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The Effects of ECS, Ether, and Pentobarbital on Single Trial Passive Avoidance Learning

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THE EFFECTS OF ECS, ETHER, AND PENTOBARBITAL ON SINGLE TRIAL PASSIVE AVOIDANCE LEARNING

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Abstract

Certain implications from a consolidation theory based on D. O. Hebb's two process theory of memory were investigated. Subjects given ECS 20, 75, or 250 seconds after a single passive avoidance trial and animals given ether or pentobarbital after a single passive avoidance trial were compared with each other and controls. It was found that only the ECS(20) and ECS(75) groups differed from the controls at the .01 level. Such results indicate that it is possible to impair memory by disrupting ongoing dynamic activity in the brain, but that the form of the disruption and the learning-disruption interval are both crucial variables. It is suggested that the disruption-retrograde amnesia phenomenon (especially using ECS) has been adequately demonstrated, but that the "how" or "why" of the phenomenon has been neglected.

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Introduction

This research is related to the consolidation of memory (consolidation being a hypothesized maturing process by which short-term or temporary memory becomes long-term or permanent memory); theories related to the consolidation of memory go back at least as far as the turn of the century. One of the first psychological theories to include the term consolidation was that of Müller and Pilzecker who published in 1900; DeCamp, in 1915, attempted one of the first physiological explanations of the process (see Glickman (1961) for a more complete review). Starting with the work of Duncan (1949), a long series of studies have shown that electroconvulsive shock (ECS) given to a subject shortly after a learning trial interferes with the subject's performance when tested the next day. The deficit found has been in terms of an inability to profit from the experience gained on the trial subsequent to the administration of ECS. These results have consistently been interpreted in terms of consolidation theory (Glickman, 1961; Madsen and McGaugh, 1961; Heriot and Coleman, 1962; Chorover and Schiller, 1965).

Many of the researchers in the field have tended to interpret their results in terms of a theory of consolidation based on D. O. Hebb's two process theory of memory. Hebb (1949; 1966) has proposed a dynamic short-term memory that consists of elec-

trical activity in the brain and a permanent or long-term memory that most likely consists of changes in the "wiring" of the brain. Hebb hypothesises that the dymanic or short-term memory consists of reverberatory circuits within the brain and that these reverberatory circuits act not only to hold short-term memory, but also act to initiate and maintain metabolic processes which result in the permanent "wiring" changes in the brain.

A major criticism of many of the consolidation studies has been that the agent used may produce an apparent memory deficit, not because the agent actually affects memory, but because it is a noxious stimulus and produces an avoidance response that conflicts with the learning task (Coons and Miller, 1960; Glickman, 1961; Lewis and Maher, 1965). Madsen and McGaugh (1961) designed a task that they felt would control for this effect. In their study rats were given a single ECS after making an exploratory response that was punished by electric shock to the feet. Madsen and McGaugh reasoned that if ECS effects performance because it is punishing, then a subject given ECS following a punished response should tend not to make the punished response (show that it has learned not to make the response) on a subsequent trial. Their results show that rats given ECS following a single learning trial on a passive avoidance task did not learn not to make the response as well as controls that received only the foot shock.

Such results support a consolidation hypothesis, but do

not directly support Hebb's position. Does any agent that disrupts ongoing brain activity (anesthetic or convulsant) produce an apparent memory impairment? The evidence seems overwhelming for ECS. A number of agents in addition to ECS have been tested in the laboratory in relation to their effects on memory. Leukel (1957) has reported that sodium pentothal injected intraperitoneally after each learning trial impaired acquisition in a maze. Pearlman, Sharpless, amd Jarvik (1961) have found that ether, pentobarbital, and metrazol shock administered within a few minutes after a learning trial impaired the retention of an avoidance response acquired in that trial when compared to sham and untreated controls. Abt, Essman, and Jarvik (1961) have also found that ether administration impairs the retention of a single trial avoidance response. Alpern and Kimble (1967) found that diethyl ether administered after a single passive avoidance trial produced a deficit, but only when the ether was heated to 100 degrees F; at room temperature (75 degrees F) no memory impairment was found. The effect of heated (potentiated) ether was not confirmed by Suboski, Litner, and Black (1968).

In this study ECS, ether (room temperature) or pentobarbital were administered to rats within a few seconds after a single passive avoidance learning trial. The latency of effect for ether and pentobarbital were determined behaviorally and two additional ECS groups were formed so that the administration of ECS coincided with the latency of effect found for ether (75

seconds) and pentobarbital (250 seconds with intraperitoneal injection). Based on Hebb's two process theory of memory, it was hypothesised (1) that the shorter the latency between the learning trial and the disruption of ongoing patterned dynamic activity in the brain (latency of effect for ether and pentobarbital; latency between the learning trial and the administration of current for ECS, since the current produces seizure immediately) the greater should be the resultant memory impairment and (2) that if ECS and ether or pentobarbital are matched in terms of latency of effect, both the anesthetic and the convulsant should produce a similar memory impairment.

Method

subjects:

Subjects were male Sprague-Dawley rats between 80 and 100 days old at the start of testing. Animals were purchased in lots of 24-30 from Abrams Small Stock Breeders located in Chicago, Illinois. Each subject was habituated to our laboratory and two animals per cage housing facilities for a period of at least ten days before testing began. All subjects were given approximately 30 minutes of group exercise daily on a 36" by 24" exercise stand until the first day of testing. Each member of the first three shipments of animals was assigned to one of four groups: control, ether, pentobarbital or ECS given approximately 20 seconds after the learning trial (ECS(20)); each of these four groups contained 16 animals that completed testing. Members of the last two shipments were assigned to one of two ECS groups designed to match either the pentobarbital or ether group in terms of latency of effect; each of these two groups contained 16 animals that completed testing.

apparatus:

A small platform (9" by 8") with four inch walls on three sides was attached to a larger chamber (18" cube). There was a small opening (6" by 2") in the wall of the chamber between the platform and the chamber as illustrated in figure #1. When a

subject stepped off of the platform into the chamber there was a drop of approximately one inch. The floor of the chamber was a grid composed of $\frac{1}{4}$" stainless steel rods spaced at $\frac{1}{2}$" intervals. There was a charge on the grid provided by a Foringer model 1154 power supply coupled with a model 1155 grid shock scrambler that was set to send approximately 0.5 milliamperes through the subject.

Figure 1 about here

ECS was administerd by passing approximately 100 volts of 60 cycle a.c. current (supplied by a Staco model 3PN 1010 variable transformer) through the subject's brain for approximately 0.2 seconds (the current was passed through a Grason-Stadler model E1100H electronic timer set to complete the circuit for 0.2 seconds). Current was administered to the subject via spring-clip electrodes wrapped in gauze and soaked in saline solution; the electrodes were attached to the subject's ears just prior to the administration of ECS.

Ether was administered by placing the subject into a 5" by 9" container with 4" high walls and a plexiglass lid. Cotton soaked with ether had previously been placed in the container. The subject was kept in the chamber until he no longer showed the righting response when placed on his back. Since the chamber had to be opened to check for the righting response,

several pilot animals were used to get an indication of about how long a subject had to remain in the ether chamber before he failed to show the righting response. These practice runs showed that a minimum of about 60 seconds was required and during the actual study all animals were checked for the righting response 50 seconds after being put into the chamber and at approximately every 5-8 seconds thereafter. After the first check for the righting response, the subject was positioned so that his nose was directly over the ether soaked cotton so that he inhaled ether fumes even when the lid to the ether chamber was open.

Pentobarbital was administerd via intraperitoneal injection of approximately 50mg/kg as indicated in table 1.

Table 1 about here

As with the ether group, the righting response was used as the measure of latency of effect for the pentobarbital group.

procedure:

The first time each subject was introduced to the testing apparatus he was placed on the platform facing 90 degrees from the opening into the chamber. This position is illustrated in figure 2.

Figure 2 about here

The latency between a subject's introduction to the testing apparatus and his stepping into the chamber (with all four feet) was measured; any subject that had a step in latency of longer than 30 seconds was discarded form the study and a previously unassigned animal from the same shipment was substituted. Once a subject had stepped from the platform into the chamber, the opening between the platform and the chamber was blocked to prevent the subject from retracing his steps and the subject was forced to experience the grid shock for approximately three seconds (measured by a Grason-Stadler model E1100H electronic timer) before being removed from the chamber.

After being removed from the chamber, ECS subjects were placed in a small (8" by 6" by 6") box where the ECS was administered. The ECS subjects received ECS as described above either approximately 20, 75, or 250 seconds after the termination of foot shock (the 75 and 250 second values were chosen to match the latency of effect of ether and pentobarbital respectively), depending upon to which of the three ECS groups they had been assigned. Subjects in the ether group were placed in the ether chamber within 5 seconds of termination of the foot shock. Subjects in the pentobarbital group were injected within 10 seconds of the termination of foot shock. All experimental subjects were placed in a "recovery" cage after treatment for 10-20 minutes before being returned to their home cage; control subjects were placed in the "recovery" cage directly after

receiving the foot shock. After 10-20 minutes in the "recovery" cage, they were returned to their home cage.

Twenty-four hours after the first trial, subjects were again placed on the platform. Again, the latency between the subjects introduction to the testing apparatus and his stepping from the platform into the chamber was measured. Subjects that remanined on the platform for 120 seconds without stepping into the chamber were recorded as having learned to avoid the shock (as having learned the passive avoidance task); those subjects that did step into the chamber within 120 seconds were again forced to experience the three second foot shock and were recorded as having not learned to avoid the shock (as having not learned the passive avoidance task).

Twenty-four hours after the second trial, subjects were given a third trial. Again, subjects that remained on the platform for 120 seconds were recorded as having learned the passive avoidance task; subjects that stepped into the chamber within 120 seconds were recorded as having not learned the passive avoidance task. The grid was not charged for the third trial.

Results

The first question to be answered is what exactly were the latencies of effect for ether, pentobarbital, and ECS. Table 2 shows the latencies of effect for each of the five experimental groups. From table 2 it can be seen that the actual lantencies

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Table 2 about here

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of effect closely approximated those aimed for (19.50, 73.06, 249.37, 73.37, & 247.81 instead of 20.00, 75.00, 250.00, 75.00, & 250.00) and that the ECS(75) and the ECS(250) groups respectively matched the ether and pentobarbital groups.

Table 3 shows the results of the first test trial. It can

Table 3 about here

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be seen from table 3 that all groups contained Ss that learned and Ss that did not learn. The Chi Square test was used to determine if there were any significant differences between the groups. The overall Chi Square was significant at the .01 level ($X^2=26.68$, $df=5$, $p < .001$). This test indicates overall significance between the groups, but reports nothing concerning any of the possible individual group differences.

The nature of several individual group comparisons seem important in reference to the original hypothesis. In order to determine the effects of the various treatments, each of five experimental groups were compared with the control group. Because of expected values of less than five per cell, the Chi Square test was not appropriate for comparing individual experimental groups with the control group (Siegel, 1956). These comparisons were made using the Fisher Exact Probabilities Test. Results are shown in table 4. From table 4 it can be seen that

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Table 4 about here

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only the ECS(20) and ECS(75) groups were significantly different from the control group at the .01 level.

A second area of interest is the relationship between the two anesthesia groups (ether and pentobarbital) and the two ECS groups (ECS(75) and ECS(250)) designed to match them in terms of latency of effect. Results of these comparisons are shown in table 5. From table 5 it can be seen that these differences

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Table 5 about here

(ECS(75)--ether; ECS(250)--pentobarbital) are not significant at the .01 level.

Results of the second test trial are shown in table 6.

It is clear from this table that all groups show that learning

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Table 6 about here

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had taken place by the second test trial. In each of the cases where an S responded on the second test trial (failed to demonstrate that he had learned the passive avoidance task) he had demonstrated learning on the first test trial and, therefore, was not punished for responding on that trial.

Discussion

The fact that the ECS(20) and ECS(75) groups were significantly different from the controls, but that the ECS(250) was not seems to fit with recent trends in ECS--consolidation research. Duncan (1949) found that ECS was effective in affecting subsequent performance even when administered as much as 15 minutes after a learning trial. This finding of ECS being effective even at relatively long learning--ECS intervals was confirmed by others (Weissman, 1964; Heriot and Coleman, 1962; Leukel, 1957) and this lead Glickman (1961) to conclude that ECS given within 15-60 minutes after a learning trial produced deficits in retention. However, more recent research seems to indicate that the period after a learning trial where ECS administration is effective is much smaller than the earlier findings indicated. King (1965) found that the effect of ECS on an avoidance response decreased rapidly with increased latency so that the effect had all but disappeared with a latency of 15 minutes. Chorover and Schiller (1965) found that impairment in retention was inversely related to the learning--ECS interval, but that the impairment had all but disappeared with intervals longer than ten seconds.

A partial explanation of these different findings would seem to involve both the type of learning task used and the

number of ECS administrations. Much of the earlier research involved maze acquisition with ECS being given daily after each trial, whereas the later research involved single trial avoidance learning with a single ECS following the one acquisition trial. The advantages of the later method have been pointed out above. However, it must be emphasized that this is not a complete explanation; Heriot and Coleman (1962) and Weissman (1964) both used the single trial avoidance task in their respective research. Alpern and McGaugh (1968) seem to have found another partial explanation of the conflicting findings concerning the maximum effective learning--ECS interval. They found that electroshock stimulation of 15 m.a. for 0.2 seconds effectively impaired memory only when given immediately following training; whereas electroshock of 8 m.a. given for 0.4 or 0.8 seconds was effective for much longer learning--ECS intervals. Another relevant finding is that of Weissman (1963); he found that the amount of current (number of milliamperes) was a crucial variable, with higher m.a. values producing the most effective impairment.

Most investigators, including this one, have found that the effectiveness of ECS in producing a memory impairment decreases with an increase in the learning--ECS interval. This trend can be clearly seen in table 3 and is reflected in the results of the Fisher Exact Probabilities Test shown in table 4.

When a comparison was made between the anesthesia groups

(ether and pentobarbital) and the ECS groups designed to match them in terms of latency of effect, it is clear that the second part of the hypothesis was not confirmed. The ECS(75) group was significantly different from the control group ($p=.0073$); whereas the ether group was not ($p=.3871$). The same trend holds true for the ECS(250) group and the pentobarbital group; although the ECS(250) group did not differ from the control group at the .01 level ($p=.0381$), it is obvious from table 3 and table 4 that the trend was there, but results for the pentobarbital group and the control group, as reported in table 3, were identical. Table 5 shows that although the differences between the anesthesia groups (ether and pentobarbital) and the ECS groups designed to match them in terms of latency of effect (ECS(75) and ECS(250)) are not significant at the .01 level, the trend is there (ECS(75)--ether, $p=.0239$; ECS(250)--pentobarbital, $p=.0381$).

Table 6 shows that all groups of experimental animals were capable of learning the task as well as controls. The second test trial was run to show that none of the experimental treatments produced some sort of change in the subjects that prevented them from learning the task at all. The second test trial also acted as control for the possibility of a decrease in step down latency as reported by Routtenberg and Kay (1965); the author is of the opinion that the results found by Routtenberg and Kay are not as relevant to the ECS--consolidation literature as the

authors of that study seem to believe, for their data shows a decrease in step down latency only for a period within six hours of ECS administration whereas in consolidation studies the step down task always follows ECS administration by at least twenty-four hours.

Results of this study can be interpreted in terms of consolidation theory, but not without some reservations. First, it appears that consolidation of neural activity (change from dynamic to structural or biochemical), if it occurs at all, must occur, or be at a stage in the process where neural activity itself is no longer necessary, within a few seconds. Second, it appears that not all means of disrupting ongoing neural activity are equally effective. This may be related to the severity of the disruption as is indicated by the findings of Weissman (1963).

Still unanswered, or nearly so, is the question of what exactly goes on in the brain when ECS is administered; that is, what is the physiological basis for experimentally induced retrograde amnesia? The beginnings of an attempt to answer this question may be found in the work of Chorover (1969). He and his associates at M.I.T. are involved in studying both the behavioral and electrocorticographic reactions to punishing foot shock and ECS under conditions similar to those commonly used in ECS consolidation studies. Their results indicate that both the electrocorticographic and behavioral reactions to ECS

are altered as a consequence of prior foot shock and that the overall frequency of such alterations declines as the foot shock-ECS interval increases. Chorover and his associates have found that the initial electrocorticographic reaction to foot shock -- the "phasic reaction"-- decreases in frequency as the interval after foot shock increases, and that the administration of ECS during the "phasic reaction" is associated closely with a performance indicative of a memory impairment. Given the extensive data demonstrating the phenomenon, more research into the "how" or "why" of the phenomenon seems to be in order.

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table 1

Table showing how mamy cc's of pentobarbital in 60mg/cc solutiion to inject in order to give the subject a 50mg/kg dosage.

weight (grams)	number of cc's
150	.125
160	.133
170	.142
180	.150
190	.158
200	.167
210	.175
220	.183
230	.192
240	.200
250	.208
260	.217
270	.225
280	.233
290	.242
300	.250
310	.258
320	.267
330	.275
340	.283
350	.292
360	.300
370	.308
380	.316
390	.325
400	.333
410	.341
420	.350
430	.358
440	.366
450	.375
460	.383
470	.391
480	.400
490	.408
500	.416

table 2

Table showing the latency of effect values for each subject and mean values for each group. All values are in seconds.

ECS(20)	ECS(75)	ECS(250)	ether	pentobarbital
20	79	258	63	229
21	73	241	56	226
28	64	248	65	360
19	73	237	75	195
24	72	241	65	206
18	69	238	59	250
13	62	259	95	270
20	75	255	100	233
20	74	264	97	279
24	81	236	91	300
22	69	270	58	300
25	81	257	55	246
16	73	237	62	198
15	81	252	80	195
13	61	244	74	223
14	82	253	79	255
312	1169	3990	1174	3965
$\bar{X}$=19.50	$\bar{X}$=73.06	$\bar{X}$=249.37	$\bar{X}$=73.37	$\bar{X}$=247.81

matches:

ECS(75) 73.06--ether 73.37

ECS(250) 249.37--pentobarbital 247.81

table 3

Table showing the results of test trial one in terms of how many subjects in each group did or did not step off of the platform into the foot shock chamber within 120 seconds. Values in this table are the ones used in statistical analysis.

	ECS (20)	ECS (75)	ECS (250)	ether	pentobarbital	control	total
responded or stepped in within 120 seconds	11	8	6	2	1	1	29
did not step in within 120 seconds	5	8	10	14	15	15	67
total	16	16	16	16	16	16	96

Chi Square = 26.68
df = 5
$p < .001$

table 4

Table showing comparisons between individual experimental groups and the control group. Probability values were determined by the Fisher Exact Probabilities Test.
yes--means the subject stepped in within 120 seconds
no--means the subject did not step in within 120 seconds

(a)

	ECS (20)	C	T
yes	11	1	12
no	5	15	20
T	16	16	32

(b)

	ECS (75)	C	T
yes	8	1	9
no	8	15	23
T	16	16	32

(c)

	ECS (250)	C	T
yes	6	1	7
no	10	15	25
T	16	16	32

(d)

	E	C	T
yes	2	1	3
no	14	15	29
T	16	16	32

(e)

	P	C	T
yes	1	1	2
no	15	15	30
T	16	16	32

a) ECS(20)--control
$p = .0003$

b) ECS(75)--control
$p = .0073$

c) ECS(250)--control
$p = .0381$

d) ether--control
$p = .3871$

e) pentobarbital--control
$p =$ _____

table 5

Table showing comparisons between anesthesia groups (ether and pentobarbital) and the ECS groups designed to match them in terms of latency of effect (ECS(75) and ECS(250)).

yes-- means the subject stepped in within 120 seconds

no--means the subject did not stepp in within 120 seconds

Probability values were determined by the Fisher Exact Probabilities Test.

(a)

	ECS (75)	ether	T
yes	8	2	10
no	8	14	22
T	16	16	32

(b)

	ECS (250)	pento-bar-bital	T
yes	6	1	7
no	10	15	25
T	16	16	32

a) ECS(75)--ether, $p=.0239$

b) ECS(250)--pentobarbital, $p=.0381$

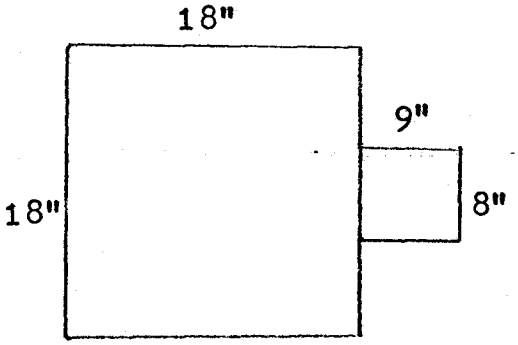
table 6

Table showing the results of test trial two in terms of how many subjects in each group did or did not step from the platform into the foot shock chamber within 120 seconds.

	ECS (20)	ECS (75)	ECS (250)	ether	pento-bar-bital	con-trol	total
responded or stepped in within 120 seconds	0	0	1	0	1	1	3
did not respond or step in within 120 seconds	16	16	15	16	15	15	93
total	16	16	16	16	16	16	96

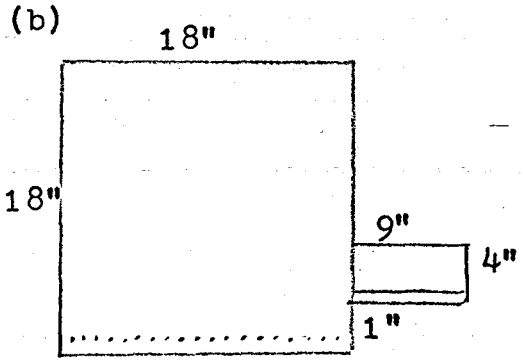
figure 1

(a)



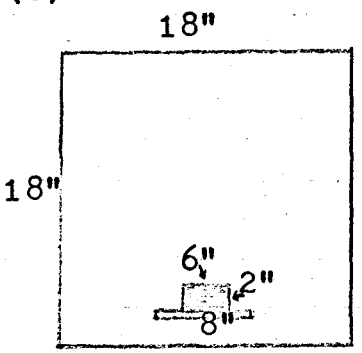
a) View of testing apparatus from above

(b)



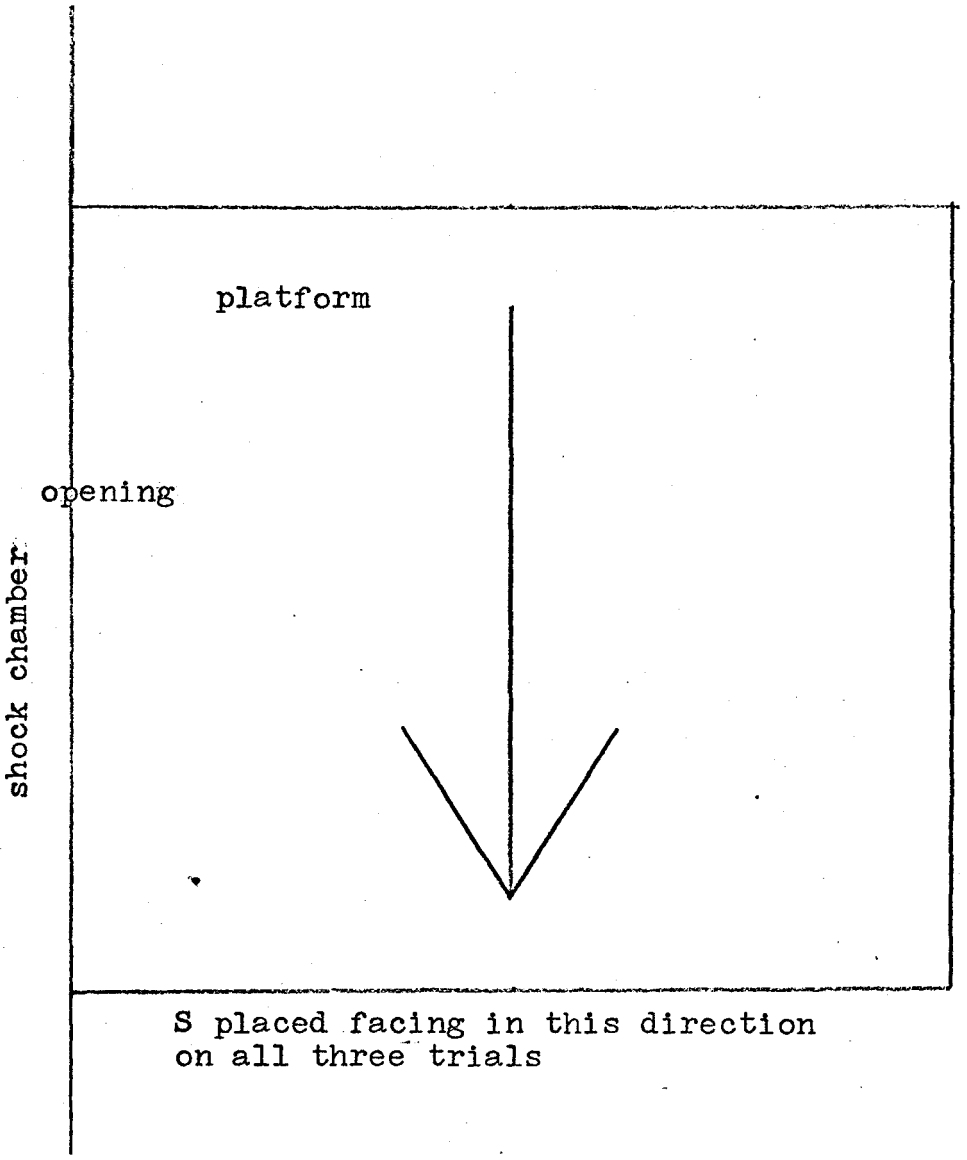
b) View of testing apparatus from the side

(c)



c) View of testing apparatus from the front

figure 2


platform
opening
shock chamber
S placed facing in this direction
on all three trials

APPROVAL SHEET

The thesis submitted by Stephen Charles Milliser has been read and approved by the director of the thesis.

Furthermore, the final copies have been examined by the director and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Arts.

June 23, 1969
Date

Richard Maier, Ph.D
Signature of Advisor