Effects of glutamic acid and aminooxyacetic acid on discrimination learning and activity in the rat

Allen Ray Branum

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THE EFFECTS OF GLUTAMIC ACID AND AMINOCXYACETIC ACID ON DISCRIMINATION LEARNING AND ACTIVITY IN THE RAT

by

Allen R. Branum

B.S., Montana State University, 1966

Presented in partial fulfillment of the requirements for the degree of

Master of Arts

UNIVERSITY OF MONTANA

1968

Approved by:

[Signatures]

Chairman, Board of Examiners

Dean, Graduate School

August 26, 1968

Date
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I. INTRODUCTION

Glutamic acid and gamma-aminobutyric acid, two closely related amino acids found in the mammalian central nervous system, have recently been implicated as possibly playing a central role in the nature of ongoing neural processes.

Glutamic acid is the immediate metabolic precursor of gamma-aminobutyric acid (GABA) (GABA being decarboxylated glutamic acid - see Appendix) and both substances are found in great quantity throughout the central nervous system (Eiduson, Geller, Yuwiler, & Eiduson, 1964). GABA is found almost exclusively in the central nervous system with respect to mammals (Elliott & Jasper, 1959). Glutamic acid is a neural excitant whereas GABA is a neural depressant. This has been established by a mass of pharmacological research. When glutamic acid is applied electrophoretically to individual neurons of the central nervous system, the spontaneous firing rate of these neurons increases. When GABA is applied electrophoretically, the activity of the neurons is either decreased or blocked (Krnjevic & Phillis, 1961). Intraventricular administration of glutamic acid produces epileptiform seizures whereas intraventricular administration of
GABA raises the threshold for chemically or electrically induced seizures (Curtis and Watkins, 1965). Also, the seizures following administration of glutamic acid can be interrupted by the subsequent administration of GABA (Wiechert & Herbst, 1966). Various hydrazides which block the decarboxylation of glutamic acid to GABA will also produce convulsions and these seizures have been convincingly correlated with decreases in brain GABA levels (Killam & Bain, 1957). Hydrazide induced seizures can be interrupted with injections of pyridoxine (vitamin B₆), the coenzyme for decarboxylation which hydrazides block, or by very high intraperitoneal doses of GABA (Kamrin & Kamrin, 1961). Although the blood-brain barrier is considered relatively impermeable to amino acids, enough of these chemicals enter the brain following intracarotid injections to produce changes in neural activity. Intracarotid injections of GABA produce a diminution of surface cortical potentials (Marazzi, Hart, & Rodriguez, 1958) and the cessation of cortical seizure activity (Hayashi, 1959a). Intracarotid injections of glutamic acid are followed by convulsions (Hayashi, 1959b). Topical application of GABA to the cortex results in a depression of the surface-negative phase of primary evoked potentials (Iwama & Jasper, 1957) while the application of glutamic acid produces spreading depression, probably because of massive
depolarization of cortical neurons (Purpura, Girado, Smith, Callan, & Grundfest, 1959).

Because of its inhibitory properties with respect to neuron activity, GABA has been considered a possible inhibitory transmitter substance (McLenna, 1963). This idea has fallen into disfavor since GABA, and glutamic acid as well, do not fit the criteria for synaptic transmitters and have been shown to produce their effects without interaction with synaptic events. The chemicals may produce their effects by altering the membrane permeability of neurons with respect to sodium, chloride, or potassium ions so as to change membrane conductance without changing membrane potential (Curtis & Watkins, 1965). GABA does not block neuron activity but only makes neurons less excitable; glutamic acid does not fire neurons but merely makes them more excitable (Kishida & Kaka, 1967).

Elliot and van Gelder (1958) first discussed the possibility that the balance of GABA and glutamic acid levels influence the level of neuronal activity in the central nervous system. McLennan (1963) pointed out that,

The picture is, therefore, that these pairs of acids, of which the depressant member is the decarboxylated counterpart of the excitatory compound, may function as humoral regulators of the general level of excitation of central neurons, and the balance in their levels could exert a wide measure of control (p. 109).

McLennan mentions "pairs of acids" because while glutamic
acid and its counterpart GABA are found in the brain in great quantity, other amino acids with similar properties are also found to some extent.

It has recently been shown that the rate of release of GABA from cat cerebral cortex increases when the ECG (electrocorticogram) shows an unaroused cortex while the release of glutamic acid from the cortex increases when the cortex is in an aroused state (Jasper, Khan, & Elliott, 1965). The activity of these substances at least within the cortex has thus been shown to correlate with neural activation.

There is much evidence that the activity of glutamic acid and GABA correlates with behavioral activation. Intraventricular injections of GABA are known to depress activity of mice, cats, and dogs (Crawford, 1963; John, Killam, Wenzel, & Tschirgi, 1960; Purpura, et al., 1959; Wiechert & Herbst, 1966). These reports are simply the gross comments of physiologists and consist of such statements as, GABA "produced loss of muscle tone and gross incoordination of movement. The animals were unable to right themselves if placed on their back or sides, and tended after a short period to fall asleep (Crawford, 1963, p. 1444)"; or, "Following the injection of GABA a certain inertia and lack of drive was noted (Wiechert & Herbst, 1966, p. 60)." Peripheral injections usually have little
effect because of the relative impermeability of the blood-brain barrier to GABA (van Gelder and Elliott, 1953) but peripheral injections of GABA transaminase inhibitors such as aminooxyacetic acid and hydroxylamine will prevent the breakdown of GABA in the central nervous system thus raising brain levels of GABA (Baxter & Roberts, 1960; Wallach, 1961). In the case of either drug, lethargic behavior was said to occur concomitantly with the elevated GABA levels (Baxter & Roberts, 1960; van Gelder, 1965).

One study (John et al., 1960) reveals that intraventricular GABA injections in cats do not affect established conditioned avoidance responses but increase the response latency. The study also showed that after intraventricular administration of GABA appetitive drive for food decreased, often producing a cessation of appetitive behavior. Oral administration of GABA has been shown to have beneficial effects upon epilepsy in humans (Tower, 1960). Strangely, it was also shown to increase the activity of rats (Czok & Lang, 1955) and to slightly (not significantly) facilitate acquisition of maze learning in rats (Blei & Levin, 1964).

With respect to glutamic acid, intraventricular injections have been shown to produce hyperactivity in mice when dose levels are below those required for seizures (Crawford, 1963). Oral administration of glutamic acid to
rats has been shown to significantly increase activity in stabilimeters (Czok & Lang, 1955; Helm-Zeller, 1956) and in exploratory situations (Hamilton & Maher, 1947; Helm-Zeller, 1956).

With the exception of those studies utilizing oral administration of GABA, the available evidence reveals GABA to be a behavioral depressant and glutamic acid to be a behavioral excitant. However, little parametric data is available demonstrating the effects of these drugs upon activity.

The further possibility exists that the activity of these chemicals may play some role in the learning process. It is well known that substances which increase neural excitability facilitate learning when administered at moderate dose levels. These include strychnine, picrotoxin, amphetamine, and others. Accordingly, substances which decrease neural excitability will inhibit the learning process. Such substances include calcium, barbiturates, ether, and carbon dioxide (Roberts, Wein, & Simon sen, 1964). The supposed effect of the excitatory drugs is to enhance neural functioning by lowering the thresholds of central neurons which comprise the "perseverating circuits" set into action when an organism is processing information. The depressant drugs, of course, should raise the neuron thresholds. Thus the neural circuitry
is made more or less efficient as the case may be (Roberts, Wein, & Simonsen, 1964). If GABA and glutamic acid are synaptic transmitters as some have hypothesized (Krnjevic & Phillis, 1963; Krnjevic & Schwarts, 1966), they may be more intimately involved in the learning process. It is suspected that specific transmitter substances may mediate discrete neural systems which have discrete behavioral correlates in aspects of the learning process (Carlton, 1963). In any case, it can be predicted that glutamic acid will facilitate learning and GABA will inhibit learning since these substances are neural excitants and depressants respectively. The present experiment is an attempt to ascertain the gross effects of these two chemicals on the learning process.

With respect to glutamic acid, a large and controversial literature was initiated by Zimmerman and Ross (1944) when they demonstrated a remarkable facilitation of maze learning in rats which were being fed supplementary glutamic acid with their diets. Albert and Warden (1944) found that glutamic acid supplements facilitated "complex reasoning" in the rat. These two studies stimulated a vast amount of research with human subjects, primarily retardates, on the cognitive effects of glutamic acid. This research has tended to show that positive IQ changes in retardates follow the oral administration of glutamic
acid if the treatment is maintained over a long period of time (3 months to 8 years) (Vogel, Broverman, Draguns, and Klaiber, 1966). However, the initial rat studies were followed by a number of experiments all of which found no effect of dietary supplements of glutamic acid upon rat learning (Hamilton & Maher, 1947; Helm-Zeller, 1956; Hughes, Cooper, & Zubek, 1957; Marx, 1948, 1949; Porter & Griffin, 1950; Stellar & McElroy, 1948; Zabarenko, Pilgrim, & Patton, 1951). Stellar and McElroy (1948) replicated the Zimmerman and Ross (1944) study and found no facilitation of learning. However, Hughes and Zubek (1956) found that glutamic acid supplements did facilitate learning in maze-dull rats whereas no effect was found with maze-bright rats. Later they were unable to replicate the effect (Hughes, Cooper, & Zubek, 1957).

However, the administration of glutamic acid has continued to have beneficial effects when administered to humans. Astin and Ross (1960) reviewed the literature and concluded that the beneficial effect of glutamic acid upon retardate intelligence could be attributed to defects in the experimental design of most of the research. Vogel et al. (1966), however, pointed out that Astin and Ross (1960) failed to consider 50% of the literature and disregarded many aspects of the glutamic acid research which are highly relevant to the conclusions drawn about its value.
After an exhaustive analysis of research to that date, Vogel et al. (1966) concluded that there are beneficial effects of glutamic acid upon the cognitive behavior not only of retardates, but normals as well. Although Vogel et al. (1966) do not commit themselves with respect to the specific effect of glutamic acid, the common denominator appears to be an increase in "drive." Repeatedly, researchers were cited as reporting that the administration of glutamic acid was followed by increased drive, concentration, attention, motivation, or persistence (Vogel et al., 1966).

These results are in keeping with the pharmacological finding that glutamic acid is a neural excitant. However, this property of the substance does not necessarily offer the physiological explanation for the results since glutamic acid is involved in a myriad of metabolic processes within brain tissue (see Appendix). The negative findings with respect to rat learning are open to numerous explanations, the most compelling of which are simply that the measures utilized probably were not sensitive enough to detect subtle changes in neural functioning or that the time between drug administration and testing was too long for direct drug effects to be evident. The studies generally involved testing the rats in multi-cued mazes with glutamic acid being administered orally with the
diet sometime after the daily test trials. In a pilot study the author found behavioral activation following intraperitoneal injection of glutamic acid which disappeared within 30 minutes and the plasma levels of glutamic acid are known to be raised for only about one hour after administration of the drug (Waelsch, 1951).

The present study is an attempt to clearly ascertain whether the administration of glutamic acid would be followed by an increase in behavioral activity and a facilitation of acquisition of a learned behavior. Also, the present experiment attempted to ascertain whether increases in brain GABA levels would depress behavioral activity and learning. Affirmative answers were predicted in each case. This study differed from previous glutamic acid studies in that the method of administration was intraperitoneal injections and testing was performed immediately after drug administration.

In demonstrating the predicted effects the blood-brain barrier posed a problem. GABA penetrates the barrier with great difficulty if at all (van Gelder & Elliott, 1958). However, there are drugs which inhibit GABA transaminase activity thus raising brain GABA levels by preventing its breakdown (Roberts, Wein, & Simonsen, 1964). Aminooxyacetic acid is one of these and it has been shown to elevate brain GABA levels (Wallach, 1961). Its behavioral
and pharmacological effects are in accordance with the observed effects of centrally administered GABA (Roberts, Wein, & Simonsen, 1964; van Gelder, 1965). In pilot work, the author found dose-specific decreases in activity in rats following AOAA administration. The possibility exists that AOAA produces its behavioral effects via some route other than its effects upon GABA but since it has been shown to significantly increase brain GABA levels, this drug, rather than GABA per se, was administered to the Ss in the present study.

Peripherally administered glutamic acid was at one time considered unable to cross the blood-brain barrier (Schwerin, Bessman, & Waelsch, 1950). Apparently glutamic acid is metabolized into something else soon after it crosses the barrier (Thompson, 1967). A behavioral change following peripherally injected glutamic acid is tenable as a central effect in terms of the Elliott and van Gelder glutamic acid - GABA balance hypothesis (1953) since the balance should be weighted in favor of glutamic acid while plasma levels of glutamic acid are raised.
II. METHOD

Subjects
The Ss were 66 Long-Evans male hooded rats obtained from Simonsen Laboratories, Inc., of Gilroy, California. Ss were 80 to 100 days of age on the first day of handling. Males were used to obviate any problems with the oestrus cycle.

Apparatus
To assess the drug effects upon learning the task performed by the S had to be simple enough that a large degree of activation would not hinder learning in keeping with the Yerkes-Dodson law, sensitive enough to detect a subtle change in cognitive functioning, and non-appetitive because John et al. (1960) found GABA to decrease appetitive drive specifically and glutamic acid is known to be capable of substituting for glucose in cell metabolism (Tower, 1959). The device chosen was the two-choice discrimination box described by Thompson and Bryant (1955) (Thompson box).

The start box, 6 x 6\(\frac{1}{2}\) x 10 inches, opened through a guillotine door into a choice chamber 20 inches in length. The floor of the start box and choice chamber was a shock grid. Two doors, 3\(\frac{1}{2}\) x 3\(\frac{1}{2}\) inches, at the end of the choice chamber opposite the start box provided entrance to a
solid-floored goal box 12 x 14 x 10 inches. Either door could be locked so as to force the S to use the opposite door. Between the two doors, a 4 x 10 inch partition extended onto the grid of the choice chamber, thus partitioning the doors from each other. The grid area immediately in front of each door could be charged independently or in unison with the main grid. The shock source was an Applegate Model 250 constant current stimulator set to deliver 1 milliamp shock. White noise was provided during the experiment at about 35 db.

Activity was measured with a circular Lehigh Valley activity box. The box was 24 inches in diameter and 17 inches deep. Its color was flat black and it had a metal mesh floor. Six invisible infra-red light beams criss-crossed the base of the apparatus and activity was measured in terms of light beam interruptions. The beam interruptions were recorded on a Varian cumulative recorder located in an adjacent room so as to minimize noise. White noise was provided during the experiment at about 35 db.

Procedure and Design

First, each S was handled 2-3 minutes per day for 5 consecutive days. Each rat was then given 10 minutes free exploration time in the Thompson box with all doors open. Ss were then pretrained in the Thompson box to push
back a card from the door of the goal box and enter the
 goal box to avoid shock. Pretraining was begun with one
goal box door locked and the other goal box door open.
The S was placed in the start box and the guillotine door
was opened after 10 seconds. As the door was opened the
E pressed a button starting a timer. The S had to leave
the start box within 5 seconds or a brief shock was de­
divered. As the S left the start box, the E pressed a
second button which stopped the first timer and started
a second timer. The S then had to leave the choice chamber
within 30 seconds or another brief shock was delivered
with pulses continuing until the S entered the goal box.
As the S entered the goal box a third button was pressed
by the E stopping the second timer. The readings of the
two timers provided measures of start latency and choice
latency respectively. The position of the locked door
was varied according to the following sequence: RLLRRLRLRLR,
with the mirror image of this sequence used on alternate
blocks of 10 trials. Once the Ss successfully ran from
the start to the goal box 10 consecutive times without the
aid of shock, a neutral card (gray, the same color as the
locked door) was brought successively closer to the open
door until the S had to push this card back in order to
enter the goal box. Pretraining continued until each S
avoided consistently by pushing back the unlocked door,
meeting a criterion of 18 avoidances in 20 consecutive trials. Since Ss were dropped from pretraining at different times according to their learning ability, all Ss received an extra 5 trials the day before discrimination training began so as to put them at approximately the same level of learning. The last 6 Ss to reach criterion were dropped from the experiment.

Following pretraining, the Ss were assigned to the 5 experimental groups. In doing this the groups were matched according to the latency measures taken during pretraining. The Ss were arranged in rank order according to the average choice latency of each S on the last 10 trials of pretraining. The top (fastest) 10 Ss were assigned to the first level of the latency factor, the next 10 Ss to the second level, etc., until all 60 Ss had been assigned and 6 levels were obtained. Within each level, the Ss were randomly assigned to the 5 treatment groups, 2 Ss per treatment by level cell, 12 Ss per treatment.

The experimental treatment groups consisted of, 1) a control group receiving injections of physiological saline (2 cc/kg), 2) a "GA low" group receiving glutamic acid injections of 20 mg/kg, 3) a "GA high" group receiving glutamic acid injections of 40 mg/kg, 4) an "AOAA low" group receiving AOAA HCl injections of 10 mg/kg, 5) an
"AOAA high" group receiving AOAA HCl injections of 20 mg/kg. All injections were intraperitoneal with the drug concentrations adjusted so as to allow the injection volume to be a constant 2cc/kg. Physiological saline was used as a carrier and the pH of the solutions was adjusted to 7 with dilute NaOH and HCl. Ss received their injections and began their trials within 5 minutes. Each S received one injection per day.

The task learned following drug administration was a black-white discrimination. The unlocked door of the Thompson box was white and the locked door was black. A correction procedure was used and multiple errors were possible on each trial. Three categories of error were recorded. A discrimination error was defined as an approach to the incorrect stimulus of closer than 4 inches and shock was delivered thereupon. Also, failure to leave the start box within 5 seconds was recorded as an error (start error) and failure to leave the choice chamber within 30 seconds was recorded as an error (latency error). All errors were accompanied by shock. Start and choice latencies were taken as described above. Five Ss, one from each treatment group, were run as a group during a designated 1 hour period of each day. Thus Ss were run at the same time every day and the members of each treatment group were distributed equally throughout the day. All
practiced was distributed with an intertrial interval of 3-4 minutes. Within any designated hour of running every S received trial 1 before any S received trial 2, etc. The order in which the 5 Ss of a given hour were run was randomized each day. Each S received 10 trials per day with the exception of the first day when they received only 4 trials. All the Ss of a given treatment group were run until all the Ss in that group reached criterion.

After a group reached criterion, it remained idle for 6 days before being run in the activity measure. Then, each S received one final injection of its drug and was placed in the Lehigh Valley activity box for 1 hour immediately following the injection. Two Ss from each group were run during each day in such a way that after 6 days the Ss of a given group had been run at all the different times of a 10 hour day.

In order that the learning trials be run blind, all drugs were mixed before the beginning of discrimination learning and coded with a number. Knowledge of the identity of the drugs was withheld from the E until the learning data was compiled.
III. RESULTS

On the first day of running three Ss of the highest AOAA dosage group died shortly after their injections reducing the N of that group to 9 Ss.\(^1\)

The data from the learning task are shown in Table 1. A Hartley's\(^2\) test (Winer, 1962)\(^3\) for homogeneity of variance performed on the trials to criterion data did not reveal significance (\(F_{\text{max}} = 5.42\)) and a subsequent one-way analysis of variance revealed significant differences between means within the data (\(F = 4.03; df = 4, 52; p < .05\)). The means are graphed in Figure 1. Duncan's new multiple range test extended for unequal Ns (Kramer, 1956) was used to compare group means. The results are presented in Table 2. All tests were two-tailed and a \(p\) value of .05 was accepted as significant. The high dosage glutamic acid group was found to differ significantly from the control group in the predicted direction. Neither AOAA group differed significantly from the control group.

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\(^1\)The exact cause of these deaths is not known but it is suspected that the high room temperature at the time of the injections may have increased the sensitivity of the animals to the drug. Twice the AOAA dose used is almost always lethal.

\(^2\)This test was performed on all data before each analysis of variance and was not significant in every case.

\(^3\)All further statistical tests are from this source unless otherwise indicated.
Significant differences were not found in the other measures taken during discrimination learning. Start errors and latency errors were virtually non-existent during the acquisition trials for all groups. Total discrimination errors were tabulated for the first 7½ trials and although the resulting means were all in the predicted direction (see Table 1) a one-way analysis of variance revealed no significant differences between means ($F = 1.81; df = 4, 52; .05 < p < 0.10$). Start and choice latency measures were scored in terms of the reciprocal of median start or choice latency for a given $S$ on a given day. In order that a two-way analysis of variance could be performed in which one factor would represent the latency levels obtained from pretraining, the missing data of the dead $S$s had to be estimated. This was done according to the method described by Winer (1962, p. 281). No factor of the two-way analysis of variance was found significant for any 10 trial block of testing or for the first 7½ trials as a block. One-way analyses of variance also did not reveal significant differences between groups. The reciprocals of the mean group latencies for the first 7½ trials are shown in Table 1. For that data, one-way analyses of variance yield $F = .60$ for start latencies and $F = .43$ for choice latencies.

The activity data were scored in terms of mean
# TABLE 1

**BEHAVIORAL MEASURES BY DRUG CONDITION**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GA High</th>
<th>GA Low</th>
<th>AOAA High</th>
<th>AOAA Low</th>
</tr>
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<tbody>
<tr>
<td>Mean Trials to Criterion</td>
<td>52.4</td>
<td>35.5</td>
<td>47.2</td>
<td>61.7</td>
<td>46.9</td>
</tr>
<tr>
<td>Mean Discrimination Errors-1st 74 Trials</td>
<td>13.4</td>
<td>9.5</td>
<td>12.4</td>
<td>17.4</td>
<td>14.6</td>
</tr>
<tr>
<td>Mean Reciprocal Start Latency</td>
<td>1.10</td>
<td>1.05</td>
<td>1.18</td>
<td>1.34</td>
<td>1.16</td>
</tr>
<tr>
<td>Mean Reciprocal Choice Latency</td>
<td>.88</td>
<td>.83</td>
<td>.82</td>
<td>.96</td>
<td>.93</td>
</tr>
<tr>
<td>Mean Light Beam Interruptions</td>
<td>722</td>
<td>770</td>
<td>701</td>
<td>373</td>
<td>448</td>
</tr>
</tbody>
</table>
### TABLE 2

**SIGNIFICANT DIFFERENCES BETWEEN TRIALS TO CRITERION MEANS (DUNCAN'S NEW MULTIPLE RANGE TEST)**

<table>
<thead>
<tr>
<th></th>
<th>GA High</th>
<th>GA Low</th>
<th>AOAA High</th>
<th>AOAA Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>p &lt; .05</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>GA High</td>
<td>N.S.</td>
<td>p &lt; .10</td>
<td>p &lt; .05</td>
<td>p &lt; .10</td>
</tr>
<tr>
<td>GA Low</td>
<td>p &lt; .10</td>
<td>N.S.</td>
<td>p &lt; .10</td>
<td>N.S.</td>
</tr>
<tr>
<td>AOAA High</td>
<td>p &lt; .10</td>
<td>N.S.</td>
<td>p &lt; .10</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

### TABLE 3

**SIGNIFICANT DIFFERENCES BETWEEN ACTIVITY MEASURE MEANS (DUNCAN'S NEW MULTIPLE RANGE TEST)**

<table>
<thead>
<tr>
<th></th>
<th>GA High</th>
<th>GA Low</th>
<th>AOAA High</th>
<th>AOAA Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N.S.</td>
<td>N.S.</td>
<td>p &lt; .001</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>GA High</td>
<td>N.S.</td>
<td>N.S.</td>
<td>p &lt; .001</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>GA Low</td>
<td>p &lt; .001</td>
<td>p &lt; .001</td>
<td>p &lt; .001</td>
<td>N.S.</td>
</tr>
<tr>
<td>AOAA High</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Fig. 1.—Mean number of trials to criterion by drug condition.
Fig. 2.—Mean number of beam interruptions by drug condition.
light beam interruptions per group for the 1 hour test period are presented in Table 1 and graphed in Figure 2. A one-way analysis of variance revealed significant differences between means \( (F = 18.25; \, df = 4, \, 52; \, p < .001) \). Duncan's new multiple range test extended for unequal Ns (Kramer, 1956) was used to compare means. The results are shown in Table 3. All tests were two-tailed and a \( p \) value of .05 was accepted as significant. The results reveal the two AOAA groups to differ significantly from the control and glutamic acid groups \((p < .001)\) but not from each other.
IV. DISCUSSION

The facilitation of learning in terms of trials to criterion shown by the high dosage glutamic acid group bears out the prediction that glutamic acid administration would facilitate acquisition. Although this prediction was based primarily on data indicating that the chemical should "activate" an organism and thus facilitate acquisition as a reflection of activation, the present experiment does not support this notion. This is because the measures taken to tap an activation effect of the drug did not show the Ss treated with glutamic acid to be more activated than the controls. There were no latency differences during discrimination learning and the activity measures did not reveal the glutamic acid groups to be significantly more active following drug administration than the control group. This data, then, points very strongly to the possibility that glutamic acid administration in some way affected the learning ability per se of these Ss.

It is not possible at this time to specify as to what biochemical property of glutamic acid may have produced this effect. However, the notion that glutamic acid may be a synaptic transmitter substance (Curtis & Watkins, 1965) or play some other very specific role in neural processes is highly tenable in view of this experiment.
Certainly if glutamic acid does affect the learning process per se it probably has a more intricate role in brain function than controlling the gross level of neural activity as Elliott and van Gelder (1958) suggested.

However, the possibility that the learning differences obtained in this experiment were due to activation effects cannot be completely ruled out. It should be noted that latency measures are not known to be a sensitive dependent variable with respect to the Thompson box. Support for this can be seen in the present experiment; the activity measures of the AOAA groups were significantly depressed but this was not reflected in the latency data taken during acquisition. It should also be noted that the activity measure taken was of a fairly gross nature and it may not have been sensitive enough to detect an increase in activity which could be reflected in a facilitation of learning. Such an interpretation would be congruent with the known pharmacology of glutamic acid but is not favored by the author.

In any case, this learning facilitation agrees with the data obtained by Zimmerman and Ross (1944), Albert and Warden (1944), and Hughes and Zubek (1956); all of whom found orally administered glutamic acid to facilitate learning in rats. This study also lends credence to the thesis of Vogel et al. (1966) that glutamic acid administration has beneficial effects upon cognitive functioning.
Future research with the drug could focus on the parameters of the drug effect and the aspects of the learning process that are particularly affected. It should be noted that the present data is equivocal as to whether or not the low dosage glutamic acid group showed a treatment effect or not. In terms of trials to criterion this group is not significantly different from either the control group or the high dosage glutamic acid group, although it is in the predicted direction.

With respect to the learning data for the AOAA groups, neither group differs significantly from the control group. Thus the prediction that increased brain GABA levels, obtained via AOAA administration, would depress acquisition was not borne out. However, the difference between the two AOAA groups approaches statistical significance (.05 < p < .10) and it seems apparent to the author that AOAA administration may have a dose-specific effect upon acquisition. It may well be that a higher dose level would depress learning while a lower dose level would facilitate learning. Such an inverted U shaped dose-response function is not unusual in behavioral drug research. This is, of course, only the author's speculation and a parametric study would be required to obtain the dose-response function for AOAA.

The activity data for the AOAA groups indicates a
clear suppression of activity following drug administration. Both groups differ significantly from the control and glutamic acid groups but not from each other. This result bears out the prediction that increased brain GABA levels will depress activity.

It is difficult, however, to specify the exact biochemical effect of AOAA upon cerebral metabolism. It may or may not be that the behavioral effects of AOAA are due entirely to increased brain GABA levels. Pharmacologically, little is known about AOAA except that it blocks GABA transaminase. This blockage, of course, raises brain GABA levels but a metabolic blockage in the brain at any point could result in an upset of many metabolic processes far removed from the metabolic cycle being tampered with.

It is evident, then, that much more research needs to be done in this area. The exact nature of the effects of glutamic acid and AOAA has yet to be fully elucidated and the physiological basis for these effects cannot be clearly specified.
The purpose of the experiment was to determine the gross effects of glutamic acid and gamma-aminobutyric acid (GABA) upon learning and activity in rats. Since GABA will not cross the blood-brain barrier, aminooxyacetic acid (AOAA), a drug known to raise brain GABA levels, was administered in its place.

It was found that intraperitoneal injection of 40 mg/kg of glutamic acid resulted in a significant facilitation of acquisition but did not increase the activity of the Ss. AOAA injections resulted in a significant depression of activity but did not significantly alter the learning rates of the Ss.

These results indicated that glutamic acid may have affected the learning process per se and suggested that AOAA may have a dose-specific effect upon learning.
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APPENDIX

GABA and Glutamic Acid Metabolism

Glutamic acid and GABA share a common metabolic pathway. Both are nonessential amino acids metabolized in the central nervous system. Apparently, the major source of brain glutamic acid is the dehydrogenation of alpha-keto-glutaric acid which is an intermediary in the tricarboxilic acid cycle (Tower, 1959). Glutamic acid may also be formed via transamination of alpha-keto-glutaric acid. Both of these reactions are reversible providing pathways for the removal of glutamic acid as well as its synthesis. Glutamic acid can also be amindated to form glutamine and this reaction is also reversible. The formation of glutamine from glutamic acid serves as an intracellular mechanism for the binding (detoxication) of ammonia (Weil-Malherbe, 1950). The importance of this reaction is not overwhelming, however, and Weil-Malherbe (1950) has stated that the ammonia-binding function of glutamic acid is "a sort of by-product and the true significance of the singular position of glutamic acid is sought elsewhere." Glutamic acid can participate in protein synthesis insofar as it can combine with a peptide in the presence of ATP to form a glutamic
acid containing peptide, i.e., glutathione (Sourkes, 1962).

GABA has glutamic acid as its immediate metabolic precursor and is synthesized via the decarboxylation of glutamic acid (see Figure 3). GABA can also be formed as a product of a transamination of glutamic acid to alpha-ketoglutaric acid (Elliott and Jasper, 1959). The principal route for the breakdown of GABA is the reverse transamination by which GABA is converted to succinic semialdehyde and glutamic acid. It is this reaction which is blocked via the inhibition of GABA transaminase by A0AA (Wallach, 1961). GABA can also be transamidated to gamma-guanidinobutyric acid or oxidated to gamma-aminocrotonic acid (Eiduson, 1964).

Succinic semialdehyde can be oxidized directly into succinic acid which is an intermediary in the tricarboxylic acid cycle along with alpha-ketoglutaric acid. Since the tricarboxylic acid cycle is the major source of energy for the cell, the fact that two of its intermediaries are but few metabolic steps removed from glutamic acid or GABA may explain why glutamic acid and GABA have been shown to support cell respiration in place of glucose (Waelsch, 1951; McKhann et al., 1960).

The metabolic cycles of glutamic and GABA are summarized in Figure 4.
Fig. 3.—Decarboxylation of glutamic acid and formula for aminooxyacetic acid (adapted from Ochs, 1965; p. 411).
Fig. 4.—Schematic Representation of the Metabolic Relationships of Glutamic Acid and GABA in the CNS (Adapted from Roberts, Wein, and Simonsen:, 1964; p. 517).