant at neither the one nor five percent level. See table IV.

With only one rat, number twenty female, was there a positive shift in handedness after application of this drug. Although this was only one rat of the total used that exhibited any curious effects, it is interesting to note that upon the application of d-glutamic acid to the left hemisphere,

II. THE DRUGS AND [illegible]

Acetylcholine. The [illegible] of [illegible] and l-choline was only [illegible], which [illegible] error. Since [illegible] the [illegible] used in the denominator to determine [illegible] is not significant at the five percent level. [illegible] level of significance is accepted, that there [illegible] larity between acetylcholine rate and [illegible] than would be expected by chance. Since there is [illegible] the experimental design [illegible] conclude that the [illegible] has occurred here.

Glutamic acid. The [illegible] was 9.30. This was significant [illegible] five percent level. See Table [illegible].

With only [illegible] positive shift in [illegible] after [illegible]. Although this was only [illegible] any curious effects, [illegible] on the application of d-glutamic acid [illegible].

right and left rear spasticity became evident fifteen minutes after the operation, March 27. A shift did not show up in the first fifty reaches even though occasional incomplete right passes were made in the feeding situation. Subsequent post-operational reaches showed a marked shift to the use of the non-preferred hand. The following day, March 28, reaches were again comparable to pre-operational reaches. On March 29, the drug was re-applied. All reaches, pre-operational and post-operational, were consistent and showed no change. See table VI. Post-mortem examination of the brain revealed no mechanical damage to cortical tissue.

In a number of other rats, re-application was made a day or two after the original operation. All these cases showed no shift in handedness.

There was no significance for any of the interaction terms.

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TABLE II

ANALYSIS OF VARIANCE OF SIX DAYS PRE-OPERATION DATA FOR TWENTY-FOUR RATS

	Sum of Squares	D.F.	Mean of Squares
Total	88,959	143	622
Rats (i)	32,798	23	1426
Days (j)	760	2	380
Orders (k)	57	1	57
(Interactions)			
i j	5,053	46	110
i k	851	23	37
j k	241	2	121
i j k	49,199	46	1070

TABLE III

FACTORIAL DESIGN AND DIFFERENCES BETWEEN PRE-AND POST-OPERATION PERIODS

Combinations	Rats	Reaches with Non-preferred Hand Pre-operation	post-operation	D
ABC	1M	4	0	4
	13M	0	0	0
AB'C	2M	30	26	4
	14M	1	0	1
aBC	7M	1	2	-1
	19M	37	49	-12
aB'C	8M	49	50	-1
	20F	3	8	-5
AbC	5M	1	0	1
	17M	3	0	3
ABc	3M	34	3	31
	15M	5	23	-18
AB'c	4M	20	10	10
	16F	39	48	-9
aBc	9M	0	0	0
	21F	26	9	17
aB'c	10M	17	0	17
	22M	25	4	21
Abc	6M	9	0	9
	18F	9	0	9
abC	11F	1	0	1
	23M	0	0	0
abc	12F	50	50	0
	24M	9	0	9

TABLE IV

ANALYSIS OF VARIANCE OF PRE-AND POST-OPERATION PERIOD DIFFERENCES

Drugs	Sum of Squares	D.F.	Mean Squares	F
Total	2597.96	23		
Acetylcholine (A)	.04	1	.04	3453 *
Glutamic Acid (B)	18.59	2	9.30	
Hemisphere (C)	425.04	1	425.04	
(Interactions)				
AB	123.58	2	61.79	
AC	165.38	1	165.38	
BC	25.58	2	12.79	
ABC	182.25	2	91.13	
Estimate of Error	1657.50	12	138.13	

* smaller variance used as denominator - significant at 5% level.

TABLE V

RECORDS OF REACHING

Days	Per-iods	Rats															
		6M		3M		4M		9M		1M		15M		5M		7M	
		Hands															
		R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
1	1	49	1	26	24	31	19	48	2	20	30	44	6	6	44	3	47
	2	47	3	11	39	26	24	50	0	26	24	47	3	1	49	5	45
2	1	49	1	21	29	32	18	47	3	27	23	49	1	1	49	9	41
	2	50	0	32	18	30	20	50	0	13	37	50	0	0	50	4	46
3	1	50	0	33	17	29	21	50	0	24	26	32	18	0	50	1	49
	2	36	14	22	28	23	27	50	0	25	25	30	20	0	50	2	48
4**	1	41	9	16	34	30	20	50	0	4	46	45	5	1	49	1	49
	2	50	0	47	3	40	10	50	0	0	50	27	23	0	50	2	48
	3	50	0	46	4	34	14	35	15	0	50	40	10	0	50		
	4	50	0	50	0	43	7	30	20	0	10	34	16				
	5	50	0	45	5	37	13	48	2								

TABLE V (continued)

RECORDS OF REACHING

Days	Periods	Rats															
		6M		3M		4M		9M		1M		15M		5M		7M	
		Hands															
		R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
5	1	46	4*	26	24*	47	3*	50	0*	0	50	39	11	0	50	1	50
	2	32	18	28	22	50	0	11	39	0	50	30	20	0	50	0	50
	3	50	0					50	0			44	6				
								50	0								

* Combination of drugs re-applied

** Operation day

TABLE V

RECORDS OF REACHING

Days	Periods	Rats															
		11F		16F		18F		12F		2M		8M		23M		10M	
		Hands															
		R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
1	1	5	45	19	31	0	50	10	40	43	7	43	7	0	50	46	4
	2	0	50	3	47	2	48	7	43	39	11	49	1	1	49	47	3
2	1	0	50	13	37	0	50	1	49	47	3	48	2	0	50	50	0
	2	1	49	4	46	27	23	0	50	42	8	16	34	0	50	37	13
3	1	0	50	4	46	30	20	1	49	45	5	36	14	0	50	48	2
	2	0	50	6	44	37	13	0	50	38	12	46	4	0	50	36	14
4**	1	1	49	11	39	41	9	0	50	30	20	49	1	0	50	33	17
	2	0	50	2	48	50	0	0	50	26	24	50	0	0	50	50	0
	3	0	50	0	50					14	36	50	0	0	50	50	0
	4			1	49					22	28					32	18

TABLE V (continued)

RECORDS OF REACHING

Days	Periods	Rats															
		11F		16F		18F		12F		2M		8M		23M		10M	
		Hands															
		R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
5	1	0	50	0	50	10	0	2	48	22	28	45	5	0	50	37	13*
	2									11	39	7	0	0	50	19	1
6	1			1	49*	50	0*			20	30						
	2			0	50	0	0			14	36						
	3			0	50												

* Combination of drugs re-applied

** Operation day

TABLE V

RECORDS OF REACHING

Days	Per-iods	Rats															
		13M		17M		24M		19M		21F		22M		20F		14M	
		Hands															
		R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
1	1	7	43	40	10	5	45	23	27	38	12	1	49	44	6	1	49
	2	12	38	32	18	29	21	35	15	35	14	47	3	35	15	14	36
2	1	3	47	32	18	32	18	29	21	44	6	37	13	18	32	16	34
	2	1	49	27	23	40	10	39	11	48	2	38	12	5	45	2	48
3	1	2	48	20	30	46	4	45	5	48	2	43	7	2	48	3	47
	2	0	50	4	46	48	2	43	7	41	9	27	23	2	48	5	45
4**	1	0	50	3	47	41	9	37	13	24	26	25	25	3	47	1	49
	2	0	50	0	50	50	0	49	1	33	9	18	1	8	42	0	9
	3	0	50	0	50	50	0	48	2			28	3	38	12	0	24
	4	0	50	0	50			50	0			43	0	36	14		
	5	0	50	0	50			50	0					46	4		
	6													35	15		

TABLE V (continued)

RECORDS OF REACHING

Days	Per-iods	Rats															
		13M		17M		24M		19M		21F		22M		20F		14M	
		Hands															
		R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
5	1	0	50	1	49*	20	30	33	17	8	0	42	8	5	45	0	17
	2	0	50	0	50	43	7	44	6								
	3					30	20	38	12								
	4					21	29	35	15								
6	1									46	4	49	1	16	34*	1	49
	2													16	34		
	3													16	34		
7	1									49	1					0	50

* Combination of drugs re-applied

** Operation Day

TABLE VI

REACHING RECORD OF RAT NUMBER TWENTY, FEMALE

Day	Hand		Day	Hand	
	R	L		R	L
1	44	6	5	5	45
	35	15			
			6 (re-application)		
2	18	32			
	5	45	pre:	16	34
3	2	48	post:	16	34
	2	48		16	34
4 (operation day)					
pre:	3	47			
post:	8	42			
	38	12			
	36	14			
	46	4			
	35	15			
	27	23			

CHAPTER V

SUMMARY AND CONCLUSIONS

Twenty-four rats were used in a factorial design to test the influence of acetylcholine and glutamic acid on handedness, when applied to the cerebral cortex, in the hemisphere homolateral to the preferred hand.

According to the analysis of variance on drugs used in this study, neither acetylcholine nor glutamic acid caused significant shifts in handedness when applied to the cerebral cortex of the albino rat. In fact, the difference between acetylcholine rats and non-acetylcholine rats was significantly less than expected from the experimental error. Since there is nothing in the experimental design to warrant this result, we are concluding that it is an exceptional occurrence which can only be accounted for by chance.

The buffer, glutamic acid, used in this study also turned out to be without significance. Only in one case, rat number twenty, female, was there a marked shift in handedness towards the use of the non-preferred hand.

[illegible]

Twenty-four male rats were used as subjects to test the influence of acetylcholine and atropine given on handedness, when applied to the cerebral cortex of the hemisphere contralateral to the preferred hand.

According to the analysis of variance employed in this study, neither acetylcholine nor atropine alone caused significant shifts in handedness when applied to the cerebral cortex of the albino rat. In fact, the difference between acetylcholine rats and non-acetylcholine rats was significantly less than expected from the experimental error. Since there is nothing in the experimental design to warrant this result, we are considering that it is an exceptional occurrence which can only be accounted for by chance.

The buffer, glutamate salt, used in this study, turned out to be without significance. Only in one case, rat number twenty, female, was there a general shift of handedness towards the side of the non-preferred hand.

Upon re-application, however, pre-operation data for the first order was the same as the two post-operational orders.

All the interaction variances were also without significance.

With the drug concentrations used in this study, neither acetylcholine nor glutamic acid, singley or taken together, seemed to have an effect in influencing the rat to a shift in hand preference. This study appears to warrant further consideration of dosages and possibly a modification of technique before glutamic acid should again be utilized in a comparable study.

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