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The Effects of Isolation on Aggressive Display in Siamese Fighting Fish (*Betta splendens*)

By

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VITA

Thomas Joseph Hinkel was born on March 4, 1945, in Philadelphia, Pennsylvania. He was graduated from Northeast Catholic High School and received his Bachelor of Arts degree in Psychology in 1967 from Saint Joseph's College, Philadelphia, Pennsylvania. In September, 1967, he entered the doctoral program at Loyola University, Chicago, Illinois, where he served as a graduate assistant and Lecturer in Psychology. He received a Master of Arts degree in Experimental Psychology in February, 1970. In February, 1973, he received the Doctor of Philosophy degree in Experimental Psychology.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The involvement of many types of variables in intraspecific aggression has been investigated over the past fifty years. The main course of research has now produced a vast body of literature emphasizing the significant effects of many environmental conditions on aggressive behavior. For example, it is widely documented that the degree of fighting or threat behavior in the males of many species can be elevated by the proximity of another individual, the presence of receptive females, territorial encroachment, overcrowding, lack of space, direct attack of a conspecific, aversive stimulation, intense heat, witholding of anticipated food, previous experience and learning (see reviews by Vernon, 1969, 1971).

A few investigators (Berkowitz, 1962; Dollard, Doob, Miller, Mowrer & Sears, 1939; Montagu, 1968; Scott, 1958) have viewed aggression primarily as a reaction to external stimuli. While acknowledging the strong influence of ontogenetic experience, any notion of aggressive behavior acting as a spontaneous drive which implicates endogenous determinants is greatly minimized. According to these authors, fighting is a learned response established as a result of encountering frustrating stimuli and serving a purely defensive function.

It cannot be denied that many exogenous factors play a crucial role in releasing attack behavior. However, one of the major points of controversy dealing with the possible causes of intraspecific aggression is whether it is determined entirely by the existing environmental stimulus pattern or if internal factors also are involved. It is clear from the issues to be discussed that there are agents other than external stimuli which at least partially determine the tendency to be aggressive. These internal variables should be taken into account in studies dealing with the causation of aggression.

Internal factors in aggression

The development of aggressiveness, both between different animal species and within the same species, can be largely genetically determined. For example, it has long been recognized that Siamese fighting fish, gamecocks, terriers and bulldogs can be selectively bred for their strong fighting tendencies. The common laboratory rat and wild Norway rat show conspicuous differences in hereditary predispositions toward aggressiveness. Scott (1966) and Southwick (1970) review several additional laboratory demonstrations of differences in fighting behavior between various mouse and rat strains, some being more easily aroused to fight and some being more capable of winning fights than other strains.

For many years it has been known that the internal biological state of an organism affects aggressive temperament. Evidence exists to indicate a positive correlation between male sex hormones and the development, performance, and frequency of fighting behavior (Collias, 1950). Males in most vertebrate species are more aggressive than females and become increasingly so during the breeding season. This difference is thought to be related to the presence of androgens. Some of the species tested, either by the administration of an androgen or by castration, include swordtail fish, anolis lizards, painted turtles, domestic fowl, night herons, doves, rats, mice, and boys (Davis, 1963).

A study by Beaman (1947) is representative of the hormone studies. The normal pattern of fighting behavior in the males of two strains of mice were observed prior to castration. Following surgery, the mice did not show aggression toward the other males. However, when testosterone was implanted in these mice, fighting behavior reappeared and persisted until the hormone treatments were discontinued. Although female mice are less aggressive than males, Bronson and Desjardins (1971) were successful in raising the level of aggression by administering testosterone to females early in life and again during adulthood at the time of testing. In another study, Tavolga (1955) castrated male gobiid fish and all indications of aggression toward other males disappeared even though courtship responses to females were unimpaired. In some males there was gradual testicular regeneration resulting in a restoration of their fighting responses toward males.

Female sex hormones may also play a role in aggression, but the exact connection remains unresolved. Guhl (1961) feels that estrogen is related to submissiveness in some species while Vandenbergh (1971) suggests that it is progesterone, in the presence of estrogen, which reduces aggression in female golden hamsters during estrous.

In addition to the involvement of the gonadal hormones in aggressive behavior, Mathewson (1961) has discovered a connection between the pituitary's luteinizing hormones and the level of aggression in male starlings. Injections of luteinizing hormone increased aggressiveness and produced a reversal of dominance while testosterone injections had no influence.

Lorenz's drive model of aggression

The evidence implicating the many possible external and internal antecedents makes it clear that the causes of aggression are complex. The exact nature and extent of the internal influences and the interaction with the external factors remain to be determined. One school of thought has invoked the conception of aggression as a drive in trying to understand this behavior. A leading proponent of this view is Konrad Lorenz who attempts to account for the dynamics of aggression by postulating an internal drive mechanism in accordance with his general model of behavior (Lorenz, 1966).

The Lorenzian model of motivation (Lorenz, 1939, 1950) proposes an "energy system" which brings about a readiness to perform an instinctive behavior pattern. When motivation increases, as when an animal is deprived of some needed object, there is an accumulation of "action specific energy" in a particular brain location. With this accumulation the animal becomes restless and exhibits appetitive behavior. When the proper stimulus appears, the neural energy is released into the appropriate motor pathways for action and the intensity of the act is directly proportional to the amount of energy that has built up. If the action pattern is not released by an appropriate stimulus, the action specific energy continues to collect resulting in a gradual lowering of the threshold necessary for release of that action. Eventually a zero threshold value may be reached and the action pattern occurs without external provocation (vacuum activity). A corollary of this phenomenon maintains that with progressive accumulation of action specific energy, increasingly less appropriate objects will produce the behavior pattern until it occurs spontaneously, that is, with no demonstrable external stimulation.

Lorenz (1966) feels that aggression is a true instinctive pattern of behavior impelled by its own action specific energy that is generated in a particular center in the central nervous system and can be dissipated only by the performance of an aggressive act. These inferences of an internal drive for aggression were based on observations that fighting behavior shows spontaneity, lowering of threshold, discharge at inadequate objects, periodic-rhythmical occurrence, and appetitive behavior.

Lorenz claims that aggressive behavior can be spontaneous, that is, it builds up from within. Fighting is not merely a reaction to certain external factors; aggression will occur even in an unchanging environment. As evidence, Lorenz (1966) cites an example of the "damming" of the aggressive instinct which appears in many cichlid fish. If a mated pair of these fish is left alone in an aquarium, the male's readiness to fight gradually increases until he vents his hostility on the only available object, the female. Placing another male in the tank, or even behind a glass partition, will enable the fish to discharge his aggressive drive at this new object and the female is safe from attack. Rasa (1969) has recently confirmed this observation that the aggressiveness of male cichlids will be intensified if they are not given the opportunity to fight or threaten other males.

Further evidence for the internal control of aggression is presented by Hinde (1970). Although territorial fighting in birds occurs when hormone level is high, there are other times, as in cold weather, when the animal may fluctuate from territorial to flocking behavior over a period of a few minutes. Hinde concludes there must be temporary central states independent of the long term hormonal states which lead to a readiness to attack.

In addition to producing an increase in the readiness to react, Lorenz (1966) maintains that the damming of the aggressive instinct also leads to appetitive behavior: "If the stimuli normally releasing it fail to appear for an appreciable period, the organism as a whole is thrown into a state of general unrest and begins to search actively for the missing stimulus (p. 50)." Although no direct evidence is offered by

Lorenz, recent studies (Baenninger, 1966, 1970; Goldstein, 1967; Hogan, 1967, 1970; Thompson, 1963, 1964, 1965) have demonstrated that domestic cocks and Siamese fighting fish will learn an operant task to gain access to stimuli that evoke fighting responses. This may be interpreted as laboratory evidence for appetitive or searching behavior since these animals actively placed themselves in a position where they could display aggressively at a conspecific, model, or mirror image.

Isolation and aggression

Another important aspect basic to the Lorenzian model is the heightening of the tendency to fight as the number of fighting opportunities decrease. The intensity of an instinctive act is thought to be related to the amount of accumulated action specific energy which in turn depends upon the length of time since that act was last performed. Thus, Lorenz expects aggression to obey a definite rule: the longer the time that has passed since fighting behavior was released, the more intense will be the response to a given aggression-releasing situation.

It is commonly known that the males of many species raised in isolation exhibit exaggerated aggressive tendencies when put together with conspecifics at a later time. A number of investigations have shown that jungle fowl cocks (Kruijt, 1964), mice (Banks, 1962; Kahn, 1954; Levine, Diakow & Barsel, 1965), and rhesus monkeys (Harlow & Harlow, 1962; Mason, 1963) reared alone will fight more vigorously than those raised in groups.

Hinde (1970), however, points out that the increased aggressiveness is probably an enduring trait induced by the developmental history of the individual. Consequently, this phenomenon would have little bearing on the question of whether fighting increases with time since the last encounter in normally reared animals.

More relevant to the topic of deprivation-induced aggressiveness are the aforementioned studies concerning the damming of the aggressive instinct in male cichlid fish in which lack of opportunity to fight causes the male to displace his pent-up aggression on the female. In addition, Lorenz (1966) cites his observations of habituation of fighting among cichlids, Siamese fighting fish and shama thrushes. A group of these animals placed together will gradually develop a high degree of mutual habituation and peaceful coexistence. However, the diminished hostile reactions can be restored if an individual is removed for a short period and afterward returned to the others.

In a similar habituation experiment, Clayton and Hinde (1968) studied the recovery of aggression in Siamese fighting fish after they had been habituated to their own mirror images. Following ten days of continuous mirror presentation, the mirror was removed for each of five isolation periods (15 minutes, 6 hours, 24 hours, 48 hours, and 4 days), interspersed by two days of rehabituation. It was found that the amount of aggressive display, as measured by the number of gill cover erections, was greater as the duration of mirror removal was increased. Recovery was slow and incomplete, however, with only 67% of the original display strength appearing after four days of isolation. This experiment provides some information about the proportionate increases in aggression with isolation, but the design does not resemble the typical situation that might be experienced by the fish. Since Siamese fighting fish persistently chase away other males of the species, one would not expect the fish to continually be in close proximity to other members of the species with only a few brief periods of seclusion. Thus, the long periods of initial mirror presentation make it difficult to generalize from the Clayton and Hinde experiment to situations which consist of relatively infrequent brief encounters between species members.

The investigations which probably come nearest to measuring the progressive enhancement of fighting with increasing time in isolation are described by Bourgault, Karczmar, and Scudder (1963) and by Welch (1967), and Welch, B. L. and Welch, A. S. (1966). Data are presented showing that individually-housed mice have shorter latencies of attack and more fights in paired encounters with the same mouse as the period of isolation increases up to fifteen weeks.

The purpose of the present investigation was to further test Lorenz's speculations, focusing on the strength of aggression as a time-dependent process. Does the suppression of fighting heighten the aggressive response to an eliciting stimulus; and is the increase in response strength in proportion to the length of time elapsed since its last release? From the evidence that has been reviewed it was hypothesized that: under unchanging environmental stimulation, the intensity of aggressive behavior is progressively increased as the period in isolation is lengthened.

In order to test the generality of the experiments with mice (Bourgault _et_ _al_., 1963; Welch, 1967; Welch, B. L. & Welch, A. S., 1966), this study compared Siamese fighting fish on the intensities of aggressive display following various time periods of isolation from display-eliciting stimuli. In addition, the selection of Siamese fighting fish allows for a more direct measure of the strength of the aggressive response. Studies of aggression in mice rely on the all or none character of the fighting behavior and the latency of attack. There is no report on the gradation of attack or fighting intensity. Siamese fighting fish will readily display toward many stimuli for long periods of time. This permits an opportunity for obtaining indices of response duration as well as the latency and frequency of attacks.

Another problem with the mice studies is the uncontrolled interaction effects of two fighting mice. In the experiment by Bourgault _et_ _al_. (1963), Welch (1967), and Welch, B.L. and Welch, A.S. (1966), the relative strength of aggression was determined by observing the number of seconds for the first attack to occur and the number of fights between a pair of mice selected from the same prior isolation condition. When each mouse is constantly reacting to what the other does, quantization of individual response strengths is a formidable task. In an attempt to improve upon the paired encounter design, the present study controlled for the paired interaction effects by presenting the Siamese fighting fish with their own mirror image (Experiment 1) and a male model (Experiment 2).

A mirror image presented a situation in which a fish interacted with another displaying male. The purpose of using a male model was to test the effects of isolation on aggressive display strength when the eliciting stimulus was relatively non-threatening, that is, the stimulus did not fight back. By comparing Experiments 1 and 2, it was hoped that it would be possible to evaluate two aspects of aggressive behavior under conditions of isolation. Experiment 1 can be viewed as a situation in which the test fish must continue to fight an attacking intruder while in Experiment 2 the stimulus does not attack. Any differential effects of isolation on the two types of aggression may be detected by the variation in stimulus conditions.

CHAPTER II

METHOD

Experiment 1

Subjects

Sixty adult male Siamese fighting fish (*Betta splendens*), obtained from a local supplier, were housed separately in adjacent one-gallon glass tanks until used in the experiment. The tanks were constantly illuminated from overhead and water temperature was maintained at 79°F - 81°F throughout experimentation. All fish were fed frozen brine shrimp daily.

Apparatus

Testing was carried out in five-gallon aquaria partitioned by opaque dividers into four equal areas measuring 12 by 14 by 17 cm. To prevent the possibility of a fish seeing another's reflections at the points of compartment subdivisions, only the end sections contained subjects. Once testing began, each fish remained in his compartment until termination of the experiment. During test periods a flourescent light was placed over the entire length of the aquarium and one end of each section was covered with a mirror. The placement of the eliciting stimulus was such that the experimenter obtained a clear lateral view of the fish when the fish was oriented toward the stimulus.

Procedure

Each subject was placed in an experimental compartment and allowed 24 hours of adaptation to the apparatus. On the second day each fish was presented with mirror stimulation for 30 minutes during which several components of the aggressive display were recorded. Each test began with the placement of the mirror when the fish was in the middle third of the tank and oriented toward the mirror.

It appears that the most indicative gesture of hostile intent in Siamese fighting fish is the gill cover erection, a spreading of the opercula and extension of the black brachiostegal membranes (Clayton & Hinde, 1968; Peeke and Peeke, 1970; Simpson, 1968). In adherence to the measures suggested by these investigators, the specific dependent variables used in this study included the frequency of gill cover erections and the total time spent with the gill covers extended. A gill cover erection begins with an extension of the opercula; it ends with a lowering of the opercula or when the fish moves away from the mirror. The reliability between the Experimenter and another observer in measuring the cumulative time of gill cover erections for the same ten subjects over the 30 minute test periods was found to be very high ($r = 0.99$). In addition to these measures, the latency of the first approach to the mirror image and the mean duration of each gill erection were recorded.

Following the initial test of aggressive display strength, the mirror was removed for one of four isolation periods; 15 minutes, 6 hours, 24 hours, or 72 hours (modified from Clayton and Hinde, 1968). The subjects were randomly assigned to one of the isolation groups and retested at the designated time. Thus, the four treatment groups, each containing 15 subjects, were arranged in a one-way analysis of covariance disign with the initial measure of aggression serving as covariate.

Experiment 2

In Experiment 1, each fish responded to his mirror image. While mirror image stimulation provides an opportunity to obtain data on display intensity in a hostile encounter between two fish, it does not control for all possible positive feedback effects. The mirror image always mimics the response being performed by the test fish. Should the stimulus provided by the image create a situation which further elicits the same response, a condition of perseverating behavior may result. To control for this reaction, a stationary model of a conspecific male was used in Experiment 2.

Subjects

Another 20 Siamese fighting fish served as subjects in Experiment 2. Pre-observation maintenance was identical to that of the subjects in Experiment 1.

Apparatus

Behavioral observations were made with the same apparatus as in Experiment 1 except for a change in the display eliciting stimulus. A red plastic model of a male Siamese fighting fish was cast from a mold of a dead fish. Moving and stationary models of conspecifics (Simpson, 1968; Thompson, 1963), and male silhouettes (Johnson, R.N. & Johnson, L.D., 1970) have been shown to evoke aggressive displays in Siamese fighting fish. The use of a model allows all stimuli to be held constant. This includes movement and orientation of the stimulus which, of course, varies from subject to subject with mirror stimulation.

Procedure

All fish were submitted to the same measurement procedures as in Experiment 1 except for one minor variation. Since responses were generally weak and of short duration, the observation periods for each fish were reduced to 15 minutes.

CHAPTER III

RESULTS

Experiment 1

The major measure of the degree of aggressivity in Experiment 1 was the amount of time that a fish spent with gill covers erected during each 30 minute mirror presentation. Table I contains the means and standard deviations of the number of minutes of gill display during the pre-isolation phase (initial test following 24 hours of visual separation from other fish) and post-isolation phase (test following the experimental isolation period of either 15 minutes, 6 hours, 24 hours, or 72 hours). No significant differences were found in pre-isolation scores , but it is clear that there was a progressive enhancement of aggressive display strength as the duration between tests increased. The post-isolation scores can be adjusted for different pre-isolation scores by use of the pooled regression equation. The adjusted percentages of post-isolation display duration were 49, 58, 68, 74 for 15 minutes, 6 hours, 24 hours, and 72 hours of deprivation respectively. The results of the analysis of covariance (Edwards, 1972) are shown in Table 2 where the F of 9.20 was significant beyond the 0.001 level.

The curves in Figure I represent the percentage of time spent with gill covers erected during each consecutive 5 minute block during the 30 minute post-isolation session. In

TABLE 2

Analysis of Covariance for Display Duration to Mirror Image

Source	SS	df	MS	F
Treatments	481.4363	3	160.4788	9.20 *
Error	959.1077	55	17.4383	
Total	1440.5440	58		

* $p < .001$

general, response strength decreased over time with the habituation effect being less for the 72 hour group. In comparison to the average pre-isolation curve, the response strength of the shorter deprivation groups (15 minutes and 6 hours) began at a lower level and gradually progressed with time to a still lower level. The curve of the 24 hour group shows a higher response rate which practically coincides with the pre-isolation average. The 72 hour deprivation group clearly demonstrated the greatest amount of aggressive display, beginning and remaining at the highest level. Thus, with longer deprivation periods, the fish respond more intensely initially and the response strength does not fall off as rapidly as with shorter isolation periods.

An additional measure of aggression recorded was the number of gill erections exhibited during the observation periods. The means and standard deviations of the pre-isolation and post-isolation gill erection frequencies are shown in Table 3. Table 4 illustrates the analysis of covariance for these data. There were no significant differences in gill erection frequency produced by the different isolation periods (F = 1.10).

Since there was a significant increase in the duration of aggressive display but no significant differences in the frequency of the display, it is reasonable to look for an isolation effect on the average duration of gill erections. Since each subject's mean display time score was based on a different frequency score, it is misleading to directly compare mean duration scores within and between groups.

TABLE 4

Analysis of Covariance for Frequency of Gill Cover Erections to Mirror Image

Source	SS	df	MS	F
Treatments	3552.76	3	1184.2533	1.10
Error	59066.06	55	1073.9284	
Total	62618.82	58		

The method used to analyze the data was first to calculate a pre-isolation to post-isolation mean duration difference score for each fish and then to convert these difference scores into ranks. The ranks for each subject are presented in Table 5. A high rank reflects a greater increase in mean display duration following the period of isolation. The scores were analyzed by the Kruskal-Wallis one way analysis of variance by ranks (Siegel, 1956). The results of this test indicated that the distribution of ranks were significantly different ($H = 10.84$, $\underline{df} = 3$, $\underline{p} < .02$). Although there was not a continuous increase in mean ranks over the four groups corresponding to the progressive increases in display duration (Table 1), only the 24 and 72 hour groups were reversed. The 15 minute and 6 hour ranks were markedly smaller than the 24 hour and 72 hour ranks. Combining the two shorter isolation periods and the two longer periods yielded mean ranks of 24.03 and 36.97 respectively, suggesting that increases in isolation periods lead to longer gill cover extensions.

The final measure of aggression used in this experiment was the latency of the initial attack toward the mirror image. Table 6 gives the means and standard deviations of response latency for the four groups. The shorter isolation periods resulted in an increase in latency from pre-isolation to post-isolation conditions while the 24 and 72 hour groups reduced the time to make their first attack in the post-isolation condition.

TABLE 5

Distribution of Ranks of Difference-Scores for Mean Duration per Response to Mirror Image

	Length of Isolation			
	15 min.	6 hr.	24 hr.	72 hr.
	3.0	2.0	13.0	1.0
	6.0	8.5	15.0	4.0
	7.0	11.0	18.0	5.0
	8.5	14.0	32.0	17.0
	10.0	20.0	36.0	28.0
	12.0	23.5	39.0	30.0
	16.0	23.5	43.0	31.0
	20.0	25.0	45.0	34.0
	20.0	26.0	47.0	35.0
	22.0	33.0	48.0	42.0
	27.0	38.0	50.0	52.0
	29.0	40.5	51.0	53.0
	37.0	46.0	54.0	55.0
	40.5	49.0	56.0	57.0
	44.0	59.0	58.0	60.0
Sum	302.0	419.0	605.0	504.0
Mean	20.13	27.93	40.33	33.60

Because many subjects showed extremely high scores, it was decided to employ a method of analysis similar to that discussed above for mean duration of display measures. Each subject's pre-isolation to post-isolation latency difference score was computed and then converted to a rank score. The ranks for each group are illustrated in Table 7 with a higher rank representing a larger reduction in latency. The Kruskal-Wallis test approaches significance ($H = 6.35$, $.10 > p > .05$) and the trend is clearly in the direction of the hypothesis with the mean rank increasing with isolation time. Combining the 15 minute group with the 6 hour group and the 24 hour group with the 72 hour group resulted in mean ranks of 25.38 for the shorter isolation groups and 35.62 for the longer isolation groups. These values were significantly different when analyzed by the Mann-Whitney U test ($z = 2.27$, $p < .02$; one-tailed test; Siegel, 1956). In summary, it was concluded that longer isolation periods produce a greater readiness to attack as measured by latency of the first response.

Experiment 2

The responses of the fish to the male conspecific model were very weak. Four fish did not respond at all to the model in either test session, with seven additional fish failing to respond in the post-isolation period. Only 3 of the 20 fish used in this experiment demonstrated an increase in aggressive display in the post-isolation session; 2 of these were in the 72 hour isolation group and the other belonged to the 24 hour

TABLE 7

Distribution of Ranks of Difference-Scores for Latency to First Response to Mirror Image

	Length of Isolation			
	15 min.	6 hr.	24 hr.	72 hr.
	1.0	5.0	3.0	10.0
	2.0	6.0	12.0	14.0
	4.0	7.0	13.0	16.5
	15.0	18.5	19.5	24.5
	19.5	8.5	22.5	28.0
	19.5	11.0	24.5	30.0
	19.5	16.5	33.0	37.5
	26.0	22.5	33.0	44.0
	28.0	28.0	33.0	47.0
	31.0	35.5	40.0	48.0
	40.0	35.5	45.0	53.0
	40.0	37.5	46.0	55.0
	43.0	42.0	49.5	58.0
	49.5	51.0	54.0	59.0
	57.0	52.0	56.0	60.0
Sum	395.0	366.5	484.0	584.5
Mean	26.33	24.43	32.27	38.97

group.

A summary of the means and standard deviations of the total time spent with gill covers erect in the pre-isolation and post-isolation test sessions is presented in Table 8. All four groups decreased in the amount of display, suggesting that the fish quickly habituated to the model. Due to the occurrence of many zero scores, the data in this experiment were analyzed by the method discussed earlier for mean duration and latency measures. Pre- to post-isolation difference scores were converted to ranks and are shown in Table 9. The results of the Kruskal-Wallis test were not significant ($H = 2.92$, $df = 3$).

The means and standard deviations of the frequency of gill cover erections during the pre-isolation and post-isolation periods are shown in Table 10. The pre-isolation to post-isolation difference score ranks are presented in Table 11. The Kruskal-Wallis test yielded results that were not significant ($H = 5.19$, $df = 3$).

TABLE 9

Distribution of Ranks of Difference-Scores for Display Duration to Model

	15 min.	6 hr.	24 hr.	72 hr.
	3.0	9.0	5.0	1.0
	4.0	10.0	8.0	2.0
	6.0	11.0	16.5	12.0
	7.0	14.0	16.5	13.0
	16.5	16.5	19.0	20.0
Sum	36.5	60.5	65.0	48.0
Mean	7.30	12.10	13.00	9.60

TABLE 11

Distribution of Ranks of Difference-Scores for Frequency of Gill Erections to Model

	Length of Isolation			
	15 min.	6 hr.	24 hr.	72 hr.
	3.0	6.5	6.5	1.0
	4.0	12.0	9.5	2.0
	6.5	12.0	15.5	9.5
	6.5	15.5	18.0	12.0
	15.5	19.0	20.0	15.5
Sum	35.5	65.0	69.5	40.0
Mean	7.10	13.0	13.90	8.0

CHAPTER IV

DISCUSSION

The results of the present study generally supported the hypothesis that the level of aggressiveness would be enhanced with increasing lengths of time spent in isolation. As measured by the total display time, mean display duration and latency of the first attack, the Siamese fighting fish in Experiment 1 became progressively more aggressive over deprivation periods of 15 minutes, 6 hours, 24 hours, and 72 hours.

The findings of this study are in agreement with the experiments of Bourgault et al. (1963) and Welch (1967) in which the degree of fighting behavior in mice was graded according to the length of isolation. The results also support the investigation of Clayton and Hinde (1968) where Siamese fighting fish that were habituated to their mirror image showed greater recovery of display strength following longer mirror removal. However, Clayton and Hinde relied heavily upon the frequency of gill cover erections as a measure of display strength. In the present study it was demonstrated that the proportion of time spent with gill covers erect is a more appropriate measure of aggression in Siamese fighting fish. Here, the number of independent gill cover erections did not vary under different isolation periods, but the total amount of display time and the mean display duration were influenced by isolation. If frequency had been the only measure of aggression, then the significant finding of the increase in the intensity of each display would have gone unnoticed.

It can be reasonably presumed that a fish is highly aggressive when it spends more time engaged in attacking the opponent (Simpson, 1968). Although a less aggressive fish may respond just as frequently with brief displays, a fish that displays for a greater proportion of the time is the one that would be labeled more aggressive. In future studies using Siamese fighting fish, the various measures of aggressive strength should be further evaluated.

A somewhat unexpected and interesting finding in Experiment 1 was the relationship of pre-isolation and post-isolation display strength levels. Not only was there an increase of aggression with isolation time, but the amount of display compared to the pre-isolation level was directly related to the length of the isolation period. The pre-isolation test of aggressive display strength followed 24 hours of visual isolation from other male fish. This period of isolation was not anticipated or intended to be comparable to the period of isolation following 30 minutes of mirror image stimulation. However, it can readily be seen in Figure 1 that the curve for the 24 hours of isolation from other fish is equivalent to 24 hours of isolation from mirror image stimulation. Furthermore, for shorter isolation periods (15 minutes and 6 hours) the curves are lower than the 24 hour isolation curves while the 72 hour group clearly responds at a higher rate and remains at a higher level in comparison to the 24 hour group. In summary, it appears that: (a) fighting at 24 hour intervals maintains the display strength at a constant level; (b) reducing the time between fights reduces display strength below that of the 24 hour level; (c) increasing the isolation period to 72 hours raises the intensity of display strength beyond that of the 24 hour level.

The finding that Siamese fighting fish are more aggressive with increased isolation periods may be very important in the social life of the species. Even small increments in display strength could be significant in deciding the outcome of territorial contests. Simpson (1968) reports that a fish's ability to win an encounter is directly correlated with the proportion of time it spends with its gill covers erect. Winners always display at a higher rate and each gill erection becomes longer with time than those of their opponents. The duration of gill erections becomes especially pronounced in the latter stages of the conflict. If this is true, then placing a fish in isolation for a considerable time should tend to make it a winner when finally paired with an opponent since isolated fish display more (Table 1), maintain a consistent level of responding (Figure 1) and have a longer average duration of gill erection (Table 5).

Experiment 2 did not prove to be a reliable test of the effects of isolation on aggressive display. Compared to mirror image stimulation (Table 1), the conspecific model (Table 8) was clearly inferior as an eliciting stimulus to which the fish quickly habituated. Perhaps this was due to color or lack of movement and reactivity. Weaker responses to models were also reported by Lissman (1932). It was demonstrated that fighting reactions in Siamese fighting fish could be elicited by crude dummies but were diminished sooner the less they resembled real fish. It must be concluded, then, that the model used in this experiment was an inadequate releaser and was not the proper stimulus to use for such a sensitive test of aggressive strength differences. It is interesting to note, though, that the only three fish to increase in aggressive display were in the 24 hour and 72 hour groups. Perhaps this is related to the claim of Lorenz (1966) that increasingly inadequate releasing stimuli are capable of evoking a response as time in isolation increases.

The results of the present study support the speculations of Lorenz that the intensity of aggressive behavior increases with the progressive lengthening of the elapsed time between hostile conflicts. Lorenz accounts for this phenomenon by postulating an endogenous accumulation of response specific behavioral motivation paralleled by an increased concentration of action specific energy in the appropriate areas of the brain. While the behavioral observations seem justified, there remains considerable controversy pertaining to the theoretical explanation of this relationship.

Animals that demonstrate a readiness to fight most certainly do so because of a cumulation of both external and internal factors. It is essential to distinguish between the stimuli which release a response and the endogenous conditions which induce the disposition to behave aggressively. Insofar as it has been demonstrated that fluctuations in fighting behavior occur under constant environmental stimuli, the potential determinants of this outcome remain to be considered.

As was discussed earlier, the gonadal hormones, in addition to activating sexual behavior in many species, are also intricately involved in aggressive behavior. As testosterone level is elevated in the males of most species, there is a corresponding increase in the probability that a fighting response will be elicited. A possibility exists that male hormones could produce a continuously stimulating effect of the central nervous system. It is generally accepted that androgens act on the neural apparatus to influence aggressive behavior and that post-puberal castration is accompanied by a diminished aggressive disposition unless testosterone replacement therapy is introduced.

According to Scott (1971), the sex hormones have a direct stimulating effect on the central nervous system and presumably a physiological mechanism is present for sexual behavior which is analogous to the blood sugar changes in hunger resulting in the searching for food. Since androgens are also involved in fighting, it is possible that testosterone causes accumulating central nervous excitability which in turn produces an enhanced inclination toward aggressive behavior. Welch (1967) suggests that the precise effect of the androgens is upon the responsiveness of the postsynaptic neural receptor sites to the presence of the neurotransmitter substances being released by stimulation. The function of the neurotransmitter substances and the level of aggressiveness will be discussed further after completing the consideration of the possible actions of other hormones.

Another endocrine which has been assigned a possible role in aggressive behavior is the pituitary's luteinizing hormone. It is difficult to separate the effects of luteinizing hormone and testosterone by observation since the secretion of luteinizing hormone is known to stimulate production of testosterone rather quickly in most species. However, in starlings there is a long period of several months between the time that luteinizing hormone is first secreted and the time that the testes have grown large enough to produce adequate testosterone for sexual behavior. It was reported earlier that Mathewson (1961) found increased fighting in starlings following administration of luteinizing hormone while testosterone affected only sexual behavior. An explanation of the significance of this relationship is offered by Davis (1963).

According to Davis, luteinizing hormone is secreted at a time when the birds are contesting for territories and presumably a high level of aggressivity would be beneficial to them at this time. Thus, well in advance of the actual breeding period, luteinizing hormone stimulates fighting behavior in starlings which promotes territorial acquisition and defense.

Perhaps, then, in species where aggression and mating occur simultaneously, the only stimulant is testosterone, whereas when they are separated by relatively long periods luteinizing hormone induces the tendency toward increased aggressiveness. However, at this time no evidence exists for any short-term fluctuations in hormone levels to coincide with increments in isolation-induced aggressiveness.

A recent line of research has been directed toward the investigation of another biochemical system that is altered during fighting behavior. This system includes the proposed subcortical neurotransmitter substances, the biogenic amines norepinephrine, dopamine (catecholamines), and serotonin (an indoleamine).

In an early study, Bourgault _et_ _al_. (1963) compared two strains of mice which differed in aggressivity and found that the more aggressive strain contained lower levels of norepinephrine and serotonin in the brain. Reis and Gunne (1965) discovered a reduction in brain norepinephrine, but not dopamine, following amygdaloid stimulation in cats whenever the stimulation resulted in rage behavior.

B.L. Welch and A.S. Welch present evidence that the enhancement of aggressiveness attributable to isolation is paralleled by changes in brain biochemistry (Welch, B.L., 1967; Welch, A.S. & Welch, B.L., 1971; Welch, B.L. & Welch, A.S. 1966, 1970). These investigators have demonstrated that mice become increasingly aggressive the longer thay are kept in isolation and the level of aggressiveness can be graded according to the amount of environmental stimulation they experience. Accompanying the isolation-induced aggressiveness are changes in the metabolism of the catecholamines and serotonin. Brain norepinephrine, dopamine, and serotonin are synthesized at a higher rate in mice living in groups than in isolates. This is presumably related to the amount of environmental stimulation since aggressivity and amine level can also be graded according to the size of the group in which the mice live. When an animal is placed in isolation, this higher rate is quickly diminished with a resultant increase in irritability and the probability that fighting will occur. The reduction of brain norepinephrine and dopamine can be lessened by permitting the mice to fight briefly for only five minutes each day.

The release of dopamine, norepinephrine, and serotonin at the synapse seems to be correlated with the level of activation and reactivity of the neurons (Welch, B.L. & Welch, A.S., 1970). The intensity of aggressive behavior may be increased by either (1) providing an adequate stimulus for release of neural transmitters in the appropriate nervous pathways, or (2) lowering the basal synthesis rate of the transmitters and thus the threshold for fighting responses. Furthermore, the stimulus-induced release of brain amines from subcortical neurons will differentially react with the existing adaptation level of the postsynaptic elements. Isolated animals may be more responsive to attack eliciting stimuli partly because the postsynaptic receptors are in a highly sensitive state and more responsive to any increment over the low spontaneous rate of neurochemical release. While activation of the organism occurs as a result of the release of the amine neurotransmitters, the neurons in the brain of mice living in groups seem to be adapted to the presence of large quantities of the activating substances (Welch, B.L. & Welch, A.S., 1970). With lower levels of environmental stimulation in isolated mice these neurotransmitters are synthesized and utilized at a lower rate. The animal will then show elevated responses to a sudden increase in stimulation and the release of brain amines.

It is interesting to note an additional discovery in the Welch's studies. In a group of mice, one or more dominant individuals tend to emerge, and the probability that an individual will display dominance in a group situation is enhanced by previous isolation experience. The similarity of heightened aggressiveness in dominant and isolated mice is also reflected in their neurochemical composition. Dominant mice are more like isolates than subordinates in that there is a decreased basal accumulation of norepinephrine and dopamine in the dominant mice (Welch, A.S. & Welch, B.L., 1971).

Marrone, Pray, and Bridges (1966) found that the aggressive display of visually isolated Siamese fighting fish could be spontaneously aroused by the introduction of norepinephrine into the water of their living tanks. It was concluded that increased gill erections and color changes are specifically evoked by norepinephrine since the aggressive displays were not induced by the highly similar epinephrine. It was also suggested that the displays were not a result of increased activation level; general activity level in the norepinephrine condition was similar to the controls. Baenninger (1968), however, claims that norepinephrine and epinephrine have the opposite effect, namely suppressing the aggressive display in Siamese fighting fish; fewer fish in the epinephrine and norepinephrine solutions displayed to mirror images than did control subjects.

A possible explanation of these conflicting results is that Baenninger used a weaker solution of norepinephrine, 48 mg/liter, compared to 70, 140, and 280 mg/liter in the Marrone _et al_. experiment. Also, while Marrone _et al_. observed their subjects following the addition of the amine solutions, Baenninger transferred the fish from their living tanks to the testing tanks and waited 17 minutes before mirror presentation. Baenninger's fish may have experienced a temporary transfer-shock characterized by unresponsiveness which the present author has observed in his laboratory. Furthermore, by the time the mirror was presented, the fish may have become adapted to the norepinephrine solution.

From the evidence presented above, there appears to be some mechanism of a gradual accumulation of aggressive disposition. However, contrary to Lorenz's postulation of a build up of a substance in the brain, enhanced aggressiveness with increasing time in isolation is more likely an effect of altered sensitivity in subcortical neurons to neurotransmitter flow. The parallel between the greater aggressivity of dominant and isolated mice and the reduction in the synthesis and utilization of brain amines and the involvement of norepinephrine in the elicitation of aggressive displays in Siamese fighting fish deserve further study. The work on biogenic amines and fighting behavior should be integrated with experiments involving direct injection of these substances in determining to what extent the changes in aggressiveness are related to the changes in brain amine level. Although the metabolic rate of brain catecholamines and serotonin are correlated with fighting behavior, it would be premature at this time to implicate the amine reduction phenomenon exclusively in aggressive behavior.

CHAPTER V

SUMMARY

This investigation tested Lorenz's hypothesis that many animals show an increased tendency toward aggression as the time of fighting deprivation is extended. Siamese fighting fish were observed to determine aggressive display strength to a mirror image (Experiment 1) and a conspecific model (Experiment 2) following either 15 minutes, 6 hours, 24 hours, or 72 hours of visual isolation. Results of Experiment 1 indicated shorter attack latencies, progressive increments in the total time of gill cover erections, and longer mean durations of each gill cover erection as the length of the isolation period was increased. No isolation-induced differences in aggressive display to the conspecific model were found in Experiment 2. Possible physiological mechanisms that might account for the enhancement of aggressive behavior with increases in isolation time were discussed.

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