DRUG ELICITATION OF THE AGGRESSIVE DISPLAY IN SIAMESE FIGHTING FISH, *BETTA SPLENDENS*

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Jay J. Singer, Department of Psychology. It was submitted to the faculty of the College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Arts.

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Abstract

Experiment I tests the hypothesis that sympathomimetic amines serve to facilitate or trigger the aggressive display of the Siamese fighting fish. Three drugs were used to test this theory: amphetamine, norepinephrine and Dibenzyline. Amphetamine was administered to a group of six females while norepinephrine and Dibenzyline were given to males.

Fish were all maintained in the laboratory for one week before any testing began. Each of the three parts of Experiment I was conducted in the same way. The Bettas were tested first with no drugs in their water to determine their baseline level of activity. Two to four days later, the fish were tested with either 40 mg of amphetamine, 70 mg of norepinephrine or 4.5 mg of Dibenzyline. Another control trial was run two to four days later and then another experimental trial after the same period of time.

Results indicated that amphetamine increased fin flaring frequency and duration in female Bettas. The norepinephrine had the effect of increasing gill plate extension frequency and duration. Also the norepinephrine increased time to habituation. Dibenzyline was shown to influence fin flaring frequency and duration. A strong adrenergic blocking agent, Dibenzyline greatly decreased the aggressive display activities but did not significantly alter general activity. The measure of general activity used was latency to feeding. All fish were deprived of food for four days and considered

to be hungry.

In Experiment II, 16 female Bettas, eight experimental and eight control, received a 25-day treatment with either methyl testosterone dissolved in alcohol or plain alcohol. All fish were tested before any treatment was given and assigned to groups on the basis of the behavioral measure of fin flaring so as to match the experimental and control groups as closely as possible. The experimental fish then were given .2 cc of methyl testosterone (1µg/cc) and the controls were given .2 cc of alcohol. The treatment in all studies was added to the water of the living tank.

After 25 days, all Ss were retested to determine what, if any, effects the testosterone had on the display activities being measured.

The hypothesis that testosterone would cause an increase in growth rate and colorfulness was not supported by the data. There were, however, significant increases in fin flare frequency and duration and in the average length of a fin flaring response. There was some tendency toward an increase in gill plate extensions as well, but this was not large enough to gain statistical significance.

Drug Elicitation of the Aggressive Display in Siamese Fighting Fish, *Betta splendens*

Until recently research in the aggressive display of Siamese fighting fish, Betta splendens, has not often included study of the female of the species (Braddock & Braddock, 1955; Pray, 1967; Simpson, 1968).

The male aggressively defends his territory; but even more interestingly, he is aggressive in all phases of his reproductive behavior from courting through mating to nesting (Collias, 1944). The male maintains the bubble nest and cares for the eggs and fry, protecting his territory by scaring away or injuring intruders.

To be on hand when invasion takes place, the male actually patrols his territory using aggressive displays and occasionally performing the ritual fight pattern. The aggressive display is considered a ritual warning and is as easily recognized as the fight pattern.

The male begins to develope aggressive behavior patterns in the third month, the same time as he begins to build his first bubble nest. From that point on, aggression and nesting become closely inter-related, with aggression peaking during nesting periods (Braddock & Braddock, 1959).

Females of the species apparently do not share this behavior with the males, but the lack of connection is

less than totally clear. Females do not often build bubble nests and when they do, more often than not, such nexts are too poorly built to serve their intended purpose.

Masculine aggression would accompany nesting in any case, but it is influenced by vision, particularly of colors (Braddock & Braddock, 1959). Bettas deepen in color during the display and fight, and Beach (1961) reports visual stimulation may elicit this change. He says the effect on many vertebrates of visual stimulation is increased glandular activity, producing hormones which act on chromatophores resulting in color changes.

No link would exist if a Betta could not see the color changes, but experimental evidence (Langler, Bardach and Miller, 1962) indicates many shallow water fish (like the Betta) can see colors. These same researchers feel sight is critically important to the life cycle of the Betta splendens.

Albino Bettas exhibit no display behavior (Simpson, 1968) and, by way of partial explanation at least, albinos historically have less acute vision than their pigmented peers.

The role of color changes although interesting, is not adequate in itself to explain the great color intensity gap between the male and the female.

Color has two effects on a Betta who views it. The fish perceiving color changes in color externally which is accompanied by elevated hormone levels internally (Lagler et al., 1962).

Cannon (1929) first set forth the theory that both fight and flight reactions are based on secretion of adrenalin and sympathin E, later identified as sympathin I.

Secretions of the adrenal glands elicit the same reactions as innervation by the sympathetic division of the nervous system. These hormones affect organs normally innervated by the sympathetic division of the nervous system.

Secretions affect those organs not by indirect influence via the central nervous system, but by direct action upon the organ itself (Cannon, 1929). The adrenal medulla is a ductless gland which is innervated by the pre-ganglionic fibers of the autonomic nervous system (Cannon, 1929).

Building on Cannon's discrimination of two distinct hormones, Von Euler suggested that they were produced by two different sets of cells in the adrenal medulla. Further, he proposed the two cell groups were controlled by two areas of the hypothalamus (Funkenstein, 1956a). Hess linked the anterior area of the hypothalamus with secretion of epinephrine and fear responses, while the posterior hypothalamus purportedly controlled the release of norepinephrine bearing a similar causal relationship to anger and agression (Funkenstein, 1956a).

Adrenalin, also known as epinephrine, "increases oxygen consumption of the brain, increases pulse rate, increases cardiac output, decreases peripheral resistance, relaxes bronchioles and decreases blood clotting time" (Funkenstein, 1956a). Noradrenalin, also called l-artenol, l-norepinephrine or l-noradrenalin, primarily increases peripheral resistance (Funkenstein, 1956a).

Funkenstein (1956a) proposed that normal humans under stress would secrete excessive amounts of substances similar to epinephrine or norepinephrine, the former if anger were directed inward (anxiety), or the latter hormone if the anger were directed outward.

Funkenstein also proposed (1956a) that norephrine was secreted under normal conditions, and that only the level of secretion could be related to aggressive anger. He further submitted that such a condition would provoke no secretion of epinephrine since outward-directed anger was not perceived as a threat to the subject.

In relating these hypotheses to mental health efforts, Funkenstein observed that patients who showed excessive secretions of substances like epinephrine had a greater probability of recovery than those showing excessive secretion of substances like norepinephrine (Funkenstein, 1954, 1955, 1956a, 1956b).

As would be expected, psychotic patients having excessive levels of substances like norepinephrine were usually classed as paranoid, exhibiting anger and aggression. Patients with high levels of substances like epinephrine, conversely, were usually classed as depressives, exhibiting fear responses. (Funkenstein, 1956b).

Funkenstein's observations of these relationships led directly to the development of the Funkenstein-Mecholyl test which was used to predict recovery potential of patients (Alexander, 1955).

Links between certain hormones and certain forms of behavior, as outlined above, may serve to explain the behavior of both sexes of the Siamese fighting fish. This continuity of mechanisms along the phylogenetic scale works either way, and predicts that noradrenalin is the trigger for aggression in both male and female Bettas.

Noradrenalin is not the only hormone which contributes to the display behavior. In the male, the presence of testosterone also may stimulate hypothalamic activity. This offers one possible explanation for the male exhibiting display behavior more often and more violently than the female.

Understanding how these behaviors differ between the sexes requires knowledge of the basic form of display, most fully described in a monograph by Simpson (1968).

Rapid swimming toward another fish, all fins fully extended and gill covers opened, is characteristic of the display pattern of the male. Only the pelvic fins remain tight against the body. The black bronchiostegal membranes can be seen clearly under the extended gill covers (opercula).

The two fish begin a series of ritual movements, one turning to face while the other turns broadside, and they alternate these positions from five to 12 times per minute. The action by one fish seems to trigger the alternate response by the other. Other activities include slow circling (carouselling) and mouth locking, with tail beating and flashing occuring frequently during facing.

Aggression displayed by Bettas during fights is similar to the behavior during courtship, the major difference being the sex of the partner: the opposite sex for courtship and usually the same sex for defense of territory (Simpson, 1968). A typical fight will last from 30 to 60 minutes, ending when one of the fish swims away. The victor usually chases his opponent for a while and the match culminates in a persisting dominance relationship (Simpson, 1968).

Male Bettas differ from females during display primarily in the frequency and duration of movements, with the females refusing to attack a fish which does not display.

Females housed together in a single tank quickly establish their own pecking order without prolonged fighting.

When a fight does occur, however, the winner retains color for as much as 30 minutes; the loser though, in addition to losing a fin or two, loses his color at once and quickly shows stripes like those often seen in the female. (Braddock and Braddock, 1955).

Winners of fights between males are decided by the relative amount of damage to each fish. If females are fighting, however, the winner of the fight is determined by one of the two simply swimming away (Braddock and Braddock, 1955).

The opportunity to display has often been used in Bettas as a reinforcer (Thompson, 1963; Thompson & Sturm, 1963). Goldstein (1967) replicated Thompson's study indicating that a mirror image could also be used as a reinforcer. Color, according to Thompson's paper, had a direct relationship to the effectiveness of the reinforcer, as indicated by differential response rates. A red male Betta responds most aggressively to a green model, moderately to a blue and least to a red, while a blue Betta reacts best to red, somewhat to green and least to blue (Thompson & Sturm, 1965).

While the display is certainly interesting, it is the underlying chemical actions taking place which are the primary variables associated with the display. To understand them, a working knowledge of the nervous system is required.

During display, there are three types of motor nerve endings directly responsible for fish movements: voluntary, sympathetic and parasympathetic. A further distinction is made between two types of sympathetic nerve endings. Those which are pre-ganglionic are classified as cholinergic since acetylcholine is the synaptic transmitting substance. The post-ganglionic nerve endings are considered to be adrenergic due to the presence of norepinephrine as the synaptic transmitter (Barlow, 1964). The latter category, is within the purview of display analysis while the former is not.

Drugs suitable for the production of aggression in Bettas include epinephrine, norepinephrine and amphetamine, all adrenergic stimulants; and Dibenzyline, an adrenergic blocking agent (Goodman & Gillman, 1960).

Epinephrine stimulates those muscles, glands and organs innervated by adrenergic fibers; the blood vessels constrict, blood pressure rises, digestion may stop, pupils dilate, and pulse rate rises.

Amphetamine acts in much the same manner, but the dexedrine is twice as strong as the levartenal form. Amphetamine stimulates the cortex and perhaps the brain stem reticular formation which often causes restlessness, increased motor activity, and in the extreme case of an overdose, twitching and color bleaching (Goodman & Gillman, 1960). These effects are not antagonized by strong adrenergic blocking agents like Dibenzyline. It is possible that adrenergic

receptors, if they exist in the brain, are not involved in the reaction to amphetamine (Randrup & Munkvad, 1963).

Norepinephrine, as opposed to epinephrine, increases color intensity and strikingly increases the frequency of gill cover extension and fin flaring (Marrone, Pray & Bridges, 1966; Pray, 1967), in male Ss with no visual stimulus. Pray further found females showed increased frequency and duration of responses to a mirror image when treated with norepinephrine.

Dibenzyline, a potent antihistimine whose effects may last as long as three days, stops the responses of effectors to epinephrine possibly by preventing the hormone from penetrating the effector cells (Goodman & Gillman, 1960). Closely related to Dibenzyline, diethylamine HCl can completely inhibit the display response, while antihistimines such as reserpine and meprobromate results in a lack of fighting without affecting swimming or appetite. Such effects last about seven days (Walaszek & Abood, 1956).

Amount of testosterone being the primary and most obvious chemical difference between male and female Bettas, it was reasoned that to administer it to females might lead to a clarification of its role in the male. Previously investigated links between behavior and masculine hormones exist. Smith and Hoar (1967) found that the display of the male stickleback was related to gonadal hormone action, while aggression in vertebrates can be stimulated by

treatment with androgens, according to Collias (1944). Human females treated with methyl testosterone exhibit masculinization and an increased sex drive (Goodman & Gillman, 1960). The rationale for the following two experiments stems from the hypothesis that the aggressive display of the Siamese fighting fish, Betta splendens is probably controlled by the hypothalamus. It is thought that the hypothalamus innervates the adrenal medulla to secrete norepinephrine during the fight reaction and epinephrine during the flight reaction. The specific hypotheses to be tested are that amphetamine, norepinephrine and testosterone will increase the concommitants of the aggressive display and that Dibenzyline will block the aggressive display. The independent variable was days and the dependent variables were frequency and duration of fin flares and gill plate extensions. In the testosterone investigation the independent variable was again days. The dependent measures were frequency and duration of fin flares and gill plate extensions, total body length, dorsal fin length, tail length and color.

Experiment I

Method

Subjects -- The Ss used in the first experiment were six female and six male Siamese fighting fish, Betta splendens. In the first study, the Ss were females, and in the other

two, males. The fish were individually maintained in one-quart bowls containing 700 ml of aged tap water, visually isolated from one another by cardboard barriers. The water was changed every four days. Each fish received a portion of Longlife Biorell flake fish food every other day. All fish were kept in the experimental room at least one week in an attempt to adapt them to the 14-hour light, 10-hour dark cycle. Light was provided in the windowless room by overhead flourescent lighting and the temperature of the room was kept at approximately 76° F. The fish were of various colors but approximately the same size, (3-5 cm.). The Bettas were obtained from a local tropical fish retailer and were in apparently good condition at the beginning of the experiment.

Apparatus -- The apparatus used for testing all Ss was a model Siamese fighting fish mounted in front of a stand. While being tested each S in his bowl was placed directly in front of the model on the stand. The model was a sacrificed Betta stuffed with dental cement and sprayed with acrylic varnish to maintain its natural color. The gill plates were red plastic semicircles attached to the sides of the model's head. Use of a model obviated some of the variance attributable to interaction effects between Ss. The model was placed so that it faced the front of the S's bowl, since facing is the first component of the pattern

to occur in the aggressive display. Responses were recorded on an Esterline-Angus time-event recorder, and trials were timed with a Grayson-Stadler timer.

Procedure -- For the studies involving amphetamine, norepinephrine and Dibenzyline the design was as follows:

1. All Ss were given both control and experimental trials.
2. Each S was first given a 15-minute control trial, defined as testing of the fish with no drug in his water.
3. Two days later the S was tested with one of the drugs in his water. The water was changed after the 15-minute trial.
4. Four days later, another control trial was conducted.
5. Two days later the second experimental trial with the same drug as the first was conducted and the water changed.

A fin flare was recorded when all except the pelvic fins were fully extended. The criterion for a gill plate extension was approximately a 90^{o} opening of the gill covers exposing the bronchiostegal membrane. Frequency and duration of responses were recorded by the experimenter.

The first treatment to be administered to the group of female Bettas was d-amphetamine or dexedrine. It was

ascertained during a pilot study conducted by the experimenter that a dosage of 40 mg in 700 ml of water would have the effect of increasing the average duration of fin flares. After adding amphetamine to the water of the S to be tested, the experimenter waited for 45 minutes for the drug to take effect. Each S in his bowl was placed on the stand and number and duration of fin flares and gill plate extensions were recorded. The experimenter was seated approximately three feet from the stand and model. Three sides of the stand were enclosed by cardboard partitions so that the fish had only the side facing the experimenter open to view the model. After each trial S was removed to fresh water and returned to a shelf. The six Ss were tested in random order over the four 15-minute trials.

The procedure for the second study, using norepinephrine with males, was the same as that for d-amphetamine except that the dosage of l-norepinephrine bitartrate was 70 mg in 700 ml of water. This concentration was chosen partly on the basis of work done by Marrone, Pray and Bridges (1966) and Pray (1967), and partly on the basis of pilot studies done by the experimenter. In addition there was no waiting period before testing the fish. Observation began immediately after administration of the drug in an attempt to duplicate the procedure of Marrone, Pray and

Bridges (1966) and Pray (1967). At the end of each regular trial, the S was observed until he had reached the criterion for habituation. Habituation was defined as the point at which the fish was displaying less than 50 per cent of any two-minute period. The habituation point was recorded for each S on each day.

The third part of Experiment I consisted of treatment with the drug Dibenzyline (SKF), a strong antihistimine. To the author's knowledge Dibenzyline has been used only in experiments with rats. On the basis of pilot studies by the experimenter, it was determined that a dosage of 4.5 mg blocked the aggressive display in males without impairing general activity or appetite. Six male Ss were used in this part of the experiment. Two of the Ss died and the experiment was continued with four Ss. On the basis of the results obtained from a pilot study the experimenter waited 15-minutes after Dibenzyline was added to the water before testing was begun. A procedure involving an operational measure of hunger for the S being tested was included in this investigation. The operational definition of hunger was four days of food deprivation. Following the food deprivation schedule, control trials were conducted and at the end of each trial the S was fed. After four more days of food deprivation Ss were retested with Dibenzyline in their water. Included in

the procedure for this study was an operational measure of hunger for the fish being tested. Food deprivation for four days was considered a definition of a hungry Betta. In the case of the Dibenzyline study, control trials were conducted and fish were fed. After 4-days of deprivation the Ss were tested. Each fish was tested under the treatment condition and then presented with food. The experimenter recorded the latency of the fish going to the surface to feed. The purpose of using the feeding test was to determine whether Dibenzyline blocks the display by blocking all activity or whether it acts to block only the adrenergic impulses hypothesized to be necessary for the display to occur. Whereas amphetamine and norepinephrine bitartrate were each soluble in water, it was necessary that the Dibenzyline be dissolved in methyl alcohol. During control trials, 4.5 mg of alcohol was placed in the water of the Ss. This drug was used last in the series since there was a possibility of permanent damage.

Results

Figure 1 shows the mean duration of fin flare responses made by the experimental and the control Ss in the amphetamine study. The analysis of variance summary table is shown in Table 1. The difference between the experimental

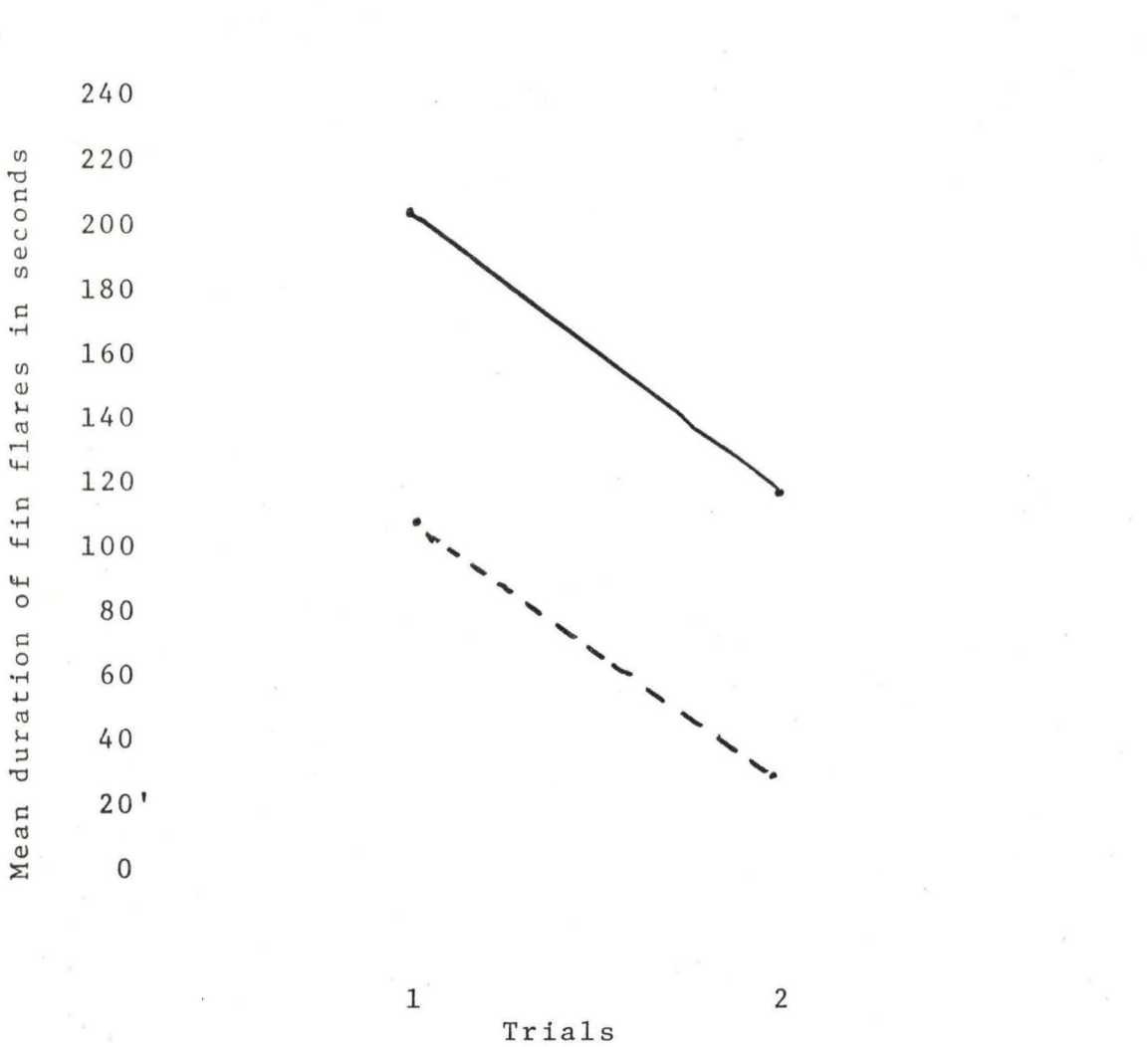

Fig. 1. Mean duration of fin flare responses for experimental and control conditions in seconds for amphetamine study.

TABLE 1

Analysis of Variance of Fin Flare Durations

Amphetamine Study

Source of Variation	ss	df	MS	F
A (treatments)	45675.375	1	45675.375	9.53 **
B (days)	39447.042	1	39447.042	8.235 *
AB	40.042	1	40.042	.008
error	71847.291	15	4789.819	

* $F = 4.54$, $p > .05$, df 1,15

** $F = 8.68$, $p > .01$, df 1,15

and control conditions was found to be significant beyond the .01 level. Differences in performance over days were significant at the .05 level. No significant interaction effect was indicated by the data.

In Figure 2 the results of amphetamine on the number of fin flares is shown. Table 2 shows that treatment conditions had significantly different effects upon the number of fin flares made by the Ss. The effects of the treatments also were significantly different over days. No gill plate extensions were recorded for any of the Ss under treatment or non-treatment conditions.

Figure 3 shows the mean duration of fin flare responses made by experimental and control Ss in the study using norepinephrine. The analysis of variance computed on this data indicated a significant interaction effect between days and treatment conditions.

Norepinephrine treated subjects decreased in response level over trials, whereas control Ss increased in response level over trials. Sign tests were employed to discover what, if any, effect norepinephrine had on the duration of fin flares. No significant difference was indicated between experimental and control Ss for trial one. Trial 2 however did yield a significant difference between the treatment and non-treatment groups. The difference between response made on trial 1 and those made on trial 2 for experimental Ss was not significant.

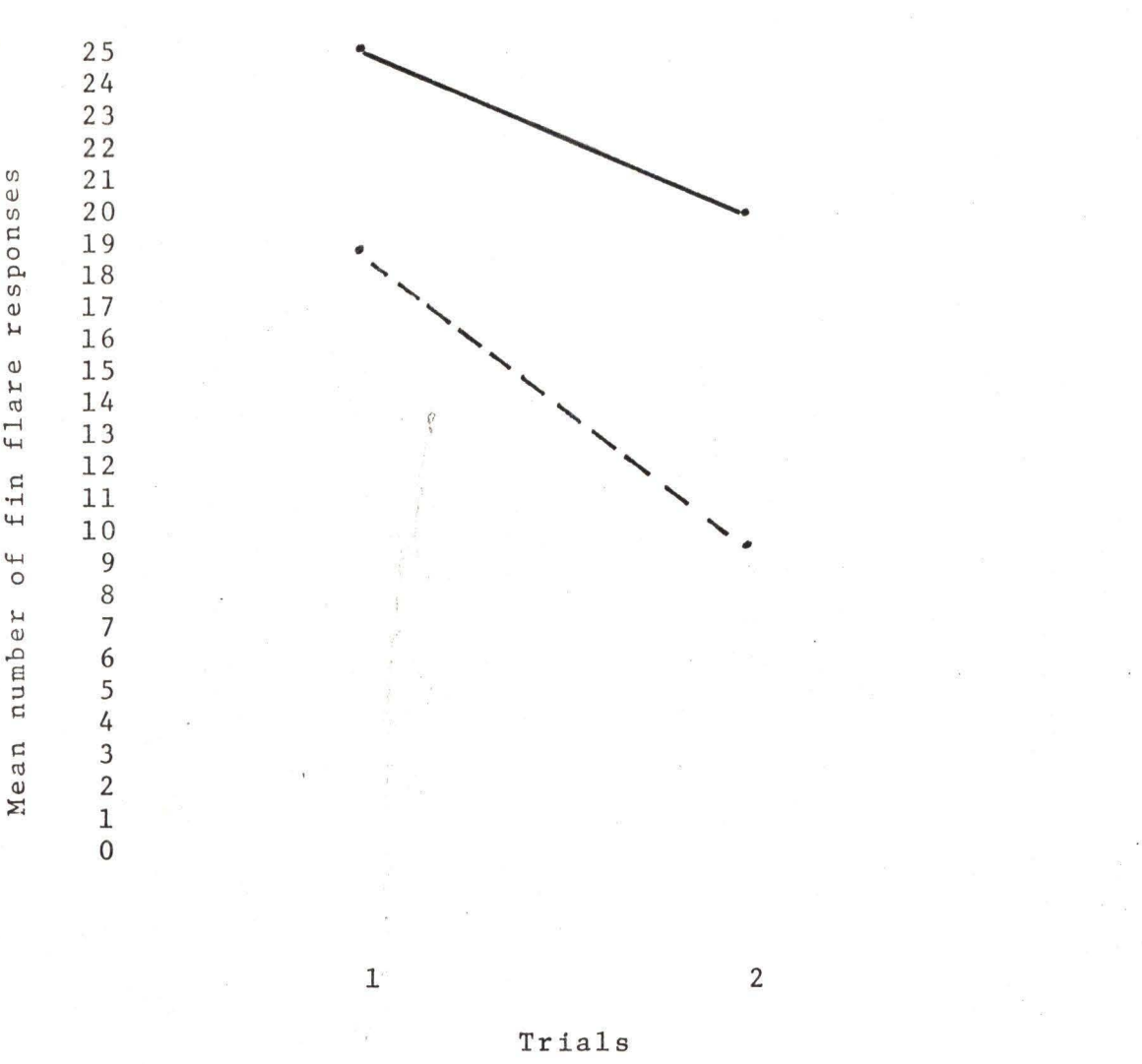

Fig. 2. Mean number of fin flares for experimental and control conditions for amphetamine study.

TABLE 2

Analysis of Variance of Number of Fin Flares

Amphetamine Study

Source of Variation	ss	df	MS	F
A (treatments)	400.166	1	400.166	6.43 *
B (days)	322.666	1	322.666	5.18 *
AB	32.666	1		
error	933.500	15	62.233	

* $F = 4.54$, $p < .05$, df 1, 15

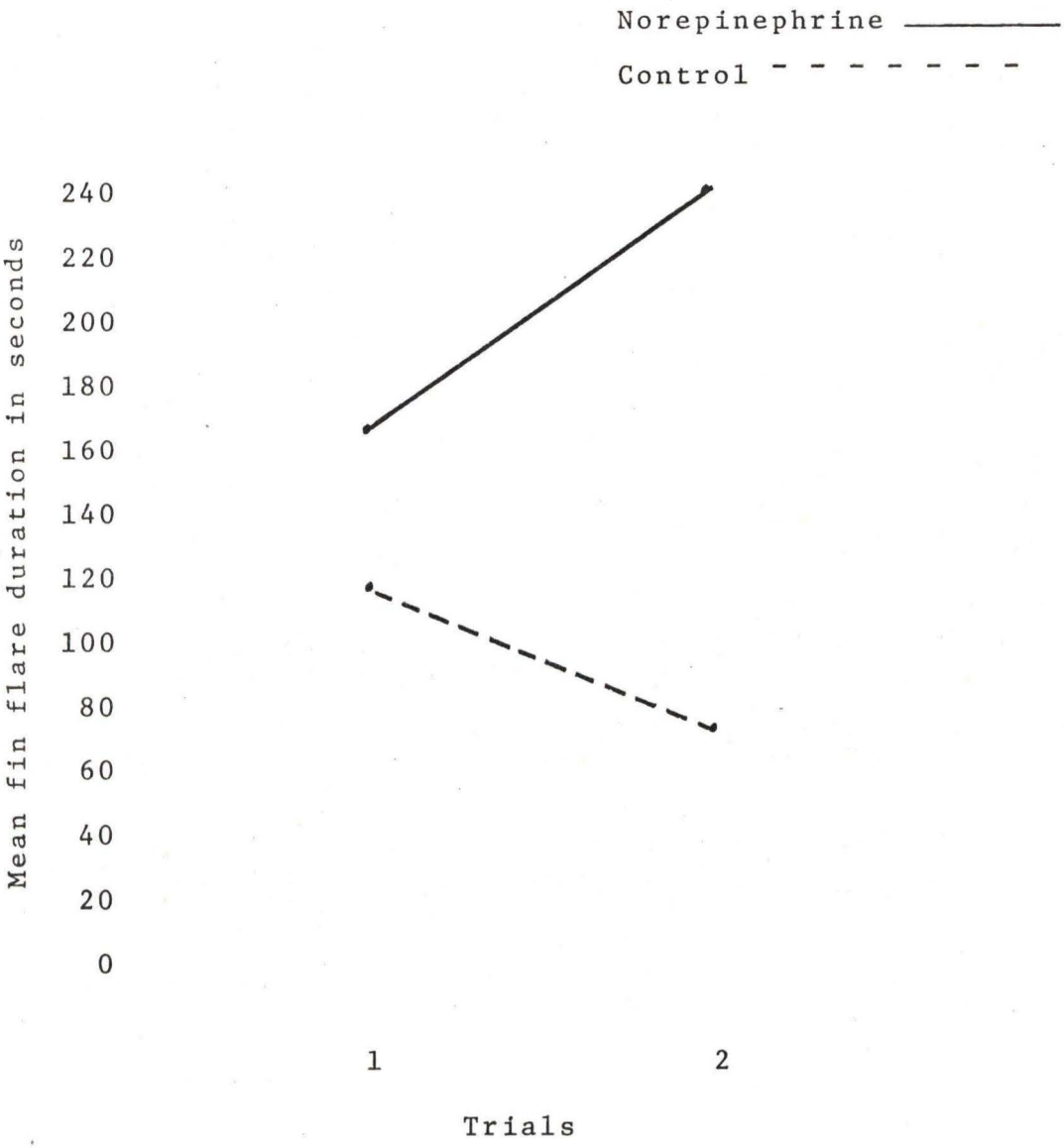

Fig. 3 Mean duration of fin flare responses for experimental and control conditions in seconds for norepinephrine study.

No significant differences were discovered at the .05 level for the effect of norepinephrine on the number of fin flares exhibited by Ss. Table 3 gives the analysis of variance summary table for number of fin flares under norepinephrine and control conditions.

Figures 4 and 5 indicate the effects of norepinephrine on the duration and number of gill plate extensions respectively. Tables 4 and 5 summarize the results of the analysis of variance for the above mentioned data. Both the number and duration of gill plate extensions increased significantly with the norepinephrine treatment. Differences on the gill plate measures for norepinephrine were significant beyond the .01 level. No significant differences were observed over days, nor was there a significant interaction effect.

In addition to the measures of fin flaring and gill plate extension, an analysis was made of the habituation point (defined as less than 50% of any 2-minute period being spent in display activity). The outcome of the habituation study is illustrated in Figure 6. The analysis of variance summary table appears in Table 6. All F ratios were significant. Both experimental and control groups decreased in habituation point from trial 1 to trial 2, yielding a significant interaction effect.

The data gathered from Experiment Ic employing Dibenzyline, include measures of fin flare frequency and

TABLE 3

Analysis of Variance of Fin Flare Number

Norepinephrine Study

Source of Variation	ss	df	MS	F
A (treatments)	281.300	1	281.3	4.35
B (days)	76.100	1	76.1	1.18
AB	101.200	1	101.200	1.11
error	775.200	12	64.6	

$F = 4.75$, $p < .05$, df 1, 12

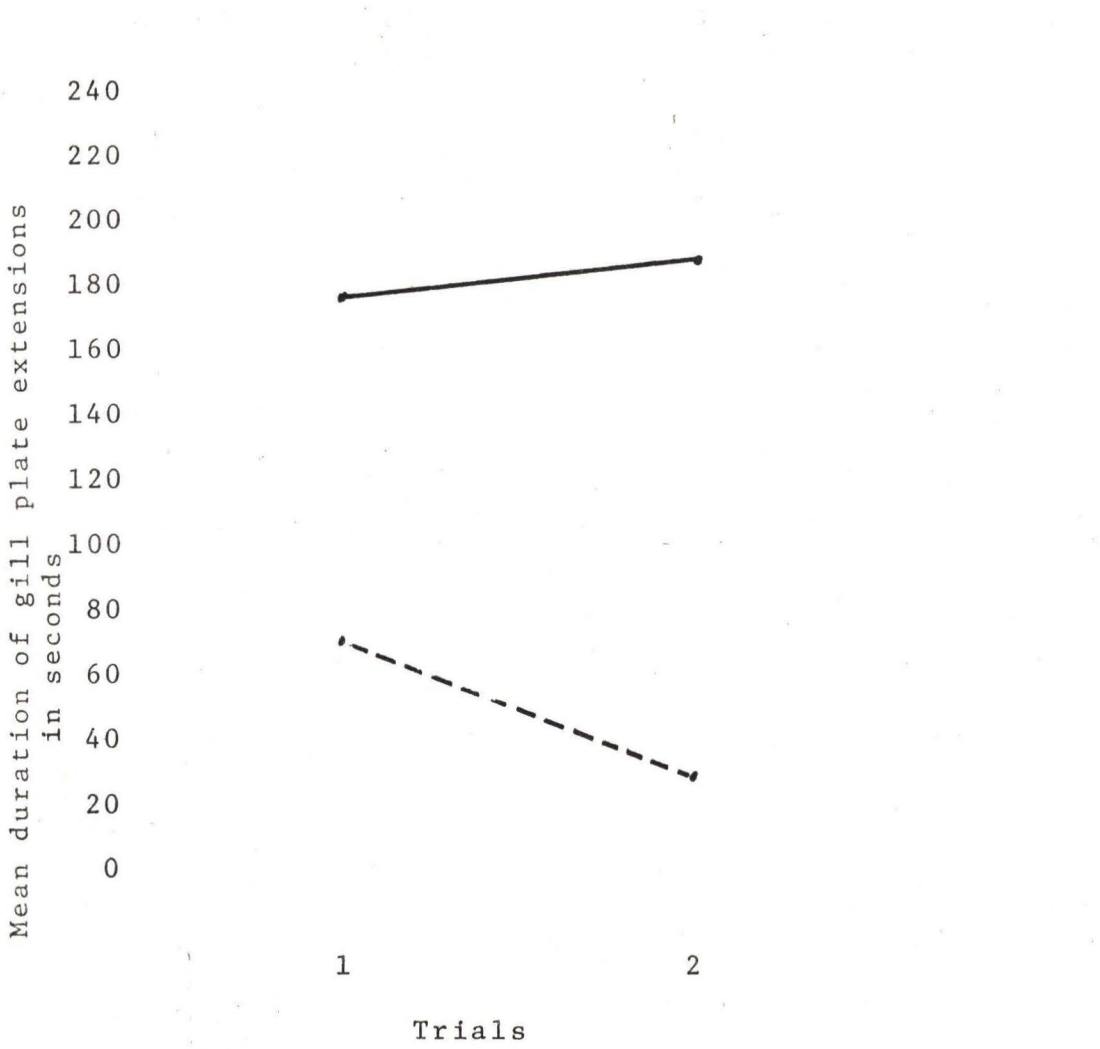

Fig. 4. Mean duration of gill plate extensions for experimental and control conditions in seconds for norepinephrine study.

TABLE 4

Analysis of Variance of Gill Plate

Extension Duration

Norepinephrine Study

Source of Variation	ss	df	MS	F
A (treatments)	89378.45	1	89378.45	28.335 **
B (days)	661.25	1	661.25	.207
AB	1940.45	1	1940.45	.600
error	38250.10	12	3187.50	

** $F = 9.33$, $p < .01$, df 1, 12

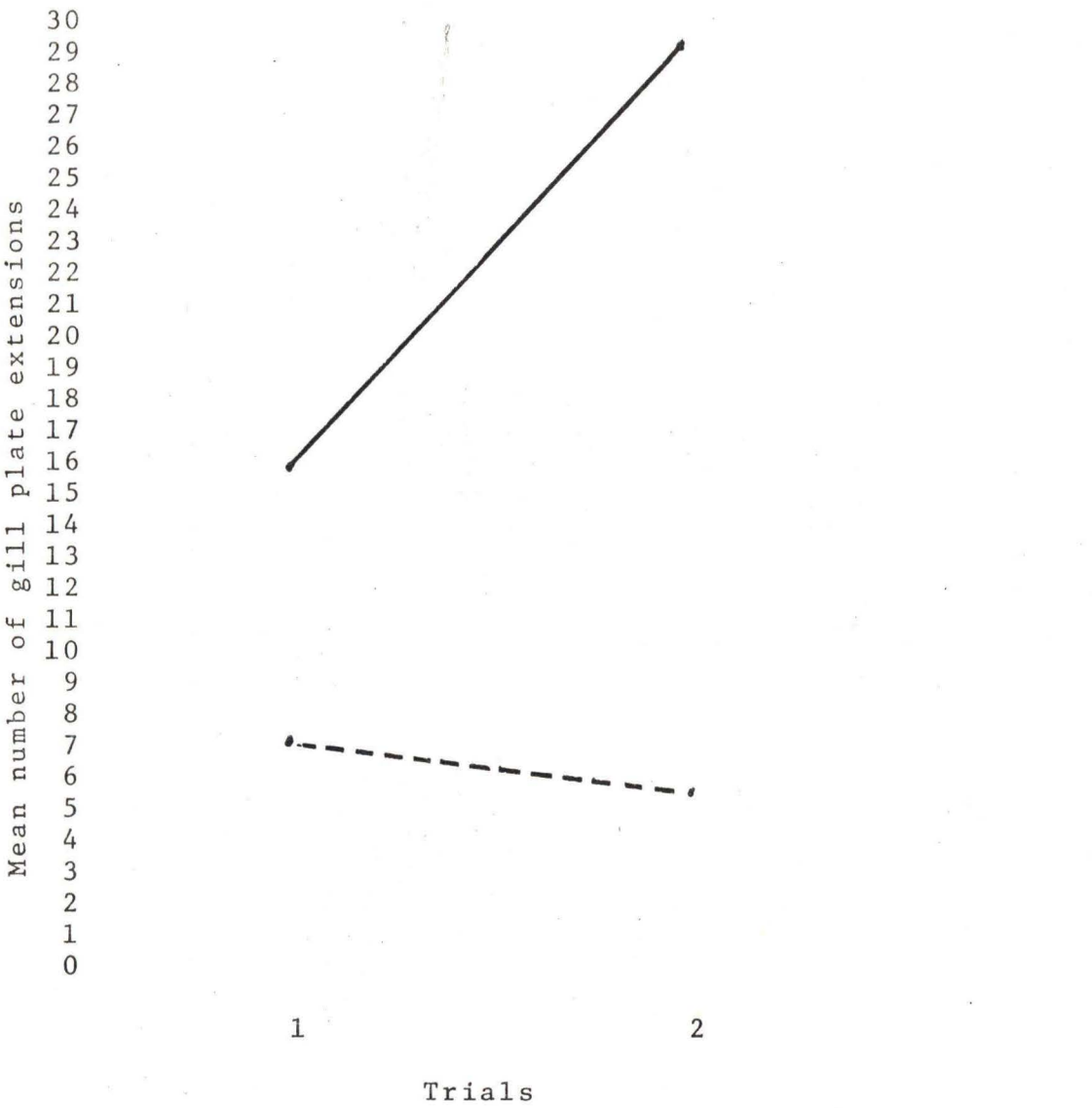

Fig. 5. Mean number of gill plate extensions for experimental and control conditions for norepinephrine study.

TABLE 5

Analysis of Variance of Gill Plate Extension Number - Norepinephrine Study

Source of Variation	ss	df	MS	F
A (treatments)	756.00	1	756.00	48.88 **
B (days)	8.00	1	8.00	.50
AB	54.90	1	54.90	3.55
error	186.35	12	15.53	

** $F = 9.33$, $p < .01$, df 1, 12

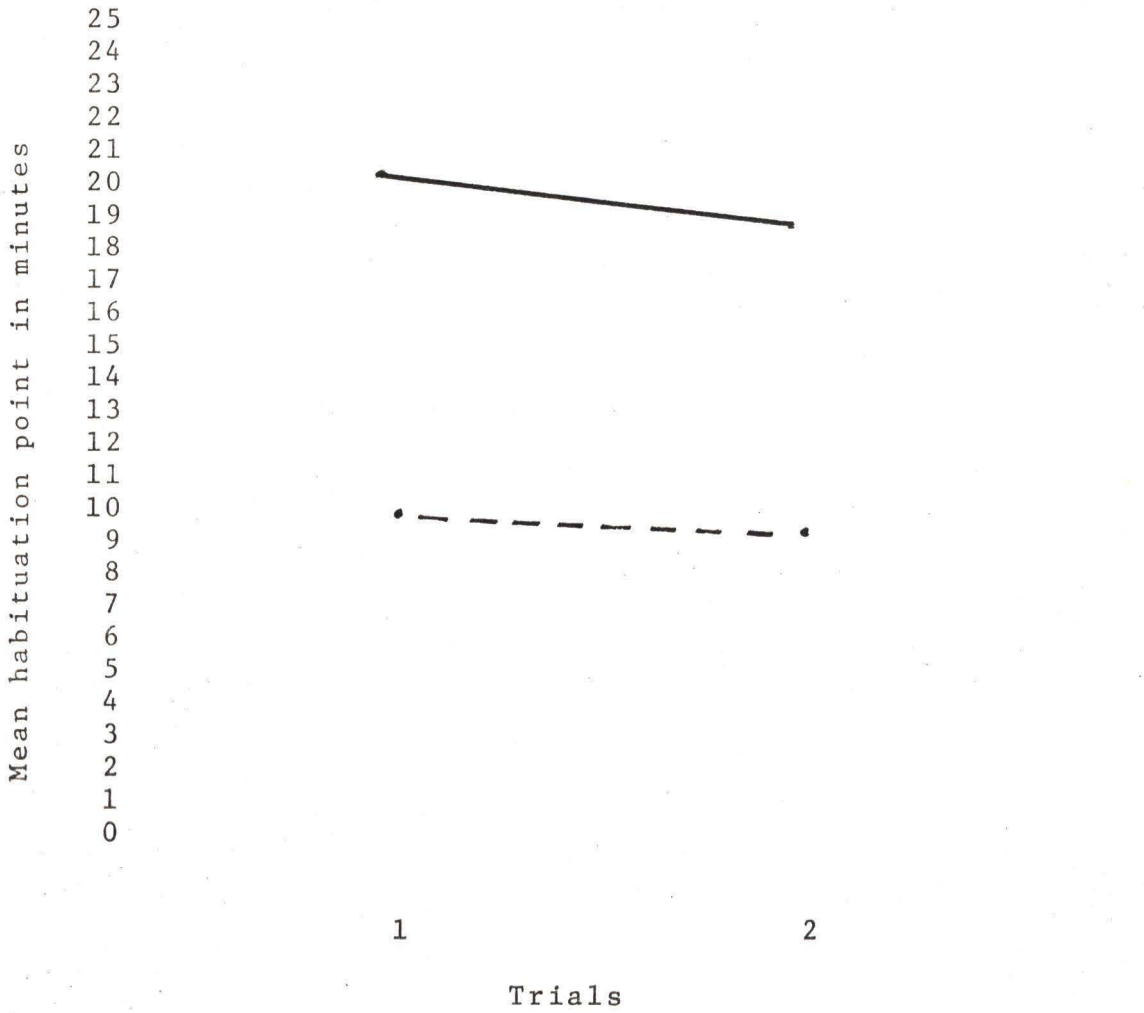

Fig. 6. Mean habituation point in minutes for experimental and control conditions in norepinephrine study.

TABLE 6

Analysis of Variance of Habituation Point

Norepinephrine Study

Source of Variation	ss	df	MS	F
A (treatments)	500.00	1	500.00	6.175 *
B (days)	995.00	1	995.00	12.292 **
AB	1001.80	1	1001.80	12.377 **
error	971.68	12	80.97	

* $F = 4.75$, $p < .05$, df 1, 12

** $F = 9.33$, $p < .01$, df 1, 12

duration, gill plate extension frequency, duration and feeding latency.

Figure 7 describes the effects of Dibenzyline on fin flare duration. In the analyses of variance summary table (Table 7) both factors A (treatments) and AB (interaction) are shown to be significantly different beyond the .01 level. The interaction effect is due to both experimental and control Ss decreasing in fin flare duration over trials.

Number of fin flares also decreased significantly under treatment with Dibenzyline. Figure 8 shows the mean number of fin flares made by experimental and control Ss. Table 8 presents the analysis of variance summary table indicating a difference between treatment and non-treatment conditions significant at the .01 level.

Gill plate extension measures for the Dibenzyline Ss are exhibited in Tables 9 and 10. Neither the duration nor frequency of gill plate extensions was significantly altered by the Dibenzyline treatment. Examination of the raw data on these measures, however, shows a definite tendency toward a decrease in responses with Dibenzyline treatment. This and the rest of the raw data can be found in the appendix.

Table 11 shows the effect of Dibenzyline on the feeding latencies of the Ss. As expected, the drug did not significantly change the amount of time before fish came to the surface to feed.

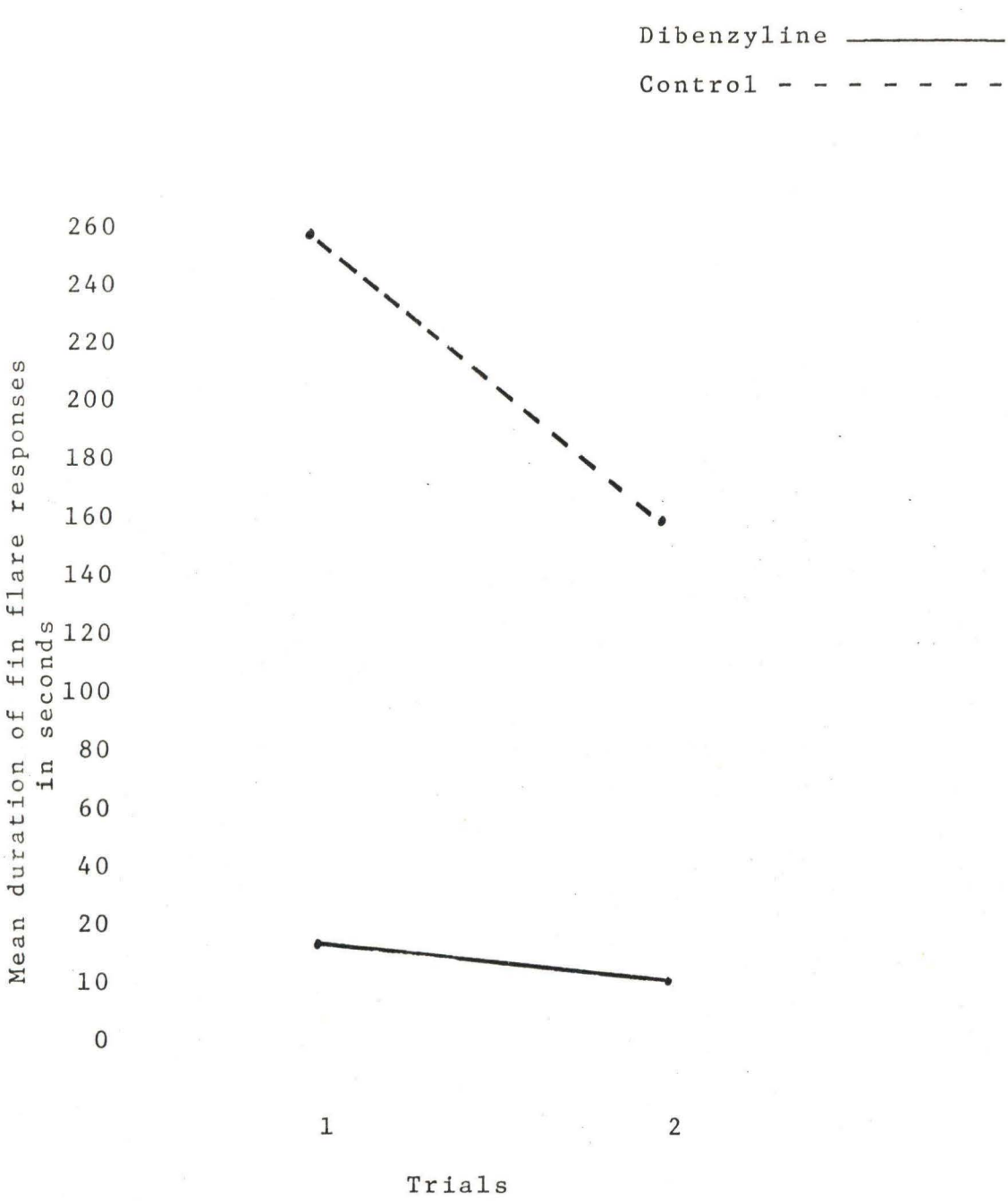

Fig. 7. Mean fin flare duration for experimental and control conditions in seconds for Dibenzyline study.

TABLE 7

Analysis of Variance of Fin Flare Duration

Dibenzyline Study

Source of Variation	ss	df	MS	F
A (Treatments)	146497.56	1	146497.56	19.62 **
B (days)	11935.44	1	11935.44	1.75
AB	299941.39	1	299941.39	40.18
error	44792.31	6	7465.38	

** $F = 13.7$, $p < .01$, df 1, 6

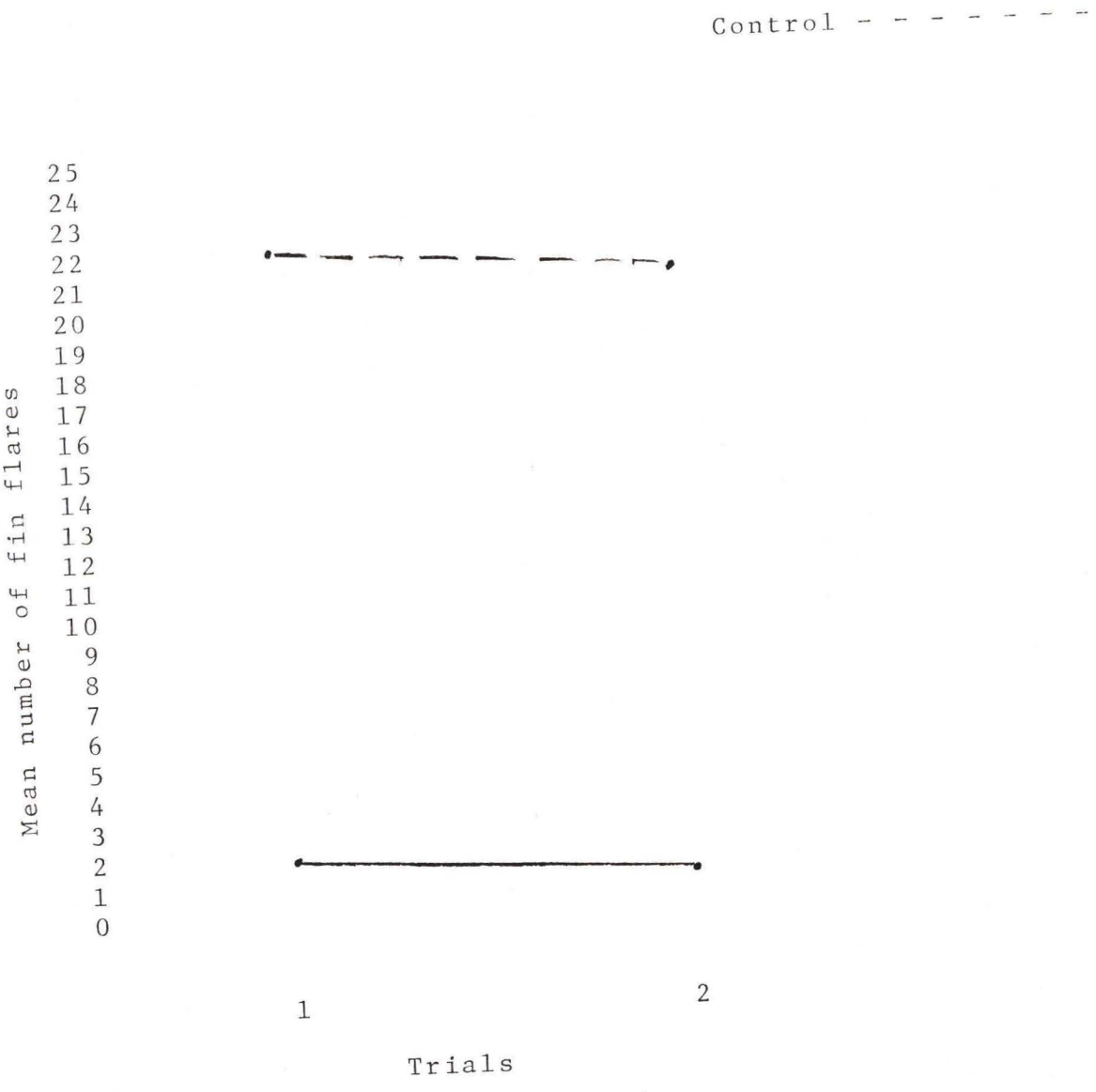

Fig. 8. Mean number of fin flares for experimental and control conditions for Dibenzyline Study.

TABLE 8

Analysis of Variance of Fin Flare Number

Dibenzyline Study

Source of Variation	ss	df	MS	F
A (treatments)	1482.25	1	1482.25	104.98 **
B (days)	2.25	1	2.25	
AB	2.25	1	2.25	
error	84.75	6	14.12	

** $F = 13.7$, $p < .01$, df 1, 6

TABLE 9

Analysis of Variance of Gill Plate Extension Duration - Dibenzyline Study

Source of Variation	ss	df	MS	F
A (Treatments)	7788.06	1	7788.06	1.41
B (days)	15687.56	1	15687.56	2.83
AB	11183.06	1	11183.06	2.21
error	33207.06	6	5534.51	

$F = 5.99, \quad p < .05, \text{ df } 1, 6$

TABLE 10

Analysis of Variance of Gill Plate Extension Number - Dibenzyline Study

Source of Variation	ss	df	MS	F
A (Treatments)	19.95	1	19.95	.963
B (days)	121.00	1	121.00	5.843
AB	64.30	1	64.30	3.105
error	124.25	6	20.70	

$F = 5.99, \quad p < .05, \quad df \ 1, \ 6$

TABLE 11

Analysis of Variance of Feeding Latency

Dibenzyline Study

Source of Variation	ss	df	MS	F
A	17.50	1	17.50	.99
B	2.50	1	2.50	
AB	3.50	1	3.50	
error	105.37	6	17.56	

$F = 5.99$, $p < .05$, df 1, 6

Experiment II

Method

Subjects -- For the testosterone experiment, 14 small but mature female Betta splendens were used. These fish were obtained from a local tropical fish retailer and were experimentally naive at the beginning of the testosterone experiment.

Apparatus -- The apparatus used for the testosterone experiment was the same as that used for Experiment I.

Procedure -- All Ss were individually maintained in one-quart bowls containing 700 ml of aged tap water, visually isolated from one another. The water was changed every four days during the one month of testosterone treatment. Diet consisted of Longlife Biorell tropical flakes fed to the fish every two days. All fish were measured on the fifth day of pre-treatment testing, and total length of fish, dorsal fin length and tail length were recorded. These measurements were made again one month later on the fifth day of post-treatment testing. Each fish was also rated prior to and after treatment on colorfulness by an independent observer. After the measures of fin, tail and total body length were taken and color ratings made, the Ss were subjected to a five-day period of pre-treatment testing. The number and duration of full fin flares and gill plate extensions were recorded for one, ten-minute trial per day. Testing took place during the light part of the light-dark cycle. Order of testing was randomized over the five days.

On the basis of the pre-treatment measures of duration of fin flares, matched pairs were established by ordering fish according to total number of seconds spent in fin flaring over all five trials. One member of each pair was then assigned to the control group and the other to the experimental group randomly. Ss in the control group received 0.2 cc of the methyl testosterone dissolved in methyl alcohol (1% g/cc) over a 25-day period. Throughout the experiment, the water was changed every four days, and the appropriate solution added.

On the 25-day, the post-treatment trials were begun. Each S was tested for 10-minutes each day and, at the end of 5-days, was again measured for physical characteristics and rated for colorfulness by the same observer who rated them the first time.

Results

All analyses in the testosterone study compare post-treatment performances of experimental and control Ss. At the beginning of the study testosterone Ss were matched on the basis of the pre-treatment fin flaring measures. Differences between pre and post treatment performance is indicated by the raw data in the appendix tables 13 - 20 A.

Figure 9 shows the effects of testosterone treatment on the fin flare duration of Ss over five trials. Analysis of the data showed a significant increase among the experimental Ss, as shown by Table 12. No difference over days, or interaction of treatment with days was indicated.

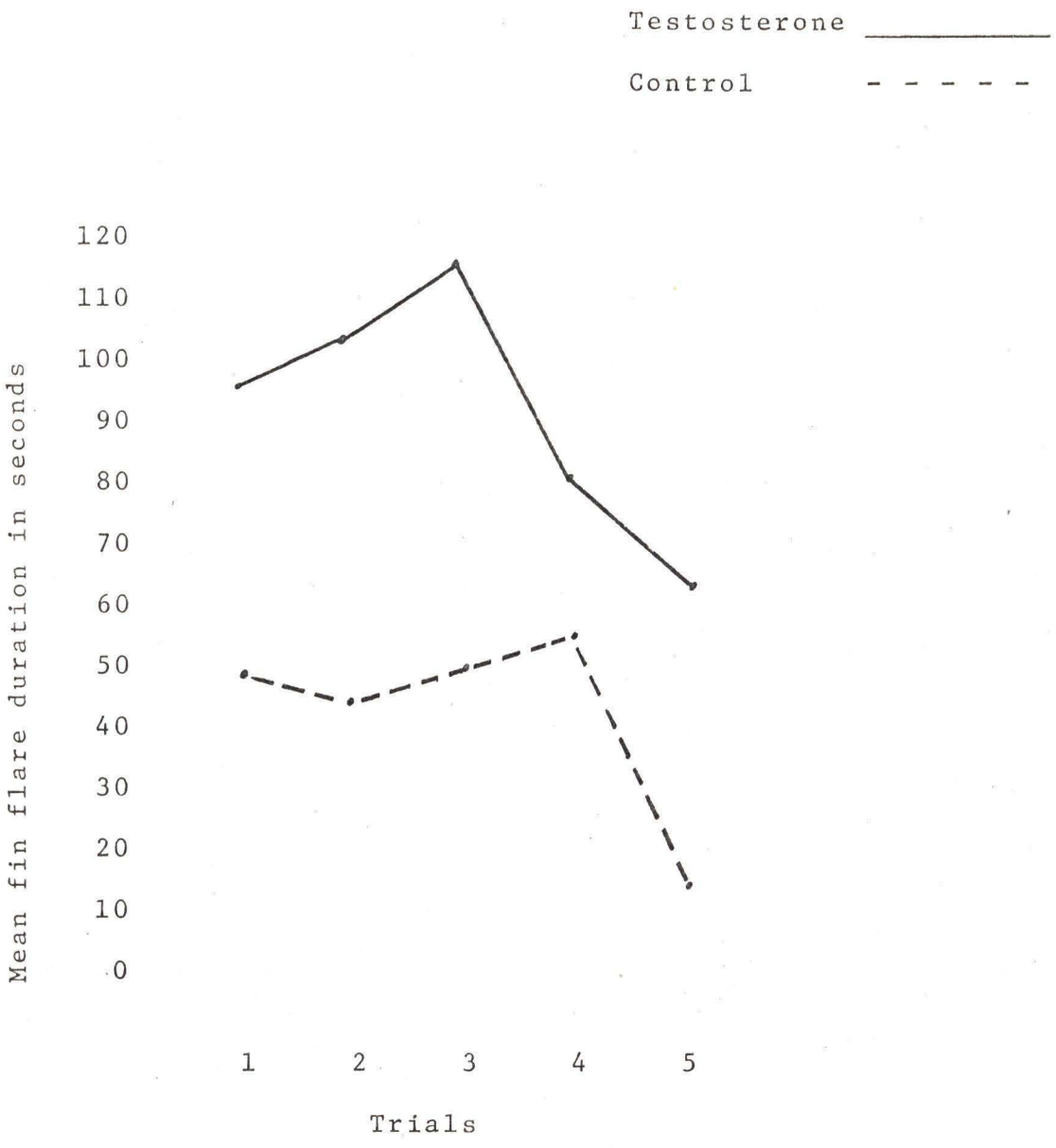

Fig. 9. Mean fin flare durations of experimental and control subjects in testosterone study.

TABLE 12

Analysis of Variance of Fin Flare Duration

of Experiment II, Testosterone

Source of Variation	ss	df	MS	F
A	33088.01	1	33088.01	26.96 **
B	11028.01	4	2757.00	2.24
AB	2317.44	4	579.36	.47
error	55214.01	45	1226.97	

** $F = 7.31$ $p < .01$, df 1, 45

Figure 10 illustrates the increased number of fin flares made by testosterone treated Ss. Table 13 indicates that this difference was significant beyond the .01 level with no differences significant for the other factors analyzed.

The mean length of fin flare responses is shown in Figure 11 and the analysis is summarized in Table 14. Testosterone seems to have a significant effect on this measure, however differences over days and the interaction effect were also significant all at the .01 level.

The data on the gill plate extensions exhibited by experimental and control Bettas is presented in Figure 12.

Physical measures taken prior to and after treatment were dorsal fin length, tail length and total body length. Each fish was also ranked on the basis of colorfulness. It was expected that the fish in the experiment I group would grow more and become more colorful than the controls. Table 15 shows the results of the analysis of dorsal fin length, indicated no significant difference.

Table 16 presents the analysis of variance summary table for the tail lengths of the subjects before and after treatment with either testosterone or alcohol.

The summary table of the analysis of total length is presented in Table 17. No significant difference was discovered between the growth of the experimental and control Ss. Pre and Post measures of course, differ significantly since all Ss grew to some extent.

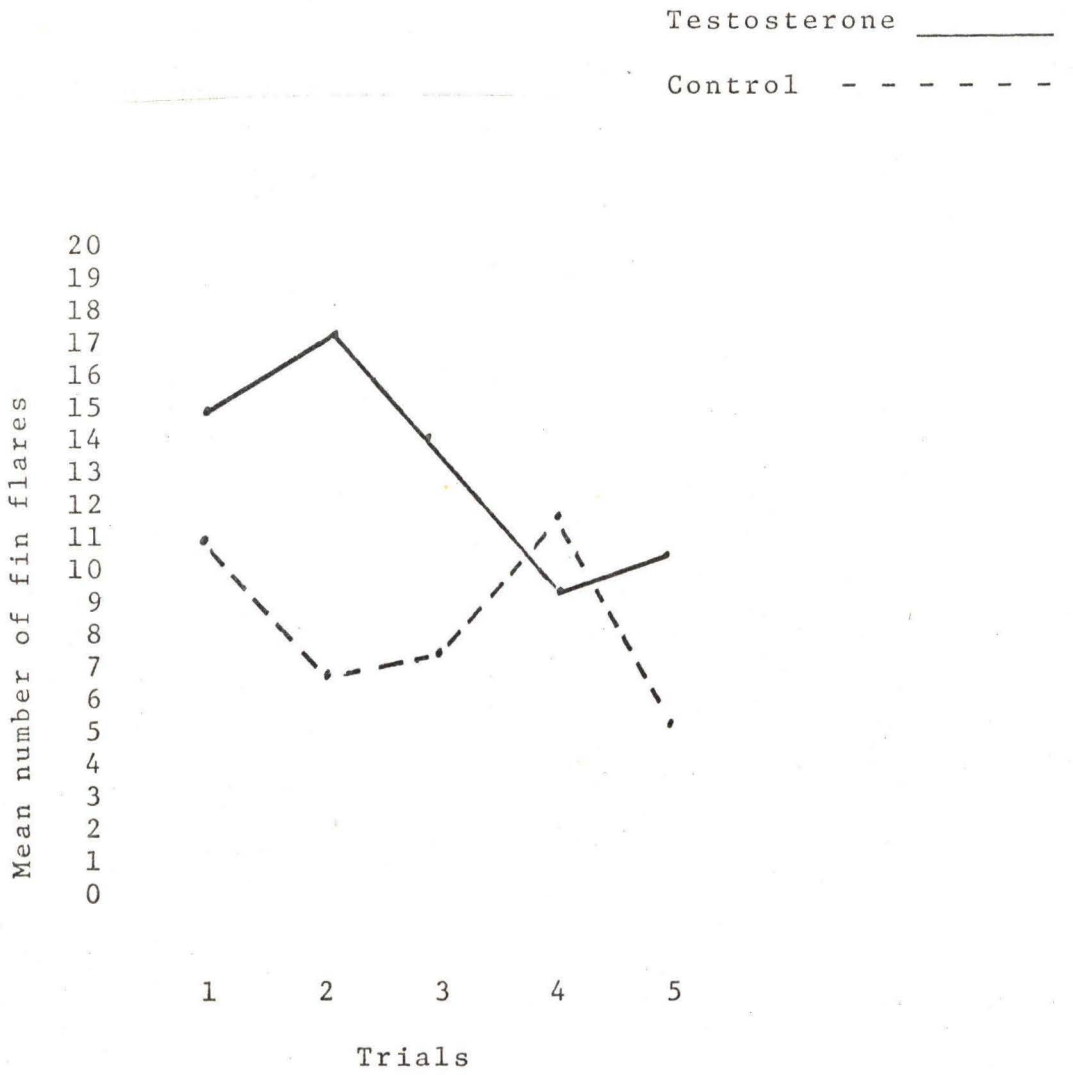

Fig. 10. Mean number of fin flares by experimental and control subjects in testosterone study.

TABLE 13

Analysis of Variance of Fin Flare Number

of Experiment II, Testosterone

Source of Variation	ss	df	MS	F
A (treatments)	340.82	1	340.82	10.12 **
B (days)	186.83	4	46.70	1.14
AB	254.76	4	63.69	1.31
error	1478.82	45	32.86	

** $F = 7.31$, $p < .01$, df 1, 45

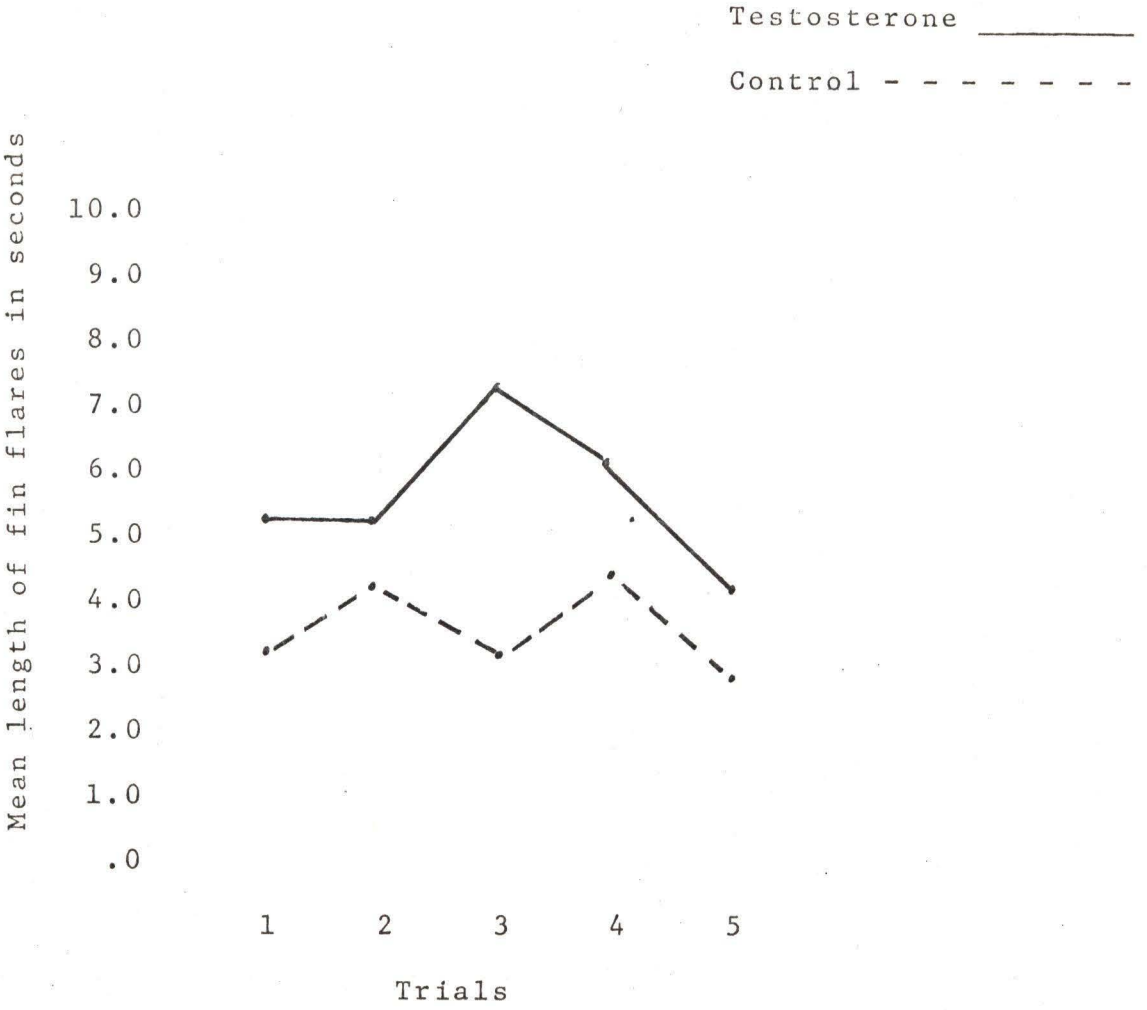

Fig. 11. Mean length of fin flare response of experimental and control subjects in testosterone study.

TABLE 14

Analysis of Variance of Average Fin Flare

Duration of Experiment II

Testosterone

Source of Variation	ss	df	MS	F
A (Treatments)	58.30	1	58.30	39.38 **
B (days)	32.32	4	8.08	5.45 **
AB	46.94	4	11.73	7.93 **
error	66.62	45	1.48	

** $F = 7.31$, $p < .01$, df 1, 45

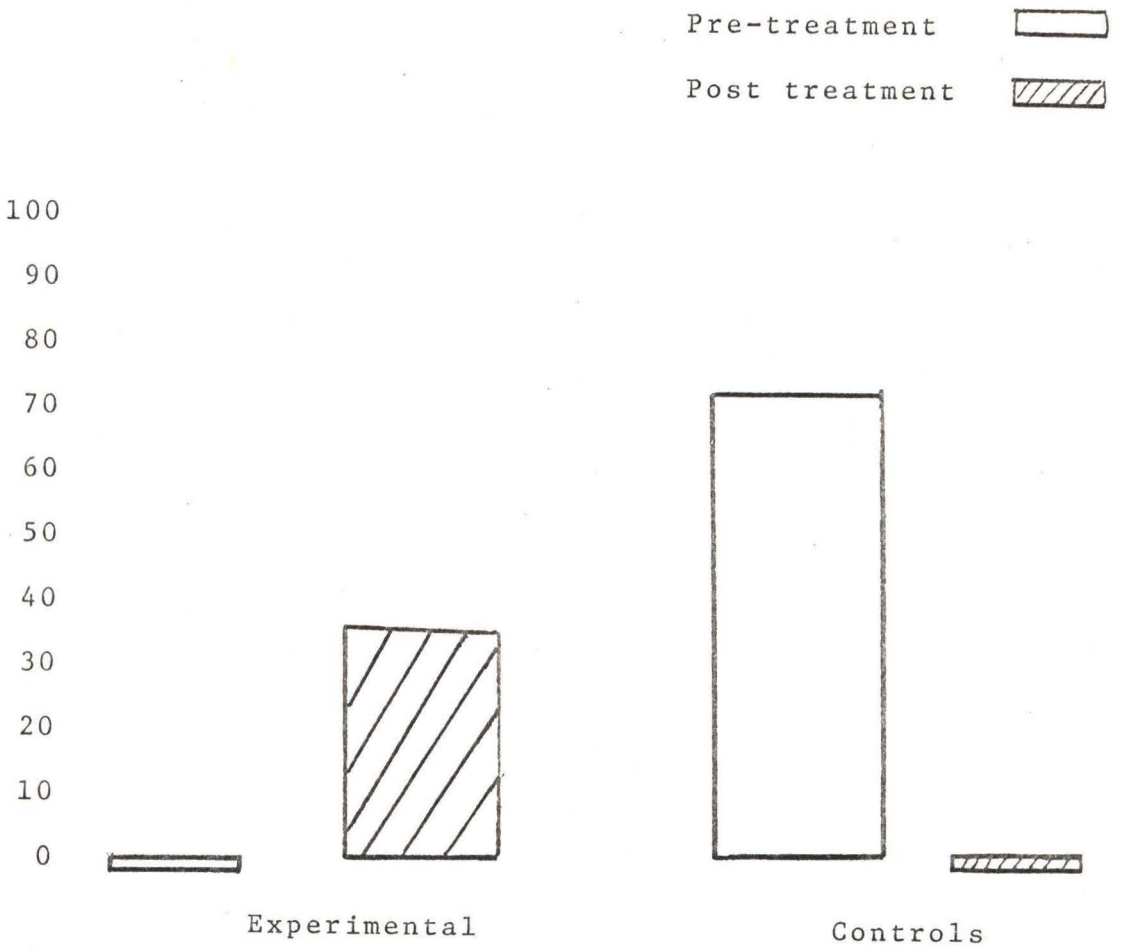

Fig. 12. Duration of gill plate extensions made by female Bettas before and after treatment with testosterone.

TABLE 15

Analysis of Variance of Dorsal Fin Length

of Experiment II

Testosterone

Source of Variation	ss	df	MS	F
A	.0003	1	.0003	.0001
B	.1103	1	.1103	.20
AB	.0142	1	.0142	
error	12.1855	33	.3692	

$F = 4.17, \quad p < .05, \quad df \ 1, 30$

TABLE 16

Analysis of Variance of Tail Length

of Experiment II

Testosterone

Source of Variation	ss	df	MS	F
A (treatment)	.7492	1	.7492	1.719
B (Pre & Post)	.6791	1	.6791	1.558
AB	1.3417	1	1.3417	3.79
error	14.0500	33	.4357	

$F = 4.17$, $p < .05$, df 1, 30

TABLE 17

Analysis of Variance of Total Body Length

of Experiment II,

Testosterone

Source of Variation	ss	df	MS	F
A (treatment	.21	1	.21	2.62
B (Pre & Post)	.37	1	.37	4.62 *
AB	.01	1	.01	.12
error	2.65	33	.08	

* $F = 4.17$, $p < .05$, df 1, 30

The ratings for color which were made for all Ss before and after their respective treatments were not significantly different for experimental and control Ss. The results of the analysis of variance, are presented in Table 18.

Discussion

Female Bettas treated with amphetamine increased fin flare duration and frequency but failed to exhibit any gill plate activity. From the results of pilot studies conducted by the experimenter, it was expected that gill plate activity would increase with amphetamine treatment. However, no gill plate activity was observed for either experimental or control Ss. Both measures of aggression should be present in order to conclude that the drug acted to increase aggression. The increased fin flaring not accompanied by gill plate extension may indicate increased activity level, not necessarily increased aggressive activity.

TABLE 18

Analysis of Variance of Color Ratings

Testosterone Study

Source of Variation	ss	df	MS	F
A	1.0208	1	1.0208	.141
B	.0208	1	.0208	.003
AB	.0208	1	.0208	.003
error	239.4375	33	7.2200	

$F = 4.17$, $p < .05$, df 1, 30

The experimenter did not feel that results obtained with amphetamine were due to improper dosage levels. Doses higher than 40 mg used in pilot studies caused Ss to shake, bleach, and exhibit jerky swimming movements. Lower dosage levels did not significantly alter any observable behavior. It would be advisable, however, in further investigation with amphetamine to include a measure of general activity. The inclusion of another activity measure might allow the experimenter to observe the effects of amphetamine on arousal in general and aggression in particular.

The experiment in which male Ss were treated with 70 mg of norepinephrine bitartrate yielded inconclusive results. The fin flare duration of experimental and control Ss differed only for trial 2. Marrone, Pray and Bridges (1966) and Pray (1967) reported high increases in fin flaring and gill plate activity with doses from 35 to 140 mg. The number of fin flares exhibited by experimental and control Ss did not differ significantly but from the experimenter's observations a tendency toward such differences did exist. The gill plate extension measures of frequency and duration increased with treatment of norepinephrine. The experimenter believes the gill plate measure to be the most significant aspect of the fish's activity during the display. The Betta rarely extends his gill plates unless he is displaying and it is unlikely that the results obtained in the norepinephrine study are due to

increased general activity. The measures of habituation latency also aid in exhibiting the importance of norepinephrine in aggression. Norepinephrine treated Ss habituated to display stimuli with significantly longer latencies than control Ss. According to Marrone et al, norepinephrine release would lead to increased aggressive behavior specifically, rather than to increased activity in general. The results of the norepinephrine experiment support that hypothesis.

The observed interaction between habituation latency and days could be the result of the general weakening or debilitation of Ss. Further investigation using more trials and different Ss for experimental and control trials could eliminate some of the problems encountered in the present experiment. It is also possible that higher doseswould lead to the fin flare increases such as those obtained by Marrone, Pray and Bridges (1966) and Pray (1967).

The dosage level of Dibenzyline was based entirely upon the pilot studies of the experimenter. Dibenzyline has been used previously with rats but has been dissolved in glucose and injected subcutaneously. The effects of Dibenzyline directly introduced into the tanks of the male Bettas differed radically with small changes in dosage levels. If not immediately removed from water containing 7-8 mg of Dibenzyline, Ss may die. A female Betta often exhibits serious impairment

of swimming and apparent difficulty of breathing in as little as 4-5 mg. The dosage decided upon for the present study was 4.5 mg.

The data collected in the Dibenzyline experiment indicates differences in fin flare measures but not in gill plate measures. The experimenter feels that Dibenzyline should not be used in a repeated measure design in the future. The drug seems to have deleterious side effects on the Betta and these effects may be relatively permanent. The side effects are indicated by the interaction effect found in analyses of fin flare responses. The effects of Dibenzyline upon feeding latency of experimental and control Ss were not different. The Dibenzyline seemed to block the aggressive display pattern without interfering with the latency to feeding. The decreases in display responses and continuation of feeding responses, yield support to the hypothesis that the blockage of adrenergic impulses would decrease aggressive behavior.

The results obtained in Experiment II supported the hypothesis that testosterone is an important part of the difference between male and female Bettas' display behavior. Previous research with sticklebacks, various vertebrates and humans indicated that treatment of females with testosterone would increase male characteristics. Male Bettas are usually larger, have longer fins and are more colorful than female Bettas, besides being more aggressive. Therefore it was

hypothesized that over a 25-day period, female Bettas treated with 2 4 g of testosterone in 0.2 cc of methyl alcohol would grow longer bodies, tails and fins than controls. It was also hypothesized that the experimental Ss would also become more colorful and display more frequently and for longer than control Ss. The desired results were obtained. Increases were apparent in fin flare frequency and duration. The average duration of fin flare responses also increased in the experimental group. Testosterone Ss exhibited more gill plate extensions after the 25-day treatment and it was noted that the entire duration of gill plate activity of control Ss was accounted for by one S. Three of the experimental Ss, which had exhibited no gill plate extensions prior to treatment increased in fin flare activity and began to show gill plate behavior after treatment with testosterone. The physical measures taken before and after treatment for both experiment and control groups indicated no differences of growth rate between groups. Tail length, dorsal fin length and total body length increased to some extent for all Ss, but not differentially for testosterone Ss. Perhaps longer treatment periods or higher dosage levels might result in differential physical changes. It would also be valuable to have a measure of general activity in future testosterone experiments.

Summary

Conclusions which may be drawn from Experiment I include:

1. Female Bettas treated with 40 mg of amphetamine in 700 ml of water increased in fin flare frequency and duration.
2. Male Bettas treated with 70 mg of norepinephrine in 700 ml of water showed significant increases in gill plate extension frequency and duration.
3. The habituation-to-display latency of male Bettas was increased by treatment with 70 mg of norepinephrine in 700 ml of water.
4. Male Bettas treated with 4.5 mg of Dibenzyline in 700 ml of water showed significantly decreased fin flaring frequency and duration.
5. Treatment with 4.5 mg of Dibenzyline in 700 ml of water did not significantly alter latency to feeding (general activity).

In general, the results of Experiment II indicate that treatment with testosterone:

1. Significantly increases the fin flaring duration and frequency of female Bettas; and
2. Increases the average length of fin flare responses for female Bettas.

From the various results reported here, it is apparent that both the sympathomimetic amines and the hormone, testosterone, play an important role in the aggressive display behavior of the Siamese fighting fish. Where the action of such substances is blocked, as with Dibenzyline, aggressive display behavior decreased significantly. In the female of the species, the display may be triggered by the release of norepinephrine but the lack of testosterone leads to a less aggressive display pattern.

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Appendix

Raw Data

TABLE 1A

Fin Fâre Durations, Experiment Ia

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
72	4	132	174
156	10	199	63
100	20	358	76
79	128	237	218
50	20	33	76
201	5	238	98

TABLE 2A

Fin Flare Number, Experiment Ia

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
17	3	22	24
14	7	17	7
26	6	33	18
16	30	33	30
14	8	10	25
27	2	34	15

TABLE 4A

Fin Flare Number, Experiment Ib

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
12	18	25	28
6	2	3	15
8	14	14	26
12	11	16	26
14	4	9	14

TABLE 5A

Gill Plate Extension Durations, Experiment Ib

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
85	29	312	208
81	32	116	172
23	52	177	178
52	31	138	296
67	8	135	65

TABLE 6A

Gill Plate Extension Number, Experiment Ib

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
8	5	22	22
10	6	10	21
3	6	18	25
7	5	16	25
6	2	13	9

TABLE 7A

Habituation Point (in minutes), Experiment Ib

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
12	13	54	37
7	7	10	12
9	12	14	14
9	11	20	24
13	5	5	8

TABLE 8A

Fin Flare Durations, Experiment Ic

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
407	62	0	2
217	187	1	0
203	201	23	0
176	151	30	17

TABLE 9A

Fin Flare Number, Experiment Ic

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
22	15	0	1
17	20	1	0
20	17	4	0
29	30	3	7

TABLE 10A

Gill Plate Extension Duration, Experiment Ic

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
292	0	18	35
2	0	0	0
90	0	0	0
78	0	56	0

TABLE 11A

Gill Plate Extension Number, Experiment Ic

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
18	0	4	7
1	0	0	0
8	0	0	0
11	0	9	0

TABLE 12 A

Feeding Latency (in seconds), Experiment Ic

Control Group		Experimental Group	
Trial 1	Trial 2	Trial 1	Trial 2
4	5	2	6
3	7	4	5
6	3	7	7
5	3	10	12

TABLE 13A

Fin Flare Duration, Experiment II

	Experimental Group					Control Group				
Day	1	2	3	4	5	1	2	3	4	5
	125	127	179	198	105	0	6	0	36	0
	133	149	108	27	45	70	87	7	18	28
	53	61	47	0	0	28	36	10	15	10
	101	87	75	93	23	91	85	62	111	17
	48	43	49	35	50	19	19	25	38	13
	120	153	204	126	145	79	40	192	101	57

TABLE 14A

Fin Flare Number, Experiment II

	Experimental Group					Control Group				
Day	1	2	3	4	5	1	2	3	4	5
	21	20	20	13	13	0	1	0	11	0
	18	27	9	5	11	20	6	4	6	7
	11	17	15	0	0	11	12	5	11	6
	16	14	16	14	6	19	15	14	18	5
	12	11	14	13	10	7	6	9	5	5
	17	22	17	19	33	16	10	24	27	11

TABLE 15A

Average Length of Fin Flare Response, Experiment II

Experimental Group					Control Group				
Day 1	2	3	4	5	1	2	3	4	5
5.95	6.35	8.95	15.23	8.08	0	6.00	0	3.27	0
6.65	5.52	12.00	5.40	4.09	3.50	5.43	1.75	3.00	4.00
4.81	3.59	3.13	0	0	2.55	3.00	2.00	4.69	1.66
4.59	6.21	4.69	6.64	3.83	4.79	5.66	4.43	6.16	3.40
4.00	3.99	3.50	2.69	5.00	2.71	3.16	2.66	7.60	2.60
4.14	6.95	12.00	6.63	4.39	4.94	4.00	8.00	3.74	3.19

TABLE 16A

Tail Length, Experiment II

Experimental Group		Control Group	
Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
.9	.9	.6	.7
1.1	1.3	.8	.9
.6	.6	1.5	1.6
.9	1.1	17	.7
. 7	.6	.6	.7
.8	1.4	1.1	1.2

TABLE 18A

Total Body Length, Experiment II

Experimental Group		Control Group	
Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
3.5	4.5	3.1	3.2
3.2	3.7	3.7	3.8
3.0	3.2	3.1	3.7
3.3	3.4	4.0	4.2
2.7	3.1	3.5	4.0
3.2	3.4	3.4	3.7

TABLE 19A

Color Ratings, Experiment II

Experimental Group		Control Group	
Pre-treatment	Post-treatment	Pre-treatment	Post treatment
12	11	6	10
7	9	3	4
4	5	1	12
8	3	4	6
10	2	9	7

TABLE 20A

Gill Plate Extension Duration, Experiment II

Experimental Group		Control Group	
Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
0	8	0	0
0	0	0	0
0	0	0	0
0	4	0	0
0	10	0	0
0	20	84	0