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The Role of the Central Nervous System Cholinergic Mechanisms in Behavior and Learning.

Final Report

Perry Black, M.D., C.M. and Pericles Spyropoulos, M.S.

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Laboratory of Neurological Sciences
Friends Medical and Scientific Research Institute
Spring Grove State Hospital
Baltimore, Maryland, 21228

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FOREWORD

The work described in this report was authorized under Project Number DA18-035-AMC253-(A), entitled "Study of the Role of Central Nervous System Cholinergic Mechanisms in Behavior and Learning". The period covered by this report extends from March 1, 1966 until August 31, 1966. The experimental data are contained in notebooks entitled "Delayed Response - Wisconsin Box (non-automated)" and "Delayed Response - Automated Situation".

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Acknowledgements

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Notices

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DIGEST

This report covers work accomplished within the past six months, and is incorporated in a summation of the entire project to date. Data have now been completed on four additional monkeys, thereby expanding the series studied to a total of 17.

Following is a summary of the overall findings:

1. In general, LSD was found to impair delayed response performance in the monkey. There is, however, a distinct differential effect of LSD on the three versions of the delayed response task studied, indicating the importance of procedural factors in training and testing. The three delay tasks under consideration are affected in the following descending order of magnitude: (1) automated, (2) indirect WGTA, (3) direct WGTA.
2. From the standpoint of dose-effect relationships, the results suggest a positive correlation between magnitude of dose and extent of functional impairment. Dose range: 10-140 mcg./Kg. In a pilot study prior to the present formal investigation, doses under 10 mcg./Kg. produced no consistent behavioral disruption.
3. A tendency toward rapidly developing tolerance was observed.
4. A "placebo effect" in response to the control agent (sterile water) was encountered in a significant number of monkeys. The implications of this observation for further exploration are discussed in the text of the report.

REPORT

Introduction

The principal purpose of this study was to investigate the possible differential effects of LSD as a function of procedural variations in performance of delayed response tasks. If such a differentiation exists, it may be inferred that performance of the tasks is subserved by diverse neural mechanisms. That differences in procedure are of importance was demonstrated by Pribram and Mishkin (1956) employing the object alternation task. In the same context, Battig, Rosvold and Mishkin (1960) showed that monkeys trained and tested in an automated apparatus manifested no deficit on a delayed alternation task after bifrontal ablation. The classical finding of post-operative impairment was seen, however, in animals trained and tested in the manually operated Wisconsin General Testing Apparatus (WGTA). This finding has been corroborated in our laboratory (unpublished data).

These procedural differences may account for some of the apparent discrepancies in the LSD literature. Jarvik and Chorover (1960) reported that LSD produced impairment in accuracy of performance of a delayed alternation task in monkeys trained in an automated apparatus. Employing the WGTA, Evarts (1958) reported no deficit in delayed response performance following LSD administration in monkeys trained to high-level criterion. Since the ability to perform delayed response or delayed alternation tasks

is affected by prefrontal ablation in animals tested in the WGTA, it may be hypothesized that the paradoxical findings reported with LSD are related to the procedural differences of manual versus automated training. In order to test this hypothesis, monkeys in the present study were trained in one or more of three types of delayed response tasks, each differing in the manner of cue presentation and environmental situation.

An additional concern of this study was that of dose-effect relationships with respect to the three tasks under consideration. Widely discrepant dose-response results have been reported by other workers. Evarts (1958) showed little or no effect on accuracy of delayed response performance in monkeys after total intravenous doses up to 950 mcg. of LSD. By contrast, Jarvik and Chorover (1960) reported impairment of delayed alternation with doses as low as five mcg./Kg. of body weight. In pilot testing prior to the present formal study, we evaluated dose effects of LSD in increments of 10 mcg./Kg. ranging from 10 to 140 mcg./Kg. The highest doses tended to produce generalized behavioral alterations such that the animal would frequently remain unresponsive in the test situations. Doses higher than 140 mcg./Kg. were therefore not employed. Having obtained this information relative to dose-limits, an investigation was then undertaken which forms the basis of the present report. The doses of LSD studied were 10 mcg./Kg., 70 mcg./Kg., and 130 mcg./Kg. Sterile water (which was used as the diluent for the LSD-25 substance) served as a control agent.

Methods

Experimentally naive monkeys in the weight range of 2.2 to 4.1 kilograms served as subjects in this investigation. Seven groups, consisting of four animals per group, were assigned to training on one, two or all three variations of the delayed response task. The various task combinations were designed to enable an evaluation of the relative significance and interaction of the respective procedural variations in relation to LSD (Table I).

Table I. Delayed response task combinations for each of the seven groups of animals.

Group	Assigned Tasks
1	Direct
2	Indirect
3	Direct + Indirect
4	Automated
5	Direct + Automated
6	Indirect + Automated
7	Direct + Indirect + Automated

The three variations of the task are described below.

1. Direct method in the WGTA. In this, the classical method of training and testing in delayed response, the animal observes baiting of one of two adjacent food cups by the experimenter, who then covers them with identical lids. Direct viewing of the food reinforcement by the subject constitutes the critical feature of this method. An opaque barrier is then interposed so that the animal is unable to view the covered cups for a six second period. At the end of the delay, the barrier is raised and the animal is permitted to respond, but in the absence of any cue.

2. Indirect method in the WGTA. In this situation, the monkey views the two adjacent cups, one covered by a lid with a grey surface and the other by a lid with a central white disk on a grey background. The cup bearing the lid with the white disk signifies that cup which will yield the food reward. In this indirect method the subject is not cued by the food. After the animal views the choices, a barrier is interposed for six seconds. During the delay period, the experimenter removes the lid with the white disk and replaces it with one having a uniform grey surface so that now both lids are identical. The animal subsequently responds in the absence of the critical cue.

3. Automated method. Employing electromechanical relay circuitry, the apparatus, as developed in this laboratory for the present experiment, cues and rewards or punishes the animal automatically. A restraining chair is employed to

enhance the probability of the animal's attention to the cueing stimuli which are presented on one of two adjacent translucent windows approximately 12 inches in front of the animal's head. The cue in this situation consists of a circular beam of light on one window for six seconds, the other remaining unilluminated; the right-left sequence is predetermined by Gellermann series. When the projectors are turned off, a six second delay period ensues. In this situation, an opaque barrier is not interposed between the animal and the windows. The animal is prevented from responding prematurely by an air-driven door which allows accessibility to the windows only at the conclusion of the delay period. The monkey then responds by pressing one of the two windows. A correct response is rewarded by food which drops into a midline food-well in front of the animal. Incorrect responses result in mild punishment, a puff of air directed at the animal's head.

Drug testing was introduced after the animal achieved a criterion performance of 90 percent correct responses per test session for five consecutive days. After meeting the criterion, a specified dose of LSD or sterile water was administered daily for five days. The animal was tested daily on each of the tasks on which it had received training, starting 15 minutes after intraperitoneal injection. The sequence of tasks on a particular day followed a predetermined order to overcome possible differential effects of the drug related to the time following administration. At the conclusion of five consecutive days

on a particular dose of the drug, testing continued until the monkey again achieved five consecutive days of criterion performance. Testing with the next dose of LSD was then begun. A counterbalanced order of drug doses was employed so that the effect of dose-sequence may be evaluated.

For the preliminary analysis of the data, statistical significance was determined by the binomial approximation to the normal distribution. The five day pre-drug sessions were used to establish the normative values for each animal. Percent correct responses over five days for any given drug dose was then compared to the animal's normative scores. Each animal thus served as its own control.

Results

Over the past six months, further progress has been made toward completion of the experimental design. Data have been completed on the performance of an additional 4 monkeys under various doses of LSD, thus bringing to 17 the total number of animals studied. The tasks on which each additional animal was trained and tested were dictated by the particular experimental group of which the monkey was a member:

Monkey #	Experimental Group
662	3
668	5
597	6
651	7

All animals were tested at the following doses of LSD: 10 mcg./Kg.
70 mcg./Kg.
130 mcg./Kg.

Sterile water (which was used as the diluent for the LSD-25 substance) served as a placebo.

Figures 1-4 graphically illustrate the compiled data on all animals studied, including the four additional monkeys evaluated over the past six months. Each of the Figures represents performance at a given dose of LSD (or sterile water placebo) with respect to the three delayed response tasks. Each graph depicts average performance of those monkeys tested at given dose on the three respective tasks.

130 mcg./Kg. (Figure 1).

The most significant behavioral changes were seen at the highest dose level, 130 mcg./Kg. but the effect was not uniform across all tasks. Performance on the three tasks was depressed on the first day of drug administration. Automated task performance remained below the 90% criterion for the succeeding days that LSD was given. The non-automated direct and indirect tasks, however, were deleteriously affected only on the first drug-day, following which the LSD effect was no longer evident.

70 mcg./Kg. (Figure 2).

At the intermediate dose-level, 70 mcg./Kg., the effect on the automated task was similar to that at the highest dose. The average indirect-task score was 5% below criterion for the first two days of drug administration after which performance improved. The direct task showed only slight initial depression by comparison with the 98.5% pre-drug level but, nonetheless, performance never dropped below the criterion level.

10 mcg./Kg. (Figure 3).

At the lowest dose, 10 mcg./Kg., the difference between the automated and the non-automated procedures was less evident. The LSD deficit was again present in the automated task but not to the same extent as seen at the higher doses. Neither of the non-automated tasks was appreciably affected.

Sterile water control (Figure 4).

With sterile water, the control agent, no alteration in performance occurred in the direct or indirect WGTA tasks. In the automated situation, however, average performance was at or slightly below criterion on four of the five days of water administration.

Table I (a) and (b) present the data relative to each subject separately. The percent alteration in performance signifies the percentage difference between the animal's pre-drug score and its subsequent performance under the influence of LSD (or of the placebo). Table I (b) contains the data pertaining to the sterile water placebo. Of particular interest is the finding of significant performance deterioration in 8 of 14 monkeys tested in the automated situation. The "placebo reaction" occurred less frequently in the non-automated situations.

In order to control for the placebo effect, it is necessary to correct the LSD data in relation to the effect of sterile water administration. Employing the sterile water data as a control produced a negligible alteration in the overall result which indicates a bone fide effect of LSD. Its action is thus independent of any placebo effect which administration of the drug may have.

SUMMARY AND DISCUSSION

With regard to the question of a differential effect of LSD on procedural factors, a clear distinction has been demonstrated between the automated and non-automated methods. The former appears to be more sensitive indicator of LSD effects. Of the two free-ranging WGTA situations, animals tested by the indirect method show somewhat greater sensitivity to LSD. Thus, LSD impairs performance on the three tasks under consideration in the following descending order of magnitude: (1) automated, (2) indirect WGTA, (3) direct WGTA.

The differential effect of LSD on versions of the delayed response task demonstrated in this study indicates the importance of procedural factors in training and testing. It is interesting to note that the differential effect of LSD appears to be directly opposite to that observed in frontally ablated animals (Battig, Rosvold and Mishkin, 1960). Conceivably the procedural differences in the apparently similar delayed response tasks may be subserved by different neural systems which are selectively affected by LSD. These findings are consistent with those of Evarts who found only minimal LSD effect on performance in the WGTA. The results of the present study are also in agreement with those of Jarvik and Chorover (1960); using an automated apparatus, they reported a decrement in delayed alternation ability. The method employed in each of the studies cited above may have accounted for the apparent disparity in LSD effect.

From the standpoint of dose-effect relationships, the results suggest a positive correlation between magnitude of dose and extent of functional impairment. This hold true, in general terms, for both the automated and WGTA situations. Although the non-automated tasks appeared relatively resistant to LSD, there was, nonetheless, a positive relationship between dose and degree of impairment.

While statistical analysis has not been completed with regard to drug tolerance effects, data shown in Figures 1 to 4 demonstrate a tendency toward rapidly developing tolerance. Maximal effect of LSD is seen on the first day, following which there is an apparent trend in the direction of criterion performance. These observations corroborate those of Jarvik and Chorover (1960) who likewise concluded that tolerance effects occurred. Evarts (1958) has suggested that the apparent improvement in performance was an effect not of tolerance but rather of

than
continued training of the animal. That drug tolerance rather/overtraining accounts for the improved performance is suggested by the fact that, in the present study, the animals had to meet a criterion of stable performance prior to the administration of the drug; additional training of itself might not be expected to improve their performance.

In order to evaluate the role played by interaction among the three tasks, it will be necessary to complete the experimental design so that full data are available on each of the seven groups of animals. It is anticipated that this will be accomplished in the future.

Statistically significant alterations in performance in response to sterile water are interpreted as representing a placebo reaction. The question is raised regarding the possibility of an "emotional effect" in response to needle puncture. Might the injection be more traumatic for the animals under conditions of the restraint imposed by the automated apparatus by comparison with the greater freedom of movement allowed in the WGTA? There is also the possibility that monkeys may differ in their inherent emotional reactivity. A similar placebo effect has been suggested in rats in a psychopharmacologic study on reserpine (Broadhurst, 1964). The placebo reaction, a common manifestation of drug therapy in humans, has been rarely reported in animals. The placebo responses observed in the present study are of considerable interest and warrant further exploration.

Legends

Figures 1-4 graphically illustrate the compiled data of the 17 monkeys studied. Each graph depicts mean performance of those subjects tested at a given dose on the three respective delayed response tasks.

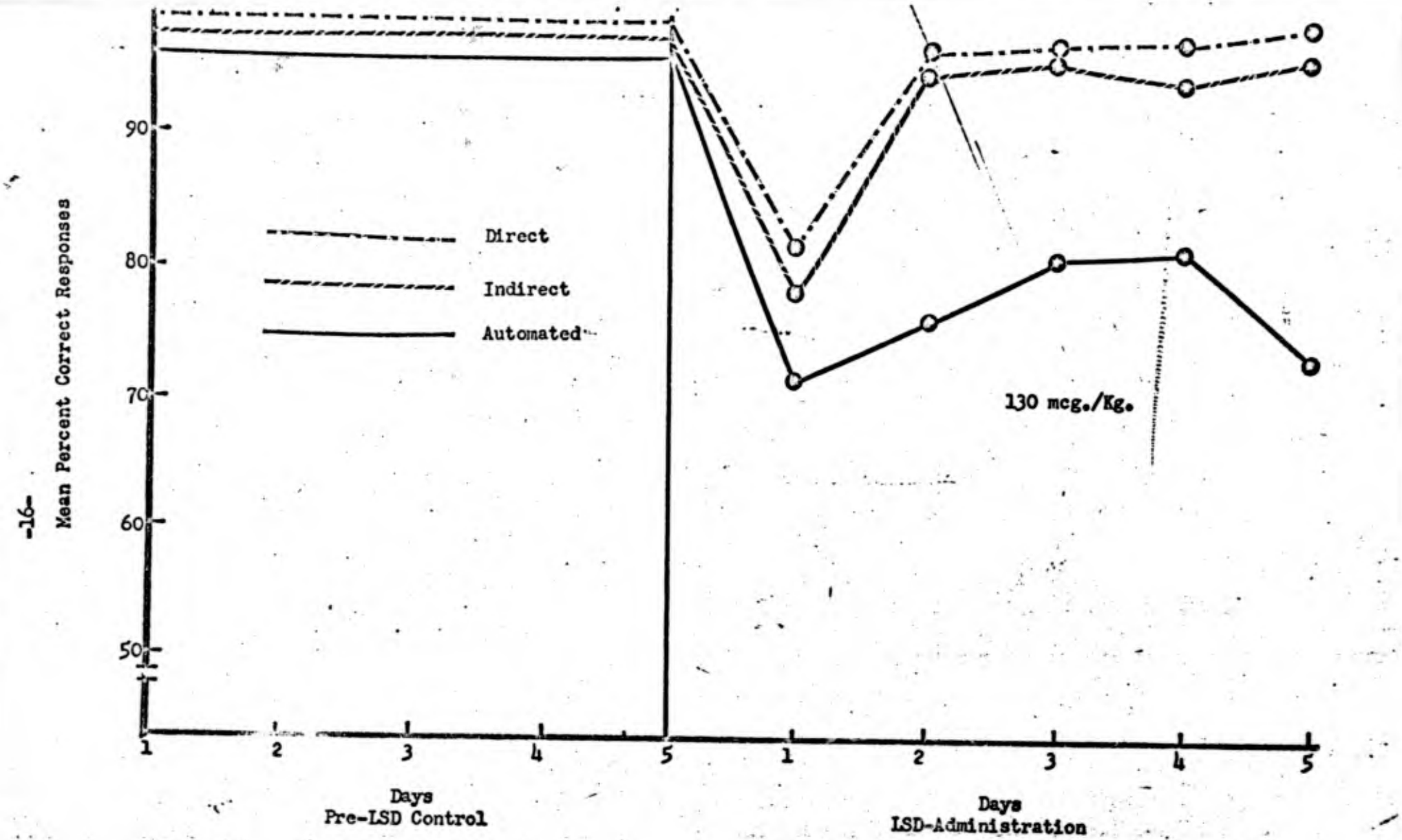
Mean Percent Correct Responses
90
80
70
60
50
Direct
Indirect
Automated
130 mcg./Kg.
1
2
3
4
5
1
2
3
4
5
Days
Pre-LSD Control
Days
LSD-Administration

Fig. 1

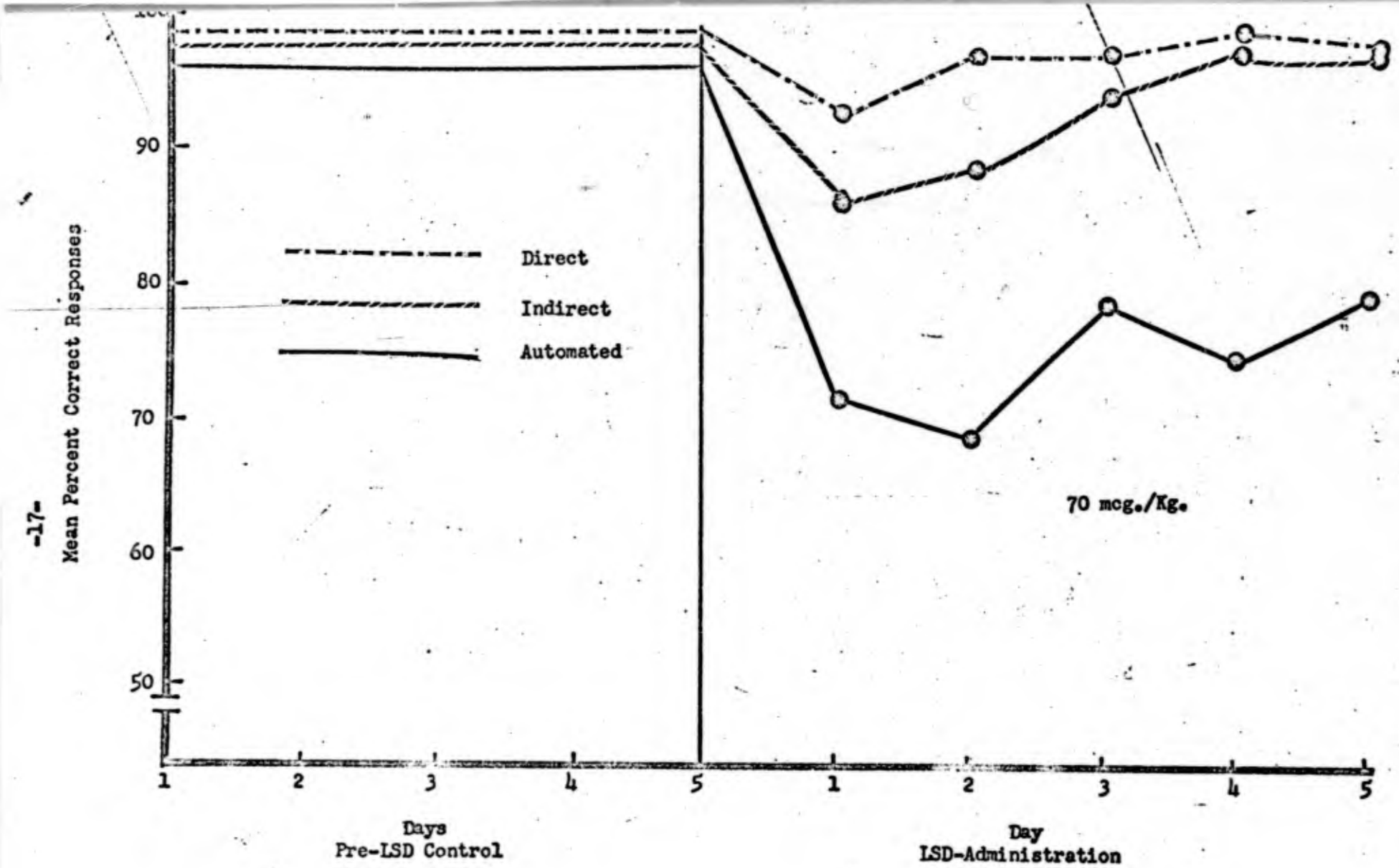
Mean Percent Correct Responses
90
80
70
60
50
Direct
Indirect
Automated
70 mcg./Kg.
1
2
3
4
5
1
2
3
4
5
Days
Pre-LSD Control
Day
LSD-Administration

Fig. 2

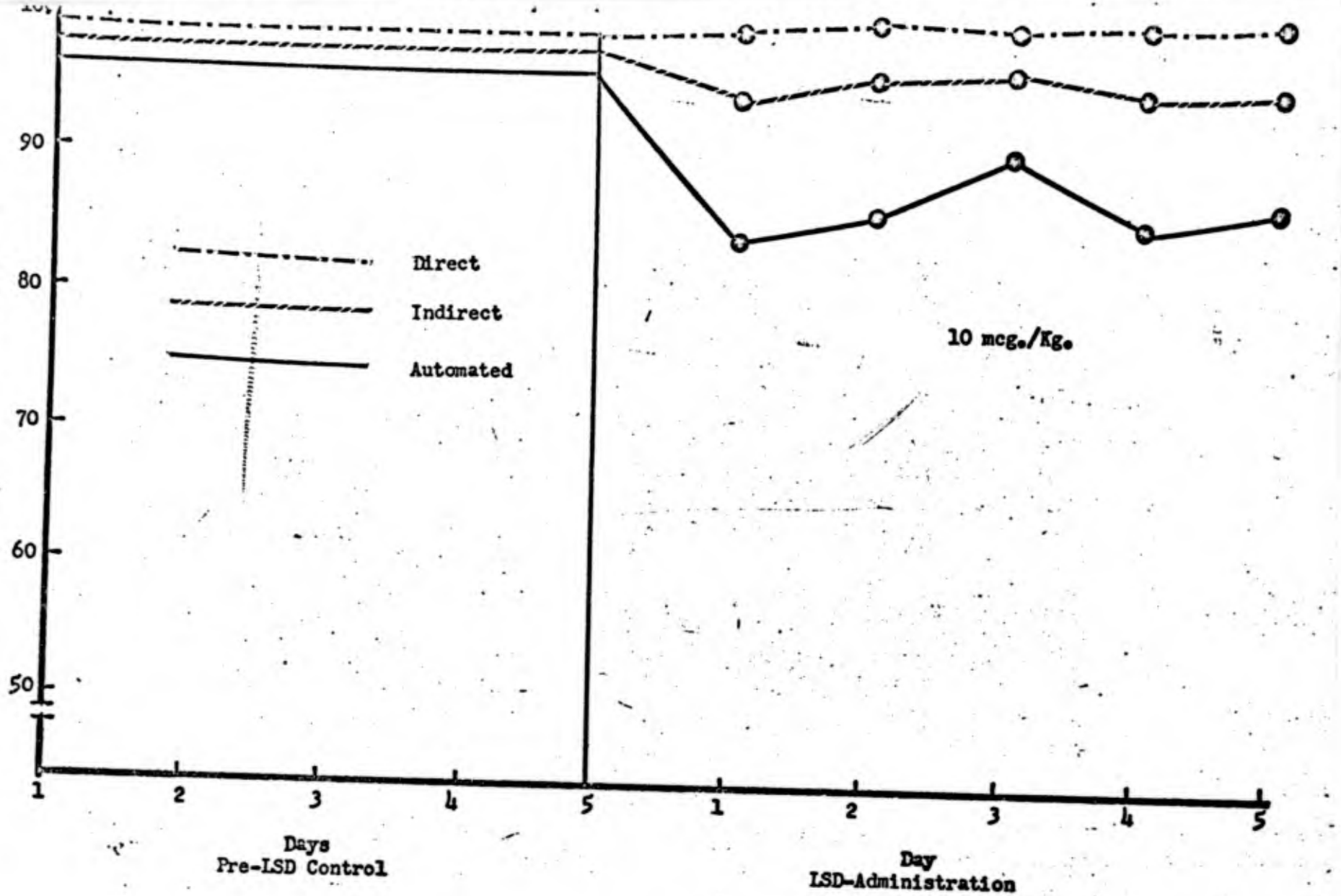
Mean Percent Correct Responses
90
80
70
60
50
Direct
Indirect
Automated
10 mcg./Kg.
1
2
3
4
5
1
2
3
4
5
Days
Pre-LSD Control
Day
LSD-Administration

Fig. 3

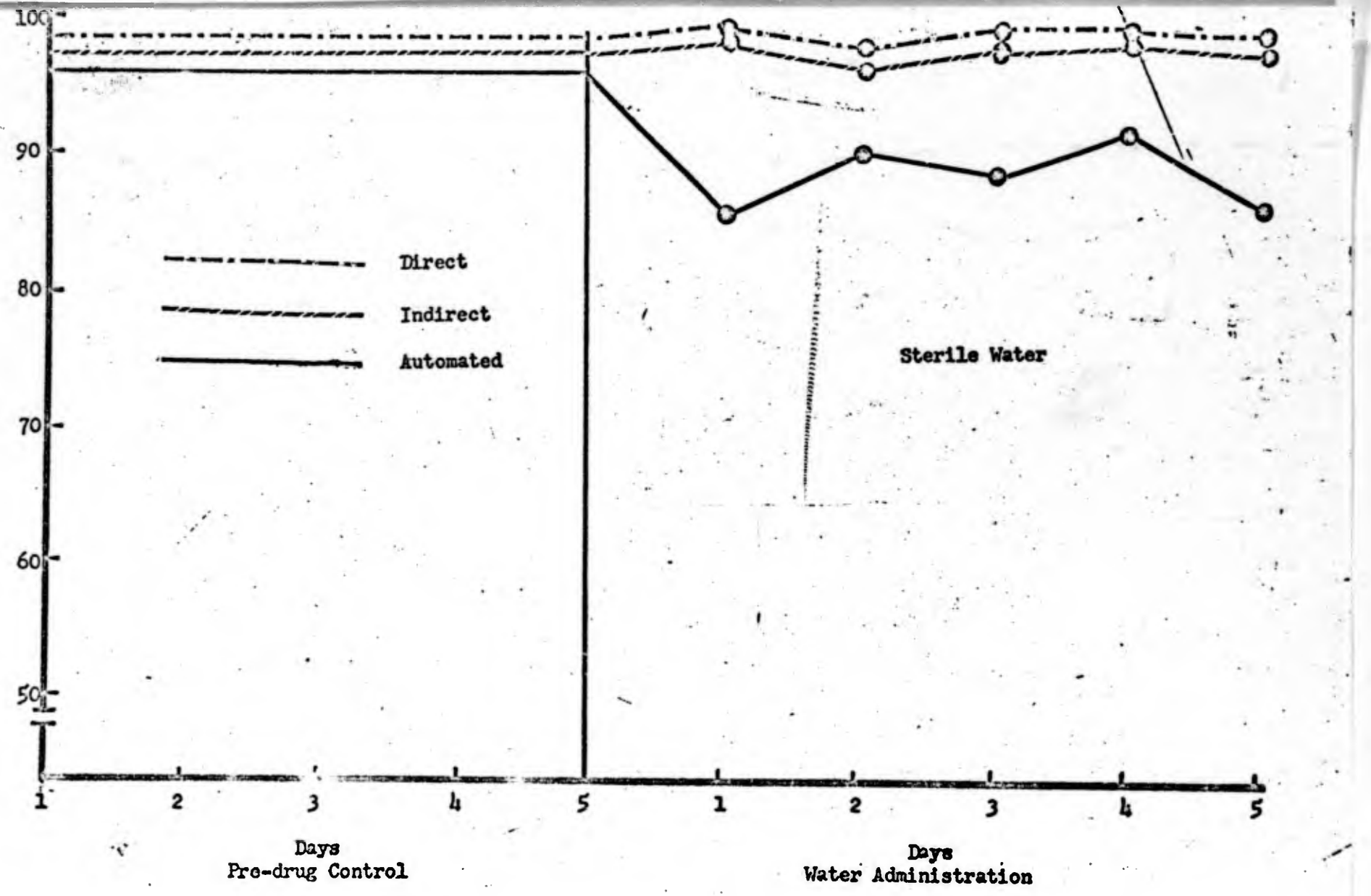
Mean Percent Correct Responses
100
90
80
70
60
50
Direct
Indirect
Automated
Sterile Water
1
2
3
4
5
1
2
3
4
5
Days
Pre-drug Control
Days
Water Administration

Fig. 4

Table 1 (a). Pre-ablation.

Dose	Monkey #	Percent Alteration in Performance		
		Automated	Indirect WGTA	Direct WGTA
130 mcg./Kg.	403	- 6.0 **	-15.0 **	-13.6 **
	449		+ 1.4	- 0.3
	515	-19.6 **	+ 2.9	- 0.8
	516	-37.3 **	-11.6 **	- 2.20
	522	- 3.3		
	538	-24.1 **	- 2.4	
	546	-13.3 **	-12.7 **	
	550		+ 0.1	- 7.7 **
	551	+ 2.7		- 7.8 **
	557		- 8.1 **	- 5.9 **
	564	- 4.3 **		- 0.1
	568	-29.0 **		
	571	-39.1 **		
	597	-10.5 **	+ 1.0	
	651	- 6.2 **	- 3.5 **	+ 0.5
	662	-37.9 **		
	668	no response		+ 1.1
70 mcg./Kg.	403	- 4.0 **	-15.0 **	+ 1.1
	449		-12.2 **	+ 0.5
	515	-15.8 **	- 2.4	- 4.8 **
	516	- 8.7 **	+ 0.4	+ 3.0
	522	-33.I **		
	538	-16.1 **	- 0.7	
	546	- 7.3 **	- 8.0 **	
	550		- 0.3	+ 0.7
	551	-37.5 **		- 6.2 **
	557		- 2.1	- 7.1 **
	564	-49.4 **		- 4.0 **
	568	-15.6 **		
	571	-19.0 **		
	597	-14.8 **	+ 4.2	
	651	-40.8 **	-12.4 **	- 0.9
	662	-42.2 **		
	668	-11.5 **		+ 0.4

** $p < .001$

Table 1 (b). Pre-ablation.

Dose	Monkey #	Percent Alteration in Performance		
		Automated	Indirect WGTA	Direct WGTA
10 mcg./Kg.	403	- 3.6 **	-16.3 **	+ 1.1
	449		- 9.4 **	- 0.3
	515	- 1.1	+ 0.9	- 0.8
	516	+ 1.3	- 0.9	+ 3.0
	522	-11.8 **		
	538	+ 1.2	- 0.4	
	546	- 7.7 **	- 1.3	
	550		- 3.1	+ 1.9
	551	+ 0.1		+ 0.2
	557		+ 1.5	+ 2.5
	564	-33.5 **		- 1.8
	568	+ 2.2		
	571	-17.8 **		
	597	-18.5 **	+ 3.4	
	651	-30.0 **	+ 1.6	+ 0.5
	662	-11.0 **		
	668	- 9.2 **		0.00
Sterile Water 1 cc./Kg.	403	- 2.3	- 1.7	+ 1.1
	449		+ 1.8	+ 0.5
	515	-10.1 **	+ 3.6	+ 0.5
	516	- 6.0 **	+ 0.4	+ 2.3
	522	- 9.1 **		
	538	- 4.8 **	- 2.1	
	546	+ 0.3	- 0.3	
	550		- 2.2	+ 0.3
	551	+ 1.3		+ 1.5
	557		+ 1.1	+ 2.5
	564	-36.8 **		- 2.4
	568	+ 2.2		
	571	- 1.0		
	597	- 4.0 **	+ 5.0	
	651	-19.5 **	+ 2.4	+ 0.5
	662	-11.0 **		
	668	- 5.1 **		- 3.2

** $p < .001$

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ABSTRACT

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1. In general, LSD was found to impair delayed response performance in the monkey. There is, however, a distinct differential effect of LSD on the three versions of the delayed response task studied, indicating the importance of procedural factors in training and testing. The three delay tasks under consideration are affected in the following descending order of magnitude: (1) automated, (2) indirect WGTA, (3) direct WGTA.
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3. A tendency toward rapidly developing tolerance was observed.
4. A "placebo effect" in response to the control agent (sterile water) was encountered in a significant number of monkeys. The implications of this observation for further exploration are discussed in the text of the report.

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13. ABSTRACT
In general, LSD was found to impair delayed response performance in the monkey. There is, however, a distinct differential effect of LSD on the three versions of the delayed response task studied, indicating the importance of procedural factors in training and testing. The three delay tasks under consideration are affected in the following descending order of magnitude: (1)automated, (2) indirect WDTA, (3) direct WDTA. From the standpoint of dose-effect relationships, the results suggest a positive correlation between magnitude of dose and extent of functional impairment. Dose range: 10-140 mcg/kg. In a pilot study prior to the present formal investigation, doses under 10 mcg/kg produced no consistent behavioral disruption. A tendency toward rapidly developing tolerance was observed. A "placebo effect" in response to the control agent (sterile water) was encountered in a significant number of monkeys. The implications of this observation for further exploration are discussed in the text of the report.

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Lysergic acid diethylamide						
Animal behavior						
Response time						
Psychochemical agents						
monkeys						