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**LA THÈSE A ÉTÉ
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THE EFFECTS OF DEFEAT AND VICTORY ON
AGGRESSION IN THE SIAMESE FIGHTING FISH
(BETTA SPLENDENS): BEHAVIOUR AND NEUROCHEMISTRY

by

ALEXANDER RNIC ¹

A DISSERTATION
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The undersigned recommend to the Faculty
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fighting fish (Betta splendens): Behaviour and Neurochemistry

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ABSTRACT

The present study investigated the effects of defeat and victory on the latency of male Siamese fighting fish to engage in aggressive display. In addition, an exploratory investigation of both fight outcomes on the brain neurochemistry of this species was also conducted. In Experiment 1 test subjects were randomly assigned to one of 4 groups and were separately defeated in fights by high aggressive stimulus animals. The 4 groups were then independently tested for their latency to display in response to a mirror image stimulus on Days 1, 2, 4 and 8 following defeat. Four hours subsequent to latency testing the brain of each subject was removed and fluorometrically assayed for norepinephrine, dopamine and serotonin concentration. The behavioural performance and neurotransmitter concentration of defeated subjects were compared to two control groups. These included subjects which did not experience defeat or were allowed social exposure to a high aggressive animal. In Experiment 2 the same methodological procedures were used to induce victory with the exception that test subjects were allowed to fight low aggressive stimulus animals. The results demonstrated that defeat decreased aggressiveness as measured by display latency i.e., longer latencies, whereas victory increased aggressiveness, i.e., shorter

latencies. In addition, no statistically significant change in display latency was found across the 8 Days of testing in either experiment, although it was noted that a trend might have emerged using larger sample sizes. In Experiment 1 both defeat and social exposure to high aggressive stimulus animals were associated with a depletion of dopamine over the 8 Day testing period. In contrast, the effect of victory or social exposure to a low aggressive stimulus animal did not significantly alter dopamine level. No trace of serotonin or norepinephrine was detected in either experiment. The effects of defeat and victory on display latency were accounted for by the relative change in strength of approach and avoidance tendencies elicited by aggressive stimuli. However, other explanations of such behavioural phenomena were also reviewed. Dopamine changes were discussed in terms of stress effects induced by exposure to high and low aggressive stimulus animals.

To my mother and father who gave everything for me.

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The act of fighting represents one of the most striking behaviours of any animal species. In an attempt to describe the nature of such behaviour, a few theorists have suggested that aggression might be best attributed to an innate mechanism found within all members of a given species (Lorenz, 1966; Tinbergen, 1951). This innate mechanism was considered not only to be reflexively activated upon the presentation of appropriate stimuli, but also determined the typography and conditions under which the behaviour would occur. To a large extent, the belief that aggression was a reflexive and predetermined process was fostered by the observation that much of animal aggression is ritualized and reliably occurs in response to specific stimulus situations. In addition, there is also a similarity between species members in the typography and appearance of the aggressive response. For example, most Siamese fighting fish will show an orderly sequence of approach and threat displays which terminate in attack behaviours (biting and butting) if confronted by a releasing stimulus in the form of an opponent (Simpson, 1968). A similar ritualization has also been observed in other animals (e.g., Eibel-Eibesfeldt, 1970).

Given such observations of response specificity and ritualization, it is not surprising that some theorists (Lorenz, 1966) have traditionally conceived of aggression as a stereotyped and rigid process which is resistant to modification. This approach, however, has been revised since environmental factors in the form of experience have been shown capable of modifying the aggressive response. As a result, more contemporary approaches no longer consider aggressive behaviour to be stereotyped and rigid to the extent once believed (Johnson, 1972).

This conclusion is supported by the finding that the strength of the aggressive response, i.e., aggressiveness, can be easily modified through various means including a process of conditioning. An example of such a conditioning process has involved the exposure of test subjects to learning situations in which the aggressive response is reinforced or punished. Using this method, aggressiveness can be increased employing food or water in rats and pigeons (Reynolds, Catania and Skinner, 1963; Ulrich, Johnston, Richardson and Wolff, 1963). Similarly, electrical stimulation of "pleasure centers" in the brain (Stachniuk, Ulrich and Mabry, 1966) or the termination of continuously present electrical shock (Miller, 1948) have also been shown to increase aggressive behaviour in rats. In contrast, the aggressive response of the Siamese fighting fish can be

diminished substantially when severe electrical shock is systematically applied in the presence of aggression eliciting stimuli (Adler and Hogan, 1963).

Other conditioning approaches including the frustration of a goal response have also been used to modify the aggressive response (Dollard, Doob, Miller, Mowrer and Sears, 1966). For example, Azrin, Hutchinson and Hake (1966) showed that frustration could be elicited in pigeons by first training them to peck at a key for food reinforcement and then disconnecting the feeding mechanism. Under these conditions of food frustration, test pigeons would aggressively attack a restrained pigeon which showed no aggressive behaviour and which was previously ignored. Dollard et al. suggest that the strength of the aggressive response in such cases is a direct function of the frequency and intensity of prior frustrating experiences.

The strength of the aggressive behaviour has also been modified by repeated exposure to aggression eliciting stimuli in the absence of any frustration or reinforcement contingency. In such cases, the Siamese fighting fish demonstrates a pronounced habituation of the aggressive response given that they are continuously or repeatedly exposed to live opponents or their mirror image (Clayton and Hinde, 1968; Figler, 1972). The mobbing response of the chaffinch shows a similar habituation if a predatory stimulus (stuffed owl) is

presented repeatedly (Hinde, 1954). In contrast, the procedure of isolating test animals in order to avoid habituation to aggression eliciting stimuli increases the aggressiveness of various species including mice (Ginsburg and Allee, 1942), dogs, birds, cats (Kuo, 1967) and monkeys (Mason, 1960).

Biological Implications of Changes in Aggressiveness

These attempts to condition aggressive behaviour have shown that aggressiveness can either be decreased or increased as a function of prior experience. It is therefore evident that aggressive behaviour can, to some extent, be characterized as a flexible behaviour system which is not wholly rigid but which can readily adapt to meet situational needs. Presumably, the capability to adapt with experience has biologically important implications for many animal species under natural conditions. For example, one method by which aggressiveness can be modified in natural environments is through defeat or victory as a function of fighting. Changes in aggressiveness as a consequence of winning or losing a fight can in turn influence factors such as hierarchical rank or size of territory. Success in these activities (hierarchy or territory) are often necessary for species-important behaviours including attraction of a mate and species propagation.

These activities are necessary conditions if the species is to survive.

Despite such biologically important implications, the following review of the effects of fight outcome indicates that the extent to which aggressiveness can be modified by fight encounters has generated little research activity. Much of the available data concerning the effects of defeat and victory on subsequent aggressiveness have been derived from observational investigations of hierarchy formation. However, the specific effects of defeat and victory on aggressiveness are often unclear in these investigations due to methodological difficulties.

Observational Investigations

In order to establish a dominance hierarchy, pairs of animals usually engage in aggressive threat or attack encounters. In its simplest case, the end result of an encounter is a dominant and a subordinate animal depending on which wins and which loses. Such fight outcomes appear to be common since victory and defeat, i.e., dominance and submission, have been observed among numerous animal species including the albino mouse (Uhrich, 1938).

Uhrich studied the dominance - subordination relationship of the albino mouse by placing groups of males in cages and observing which of the combatants

would win or lose relative to each other. The results of these observations indicated that test groups formed several different types of hierarchies dependent upon which animals lost or won successive encounters.

Although the reason for the development of different types of hierarchies is unknown, the most commonly observed hierarchy was exclusive dominance by one of the males and complete subordination by all other animals in the group. Another type of frequently observed hierarchy was linear. In this situation, one animal dominated all others, whereas the second highest ranking animal dominated all other animals except the highest ranking individual.

The importance of these observations is that they indicate that winning or losing modifies the degree of aggressiveness exhibited by test subjects. For example, defeated subjects demonstrated fleeing responses and a complete cessation of fighting in response to the victor and in some cases other subordinate animals. In contrast, these behaviours were not exhibited by the dominant members of any given hierarchy. However, such changes in aggressiveness are not permanent since Uhrich noted that the stability of a hierarchy characterized by non-fighting varied from a few days to a few months. Towards the end of these periods there was a shifting in rank as a function of renewed fighting.

Similar findings have been noted in other widely disparate species. Some of these have included grain beetles (Howard, 1955), fish (Frey and Miller, 1968, 1972; Kuo, 1960), geese (Jenkins, 1944), chickens (Guhl, 1956; Schjelderup-Ebbe, 1935), cattle (Schein and Fohrman, 1955), rats (Grant and Chance, 1958), monkeys (Sade, 1967; Yerkes and Yerkes, 1935) and other vertebrates (see Allee, 1938, 1942; Collias, 1944; Etkin, 1967; Wynne-Edwards, 1962). As in the Urich study, the results of these investigations indicated that lower status subjects will display a submissive posture in contrast to the behaviour of dominant members. Furthermore, the submissive posture, irrespective of species, is observed as the absence of any overt threat and attack behaviours and the appearance of avoidance behaviours on the part of defeated test subjects. On the basis of these observations, a change in aggressiveness would appear to be a consequence of losing as opposed to winning in hierarchical situations. However, Urich (1940) questioned this conclusion since the reason for behavioural changes following fighting can often be unclear in such situations.

For example, Urich observed that the experience of past wins or losses seemed to provide some evidence as to whether or not a test mouse would emerge as a winner or loser in subsequent fights. Urich's conclusion was based on the finding that animals with a total of 12

defeats lost 9 of their next 12 fights. Conversely, animals with 32 past victories won 20 of the succeeding 32 combat encounters. Such data, however, were considered problematical by Uhrich since it was not clear whether or not the subsequent fight performance of test subjects was due to the conditioning effect of prior wins or losses on aggressiveness, or the inherent individual level of aggressiveness (genetically endowed). In order to circumvent such difficulties associated with observational investigations, a few attempts have been made to examine the effects of fight outcome under controlled experimental conditions.

Experimental Investigations

Experimental investigations of fight outcome have been concerned with the effects of winning or losing on the physiology and behaviour of test subjects. In such studies, test subjects are first allowed to engage in a single or series of fights until one subject emerges as the loser or winner on the basis of predetermined criteria. The effects of fight outcome are then evaluated by either obtaining a measure of physiological change (e.g., Eleftheriou and Church, 1968; Eleftheriou, Church, Norman, Pattison and Zolovick, 1968; Taylor and Costanzo, 1975) or by exposing subjects to an aggression test. Aggression tests have involved fight performance

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in response to other conspecifics (Frey and Miller, 1972; McDonald, Heimstra and Damkot, 1968), frequency of approach (Meliska, Meliska, Hoyenga, Hoyenga and Ward, 1975; Phillips, 1973), and an operant response which produces an aggression releasing stimulus such as the subject's own mirror image (Baenninger, 1970).

The results of tests such as these clearly show that fight outcome is correlated with changes in aggressive behaviour and physiology.

Physiological changes. Exposure to aggressive encounters as well as a variety of other stressors (e.g., electric shock) have consistently been observed to result in a broad spectrum of hormonal and neurochemical changes (see review in Stone, 1975). In aggressive encounters, subordinate animals demonstrate greater increases than do dominants and/or controls in adrenal size (Barnett, 1955; Davis and Christian, 1957), blood plasma corticosterone, pituitary and plasma thyrotropin hormone (Bronson and Eleftheriou, 1964, 1965a, 1965b). In addition, subordinate animals also show decreases in body and seminal vesicle weight (see Bronson and Eleftheriou) which are not evident in dominant animals.

Several studies have also suggested that exposure to aggressive encounters are associated with stress induced alterations in the concentration of neurotransmitters such as norepinephrine, dopamine and serotonin (biogenic amines). Reis (1974), in a

discussion of the pharmacological basis of affective aggression, indicated that increases in norepinephrine and dopamine levels are positively correlated with increases in aggressive behaviour, whereas decreases in aggressive behaviour are positively correlated with serotonin.

Supporting evidence. The finding that increases in norepinephrine are associated with heightened aggressiveness is supported by the observation that the injection of norepinephrine precursors (e.g., L-DOPA) facilitates aggressiveness in test subjects (Everett and Borcharding (1970). In turn, Stolk, Conner, Levine and Barchas (1974) showed that aggressive behaviour (fighting) can also influence changes in neurotransmitter concentration. Stolk et al. noted that levels of norepinephrine (and dopamine) were elevated among fighting animals, but were low among rats which exhibited little fighting. In contrast, the depletion of norepinephrine and dopamine by α -methyl-para-tyrosine (α -MpT) was found to be associated with decreases in fighting behaviour (Welch and Welch, 1970). Further to this point, it has also been demonstrated that norepinephrine depletion, induced by tyrosine hydroxylase inhibition, was exaggerated following fights, suggesting a direct relationship between aggressive encounters and norepinephrine utilization (Welch and Welch, 1965, 1970, 1971).

In reference to the relationship between dopamine and aggressive behaviour, the available data suggests that high levels of dopamine, in addition to norepinephrine, are associated with increased aggression (Modigh, 1976). This is because reduction of dopamine by α -MPT is associated with decreases in aggressiveness of test mice (Lycke, Modigh and Roos, 1969). In contrast, serotonin is associated with decreased aggressiveness since blocking of serotonin synthesis by PCPA (p-chlorophenylalanine) results in an increase in shock induced fighting among rats (Sheard, 1970).

Relationship between behaviour and neurochemistry.

Although these studies clearly suggest that neurotransmitter concentration and aggressive behaviour are correlated, it is also evident that comparatively few investigations have been conducted examining the nature of the relationship between both measures. As a result, it is often unknown whether changes in neurotransmitter concentration directly affect behaviour or whether some intervening mechanism is affected which in turn influences the behaviour in question (Reis, 1974). Similarly, under naturalistic conditions, it is unknown whether changes in behaviour alter neurochemistry or whether changes in neurochemistry alter behaviour (Eleftheriou and Church, 1968).

In addition, the exact influence of aggressive behaviour on neurochemical concentration can be considered

to be complex since Stolk, Conner, Levine and Barchas (1974) showed that the depletion of norepinephrine and dopamine as a function of stressful footshock can be prevented if rats were allowed to fight while receiving such stress. Neurochemical analysis carried out at various intervals after the stress as well as after varying amounts of stress suggested that fighting prevented neurochemical changes by possibly acting as a coping mechanism. This finding is surprising considering that fighting in the absence of other stressors (e.g., footshock) has been shown to alter but not prevent changes in neurochemical concentration (Welch and Welch, 1970).

Effects of fight outcome on neurochemistry. Since the exact relationship between neurochemistry and behaviour in many cases appears to be a complex one, it is not clear from the aforementioned studies whether the outcome of an aggressive encounter, i.e., defeat versus victory, will differentially influence biogenic amine activity. Furthermore, this lack of clarity is compounded by the finding that only a few studies have specifically explored the relationship between fight outcome and neurochemistry. One example, however, has been provided by Eleftheriou and Church (1968) who showed that defeat by aggressive animals served to increase norepinephrine and decrease serotonin levels in mice. This finding suggested that there may be a reciprocal relationship

between these neurochemicals which in some manner was induced by the stressful experience of defeat.

Nevertheless, it should be noted that the direction in which these neurotransmitters change as a function of fights is unclear since this reciprocal relationship has not been observed in other studies dealing with this issue (Welch and Welch, 1971). Moreover, there has also been some disagreement concerning the direction in which neurotransmitters change following fights. For example, norepinephrine and dopamine have been observed to increase in one experiment, and decrease in another under relatively similar fight conditions (Welch and Welch, 1971). Although the reason for this difference is not clear, Welch and Welch (1969) suggested that stress levels may have been greater in one situation than in the other, thereby producing different neurochemical effects. In addition, since both of these neurotransmitters have also been shown to decrease and increase in concentration as a function of the interval between application of the stressor and neurochemical analysis (Stolk et al., 1974), it is possible that differences between both studies as to the direction of neurochemical change may be a function of when the neurochemical analysis was performed. In any event, this conclusion requires further testing.

Although the effects of defeat on norepinephrine and serotonin have been investigated, the effects of fight outcome on dopamine remain yet to be explored. Consequently, the relative absence of experimental data combined with the variable results of existent findings preclude a definitive conclusion concerning the neurochemical effects of fight outcome.

Behavioural changes. More specific findings, however, are available concerning the behavioural effects of losing or winning since the outcome of an encounter is correlated with similar changes in aggressiveness across different test species. In test subjects which have been defeated this change is often observed in the reduction of threat and attack behaviours in contrast to those subjects who have emerged as victors. This change in aggressive behaviour has been observed among several species including mice (Ginsburg and Allee, 1942), birds (Ratner, 1961), and fish (Baenninger, 1970; Meliska, Meliska, Hoyenga, Hoyenga and Ward, 1975).

However, the conclusion that fight outcome produces a change in aggressiveness may not be warranted in all of these studies (Baenninger, 1970, 1970; Meliska et al., 1975), because of the problem of sample bias previously noted in observational investigations of dominance hierarchy. For example, Baenninger (1970) allowed Siamese fighting fish to engage in aggressive behaviour until test subjects could be identified as

dominants or submissives. These subjects were then exposed to an aggression test in which dominant and submissive subjects learned to swim through a ring in order to gain visual access to an aggression eliciting stimulus (mirror image). The results suggested that fight experience modified aggressiveness since dominant animals sought out aggression eliciting stimuli significantly more often than did submissiveness.

Another study investigating the effects of defeat and victory in the Siamese fighting fish has also demonstrated similar effects (Meliska, Meliska, Hoyenga, Hoyenga and Ward, 1975). Meliska et al. allowed pairs of male Siamese fighting fish to fight until a winner and loser emerged. In order to assess the effects of fight outcome, each of the winners and losers were later exposed to an aggression test in which the time spent in approach and threat display in response toward either its own pair member or another combat experienced fish was recorded. These stimuli fish were protected behind a protective transparent barrier. The results of this aggression test showed clear differences in the aggressiveness of winners and losers, since victorious subjects approached and displayed significantly more than did defeated subjects in response to both its own pair member or another combat experienced fish.

The difficulty with both the Baenninger and Meliska et al. studies, however, is that defeated subjects

may have initially lost in aggressive encounters because they were inherently low in aggressiveness. Conversely, subjects which emerged as victors may have been highly aggressive in nature (see Baenninger, 1970). Therefore, the difference in aggressiveness of winners and losers following combat (during the aggression test) may have been due to previously established aggressive tendencies rather than to experience with winning or losing per se.

Although both studies did recognize this possibility, Meliska et al. attempted to ascertain if, in fact, the behavioural effects demonstrated by winners and losers in their study was a function of fighting or previously established tendencies. In order to experimentally resolve this issue, Meliska et al. first exposed fight inexperienced Betta to the aggression test in order to record time spent in approach and threat display towards another fight inexperienced fish placed behind a partition. On the following day, these test subjects were placed together in pairs in order that defeat and victory would occur as a function of fighting. The results showed that the winner of these fights had also previously exhibited greater approach and threat display scores than did defeated subjects during aggression testing. Furthermore, these aggression scores for winners and losers prior to combat did not differ significantly from the aggression scores obtained for winners and losers following combat. This finding demonstrated that the prior

experience of victory or defeat as a function of combat was not a necessary condition for differences in approach and display durations, i.e., aggressiveness, to appear between dominants and submissives. This is because "dominant fish always exceeded subordinates on these dependent measures regardless of whether fish had received combat experience immediately prior to the test" (p. 137). The value of this demonstration is that it shows how groups (winners and losers) which are biased in their degree of aggressiveness prior to an aggression test, may lead to inappropriate conclusions concerning the effects of defeat and victory on subsequent aggressiveness. In addition, it should also be noted, that although the presence of sample bias in Meliska et al.'s study suggest that fight outcome did little to influence approach and threat display durations, this does not mean that aggressiveness cannot be modified by defeat and victory in this species.

Alternative Methods of Experimental Investigation

Since the presence of sample bias can influence the interpretation of any study dealing with the effects of defeat and victory, alternative procedures have been attempted in order to circumvent this problem. One such procedure has been developed by Ginsburg and Allee (1942). They avoided the problem of sample bias by first

allowing albino mice to form a dominance hierarchy through a round-robin series of fights. As a result, they obtained an index of aggressiveness for each subject, as measured by social rank, prior to any experimental treatment (e.g., defeat or victory). Albino mice which were dominant and presumably high aggressive were then defeated by exposing them to a high ranking combatant from another strain known to be more aggressive, e.g., C-57 Blacks. Conversely, submissive mice (low aggressive) were made victorious by providing test subjects with a territory, and allowing these subjects to defeat newcomers who had previously experienced a series of defeats. In some cases, victory was also induced in submissive mice by isolating them for an extended period and then permitting exposure to other low ranking combatants.

The results of these experimental manipulations showed that fight outcome modified aggressiveness as measured by social rank when test subjects were reintroduced into the hierarchy. This is because victory served to elevate the social rank of previously submissive animals, whereas defeat served to lower the social rank of previously dominant animals. The methodology used by Ginsburg and Allee (1942) avoided the possibility that the behavioural performance of test subjects was a function of previously established aggressive tendencies (sample bias) since the social

rank of a test subject following victory or defeat could be compared to the same subject's rank prior to fighting. Therefore, any change in aggressiveness as measured by social rank could only have resulted as a function of the treatments of defeat or victory.

Using an experimental paradigm similar to Ginsburg and Allee, other studies employing fish (McDonald et al., 1965), and birds (Ratner, 1961) as experimental subjects have also concluded that fight outcome in itself can modify aggressive behaviour. For example, McDonald et al. attempted to determine if the aggressiveness exhibited by dominant and submissive fish (Lepomis cyanellus) during previous fights, could be modified using a conditioning procedure. In order to accomplish this objective, pairs of fish were allowed to fight on three separate occasions until a winner (high aggressive) and loser (low aggressive) emerged. Following this period, the aggressiveness of these test fish was modified by exposing dominants to a larger fish which had a high probability of inducing defeat. In contrast, submissive subjects were exposed to smaller fish in order to facilitate victory. The results showed that the aggressiveness of dominant subjects decreased following exposure to a larger fish, whereas the aggressiveness of formerly submissive animals increased following exposure to a smaller fish. This change in aggressiveness was evidenced by the finding

that when previously dominant and submissive fish were allowed to fight again in their original pairs, there was a reversal in dominance and submission in many cases.

In reference to the effects of defeat, the results of Ratner (1961) using birds as experimental subjects also showed a decrease in aggressive behaviour similar to that found in fish and mice. Ratner (1961) allowed groups of White Leghorn chickens to form hierarchies characterized by linear pecking orders. Subjects which occupied intermediate ranks (intermediate aggressiveness) were then temporarily removed from the hierarchy and exposed to defeat by a high ranking despotic bird. When these test subjects were reintroduced into their respective hierarchies, all but one subject showed a decrease in hierarchical rank. This decrease in hierarchical rank suggested that defeat served to decrease the aggressiveness of previously intermediately ranked birds. Ratner, however, did not test the effects of victory on the aggressiveness of domestic fowl.

Problems associated with alternative methods.

Although the procedures used by Ginsburg and Allee (1942), McDonald et al., (1968) and Ratner (1961) have avoided the problem of sample bias, one must be cautious in interpreting the results of these studies. There are several reasons for adopting such a cautionary viewpoint. First, none of these studies used adequate control

procedures in order to ensure that the effects of winning or losing in a fight were not simply due to factors associated with the physical presence of another conspecific. Under such conditions, the effects of fight outcome may not necessarily be due to defeat or victory, but may be more related to social experience and/or other related stimuli such as odour or vocalizations. Second, the decreased aggressiveness of subjects exposed to defeat may also be due to fatigue as a function of fighting. Thus far, procedures have not been developed in order to control for this possibility. Third, the design of the studies which have been described are limited in the extent to which the behavioural effects of defeat and victory can be generalized to other normally distributed populations. For example, in those studies described by Ginsburg and Allee, (1942) and McDonald et al. (1968), test subjects were first allowed to establish a dominance relationship through a series of fights. Dominant subjects (high aggressive) were then selected on the basis of these fight outcomes and exposed to defeat by other combatants. In opposition, low ranking members were selected for treatment procedures which would induce victory.

However, the difficulty with these procedures is that they assess the effects of defeat or victory using sample populations which are of a narrow range of aggressiveness (e.g., high or low). Because of this

feature, any generalization of the effects of fight outcome (defeat or victory) to populations which demonstrate a broad range in aggressiveness from high to low is not readily justified.

A simple method of avoiding this problem of generalization would be to randomly select two independent groups of fight naïve animals. It is assumed that the procedure of random selection ensures that the degree of subject aggressiveness is distributed from high to low in such groups. Following this procedure, one of the randomly selected groups would be exposed to defeat, and the other victory. Under these conditions, the extent to which the effect of winning or losing would generalize to other randomly selected populations is greatly increased. In addition, this procedure would also avoid the problem of sample bias, since groups exposed to victory or defeat would not be differentially biased in the degree to which they exhibited aggressiveness prior to fights.

Thus far, only two studies have employed this procedure in examining the behavioural effects of fight outcome (Kahn, 1951; Scott and Marston, 1953). This relative absence of studies is unexpected given the observation that the procedure as described is a relatively simple one which also avoids many of the problems (sample bias) associated with studies of defeat and victory. Furthermore, it is surprising that this

methodology has only been used to investigate the effects of defeat and not victory.

In both studies, the effects of defeat were tested by first training high aggressive "fighters". The function of these fighters was to defeat randomly selected groups of test subjects which had no previous fight experience. These fighters were trained by providing them with a history of wins since they were allowed to repeatedly attack victim mice which could not retaliate or escape. Subsequently, the trained fighters were shown to be capable of defeating any fight inexperienced subject irrespective of whether the test subject was of high or low aggressiveness. Sample bias was avoided here, since groups of animals randomly distributed in aggressiveness invariably experienced defeat. In addition, since there was no bias in subject aggressiveness within groups, the results of these studies may be generalized to other populations.

However, this approach is also not without problems, since typically only a few fighters are used to defeat many test subjects and usually on more than one occasion, both between and within days (see Scott and Marston, 1953; Kahn, 1951). Therefore, if groups are defeated consecutively, as in Kahn (1951), the fight experience of trained fighters may differ between groups. This would lead to a problem of stimulus constancy. As

Kahn (1951) most aptly notes "... as the experiment progressed, the fighters became more fierce, particularly after encounters with the younger animals" (p. 119). If not controlled, this factor may produce artifactual differences in aggressiveness between groups. One method of dealing with the problem of stimulus constancy would be to counterbalance the fight experience of "fighters" between test groups.

Apart from the issue of stimulus constancy, the results of the studies which have employed trained fighters have clearly not only avoided the problem of sample bias but have also shown that defeat serves to lower aggressiveness in groups of randomly selected mice. However, the extent to which this conclusion applies to other animal species, especially those which are non-mammalian, requires further investigation. It is therefore of interest to ask if other species such as fish would also demonstrate similar changes in aggressiveness following treatment effects of defeat or victory.

The Use of Betta Splendens as Test Subjects

The use of fish as experimental subjects may be of value in the study of defeat and victory since the aggressive behaviour of some fish species is more stereotyped, less complex and therefore easier to quantify than

mammalian aggressive behaviour. One such species in which aggressive behaviour is not only highly ritualized but also easily measured is Betta splendens, more commonly known as the Siamese fighting fish.

The Siamese fighting fish, a member of the anabantid family, represents one of the most popular species used by tropical aquarists and researchers. This particular species is approximately 6.0 - 9.0 cm in length and is characterized by long undulating fins which come in a variety of pure or mixed colours and tones (reds, greens, blues and yellows). The graceful beauty and colouration of this fish in addition to its extreme aggressiveness first prompted its introduction to San Francisco from Siam in 1927 (Innes, 1966). Although the Siamese fighting fish became an immediate commercial success with ichthyologists and hobbyists, it was not until later that the Siamese fighting fish became of interest to the scientific community engaged in research on aggression.

However, in order to understand the aggressive nature of Betta splendens, it is necessary to examine the physical and social background from which this species derives. The original Siamese fighting fish were found in the warm tropical pools and shallow rice paddies of South-east Asia (Goldstein, 1971). In such physical surroundings the Siamese fighting fish fed primarily on mosquito larvae although it has been known to thrive on

commercially available sources of brine shrimp (Artemia salina), free swimming small animals (Chaoborus larvae, Daphnia), tubifex worms (Tubifex tubifex) and white worms (Enchocheatu albinus). A unique feature of the physical environment was that many of these pools or rice paddies represented stagnant bodies of water in which the amount of available dissolved oxygen was quite low (Carter, 1957). As a result, the Siamese fighting fish possesses an auxiliary breathing device known as the labyrinth organ (a primitive lung) which allows it to breathe atmospheric air. Simpson (1968) reports that adult domestic fish rise to the surface and gulp air approximately once every minute when the water temperature is maintained at 27 °C. Although the Siamese fighting fish is capable of obtaining its entire oxygen requirements in this manner (Lissman, 1932, as reported in Simpson, 1968), the conventional gill system is still functional in extracting oxygen from water. Innes (1966) verified this observation by reporting that a related species (Paradise fish) will survive in air-free sealed bottles for up to 12 hr without distress. A similar conclusion has been reached by the author with the exception that Siamese fighting fish become totally inactive and lie on the bottom if required to breathe solely using the gill system.

It should be noted, however, that it was the

ability of Siamese fighting fish to breathe atmospheric air which facilitated its transition to artificial tank environments which are characteristically low in oxygen content unless otherwise aerated. With the discovery that Siamese fighting fish would thrive under artificial conditions and continue to show a high degree of aggressiveness when visually exposed to opposing conspecifics, an important development occurred in the genetic history of this species. According to Dr. Hugh Smith, an ichthyologist, numerous individuals in Bangkok recognized the commercial value of raising Betta splendens under artificial conditions for the purpose of staging fights and wagering upon which fish would win or lose (as reported in Innes, 1966). To a large extent, this activity closely resembled the "cock fighting" of Occidental cultures in which subjects were selectively bred for aggressiveness in the hope that they would win in aggressive encounters. A similar process of selective breeding was conducted in the Siamese fighting fish since winners of fights were allowed to engage in reproductive activities, whereas losers were discarded. Consequently, domesticated Betta would appear to be more aggressive than its natural counterpart. This increase in aggressiveness has been verified by Dr. Smith who reported that the natural variety of Betta splendens rarely fight for more than 15 min, whereas

selectively bred fish may fight for up to as long as 6 hr. Other differences have also been noted between the natural and domestic form of Betta splendens. For example, Simpson (1968) noted that the natural Betta confined to tanks swim constantly with their vertical fins erect as if they were patrolling a certain territorial area. In contrast, domestic fish do not show a similar patrolling behaviour but periodically swim to and fro along one of the tank walls. This particular pattern of swimming does not necessarily suggest that domesticated Betta are not territorial since Goldstein (1975) has shown that male Betta exhibit a large personal space which is actively defended. Exclusive of territoriality Simpson (1968) also suggests that natural Betta show more ongoing changes in colour and pattern than do the domestic variety. It is possible that such changes may serve as indicators of the fish's internal states (Simpson, 1968). These particular differences between both varieties, however, remain to be systematically explored and verified.

Nevertheless, given that the primary difference between the natural and domesticated Betta lies only in the degree of subject aggressiveness, it is of value to examine the course of fighting in this species so that the outcome of defeat or victory can be accurately described.

Fighting Behaviour

The fighting behaviour of the male Betta has been described, in part, by Lissman (1932), Smith (1945), Forselius (1957) and Simpson (1968). Although there has been no complete account of the fighting behaviour of Betta splendens under naturalistic conditions, the following discussion will attempt to synthesize the available literature in order to provide a description of the sequence of events from the moment of first contact to the outcome of defeat or victory.

One of the characteristics of the fighting behaviour of Betta splendens is that such behaviours are highly stereotyped and ritualized in appearance. As a result, the aggressive behaviours of this species can be divided into relatively stable units of behaviour which follow a predetermined sequence. In this respect, the fighting behaviour of Betta splendens can be viewed as a fixed action pattern (FAP) similar to that described by Lorenz (1966).

For purposes of description, such a fixed action pattern can be categorized into two behaviour classes representing threat displays and attack behaviours. Within each behaviour class there are a number of smaller behavioural units which are described in the following.

Threat displays. Upon detecting an intruder in the immediate vicinity, the Siamese fighting fish demonstrates a slow gliding approach which culminates

in an extension of the gill covers at right angles to the body. This usually occurs within 4 - 6 cm of the opponent. Simultaneous to gill cover erection, dorsal, ventral, and caudal fins become fully erect and spread to their fullest extent. However, both combatants do not usually engage in face to face confrontations with the gill covers fully erect. Rather, one of the antagonists engages in gill cover erection, whereas the other turns broadside to the opponent and then engages in a vigorous tail beating. This broadside orientation is known as a lateral display (Lissman, 1932; Forselius, 1957). Full gill cover erections do not occur while the subject is engaging in lateral display (Evans et al., 1956). When viewed from above, the position of both antagonists is in the shape of a "T" with both subjects alternating between frontal and lateral display orientations. Occasionally, both subjects will engage in simultaneous lateral displays and assume a parallel head to head or head to tail position while engaging in tail beating. Following these threat displays which are primarily non-contact in nature, attack behaviours in the form of biting and mouthlocking progressively begin to appear.

Attack behaviours. While both combatants are circling about the tank in either frontal or lateral display modes, attempts are made to bite at the fins or lateral side of the opponent. This biting, often accompanied by butting, can lead to major physical

damage in addition to a severe tearing and tattering of the fins. In fights observed by the author, it has been noted that some fish are aggressive to the extent that they will tear sections of fin from the opponent and devour such finnage while the opponent looks on.

Occasionally, fights will become prolonged especially in those cases where the combatants are evenly matched. Should this occur, mouthlocking behaviour results in which each subject clenches the other's upper or lower jaw. They then engage in a tugging and twisting about the long axis of the body while slowly sinking to the bottom. This particular manoeuvre may represent a test of strength since it deprives the opponent of atmospheric air and increases the risk of drowning (Lissman, 1932; Smith, 1937). Both opponents usually remain in this position in excess of 1 min before breaking contact and returning to the surface for air.

These threat and attack behaviours continue until one of the combatants is eventually defeated. Defeat on the part of one of the subjects occurs abruptly and is characterized by an immediate cessation of threat displays and the appearance of submissive postures.

Submissive postures. Defeated subjects flee from the encounter with the victor in close pursuit. During this period the fins of the defeated subject become flaccid and there is a paling (bleaching) of body colouration with two dark horizontal bands appearing

on the lateral sides. Should the defeated subject be unable to escape the victor of the fight, the subject will remain motionless in a corner or assume a vertical head-up orientation near the surface. Tinbergen (1951) in describing stickleback behaviour, and Forselius (1957) in describing anabantid behaviour, indicate that the vertical head-up position may be demonstrative of fear. Such head-up orientations appear to inhibit further attack on the part of victors, although the winners of such fights will continue to display to defeated subjects. Defeated combatants will usually die if not removed from tanks in which the victor is continually present. Whether test subjects die from physical damage or from some other factor, such as stress is unclear in these cases.

Fighting and Reproductive Activities

The preceding discussion has described the fighting behaviour of the Siamese fighting fish in response to other males. One of the striking aspects of this species is that similar fighting sequences are directed toward females of the species during preliminary reproductive periods (Robertson and Sale, 1975).

The male Siamese fighting fish signals reproductive readiness by constructing a floating nest made from bubbles. The bubble nest is formed from individual mucous covered bubbles which are blown by the male into a

compact cluster ranging from 8 to 10 cm in diameter. Following completion of the nest, initial contact with a female introduced into the tank is decidedly aggressive with the male engaging in characteristic display and attack behaviours similar to that directed toward other males. The reason for the similarity of aggressive behaviour directed toward both sexes is not entirely clear. However, Rnic (1977) in a preliminary investigation of this issue suggested that male Siamese fighting fish may not initially recognize females as sexual targets. This may occur since male Betta are raised in isolation prior to and following the development of secondary sex characteristics, resulting in a pronounced lack of developmental social experience with other male or female stimuli. Test males may therefore only respond to similar aggression releasing features found in both male and female stimuli and thereby fail to recognize females as sexual targets. Nevertheless, as contact is maintained between male and female stimuli, the aggressive behaviour of the male comes to incorporate reproductive components. This would seem to indicate that the male Siamese fighting fish responds to sexual aspects of the female and in so doing can eventually discriminate the female from other male conspecifics. 7

A key element of the reproductive behaviour of Betta splendens involves the leading of the female from the tank bottom to a point immediately below the

floating bubble nest. This leading is accomplished by the male's approaching the female and then swimming in the direction of the nest in a zig-zag pattern. This behaviour is repeated until the female follows. Once directly below the nest, the male wraps his body about the female in the shape of a "C" and the female releases her eggs. Presumably, the eggs are also fertilized at this point by the male. Subsequently, the male releases his nuptial clasp and collects the eggs in his mouth. These eggs are then blown into the bubble nest. With the exception of "leading", this repertoire of behaviours is repeated until the female's supply of eggs has been exhausted. Since the male cares for the developing eggs in this species, there is a resurgence of aggressive behaviours at the end of the reproductive cycle in order to drive off the female. If not removed from the tank, the female is usually killed by the aggressive attacks of the male. The developing young hatch in approximately 2 wk and remain under the care of the male parent until old enough (approximately 1 month) to feed independently.

Although the importance of the fighting behaviour of Betta splendens remains yet to be determined under naturalistic conditions, it is evident that aggressive behaviour represents both a prominent feature and a primary method of social interaction between other males and females of this species. In addition, the reliability.

and ritualized aspect of the fighting behaviour of Betta splendens in the presence of aggression eliciting stimuli has prompted numerous investigators to experimentally explore methods of either controlling or altering the aggressive response. These explorations have provided further insights into the nature of aggression and fighting in the Siamese fighting fish.

Experimental Investigations of Aggressive Behaviour in Betta splendens

Research into the fighting behaviour of Betta splendens has been facilitated by the finding that the aggressive response can be reliably elicited by a variety of readily available stimuli. These stimuli not only include live males (e.g., Peeke and Peeke, 1970), but also their mirror image (Rnic, 1976; Thompson, 1963), motion pictures of Betta (Turnbough and Lloyd, 1973), paper cut-outs (Johnson and Johnson, 1973) and models of male Betta graded in the degree of "realness" (Lissman, 1932; Hess, 1952). Although test subjects have been found to approach and display to all such stimuli, the frequency of the response is clearly related to the degree that the stimulus approximates the physical appearance of a live male Betta. This was shown by Lissman (1932) who presented seven models graded in their "realness" to a male Betta and recorded

the frequency of displays to each stimulus model. A continuum of response frequency was found with test fish displaying to "real" models more often than unrepresentative models. Hess (1952) later confirmed these results, although a fewer number of models were employed.

Lissman (1932) and Hess (1952) also showed that the intensity of aggression directed toward such stimuli could be altered by systematically varying water temperature. For example, Lissman's study demonstrated that certain temperatures were more effective in eliciting the aggressive display since all models evoked a response at 28°C , whereas two of the most unrepresentative models failed to elicit a response at 22°C . Hess (1952) replicated Lissman's efforts with the exception that a wider range of temperature levels was used (15°C , 20°C , 25°C , 30°C , 35°C). The results clearly showed that the total time spent in aggressive display increased as temperature increased with the highest rates of responding being elicited by the most "real" model. Although Hess found that temperatures as great as 35°C would support high rates of responding, most of the studies cited in this review of Siamese fighting fish literature have used a temperature range of $26 \pm 1.1^{\circ}\text{C}$. This temperature range appears best suited for the maintenance of Siamese fighting fish for long periods, and it is not surprising that this temperature range has also been found to facilitate reproductive

behaviour in this species (Innes, 1966).

In addition, the observation that test males will actively respond to a wide range of Betta-like stimuli also prompted many investigators to determine if the display response could come under the control of either operant or classical conditioning procedures.

Conditioning

Operant studies. Thompson (1963) first showed that the opportunity to engage in aggressive display would serve as an effective reinforcer for the Siamese fighting fish. Thompson's procedure required a male Betta to swim through a ring in order to receive a 20 sec exposure to an aggression eliciting stimulus in the form of its own mirror image. The importance of this study was that it clearly indicated that the opportunity to engage in aggressive behaviour could be a reinforcing event. Glickman and Schiff (1967), in accounting for such results, suggested that the reinforcing propensity of aggression is a biologically important aspect of animal behaviour since it ensures that fighting responses will occur in future situations. If the opportunity to engage in aggressive behaviour was aversive, then it is unlikely that aggressive behaviour would continue to occur in biologically important situations such as defence of territory and protection of food resources. In contrast, the reinforcing aspect of aggression

encourages fighting behaviour to consistently reappear in those stimulus settings which may threaten the survival of a species member. In so doing, the reinforcing nature of aggressive behaviour serves a biologically adaptive purpose.

Since the appearance of Thompson's study, numerous attempts have been made not only to verify the finding that aggression is a reinforcer, but also to show that other types of eliciting stimuli can be used as reinforcing agents. In this respect, mirror image has been used to reinforce operant behaviour (Goldstein, 1967, 1971; Hogan, 1967; Rhoad, Kalat and Klopfer, 1975), in addition to the presentation of other live males (Goldstein, 1971), and motion pictures of male Betta (Turnbough and Lloyd, 1973). Similarly, Rnic (1977) provided the first published account showing that the aggressive display elicited by female Betta could serve as reinforcement in an operant ring swimming task.

Other studies have also examined the effectiveness of other reinforcers such as food (in addition to mirror image) in Betta splendens under continuous reinforcement (Hogan, 1967) and fixed ratio schedules of reinforcement (Hogan, Kleist and Hutchings, 1970). Although it is not readily possible to accurately compare two qualitatively different reinforcers, food reinforcement did produce higher levels of responding than did mirror image in both studies. This difference in response level for each

reinforcer suggested that food might be preferred to a greater degree than aggression eliciting stimuli in the Siamese fighting fish. Fantino, Weigle and Lancy (1972) tested this suggestion by exposing test subjects to a two-choice discrimination situation in which subjects could select either food or a brief exposure to mirror image. The results clearly showed that mirror image stimulus was preferred by Betta splendens even though some subjects were moderately food deprived. This finding, however, requires additional investigation since Hogan (1972) in a subsequent study showed that food and not mirror image was preferred.

Classical conditioning. Only two studies have investigated the possibility of classically conditioning the aggressive display of the Siamese fighting fish. In both cases (Adler and Hogan, 1963; Thompson and Sturm, 1965) it was found that the display response could be elicited by a previously neutral stimulus. In Thompson and Sturm's study, the onset of a red light served as the conditioned stimulus, whereas in the Adler and Hogan study, a mild electric shock (1.0 v) served as the conditioned stimulus. However, since electric shock has been shown to elicit aggressive fighting in rats (Ulrich and Azrin, 1962), it is not known to what extent electric shock may have in fact served to energize the aggressive display in the Adler and Hogan study. In any event, the finding that the aggressive response of

Betta splendens can be classically conditioned supports Berkowitz's (1964) suggestion that stereotyped aggression can be reflexively elicited by environmental stimuli.

Habituation

Studies which have examined the operant behaviours of Betta splendens during sustained training periods have noted a decrease in operant rate and display frequency as the number of exposures to the aggression eliciting stimulus (mirror image) increased (Rhoad, Kalat and Klopfer, 1975; Thompson, 1963, 1969). Although test subjects continued to show a display response at the end of training, the decrement in display frequency has been attributed to a process of habituation. In the Siamese fighting fish, the habituation of the aggressive display was first recorded by Smith (1945) and Forselius (1957) who noted that male Betta would cease to display if left in adjacent jars for long periods of time. Several contemporary studies have examined the habituation of the aggressive response in Siamese fighting fish under more controlled conditions. These studies have been accomplished by exposing subjects to mirror image stimuli or live conspecifics (behind glass barriers) either under conditions of continuous (Baenninger, 1966; Clayton and Hinde, 1968) or repeated daily exposures (Figler, 1972; Peeke and Peeke, 1970; Shapiro and Schuckman, 1971). Under both types of conditions, habituation of the response

is characterized by a decrease in the frequency and/or duration of the selected display component. However, this finding may not necessarily apply to all aspects of the display. For example, Clayton and Hinde exposed test Betta to their mirror image for 10 consecutive days and recorded gill cover erection frequency, gill cover erection duration, tail beating, biting and air gulping frequency. After the second day of continuous exposure it was found that gill cover erection and tail beating frequency showed a marked habituation, but gill cover erection duration and biting frequency actually increased.

Recent evidence also indicates that under conditions of prolonged exposure to mirror image (as found during habituation procedures), display eliciting stimuli may come to acquire negative reinforcement or aversive properties. This was shown by Baenninger (1970) who demonstrated that if subjects were exposed to their mirror image for 48 consecutive hr, they would subsequently learn to remove the ever present eliciting stimulus using an operant response. Rhoad, Kalat and Klopfer (1975) also observed that subjects repeatedly exposed to mirror image stimuli would actively avoid responses which would produce the stimulus. Such evidence suggests that if Betta are exposed to aggression eliciting stimuli for long durations, the reinforcing properties of such stimuli wane and may gradually

incorporate other motivational properties. This conclusion, however, must remain tenuous until further tests are made.

Effect of Drugs

Although habituation procedures have been clearly shown to reduce the aggressiveness of Betta, other factors such as chemical agents, also produce a similar effect on the fighting response. The most common drug used to alter the aggressive display has been the hallucinogenic drug LSD-25 (Abramson and Evans, 1954; Evans, Geronimus, Kornetsky and Abramson, 1956). These studies introduce test subjects to the hallucinogenic compound by bathing them a solution of LSD-25 and then exposing these subjects to a non-treated opponent. The results show not only abnormal swimming patterns (e.g., swimming backwards, barrel rolling), but also a general quiescence in which treated subjects demonstrate no aggressive responses. However, LSD treated fish can be aroused to engage in aggressive display by the attack of another male, although such counter attacks are not usually sustained. Turner (1956) confirmed these observations, and also showed that LSD treated fish were less responsive to food in that they would feed but not to the same extent as non-treated controls.

Since LSD-25 would affect aggressiveness, Evans, Abramson and Fremont-Smith (1958) performed an interesting

experiment in which they attempted to investigate the effects of LSD on the social behaviour of this species. In order to accomplish this objective, Evans et al. allowed dominance hierarchies to be formed by juvenile male and female Betta. Upon the formation of a stable hierarchy, the lowest ranking male and female were treated with LSD. It was found that within 1 or 2 days the lowest ranking fish, whether male or female, ascended to the top of the hierarchy. This particular finding is surprising considering that LSD appears to reduce aggressiveness and that male Betta do not usually form stable hierarchies because of their extreme fighting propensities. McDonald and Heimstra (1964) in a later study attempted to replicate these results, but were unable to do so since test subjects fought to the death before a hierarchy could be established.

Since most studies show that LSD-25 serves to alter the aggressiveness of the Siamese fighting fish, Walaszek and Abood (1956) attempted to determine if similar effects would be produced by other classes of drugs. In order to assess the effects of other drugs on aggressiveness, Walaszek and Abood (1956) exposed test fish to analgesics (morphine sulphate, sodium salicylate), hypnotics (phenobarbital, sodium thiopental), tranquilizers (reserpine, chlorpromazine, meprabamate), and antihistamines (benadryl, phenergran, pyribenzamine, atarax). In all cases, except analgesics, it was found

that these drugs suppressed aggressive behaviour in the presence of another male combatant. In contrast, however, the analgesics were found to produce hyperactivity and increased aggressiveness.

Effects of neurotransmitters. Several studies have also examined the effects of neurotransmitters such as epinephrine and norepinephrine bitartrates on the aggressiveness of Betta splendens (Baenninger, 1968; Marrone, Pray and Bridges, 1966). In order to test both neurotransmitters, Marrone et al. immersed subjects in solutions of either norepinephrine or epinephrine. The results showed that one-half of the test subjects in the norepinephrine group showed spontaneous gill cover erections even though no aggression eliciting stimulus e.g., mirror image, was present. A similar effect was not observed for the epinephrine group. The importance of Marrone et al.'s work was that it seemed to provide additional empirical evidence for Funkenstein's (1955) suggestion that aggressive behaviour is associated with norepinephrine, whereas submissive behaviour is associated with epinephrine. However, it should be noted that Baenninger (1968) in a further more detailed study of the effect of these neurotransmitters on aggressive behaviour did not discover the spontaneous aggressive displays described by Marrone et al. Rather, it was found that both norepinephrine and epinephrine actually decreased display frequency to a mirror image stimulus.

The reason for the discrepancy between both studies is unclear although methodological differences may have been responsible.

Effects of Defeat and Victory

The preceding discussion has attempted to demonstrate not only the extreme aggressiveness of Betta splendens, but also the diversity of methods which have been generated to either control or modify the aggressive response. These methods have ranged from conditioning procedures to stimulus factors, temperature, habituation and drug applications. There has been little research conducted on the extent to which aggressiveness can be modified by winning or losing in a fight. This is surprising since fighting represents both a predominant feature and method of social interaction in this species. Thus far, only a few studies, as previously described, have specifically investigated the effects of defeat and victory in the male Betta (Baenninger, 1970; Meliska et al., 1975). Although their results show that the experience of winning or losing increases or decreases subsequent aggressiveness respectively, neither study has adequately dealt with the problem of sample bias. It is for this reason, in part, that the effects of fight outcome should be examined without the possibility of sample bias in the Siamese fighting fish.

In addition, there are several reasons why the male Siamese fighting fish may serve as an appropriate test species in the study of defeat or victory. First, the most striking and primary feature of the aggressive response as previously described, is the frontal display, in which the operculum extends at an approximate 90° angle relative to the body. Since the threat display occurs prior to the appearance of other related display and attack components, such as tail beating and biting, it is evident that gill cover erection is directly related to aggressive behaviour in Betta splendens. Furthermore, this display response is easily recognized on the part of the experimenter and therefore easily detected and measured under laboratory conditions. Second, domesticated Siamese fighting fish are typically raised in isolation well before the development of secondary sex characteristics and overt aggressive behaviour (see Rnic, 1977). The reason for this is to protect individual members from physical damage and possibly death as a function of fights. When male Betta are exposed to aggression eliciting stimuli, e.g., mirror image, in later adult life, the pattern of aggressive behaviour is remarkably similar and highly stereotyped between species members. Presumably, this is because there has been little opportunity for factors such as "frustration" or reinforcement contingencies to modify the appearance or strength of the aggressive response.

Since such Siamese fighting fish are naive in fight experience, one can examine the modifying effects of defeat or victory without concern for the possibility that treatment effects between subjects may be altered by the differing histories of test subjects. Second, the typography of defeat and victory have been recorded in this species (Goldstein, 1975). Under fight conditions, the defeated subject can be readily identified from that of the victor by characteristic behaviour and/or colour changes. The ability to easily discriminate the winner from the loser is considered to be an important criterion when examining the effects of fight outcome. Third, once an aggressive encounter begins between two Siamese fighting fish, a dominant-subordinate relationship also usually results within a short period of time (see Meliska and Meliska, 1976). The brevity of time required for a loser or winner to emerge is of practical value when examining the effects of defeat or victory in a laboratory setting.

Purpose of Study

In the preceding discussion it has been shown that experience of defeat and victory is correlated with the subsequent aggressiveness of test subjects. This in turn may have biologically important implications for the species in question. However, it has been seen that there are several methodological difficulties (sample bias and stimulus

constancy) associated with the experimental investigation of both fight outcomes. The purpose of the present study was to examine separately the effects of defeat and victory in the Siamese fighting fish while eliminating these problems. This approach was adopted not only to elaborate upon the mechanisms which mediate aggressive behaviour, but also to provide specific information on the effects of defeat or victory on the aggressiveness of this test species.

Although the purpose of the present study was primarily behavioural, a secondary objective was to determine whether victory or defeat was associated with certain concentration levels of norepinephrine, dopamine and serotonin in the neural tissue of Siamese fighting fish. This was done because these biogenic amines have been implicated in the aggressive behaviour of mammals, but there has been no equivalent investigation conducted in teleost species.

Experiment 1

One of the difficulties in using test subjects from a highly aggressive species is in the selection of a stable dependent measure which not only can detect small treatment effects but which is also appropriate for the species in question. In the Siamese fighting fish, exposure to other conspecifics and fights following victory

or defeat is not a recommended dependent measure. This is because Siamese fighting fish are aggressive to the extent that they can frequently inflict serious physical injury often resulting in death. If the purpose of a study is to examine the effects of defeat or victory on subsequent aggressiveness, physical injury can only serve to confound the results. It is for this reason, in part, that researchers have attempted to use the aggressive display as opposed to the fighting behaviour of Betta splendens as a dependent measure of treatment effects.

Aggressive Behaviour and Test Methodology

The preceding review of experimental investigations of aggression in the Siamese fighting fish indicates that the aggressive display of Betta splendens has been employed in two different types of test methodologies. These methods involve operant response rate and frequency or duration of a display response in the presence of a continually available stimulus. As previously described, operant methodologies require a response of swimming through a ring which is reinforced by the opportunity to engage in aggressive display in response to an eliciting stimulus (Thompson, 1963). Conversely, continually available eliciting stimulus methods entail exposure of the subject to the eliciting stimulus and the subsequent recording of the frequency and/or duration of the selected display

component over a series of trials or days. This method has been used most notably in the examination of the habituation of intraspecific aggressive behaviour (e.g., Clayton and Hinde, 1968).

Investigations have used either operant or continually available eliciting stimulus methods in experimental studies of aggression in order to establish baseline performance levels against which to compare treatment effects (e.g., Abramson and Evans, 1954; Baenninger, 1970). However, the reliability of the display measure in either type of methodology may be questioned. This is because where the eliciting stimulus has been continually present, subject position has not been standardized with respect to the eliciting stimulus. Under these situations, experimental subjects may detect and approach eliciting stimuli from various distances and orientations. Subjects are also free to engage in other behaviours (e.g., nest building) which may interfere with the frequency and/or the duration of the display. These factors can produce undesirable variability in display response measures and may interfere with detection of the effects of experimenter imposed treatments. Comparison may therefore be difficult between baseline control and experimental conditions, especially where treatment effects are expected to be small.

Operant techniques share comparable difficulties, although a ring swimming response does ensure exposure to an eliciting stimulus from a relatively fixed distance

and orientation. Unfortunately, the subject remains in a free swimming state during non-response periods and is allowed to engage in other behaviours. These extraneous behaviours may account for the great variability often observed in the rate of responding over days, between and within subjects (Hogan, Kleist and Hutchings, 1970). In Hogan, Kleist and Hutchings' study, they point out that the variability between and within their subjects in operant response rate was sufficiently large to preclude the detection of experimenter imposed treatments (i.e., varying the duration of mirror image reinforcement).

Such variability can, however, be minimized by standardizing the position of the subject relative to the eliciting stimulus, ensuring exposure to the stimulus source at a constant distance, orientation, intensity and limiting the frequency of interfering behaviours. Development of a standardized positioning technique would also allow the experimenter to examine other display features (exclusive of frequency or duration), not readily possible with the procedures as described. For example, standardization of position would allow one to measure the latency to engage in aggressive display in response to the presentation of an aggression eliciting stimulus. Using such a method, Rnic (1976) has demonstrated that if experimental subjects are restrained in a standardized testing position relative to an aggression eliciting stimulus, the latency to display remained stable between and within days, given a

constant duration of exposure to that stimulus (mirror image). This finding suggests that latency to display would serve as an effective and stable dependent measure against which to compare treatment effects. Consequently, a procedure which standardized position and used latency to display as the dependent measure was employed in Experiment 1 to assess the effects of defeat in the Siamese fighting fish.

Method

Subjects

Forty-eight experimentally naive male Siamese fighting fish were used as test subjects during Experiment 1. The subjects were approximately 8 - 12 months in age and were sexually mature adults at time of testing. On the basis of observed continual swimming activity, willingness to feed, and bright colouration, the subjects were considered to be healthy and active by the experimenter at the beginning of the study. These subjects also demonstrated vigorous displays to a hand held mirror. All subjects were purchased from South-east Asia through a commercial dealer.

Apparatus

Housing and maintenance materials. The housing facilities consisted of forty-eight cylindrical 32 oz

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containers (Lab-Tek). The containers were constructed of white plastic and were 12.70 cm in height and 12.06 cm in diameter at the opening. Each container was provided with aquarium gravel (1.0 cm in depth), an air stone (Universal) and a translucent white cover. A 1.25 cm diameter hole was bored in the center of each cover in order to allow additional light to enter the container. The centrally bored hole also allowed air emitted by the air stone to escape from the container without permitting subjects to escape by jumping. The home containers were evenly distributed into separate 21 litre water filled tanks (5 containers per tank). These tanks were each provided with a single aquarium heater (Wil-nes) and a glass cover (3/16") in order to minimize temperature fluctuations and water loss through evaporation. All tanks were positioned on a 3 level holding rack equipped with 40-w light bulbs.

To remove or place subjects in each home container, a curved net was constructed which matched the interior surface of the home containers. This allowed the subjects to be caught more efficiently than allowed by conventional rectangular nets. Much of the trauma associated with the netting of experimental subjects was thereby avoided. The food used to maintain subjects was frozen brine shrimp (San Francisco Brand).

Two testing units were employed to determine the effects of defeat on the dependent measure (latency to

display). Other apparatuses were used to select stimulus animals through an operant technique for the purpose of defeating test subjects. In addition, separate aquaria were also employed as fight tanks in which test subjects experienced defeat.

Testing apparatus. The apparatus in which defeat effects were assessed consisted of two restraining cages (1/2" Plexiglas) in which the position of subjects was standardized during testing. The back of each cage was composed of clear Plexiglas overlaid by a thin layer of white Plexiglas. The sides were constructed of two parallel Plexiglas walls extending at right angles from the rear of the cage. Since the apparatus was inserted into an experimental aquarium (18 x 18 x 12.5 cm), the transparent end wall of the tank served as the front of the cage. A small hole (0.60 cm in diameter) was bored in the back of each restraining cage in order to allow the circulation of water. The height and width of the restraining cages were 4.2 and 2.5 cm respectively. In order to facilitate placement of each subject within the testing situation, the top of each cage was extended 4.0 cm vertically above water level and widened to a maximum width of 10 cm. By use of a silicone sealant, aquarium gravel (natural colour) was also fixed to the floor of the cage.

Two rails mounted directly below each cage served to support the apparatus while immersed to a depth of

4.2 cm in the experimental tank. In the testing position, the restraining cage was centered widthwise and placed firmly against one end of the aquarium. To help insert or remove subjects, the supporting rails allowed the open end of each cage to be moved away from the tank wall or to be locked in position. In order to maintain optimum water quality, two external Dynaflo filter pumps (Metaframe) were used, employing activated charcoal and glass wool as filtration agents. Since each filtration pump was located approximately 62 cm from the tanks, interconnecting flexible tubing (1.25 cm in diameter) provided for intake and exit of water. A heater (Wil-nes) inserted into each motorized filter was used to maintain constant temperatures.

A two-way mirror (25.5 x 25.5 cm) was placed on the exterior side of both experimental tanks directly opposite to the restraining cage. Eight frosted 60-w bulbs were placed within a single fan cooled box enclosure mounted directly behind both mirrors. The two-way mirror acted as transparent glass when the light was on and as a true mirror when the light was off. However, the system was designed such that one mirror could operate independently of the other. Although the two tanks were situated adjacent to each other, they were visually isolated by white Plexiglas dividers positioned on the right and left sides of both tanks. Plate 1 shows the position of both tanks relative to

Plate 1

Experimenter's view of both testing apparatuses.
The mirror image device is shown positioned in
front of both units.

each other and the mirror image apparatus.

For purposes of monitoring each subject's behaviour, a conventional mirror (20 x 48 cm) was placed 20.5 cm above both restraining cages and was positioned at a 22° angle respective to the horizontal plane. This mirror placement allowed the experimenter to observe each subject in the testing apparatus while seated directly behind either experimental tank (Fig. 1).

Lehigh Valley programming equipment was used with an interfaced Lafayette electric stop clock calibrated in 0.01-sec intervals. Depression of a stimulus button held by the experimenter simultaneously activated both the mirror condition for a preset duration and the electric clock. Release of the stimulus button, based on behavioural criteria established by the experimenter, terminated the electric clock and provided a measure of latency in 0.01-sec intervals from the onset of the mirror-image condition. The preset duration of the mirror-image condition was not affected by release of the stimulus button. In conjunction with an independent programming unit, a second Lafayette electric stop clock and stimulus button was provided so that the latency to display could also be recorded by an independent observer to establish inter-observer reliability measures.

Operant apparatus. Two operant tanks were used in order to select stimulus animals for the purpose of

defeating test subjects. Each tank (Plate 2) was constructed of transparent Plexiglas (1/2") with inside dimensions of 18 x 18 x 12.5 cm. The tanks were situated adjacent to each other but were visually isolated using white Plexiglas dividers positioned on the right and left sides. A heater and continuously operational Dynaflo filter pump was also located externally to each tank.

The response mechanism for each tank consisted of a single funnel-shaped glass ring, 3.5 cm in length. The diameter of the ring was 2.5 cm at the largest opening and 2.0 cm at the smallest opening. Each ring was covered with a black enamel coat and was provided with an infrared photo-cell system. Electrical cables connecting the photo-cell system to the programming equipment were placed through two glass tubes fused at right angles to each response ring. Both tubes exited on the right and left sides of each tank through two water-tight outlets. Using both glass tubes as support, each ring was then centered in the tank (4.0 cm from the bottom) with the small opening of the response ring being placed 5.0 cm from the end wall of the operant tank. A two-way mirror identical to that used in the testing apparatus was placed on the exterior side of each operant tank nearest the response ring.

The operant program was maintained automatically by a Lehigh Valley programming unit. In both mirror-image

Plate 2

Operant apparatus

reinforcement tanks, interruption of the photo-cell system by a response of swimming through the ring deactivated the light bulbs behind the respective mirror for a 20 sec period. This allowed the two-way mirror to act as a true mirror. The mirror duration was fixed at 20 sec and could not be prolonged by the subject either remaining within the ring (continued interruption of photo-cell system) or making additional responses during that period.

Defeat induction apparatus. The apparatus in which test subjects experienced defeat consisted of twelve rectangular tanks (30.5 x 15.3 x 20.4 cm) constructed of glass. The tanks were arranged on a laboratory table in four sets of three tanks. Four of the tanks (one in each set) were also divided into two equal parts using a clear Plexiglas divider perforated with 0.60 cm diameter holes. Each tank was visually isolated from the other using white styrofoam dividers. Aquarium gravel was maintained at a depth of 3.0 cm. To ensure constant water temperature and quality, all tanks were provided with an independent heater (Aqua-matic) and an external air driven charcoal filter (Metaframe).

Procedure

The forty-eight subjects were maintained in the home containers at a temperature of 26.6 ± 1.1 °C for

a duration of 10 days prior to testing. A 16/8-hr light-dark cycle with a 3-hr dawn-dusk condition was in effect at all times. During this period and subsequent testing, subjects were maintained on twice daily feedings of brine shrimp (0800 and 1700 hr). A regular feeding schedule was established whereby subjects were individually trained to accept food from a syringe held by the experimenter. Feeding behaviour was facilitated by conditioning each subject to accept food following the onset of a signal. In the present study, the signal consisted of the termination of the air stone in each container for a few min prior to feeding. Use of a syringe during feeding not only helped to equate the amount of food received by each subject, but also helped to reduce the amount of uneaten food allowed to remain in each container. To minimize the possibility of disease arising from an accumulation of food and feces, the water in each container was regularly replaced with aged charcoal purified water once every 4 days. However, only three-quarters of the container was replenished in order to avoid netting and moving of the subject. The water was medicated using Malachite Green (Tetra-Werke).

Experimental design: General outline. Table 1 shows the design of the study. One day prior to the experiment the 48 test subjects were randomly assigned to one of three experimental conditions representing one

TABLE 1

Design of Experiment 1

Days	Experimental Conditions		
	Treatment	Controls	
	D	SDC	DC
1	1	17	33
	2	18	34
	3	19	35
	4	20	36
2	5	21	37
	6	22	38
	7	23	39
	8	24	40
4	9	25	41
	10	26	42
	11	27	43
	12	28	44
8	13	29	45
	14	30	46
	15	31	47
	16	32	48

D = defeat

SDC = social experience

DC = no defeat or social experience

Arabic numerals = identification number of test subjects in each group

1

treatment and two control procedures. The treatment procedure involved the defeat (D) of test subjects, whereas the control procedures involved those subjects which were not defeated (DC) or which had social experience (SDC) similar to that of defeated subjects. Within each of these experimental conditions, the test subjects were randomly divided into 4 groups of 4 subjects each. These groups were employed to evaluate the effects of the treatment and control procedures at 1, 2, 4 and 8 days subsequent to their occurrence. Only 4 subjects were used in each group since it was anticipated that predominant treatment effects would become evident even with small numbers of subjects. This was because previous work (see Rnic, 1976) indicated that the dependent measure (latency to display) was sensitive to the extent that small changes in the treatment condition could be readily detected using this sample size.

Since an independent group was used to assess the treatment and control effects on each testing day, the design had no repeated measures variable. A logarithmic progression of days was used since it was anticipated that treatment effects might be most pronounced soon after defeat rather than within longer periods following defeat.

Selection of stimulus animals used to induce defeat: In order to ensure that subjects experienced

defeat, highly aggressive stimulus animals were used as fight combatants. These stimulus animals were selected from an independent group of 50 Siamese fighting fish which were housed and maintained under conditions identical to those experienced by experimental test subjects. However, it should be noted that this group of 50 stimulus animals was originally selected from a larger sample of 98 Siamese fighting fish. Prior to the experiment, these 98 subjects were randomly assigned to one of two groups with 50 and 48 members in each group respectively. The group with 48 members served as the experimental test subjects, whereas the remaining 50 fish served as a sample pool from which high aggressive stimulus animals were chosen. The selection of stimulus animals was performed using a modification of Goldstein's (1971) procedures. Goldstein had demonstrated that the probability of winning a fight could be predetermined by operant rate in the Siamese fighting fish. The higher the operant rate, the greater the probability of victory in subsequent fights.

In the present experiment, selection of stimulus animals was accomplished by exposing all 50 members of the stimulus population to an operant task in which a response of swimming through a ring produced its mirror image for a 20 sec period. Stimulus animals were trained to perform the operant task by separately placing them in each of the operant tanks and allowing them to

discover that swimming through the ring led to mirror image presentation. Subsequent to the first response, stimulus animals were permitted to remain in the tank until a total of 10 responses had been made. Upon reaching this criterion, stimulus animals were considered to have learned the operant task.

Using both operant tanks simultaneously, pretrained stimulus animals were then tested for 1 hr/day for 2 consecutive days. Following this period, the 8 subjects which indicated the highest operant rate during the last hr of testing were selected as high aggressive stimulus animals (see Fig. 2). These animals were used to defeat test subjects (D), or to act as social stimuli for SDC control subjects. Eight subjects which demonstrated the lowest operant rate during the last hr of testing were also identified at that time as low aggressive stimulus animals. These animals were used during Experiment 2. Fig. 2 represents the distribution of stimulus subjects per response rate category. The stimulus animals indicative of high and low aggressiveness were selected from the upper and lower ends of this distribution respectively.

Defeat methodology. Following the operant selection procedure, the 8 high aggressive stimulus animals were randomly assigned as stimuli for one of two experimental conditions (D & SDC). The defeat control condition (DC) did not require exposure to high

Fig. 2. Frequency of stimulus animals per operant response category. The solid black line represents a normal distribution about which the data points (subject frequency) are clustered. This shows that stimulus animals approximated a normal distribution on the basis of operant rate. Stimulus animals indicative of high and low aggressiveness were selected from those subjects having the highest and lowest operant rates respectively. The solid black line was visually fitted to the data points.

aggressive stimulus animals and therefore was not assigned stimulus combatants.

On each of the 4 defeat induction days, the 4 stimulus animals (assigned to the D treatment condition) were required to defeat 4 test subjects. Since such experience may produce a progressive change in the degree of aggressiveness of stimulus animals, the fight experience of stimulus animals was counterbalanced between the 4 test groups using Latin Square orders (Edwards, 1968). This procedure removed any systematic bias between groups within treatment condition relative to the fight experience of stimulus animals. Stimulus constancy was thereby ensured between groups.² It was not deemed feasible to defeat each test subject with a new stimulus animal since high aggressive animals occur infrequently in any given population (see Fig. 2).

Although the stimulus animals selected were considered to have a high probability of defeating test subjects, it was considered possible that during the induction of defeat a stimulus animal might be paired with a test subject of equal or possibly greater aggressiveness. This could result in a failure of test subjects to experience defeat. In order to prevent this outcome, stimulus animals were allowed to occupy their respective tanks (defeat induction apparatus) for a 24-hr period thereby forming a territory prior to the insertion of the test subject. Braddock

(1949) indicated that the probability of losing a fight is decreased if the encounter takes place in the subject's home territory. Furthermore, this procedure of allowing stimulus animals to remain in their tanks for 24 hr prior to their fights also eliminated the possibility that stimulus subjects would fail to defeat test subjects because of fatigue effects. This was because only 1 fight per day was allowed. As a consequence, the defeat of test subjects was spread over a 4 day period with approximately 24 hr intervening between fights.

Fights were initiated by the experimenter inserting a test subject into one of the defeat induction tanks occupied by the appropriate stimulus animal. The combatants were then allowed to engage in aggressive threat and attack behaviours until the test subject was defeated. Defeat on the part of the test subject was evidenced by the appearance of fleeing behaviour during which the test subject actively avoided the stimulus animal. Upon observation of fleeing behaviour, the loser was allowed to remain in the defeat induction apparatus with the stimulus animal for a 3 min period in order to ensure that defeat had occurred. Longer periods were not used in order to ensure that test subjects would not suffer injury as a function of prolonged fights.

Following each fight, the respective defeat induction tanks were drained, cleaned and provided with

fresh water and filtration materials in preparation for the next day's fights. Replacement of the tank water controlled for changes in chemistry as a function of aggressive encounters. Such changes have been shown to suppress subsequent fights when the same tank water is used (see Baenninger, 1968). Upon cleaning all tanks, the stimulus animals were reintroduced into their respective tanks in order to await the next day's fight.

Control conditions: Further considerations. To assess the effects of defeat, a control condition was employed in which the defeat procedure was not instituted. Subjects in this condition (DC) were introduced into the defeat induction apparatus in the absence of any stimulus animal. To equate treatment and control conditions temporally, the time that each control subject was kept in the defeat apparatus was yoked to durations of one of the subjects undergoing defeat. Yoking was accomplished by randomly pairing control subjects with test subjects which were to be defeated. Each control subject was allowed to remain in an adjacent tank only as long as the test member with which it was paired. Test subjects and their respective control members were run simultaneously.

This procedure, however, did not allow the experimenter to remove all subjects at the same time, since the defeated subject and its yoked control member could not be netted simultaneously. In order to eliminate any systematic bias caused by subjects in

one condition remaining in the defeat apparatus for slightly longer periods (e.g., 10 sec) than subjects in another condition, the order in which test subjects were removed was random. This was accomplished by preparing a daily list specifying the order in which test subjects were to be removed. Similarly, since it was not possible to introduce subjects in D, SDC and DC groups to the defeat induction tanks simultaneously, a similar randomization procedure of subject insertation was performed.

In order to control for the possibility that mere social experience with the stimulus animal may itself cause behavioural effects, an additional control condition (SDC) was used. These control subjects (SDC) were exposed to stimulus animals, but defeat was not allowed to occur. This was accomplished by physically separating both test subject and the stimulus animal in tanks divided by a perforated clear Plexiglas partition. This arrangement allowed both subjects to engage in threat behaviours in the presence of odours, sounds, and tail beating pressure waves typically found in aggressive encounters. Duration in the defeat induction apparatus was determined as in the DC control condition, i.e., yoked to a D subject.

Behaviour of D and SDC test subjects during defeat induction. During treatment and control procedures it was considered possible that social exposure to high aggressive

stimulus animals may also induce defeat on the part of SDC control subjects. In order to determine if this occurred, the behaviour of D and SDC subjects was compared during defeat induction.

This was accomplished by randomly selecting one D subject and its yoked SDC control subject for observation on each of the 4 defeat induction days. Since D and SDC subjects were run simultaneously, the experimenter observed one tank (e.g., D subject), whereas a second individual was used to observe the remaining tank (e.g., SDC subject). During these periods, six dependent behavioural measures which were considered indicative of defeat, or an absence of defeat were recorded on a check list. These measures involved the frequency of approach, gill cover erection, fin erection, withdrawal, lateral banding and colour bleaching. The total number of each of the observed behaviours was tabulated at the end of encounters in both D and SDC tanks. Table 2 defines the observational typography of each of the dependent measures.

Additional observations on test subjects. Test subjects were also assessed for the latency to display 2 days prior to the introduction of treatment and control conditions. This was done, not only to allow an adaptation to the test apparatus, but also to provide a pretest measure of display latency against which to compare treatment effects.

TABLE 2

Description of behaviours recorded in subjects undergoing defeat (D) or social exposure control (SDC) conditions.

Behaviour	Description
Approach	<ul style="list-style-type: none"> -characterized by a slow gliding movement directly towards the stimulus. -such approach is often preceded by a momentary pause in movement. -gill cover and fin erection are not typically evident during the initial phases of approach.
Gill Cover Erection	<ul style="list-style-type: none"> -extension of both gill covers at approximate right angles to the body. -gill cover erection usually occurs at the end of the approach sequence.
Fin Erection	<ul style="list-style-type: none"> -erection and full spreading of dorsal, ventral, anal and caudal fins. -fin erection occurs either prior to the approach sequence or during the approach sequence prior to gill cover erection.
Withdrawal	<ul style="list-style-type: none"> -swimming activity in the direction opposite to the stimulus animal. -withdrawal may also be observed as momentary backward swimming due to rapid frontal approach by the stimulus.
Lateral Banding	<ul style="list-style-type: none"> -the appearance of two black bands on both sides of the body extending from posterior to the gill covers to the base of the caudal fin.
Bleaching	<ul style="list-style-type: none"> -generalized paling in body colouration.

During testing each subject was transferred to one of the standardization apparatuses for a 10 min period. Each subject was then exposed to 2 consecutive presentations of mirror image (10 sec in duration) separated by an interval of 2.50 min given that the subject was visually oriented towards the mirror. Should the subject not be correctly oriented (e.g., air breathing), mirror image presentation was momentarily delayed until the subject oriented itself correctly. Latency to display was recorded only during the second mirror image presentation. This was done since the first display latency was not considered reliable due to the possible "startle" effects of the initial presentation of the display eliciting stimulus. These startle effects are observed as reflexive darting movements which typically occur only during the subject's initial response to the mirror image. Startle responses are not often seen after subsequent mirror image exposures. Each presentation was started when the experimenter depressed the stimulus button which was released upon the observation of a full frontal display, i.e., gill cover erection (see Plate 3). Latency to display as indicated by the electric stop clock was then recorded.

Testing procedure. One day subsequent to treatment (D) and control (DC and SDC) procedures, each subject was assessed for the latency to display following the onset of mirror image using one of the two testing

Plate 3

The aggressive display (gill cover erection) of
Betta splendens as observed in the testing
apparatus.

apparatuses.

In the test situation as described, the experimental apparatus was used consecutively by test subjects. This procedure, however, may have lead to potential problems since changes may occur in water chemistry as a function of repeated subject use. Such changes may have deleterious effects on the performance of subsequent subjects using the same apparatus (see Baenninger, 1968). It was therefore considered important that each tank remain vacant for a short period after use so that possible changes in water chemistry could be corrected using the charcoal filter system. This was accomplished by alternating the use of two tanks such that one was used for testing while the other was simultaneously undergoing filtration. Alternating the use of two tanks also substantially decreased the interval between the testing of subjects since there was no waiting period before a test subject could be placed into one of the tanks. In addition, as a consequence of alternating tanks, there was no systematic bias between groups concerning which of the two testing apparatuses was employed. This was because the use of the two units was counterbalanced within groups so that one-half of a group used one test apparatus whereas the remaining half used the other.

Each subject was exposed to 4 presentations of mirror image during latency testing. Multiple mirror image presentations were used to ensure that the

measure of display latency was reliable, and to control for the possibility that failure to display on the first mirror image presentation may be a function of some factor other than defeat (e.g., failure to attend to the mirror).

During testing, the duration of each mirror image presentation was set at 15 sec instead of 10 sec as in pre-experimental latency testing. The duration of mirror image presentation was extended to 15 sec in order to give test subjects more opportunity to display during testing, especially if the effect of defeat was to increase response latency. To prevent potential sensitization effects due to the initial exposure to mirror image and to avoid possible temporal conditioning effects, mirror image presentations were separated by a random choice of durations ranging from 1-5 min. A single composite latency score was then obtained for each subject by determining the mean latency required to display in response to the mirror image. The persistence of defeat effects was assessed by testing independent groups for each treatment and control condition at 1, 2, 4 and 8 days subsequent to defeat.

During latency testing, one subject randomly selected from each of the twelve groups was observed for latency to display by both the experimenter and an independent observer. The independent observer was not aware of the treatment or control condition of test

subjects. A correlation (Pearson product moment) of 0.89 between the experimenter and independent observer indicated that the observations of the experimenter were reliable. Not all test subjects were observed in this manner since this would have required an independent observer to be present during the entire duration of the experiment.

Biogenic amine assay procedures. Four hours subsequent to testing in the standardization apparatus, each subject was sacrificed and the brain removed for assay purposes. The 4 hr delay was used to allow all subjects to be tested before surgical procedures were instituted. The removal of brains was performed in order to determine whether treatment conditions produced changes in the concentration of various neurotransmitters. Termination of each subject was accomplished by placing each unanesthetized test animal upon an ice-cooled petri dish to minimize tissue degeneration and severing the spinal cord immediately posterior to the skull using dissecting scissors. Iris scissors were then used to make two bilateral cuts extending from the severed spinal cord to the posterior orbit of each eye. These cuts allowed the skull to be pulled away from the trachea and lower jaw using forceps, thereby exposing the ventral surface of the brain case. The brain was then removed by breaking open the brain case with the iris dissecting forceps and sectioning the

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spinal cord posterior to the cerebellum and the optic nerves. Each brain sample was placed into a separate plastic vial and immediately frozen in a canister (Lilly) of liquid nitrogen.

The concentration of each neurotransmitter per gram of neural tissue was determined using fluorometric assay techniques established by Lavery and Taylor (1968), and Maickel, Cox, Saillant and Miller (1968). Each sample was removed from the liquid nitrogen, weighed, homogenized in cold acidified butanol and then centrifuged. The biogenic amines were subsequently extracted with 0.1N HCL by the addition of heptane and the HCL to an aliquot of the butanol supernatant and centrifuged again. This procedure allowed approximately 50-60% of available biogenic amine material to be recovered from neural samples for fluorometric analysis.

Prior to determining amine concentration, the norepinephrine samples were allowed to remain at room temperature for 20 min. During the same period, dopamine samples were heated to 100 °C for 10 min, whereas serotonin samples were heated to 65 °C for 10 min. The fluorescence of dopamine was then read on an Aminco-Bowman Spectrophotofluorometer (model D22562155) with ~~excitation~~/emission wavelengths being set at 330 nm/390 nm. The same procedure was followed for norepinephrine and serotonin samples with the exception that the excitation/emission wavelengths were set at 380

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nm/480 nm and 360 nm/470 nm respectively.

Results

Statistical Methods of Analysis

The results of Experiment 1 were subjected to an analysis of covariance (ANCOVA). In those cases where analysis of covariance was not deemed appropriate, analysis of variance or non-parametric statistics were used. Since pretest latency score distributions were observed to be skewed in the direction of short latencies, raw data were transformed to reciprocal scores, $(1/X)$. This was done to assist in normalizing the distribution of latency data for statistical purposes (see Crespi, 1942; Edwards, 1968).

Analysis of covariance was used as the primary method of analysis since ANCOVA allows a reduction in the magnitude of the error variance not possible using simple analysis of variance (Winer, 1971). A reduction of the experimental error is accomplished by taking into account the regression of the dependent variable on a covariate (Edwards, 1968; Ferguson, 1966). In the present experiment, the pretest measure of latency to display was used as the covariate.

Experimental Findings

The results of Experiment 1 were two-fold.

First, defeated subjects demonstrated longer display latencies (slower to respond) during testing that did control subjects (SDC and DC). Second, both defeated (D) and social exposure (SDC) groups demonstrated an equivalent depletion of dopamine concentration in comparison to the control group (DC). In addition, no evidence of serotonin (5HT) or norepinephrine (Ne) was found in the neural tissue of either treatment or control subjects. These findings are described in greater detail below.

Effects of defeat. Figure 3 shows the effects of defeat (D) on display latency in test subjects as opposed to subjects undergoing social exposure (SDC) and no social exposure (DC) procedures. Analysis of covariance (Appendix B) indicated that there was a significant difference between Experimental Conditions D, SDC and DC ($F = 5.290$, $df = 2/35$, $p < .01$), although there was no statistically significant change in behaviour across Days 1, 2, 4 and 8 ($F = 0.365$, $df = 3/35$, $p > .25$). In order to determine which of the three Experimental Conditions were significantly different from the other, the Experimental Conditions were collapsed across Days. A Newman-Keuls test (Winer, 1971) indicated that defeated subjects (D) were significantly slower in responding to the aggression eliciting stimulus ($p < .05$) than were subjects in either control group (SDC and DC) which did not differ from each other. Comparison of pre and post treatment

Fig. 3. Mean reciprocal latency for defeat (D), social exposure (SDC) and defeat control (DC) subjects on Days 1, 2, 4 and 8 following defeat induction procedures.

latency also indicated similar defeat effects. For example, both SDC and DC groups showed little change in display latency prior to ($1/\bar{X} = 0.48$ sec) and following ($1/\bar{X} = 0.47$ sec) control procedures. In contrast, D subjects showed a substantial slowing of the response ($1/\bar{X} = 0.25$ sec) when compared to their own pre-test measures ($1/\bar{X} = 0.42$ sec) of display latency.

A comparison of the means for each of the Experimental Conditions across Days or for each of the Days across Experimental Conditions was not called for as the Days X Experimental Condition interaction was not significant ($F = 0.355$, $df = 6/35$, $p > .25$). The mean and standard deviation for each group is shown in Appendix C.

Behaviour of D and SDC test subjects during defeat induction. Each of the six measures comparing D and SDC behaviours (gill cover erection, fin erection, approach, withdrawal, lateral banding, bleaching) were analyzed separately using non-parametric statistics, i.e., Mann-Whitney U test. This approach was adopted since the data for each of the dependent measures were in the form of frequencies. The Mann-Whitney U test was selected since it is considered to be the most powerful of the non-parametric tests (Siegal, 1956).

The results (Fig. 4) indicated that social exposure to high aggressive stimulus animals did not serve to defeat SDC control subjects. This is shown

Fig. 4. Mean frequency of approach, gill cover erection, fin erection, withdrawal, lateral banding and bleaching for defeat (D) and social exposure (SDC) groups.

in the observation that SDC subjects demonstrated no submissive behaviours in the form of lateral banding ($p < .05$) and bleaching ($p < .05$). Conversely, D subjects invariably showed these submissive behaviours when directly exposed to stimulus animals. Similarly, SDC subjects also showed significantly fewer occurrences of withdrawal behaviour in the presence of a high aggressive stimulus animal than did defeated subjects ($p < .05$).

However, the groups did not differ significantly from each other in gill cover ($p > .24$), fin erection ($p > .24$), or approach ($p > .057$) frequency.

Although subjects socially exposed (SDC) to stimulus animals showed no evidence of defeat, the typography of aggressive behaviour of D and SDC subjects was observed to be similar during early phases of aggressive contact. During defeat induction procedures all test subjects engaged in ritualized threat displays which progressively came to incorporate physical attack behaviours on the part of D subjects. The sequence of appearance of these behaviours was predictable and was in close agreement with previous observational pilot data collected on the fighting and defeat behaviour of this species (Appendix D). In sequential order of appearance, the threat behaviours of Betta splendens include gill cover erection, fin erection, and lateral display, whereas attack behaviours include biting and mouthlocking. Although mouthlocking

was not observed in Experiment 1, Appendix D defines the observational typography of each of the behaviours. Presumably, mouthlocking was not observed in the present study since this behaviour usually occurs between opponents which are evenly matched and only after prolonged encounters. No attack behaviours could occur in SDC subjects because of the separating partition.

High aggressive stimulus animals demonstrated aggressive behaviours similar to that of test subjects. However, following the first few gill cover erections, stimulus animals often dispensed with ritualistic threat behaviours and immediately engaged in attack. Under these conditions D test subjects were defeated in a mean of 6.83 min. Defeat was not evidenced as a progressive change in fight behaviour, but rather as a sudden transition from fighting to fleeing. During this period, i.e., fleeing, stimulus animals retained full fight colouration while in pursuit whereas test subjects demonstrated lateral banding and bleaching. Test subjects also demonstrated a motionless head-up and tail-down position if trapped in a corner of the defeat induction tank. Stimulus animals did not attack defeated subjects when this occurred.

Fluorometric assay. Analysis of covariance (Appendix E) showed that the covariate did not regress significantly on dopamine scores and thereby had no significant effect in reducing variance due to

experimental error ($F = 0.255$, $df = 1/32$, $p > .25$).

Consequently, ANCOVA was not considered an appropriate method of analysis and analysis of variance was employed.

Figure 5 demonstrates that dopamine concentration was significantly altered (Appendix F) depending upon the Experimental Condition ($F = 4.19$, $df = 2/33$, $p < .05$). Since the Days X Experimental Condition interaction was not significant ($F = 0.67$, $df = 6/33$, $p > .25$), a comparison of the means at all points was not justified. However, when the Experimental Conditions were collapsed across Days, a Newman-Keuls test indicated that dopamine concentrations were significantly depleted in D and SDC ($p < .05$) subjects in contrast to control subjects (DC) not exposed to stimulus animals. Subjects in D and SDC groups did not differ significantly from each other. There was no evidence of a significant recovery of dopamine levels over the 8 Days of testing following initial exposure to stimulus animals in D and SDC conditions ($F = 0.63$, $df = 3/33$, $p < .25$). Appendix C shows the mean and standard deviation of each of the groups.

Correlation of neurochemical and behavioural data.

The absence of a significant regression (Appendix E) indicated that dopamine concentration in control and treatment conditions did not covary significantly with pretest latencies. Similarly, in a separate analysis (Pearson product moment) it was shown that dopamine concentration did not correlate significantly ($r = .06$).

Fig. 5. Mean dopamine concentrations for defeat (D), social exposure (SDC) and defeat control (DC) subjects on Days 1, 2, 4 and 8 following defeat induction procedures.

with display latencies i.e., aggressiveness, obtained during testing ($p > .05$).

Experiment 2

Experiment 1 investigated the effects of defeat in terms of latency to display and biogenic amine concentration. Experiment 2, however, was designed to assess the effects of victory on both measures using the methodological principles outlined in Experiment 1. In addition, Experiment 2 had the purpose of testing whether or not the effects observed in Experiment 1 were due solely to the effect of defeat and not the effect of combat per se.

Method

Subjects

Forty-eight experimentally naive subjects similar to those employed in Experiment 1 were used.

Apparatus

Experiment 2 was conducted using the same apparatus and materials described in Experiment 1. The operant rate apparatus, however, was not used. The defeat induction tanks of Experiment 1 are referred to as victory induction tanks during Experiment 2.

Procedure

The procedure of Experiment 2 replicated that of Experiment 1 with the exception that test subjects were exposed to stimulus animals of a low aggressiveness as opposed to high aggressiveness. This was done to ensure victory on the part of experimental test subjects.

Low aggressive stimulus animals had been previously selected in Experiment 1 from the same sample that high aggressive stimulus animals were derived using the operant rate procedure (see Fig. 2). The probability of victory in test subjects was maximized by placing experimental test subjects within the respective victory induction tanks for a 24-hr period in order to allow territorial formation.

During Experiment 2 a measure of inter-observer reliability was obtained as in Experiment 1. A correlation of 0.91 between those latencies recorded by the independent observer and the experimenter indicated that the method of recording latency data was reliable.

Since norepinephrine and serotonin were not found in Experiment 1 the volume of these amines recovered from neural tissue samples during assay procedures was increased in Experiment 2. As a result, approximately 90-95% of available amines were extracted from brain samples. This was done in an attempt to control for the possibility that the absence of norepinephrine and serotonin may have been due to insufficient quantities of these amines being extracted during fluorometric assay.

Results

Experimental Findings.

The results of Experiment 2 were analysed using the same statistical procedures which were described in Experiment 1. Two findings were evident. First, the effect of victory was found to significantly decrease the latency to display in response to the onset of the mirror image stimulus. Second, no significant differences were found in dopamine concentrations between V, SVC, and VC Experimental Conditions. In addition, serotonin and norepinephrine were not found.

Effects of victory. Figure 6 shows the effects of victory on display latency. Analysis of covariance (Appendix G) indicated that victory induction procedures produced a significant difference among Experimental Conditions (V, SVC, VC) in display latency ($F = 3.395$, $df = 2/35$, $p < .05$). No evidence of a significant change across Days was found ($F = 1.387$, $df = 3/35$, $p > .25$) although Fig. 6 does indicate that the mean latency for V subjects changed appreciably during testing between Days 1 and 4. Appendix H shows the mean and standard deviation for treatment (V) and control groups (SVC and VC).

Since a significant Days X Experimental Condition interaction was not present ($F = 1.832$, $df = 6/35$, $p > .10$) a statistical comparison of all the means was not justified. However, in order to determine which of the

Fig. 6. Mean reciprocal latency for victory (V), social exposure (SVC) and victory control (VC) subjects on Days 1, 2, 4 and 8 following victory induction procedures.

treatment and/or control groups were significantly different, the Experimental Conditions (V, SVC and VC) were collapsed across Days and a Newman-Keuls test was applied. The Newman-Keuls test indicated that subjects which had experienced victory (V) responded with shorter latencies ($p < .05$) than did either control group (SVC, VC). The control groups did not differ significantly from each other. A comparison of pre and post treatment measures of display latency also showed similar victory effects. That is, SVC and VC groups demonstrated little change in display latency prior to ($1/\bar{X} = 0.32$ sec) and following ($1/\bar{X} = 0.34$ sec) control procedures. In contrast, subjects exposed to victory showed a decrease in display latency ($1/\bar{X} = 0.46$ sec) when compared to pre-test measures of the display response ($1/\bar{X} = 0.36$ sec) for the same subjects.

Behaviour of V and SVC test subjects during victory induction. The performance of both V and SVC groups are shown in Fig. 7. No significant difference was found between groups concerning the frequency of approach ($p > .100$), gill cover erection ($p > .171$), and fin erection ($p > .343$). However, SVC subjects demonstrated a marginally higher frequency of withdrawal response than did V subjects ($p < .05$). Neither group demonstrated any submissive behaviours in the form of lateral banding ($p > .343$) and bleaching ($p > .343$). In addition, V and SVC subjects also exhibited

Fig. 7. Mean frequency of approach, gill cover erection, fin erection, withdrawal, lateral banding and bleaching for victory (V) and social exposure (SVC) groups.

similar aggressive behaviour patterns in response to low aggressive stimulus animals during victory induction procedures. However, mouthlocking was not observed (see Appendix D). Low aggressive stimulus animals only engaged in threat displays after test subjects approached and displayed. Fights were short in duration ($\bar{X} = 5.16$ min) with stimulus animals typically fleeing to a corner where a head-up, tail-down submissive posture was adopted. Further attacks by victorious test subjects (V) were not observed following the appearance of submissive behaviour. In the social exposure tanks (SVC) low aggressive stimulus animals did not show similar defeat behaviours because of the protecting barrier. However, as the duration of social exposure increased these animals would often retreat to the back of the tank when approached and displayed to by a test subject (SVC) near the partition.

Fluorometric assay. The mean dopamine concentrations for subjects in each experimental condition are shown in Fig. 8. Since the effect of the covariate (Appendix I) was not found to be significant ($F = 1.118$, $df = 1/35$, $p > .25$), analysis of variance was used as the statistical procedure. Analysis of variance (Appendix J) showed no evidence of a significant difference in the dopamine concentration of test subjects among Experimental Conditions ($F = 0.17$, $df = 2/36$, $p > .25$) or across Days ($F = 1.64$, $df = 3/36$, $p > .25$). The

Fig. 8. Mean dopamine concentrations for victory (V), social exposure (SVC) and victory control (VC) subjects on Days 1, 2, 4 and 8 following victory induction procedures.

general increase in dopamine levels over that of Experiment 1 was considered ~~to be~~ a function of the more sensitive amine recovery procedure used, and of normally occurring changes inherent in the sensitivity of the spectrophotofluorometer.

Correlation of neurochemical and behavioural data.

The absence of a significant covariate effect (Appendix I) indicated that pre-experimental measures of display latency did not covary significantly with dopamine concentration in control and treatment subjects. The same effect is reflected in the finding that dopamine concentration did not correlate ($r = -.20$) significantly with latency scores taken during testing ($p > .05$).

Discussion

The results of Experiment 1 and 2 showed that the behaviour and neurochemical concentration of the male Siamese fighting fish can be modified by the experience of defeat and victory. Since test subjects were randomly distributed in aggressiveness within groups, the observed behavioural and neurochemical changes cannot be attributed to sample bias. Furthermore, a lack of stimulus constancy cannot account for these results since the design of the study ensured that the influence of stimulus animals on test groups was equal. This was because stimulus animals (characteristic of a narrow range of

aggressiveness) were randomly divided between treatment and social exposure conditions and were counterbalanced within groups relative to fight experience.

In addition, the design of Experiments 1 and 2 ensured that the observed changes in behaviour (display latency) were specific to the treatment effects of defeat and victory.

Behavioural Effects of Defeat and Victory

In Experiment 1 the increase in display latency by defeated subjects is considered specific to the experience of defeat since this effect cannot be attributed to either (a) social exposure to high aggressive stimulus animals or (b) fatigue effects as a function of fighting. Social exposure is not considered responsible for poorer display performance because test subjects which were exposed to high aggressive stimulus animals but were protected from defeat (SDC), showed no significant increase in display latency. Physical fatigue as a function of fighting also cannot conclusively account for poorer display performance, since SDC subjects did engage in threat behaviours and increased locomotor activity similar to that of defeat subjects with the exception of fleeing (Fig. 4). Furthermore, test subjects of Experiment 2, which fought in aggressive encounters but emerged as victors instead of losers, showed the opposite change in behaviour, i.e., decrease in display

latency. This observation argues against the possibility that fatigue was responsible for the behavioural effects of defeat since it is not probable that fatigued subjects would improve in performance.

Although defeat and victory served to alter display latency, the extent to which the change in aggressiveness persisted over time is not entirely clear in the present study. For example, Fig. 3 shows some improvement in display latency across the 8 days of testing, although this trend was not statistically significant. The detection of a significant "Days" effect may have been precluded by the large variance of reciprocal scores (Appendix C), combined with the small sample size of test groups. The inability to detect a significant "Days" effect due to large group variance and small sample size must also apply to the results of Experiment 2 concerning the persistence of victory effects (Appendix H). That is, Fig. 6 shows a decrement in display latency across days, but this effect is not statistically significant.

Nevertheless, Experiment 2 did show that the effect of victory was to decrease display latency upon the presentation of an aggression eliciting stimulus in the form of mirror image. This decrease in display latency was also specific to the effect of victory since social exposure to a stimulus animal (SVC) or fight outcome other than victory (see Experiment 1) did not serve to

produce a similar decrease in response latency.

It should be noted, however, that victory did not decrease display latency, i.e., aggressiveness, to the same extent that defeat increased display latency. This effect is also observed in the comparison of pre and post test latency measures for subjects of Experiments 1 and 2. Although it is difficult to compare the effects of both fight outcomes since they are qualitatively different phenomena, it is plausible that the extent to which display latency could decrease (Expt. 2) may have been limited by the type of dependent measure used. In other words, there is an upper limit (ceiling effect) in the time within which a display can occur following the presentation of mirror image. This upper limit is determined not only by the time required to detect the aggression eliciting stimulus, but also by the time required to execute the resultant motor sequence controlling the erection of the gill covers. The same restriction does not apply to defeated subjects since there is no comparable limitation on the amount of time that can elapse before a display occurs.

In addition to the finding that defeat and victory produced different effects of differing magnitudes in the present study, the discovery that defeat decreases aggressiveness as measured by display latency in Betta splendens is consistent with those studies which previously investigated the effects of

defeat in other species without the problem of sample bias (e.g., Ginsburg and Allee, 1942; Kahn, 1951; Scott and Marston, 1953).

Experiment 1 shows a similar effect in fish, and Experiment 2 extends this finding by demonstrating that the experience of victory serves to enhance aggressiveness.

Given these effects of defeat and victory on the aggressiveness of Betta splendens it is important to postulate the mechanism or mechanisms which can account for such behavioural changes. Attempts to do so, however, are difficult since the purpose of the present study was to explore and document a specific phenomenon, rather than test the validity of a specific explanatory hypothesis. In this respect, Simpson (1968) noted that it is relatively simple to achieve results in studies of animal behaviour, but these results are often difficult to satisfactorily explain. It is because of this difficulty concerning the explanation of behavioural phenomena that Simpson (1968) concludes that many studies can only provide a summary of explanatory interpretations. In reference to the present study, it is difficult to avoid following a similar course of action. Nevertheless, it is possible to identify some of the more plausible hypotheses and to show how each of these can best account for the available data.

Stress effects. When subjects are exposed to aversive or potentially life threatening stimuli such as electric shock or burns, adaptive behaviours occur in response to such stimuli. At first, these responses appear in the form of species typical alarm reactions which are observed as defensive behaviours such as withdrawal (Bolles, 1970, 1971). The function of these behaviours is to allow the animal to deal effectively in the removal of the stressor. Furthermore, physiological adaptation may also occur in order to maintain the body's physical equilibrium necessary for life sustaining processes. However, should the stress be severe or prolonged in nature, changes occur which do not appear to serve any adaptive function. These responses, as previously described, involve a broad spectrum of effects ranging from adrenalin release (Barnett, 1955) and changes in neurotransmitter concentration (Welch and Welch, 1970), to a general disruption of adaptive behaviours (Barry and Buckley, 1966; Selye, 1971). Behavioural changes which have been observed include long term changes in emotionality (Campbell and Campbell, 1962) and deficits in active avoidance tasks (Seligman, Maier and Solomon, 1971) on the part of stressed animals.

Since exposure to an opponent in an aggressive encounter may also be considered a potentially life threatening situation, it is possible that stress as a function of fighting can induce behavioural changes. In

the present study, defeat is presumably a stressful experience since test subjects are physically beaten by high aggressive stimulus animals in a fight. Under these stimulus conditions, it is possible that subsequent aggressive behaviour is disrupted as measured by longer display latencies. Stress effects may have also been present in Experiment 2 in which animals were victorious in fights because the very act of fighting could involve stressful stimulation. However, it should be noted that stress effects do not easily account for the results of Experiment 2 because the effects of victory were to enhance rather than disrupt display latency. Nevertheless, this does not necessarily mean that stress was not present in such situations since other factors may have been involved as well. Some of these other factors including possible neurochemical effects are discussed in the following sections.

Conditioning. Many of the studies which have examined the effects of fight outcome on subsequent aggressive behaviour have favoured a conditioning approach. In such an approach, the effects of losing or winning are interpreted in terms of learning principles (Baenninger, 1970; John, 1951; Ratner, 1961; Scott and Marston, 1948). For example, defeated test subjects are considered to demonstrate submissive behaviours (unconditioned response) as a function of attacks (unconditioned stimulus) by stimulus animals. Since

these attacks are associated with high aggressive stimulus animals, these animals acquire conditioned stimulus properties. Subsequently, when defeated test subjects are exposed to aggression eliciting stimuli during the aggression test, the submissive response generalizes to the conditioned stimulus, i.e., opponent (Ratner, 1961). Conversely, in the case of subjects who emerge as victors, it is the dominant behaviour of these subjects which generalize to the aggressive eliciting stimulus (conditioned stimulus) during testing.

In the present study, the conditioning approach is capable of describing the increased and decreased display latencies exhibited by losers and winners respectively. However, in order to conclusively support a conditioning approach, it would have been required that the latency of winners and losers be determined during the latter part of fights. This procedure would be necessary in order to determine if the latency of test subjects had become slower or faster as a function of fighting. If such a change in display latency (unconditioned response) had occurred at the end of aggressive fights, then it would not be entirely unexpected to find that such responses become associated with stimulus animals (conditioned stimulus), and subsequently generalize to the test situation. Unfortunately, it was not possible to determine the display latency of test subjects during fights. This was because

it was considered improbable that subjects could be provided with eliciting stimulus onsets, in order to record display latency while engaged in combat with a stimulus animal. As a result, it is not known if winners and losers changed in their display latencies toward the ends of their fights and if these changes became associated with stimulus animals (conditioned stimulus). However, it should be noted that submissive subjects were not observed to display toward the end of fights with high aggressive stimulus animals. This could be taken to mean that there was a dramatically large increase in display latency on the part of losers, which was not evident on the part of winners in Experiment 2.

On the basis of this evidence, albeit indirect, it is not implausible that increases and decreases in response latency became conditioned to stimulus animals and were subsequently elicited by the mirror image during testing.

The role of fear in conditioning approaches. The conditioning approach to behaviour represents a stimulus-response model in which specific responses (e.g., decreased latency) become associated with specific stimuli (e.g., aggression eliciting stimulus). Such an approach does not elaborate upon the possibility that specific central states might also be elicited by specific environmental stimuli such as defeat. In exploring this issue, Mowrer (1960) and Rescorla and Solomon (1967) suggest that when an animal is exposed

to an aversive stimulus such as electric shock, a state of fear is elicited within the animal. The affected subject is then considered to possess an acquired drive which the animal seeks to reduce by performing an instrumental response which allows an avoidance of the aversive stimulus. Since such a model was dependent upon one factor (classical conditioning) to instill fear and upon another (instrumental learning) to alleviate fear, it was commonly referred to as two-factor theory (Rescorla and Solomon, 1967). Miller (1948) provided an example of two-factor theory in which rats were first exposed to electric shock in an enclosed compartment. Subsequently, these animals learned an instrumental response (rotating a wheel or lever pressing) in order to escape from the compartment in which they were previously shocked. According to Mowrer (1960), such behaviour can easily be explained if one assumes that shock elicits fear and that the instrumental response reduces fear. In other words, reinforcement for the operant response is the reduction of fear rather than avoidance of shock itself. This conclusion supports the notion that fear may play an important role in modifying behaviour.

Problems of definition. The difficulty with the concept of fear, however, is that it represents a hypothetical construct which is not amenable to direct observation and measurement since it occurs within the organism. Therefore, any characterization of fear is

possible only through operational means. In attempting to characterize fear, Hinde (1970) suggested that fear can be detected by the presence of overt behaviours which occur in the presence of potentially aversive stimuli (e.g., electric shock, predators). These fear responses may be divided broadly into those which involve fleeing, and those which involve immobility responses such as freezing. For example, species such as rats will demonstrate a fleeing response when exposed to shock (Miller, 1948), whereas domestic fowl demonstrate a freezing response in the presence of a predator (Ratner and Thompson, 1960). Whether freezing or fleeing occurs, depends to a large extent on the innate propensities of the species and the intensity of stimulation.

Given that fear can be detected on the basis of behavioural actions, it is possible that such a central state may have influenced the latency measures of test subjects in Experiment 1 and 2. This is because fear responses in the form of fleeing were invariably observed in test subjects during defeat. Furthermore, immobility responses and attempts to flee (thrashing) were also occasionally observed in the standardization apparatus during testing although these responses were not systematically recorded. Presumably, these fear responses arose out of contact with high aggressive stimulus fish and subsequent physical attacks leading to defeat. In many respects, the process of defeat can be likened

to other stimuli such as electric shock which test animals attempt to avoid. This is not surprising since it has been shown that fish will flee from electric shock (Behrend and Bitterman, 1963) as well as other dominant subjects (Frey and Miller, 1968).

Since fear can be associated with aggression eliciting stimuli on the basis of behavioural evidence, it is possible that two opposing drives are elicited within the organism when defeated subjects are exposed to mirror image stimuli during testing. On the one hand there is an innate predisposition to attack an opposing Siamese fighting fish; on the other, there is an arousal of fear which motivates the animal to flee from the situation. When both of these drives are elicited simultaneously, it is possible that the fear response (tendency to flee or make no response) interferes with the drive to attack. The end result is an increase in display latency, the magnitude of which is determined by the strength of the fear response.

In contrast, fear responses in the form of fleeing or immobility were never observed in subjects who emerged as victors as a function of fights in Experiment 2. The absence of an acquired drive (fear) would not interfere with the drive to attack, and therefore would not interfere with the display response. In addition, the absence of fear in conjunction with the reinforcing effects of winning a previous fight, may have strengthened

the predisposition to attack an opposing conspecific. Under these conditions, it is not unexpected to find that the latency to engage in aggressive display decreases in response to mirror image stimuli.

Fear and conflict. The use of central drive states to describe behaviour differs from the pure conditioning approach since it suggests that there are two opposing forces which operate to modify overt behaviour. European ethologists (e.g., Tinbergen, 1951) have long recognized that aggressive behaviour can be described in terms of tendencies to flee or attack which are in opposition or conflict with each other. However, they have adopted such a conflict approach without dependence upon concepts such as interference proposed by a conditioned fear approach.

In discussing conflict theory, Hinde (1970) has provided a definitive account of this model and its relationship to the control of aggressive behaviour. Given the findings of Experiment 1 and 2, it is apparent that the conflict approach may be directly applicable to the results of the present study.

Conflict Approach

When two animals confront each other in an aggressive encounter, an approach-avoidance conflict is elicited in both subjects. On the one hand, there is a tendency to attack (approach) the conspecific for

purposes of territoriality and dominance. However, simultaneous to the arousal of the approach tendency, there is also the arousal of an avoidance component (fleeing) as a function of the threat display of the opposing conspecific. It is the relative strength of both of these tendencies which either drives the animal forward to attack (approach) or retreat (fleeing) from the opponent.

However, before discussing the ability of the conflict approach to describe behaviour, it is important to make clear what is meant by "tendency" since the conflict model is highly dependent upon this term.

Definition of tendency. As in the case of fear, the term "tendency" describes a central state which is not directly available to observation. In such a description, tendencies are hypothetical constructs which can only be operationally defined on the basis of what an animal does in a certain situation. It is assumed that tendencies are present when we observe that animals behave in a specific manner. When a test subject moves toward another animal, it can be concluded that the subject is experiencing a tendency to approach. When an animal runs away from another conspecific, it is highly probable that the animal is experiencing a tendency to avoid. When both of these tendencies are simultaneously present, a conflict state is aroused within the animal. Whether an animal will flee, attack,

or simply engage in aggressive display in the presence of another conspecific, is a function of the relative strengths of both tendencies.

. Use of the conflict approach in describing behaviour. Using this operational approach to behaviour, numerous investigators have found the conflict model to be of value in describing behavioural phenomena which are particularly difficult to explain using other approaches such as conditioning. One such case is pendulum fighting exhibited by pairs of adjacently placed animals. Hinde (1952) gives the example of the Great Tits which form adjacent territories separated by a narrow no man's land. Should one Tit encroach upon the other's territory, the intruder is immediately attacked and pursued. However, as the pursuer encroaches upon the fleeing subject's territory, the situation is reversed and the fleeing animal attacks and pursues the intruder. Should both animals encounter each other on the borders of their respective territories (no man's land), neither approach nor fleeing behaviours occur. Rather, aggressive displays (head up or forward) are observed in the absence of direct combat.

Such pendulum fighting can readily be described by a conflict model in which there is a continual shifting of approach (attack) and avoidance (fleeing) strengths. When subjects occupy their own territories, the approach tendency is greater than the avoidance

tendency since these subjects will readily attack and pursue any intruder. However, once the pursuing animal encroaches upon the other's territory, the avoidance tendency increases in strength since the subject now begins to flee. Since this sequence repeats itself, it has become known as pendulum fighting because combat swings from one subject's territory to the other.

Threat displays at the boundary between territories is considered to be a direct result of conflict, where the tendencies to attack and flee are approximately of equal strength. Ethologists such as Manning (1969) consider the threat display to be an inevitable result of conflict when neither attack nor fleeing tendencies are strong enough to "find separate expression". In such cases, the display response is not only considered to be an ambivalent intermediate response, but also an indicator of the conflicting tendencies occurring within the organism.

The conflict approach to behaviour has also proven itself to be popular in describing the behaviour of other animal species. For example, the domestic cat demonstrates some postures which are characteristic of pure attack or fleeing, in addition to a wide range of intermediate postures indicative of the mixed elements of attack and flight. In a classic study, Leyhausen (1956) showed that if cats were exposed to a rival, the threat postures of the cats varied with the extent to

which the tendencies of attack and fleeing were elicited. In other words, more submissive escape postures were observed when test subjects were exposed to a dominant rival than vice-versa.

The presence of these ambivalent behaviours are strongly suggestive of the possibility that animals experience conflict between attack and escape when exposed to an opposing conspecific. In addition, this phenomenon appears to be phylogenetically wide, since Hinde (1970) noted that ambivalence (conflict) has been observed in diverse other animals including rats (Grant, 1963), primates (Hinde and Rowell, 1962; Rowell and Hinde, 1962), birds (Tinbergen, 1952, 1953, 1959), and fish (Baerends et al., 1955; Barlow, 1962a, b, 1963; Forselius, 1957; Kleenleyside and Yamamoto, 1962; McKenzie, 1969; Morris, 1954, 1958).

Although the conflict hypothesis can describe behaviour as the shifting strengths of approach and avoidance tendencies, ethologists have been reluctant to specify exactly what causes the strength of each tendency to change relative to the other. This reluctance, no doubt, has arisen from the recognition that the causal factors which influence behaviour are complex and may often go undetected by the experimenter.

Causal factors. Nevertheless, the explanation of any behavioural phenomenon using the approach-avoidance model would be incomplete if no attempt was made to

specify the causal factor which alters the strength of either tendency. For example, in the present study, differences between Experiments 1 and 2 in aggressiveness as measured by display latency could easily be described by the differing strengths of approach and avoidance tendencies. However, the explanatory power of this interpretation is minimized if one cannot suggest a probable causal factor which increases or decreases the relative strengths of each tendency.

Although various unknown factors could serve as a causal agent in the present experiment, a plausible suggestion is that fear acquired as a function of fighting (see Conditioning) may serve to alter the tendency to avoid (i.e., flee) the opponent. This suggestion is supported by the observation that behavioural indices of fear in the form of fleeing were clearly evident on the part of test subjects in Experiment 1. Furthermore, Tinbergen, (1951), suggested that it is fear of the opponent acquired as a function of fighting which counterbalances the tendency to engage in aggression. This conclusion would support the notion that fear can be conceived as a causal factor in an approach-avoidance context.

Therefore, given the possibility that test subjects do acquire fear in combat situations, it would not be unlikely to find that the conflict and fear conditioning approach are in some ways related and dependent upon

each other. This suggestion is further elaborated upon in the following discussion of approach-avoidance conflict and its relationship to the performance of test subjects in Experiments 1 and 2.

Approach - Avoidance Conflict and the Behaviour of Betta Splendens

On the basis of the previous evidence concerning the significance of threat displays, it is apparent that the aggressive threat display of Betta splendens, i.e., gill cover erection, can also be viewed as an ambivalent response between the tendency to attack and the tendency to flee. Given that conflict mediates the display in this species, it would be expected that changes in the relative strength of attack and fleeing tendencies may alter the latency within which an aggressive display occurs. Furthermore, the relative strengths of both approach and avoidance tendencies might be expected to depend upon the degree of fear induced by the aggression eliciting stimulus.

In the case of defeated subjects, it is possible that aggression eliciting stimuli become associated with fear through a conditioning process, as previously described. The fear response develops as a function of attack and defeat (unconditioned stimulus) by a high aggressive stimulus animal and is evidenced by the presence of fleeing behaviour. Subsequent to defeat,

exposure to an aggression eliciting stimulus (mirror image) during testing may serve as a conditioned stimulus which reinstates the fear response. As a result, the fear response may in turn alter the strength of the tendency to flee, which in turn automatically determines the strength of the approach tendency.

In the Siamese fighting fish, the approach tendency is of greater strength than the avoidance tendency since Betta splendens readily approach and display to aggression eliciting stimuli. However, in defeated subjects, the possibility of a fear response associated with aggressive stimuli could increase the strength of the avoidance tendency relative to the approach tendency. Consequently, test subjects (D) are slower in displaying to the mirror image than subjects in either control condition (DC and DC).

The approach - avoidance hypothesis can also account for the finding that victory produces shorter response latencies, i.e., increased aggressiveness. Victory over an opponent is considered to be a reinforcing event since it allows the victor to retain territorial rights. Furthermore, it has also been shown that allowing animals to experience a series of wins will reinforce an increase in aggressiveness (Scott and Marston, 1948). Under these conditions the reinforcing effects of winning may become associated with aggression eliciting stimuli. In addition, the absence of submissive behaviour suggests that fear is not associated

with aggression eliciting stimuli, i.e., stimulus animals. The relative absence of fear (and its influence on the avoidance tendency) combined with the reinforcing effects of victory may serve to facilitate display performance through an increase in the approach tendency (aggression) relative to the avoidance tendency (escape). This produces shorter display latencies in response to the mirror image stimulus during testing.

The modification of both approach and avoidance tendencies as a function of prior experience and fear may also account for those changes in aggressiveness reported in other Siamese fighting fish studies using different treatment methods. Baenninger (1970) demonstrated that subjects continuously exposed to mirror image stimuli would learn an operant response to terminate such stimuli for short durations. Similarly, Rhoad, Kalat and Klopfer (1975) showed that after repeated exposures to reinforcing mirror image, subjects tended to avoid making approach responses which would produce the opponent as represented by the mirror image. In both cases, aggression eliciting stimuli can be conceived as aversive and fear inducing since subjects are unable to defeat or drive off an opponent. Under these circumstances, the dependent measure would be expected to show a decrement in response rate since the strength of the avoidance component would have increased relative to the strength of the

approach component.

Additional Evidence in Support of the Conflict Approach

In the previous discussion, the presence of an approach - avoidance conflict in the Siamese fighting fish was supported by the observation of threat display, approach and fleeing behaviours. All of these behaviours have been considered by ethologists to be behavioural manifestations of conflict (Manning, 1969). However, Hinde (1970) suggests that the presence of an approach - avoidance conflict can also be indirectly substantiated by other forms of evidence. These include a neurological substrate for the mediation of approach - avoidance tendencies and the presence of bodily changes indicative of stress.

Neurological substrate. According to von Holst and von Saint Paul (1963), approach and avoidance can be experimentally examined by the simultaneous stimulation of two neural areas, or the stimulation of one neural area combined with the presentation of an opposing external stimulus. This suggests that neural pathways involved in aggressive behaviour may be discretely organized into those which mediate attack and escape. Although there has been no study specifically investigating this issue in the Siamese fighting fish, a few studies have indicated that a neural organization of discrete approach - avoidance pathways is highly

probable. This conclusion is supported by Roberts and Kiess (1964) and Wasmann and Flynn (1962) who localized discrete pathways mediating pure attack behaviours (approach). In addition, pathways mediating pure flight behaviours (avoidance) have been identified by Hunsperger (1956) and Ursin and Kaada (1960). When both attack and fleeing tendencies are simultaneously elicited through electrical intra-cranial stimulation, neither attack nor fleeing behaviours appear. Rather, behaviour is transformed into a new affective reaction which is observed as the aggressive threat display. In the chicken, simultaneous stimulation of attack and fleeing pathways result in a puffing of feathers and threat screeching (von Holst and von Saint Paul, 1963). In other words, an aggressive threat display occurs similar to that found when a predator or conspecific conventionally elicits conflicting attack and escape behaviours under naturalistic conditions. The appearance of the display itself is indicative of a conflict situation.

Although such neurological investigations have only been conducted in mammals and birds, it is possible that aggressive stimuli may also activate discrete approach and avoidance pathways in the brain of the Siamese fighting fish. One method to determine whether or not this occurs would be to localize those approach and avoidance pathways which mediate aggressive behaviour in Betta

splendens through the use of recording electrodes (Gusel-nikov, 1964). The implantation of such electrodes could be facilitated using the standardization apparatus of the present study. This is because test subjects would be held to a fixed position relative to the eliciting stimulus while attempts were made to localize approach and avoidance pathways. Upon localization of both pathways, electrical stimulation through the same electrodes should lead to the appearance of the threat display (gill cover erection), since conflicting tendencies are aroused. Similarly, it should also be possible to vary the latency of the display depending on the relative strength of stimulation of each pathway. For example, if the pathway mediating approach is stimulated more intensely than the avoidance pathway, then the aggressive display may show a decrease in latency, and vice-versa. These behavioural effects, however, require additional investigation, and the development of highly refined implantation techniques in order to allow such research into the teleost brain. Advances in this area may provide additional support for the conflict approach to aggressive behaviour. Similarly, the presence of physiological stress effects which are correlated with fighting may also provide supportive evidence for the presence of conflicting approach and avoidance tendencies.

Stress. Ethologists such as Manning (1969) have considered stress to be associated with conflict in the

form of simultaneously present approach and avoidance tendencies. This is because the physiological responses which occur in a conflict situation (e.g., norepinephrine depletion) are similar to those found when subjects are exposed to stressors such as thwarted escape, cold, burns, etc. (Barnett, 1964). However, this correlation between stress and conflict does not necessarily imply that stress induced physiological responses automatically signal the presence of conflict. It is clearly possible that stress effects can occur without the induction of conflicting approach - avoidance tendencies as in the case of receiving a burn. Conflict between opposing behaviours may be sufficient to induce stress, but it is not a necessary condition. In any event, there are several reasons to believe that stress may have been responsible for changes in dopamine concentration observed in the present study.

First, test subjects in Experiment 1 were exposed to stimulus animals of a highly aggressive nature. Consequently, attacks on test subjects (D) were observed by the experimenter to be unusually intense without the ritualistic preflight displays often observed in Siamese fighting fish encounters (Smith, 1945). The intensity of attack is substantiated by the finding that fights were exceptionally brief in duration ($\bar{X} = 6.83$ min) in comparison to other aggressive encounters ($\bar{X} = 78-95$ min) which have been observed between male Betta (Meliska and

Meliska, 1975). Second, the design of the defeat induction situation also ensured that test subjects could not initiate alternative responses such as escape, which would have prevented defeat from occurring. Under such conditions, the inability to escape or exercise control over the outcome of a particular situation would in all probability represent a stressful situation. Considering that changes in neurotransmitter concentration have been thought to be a function of stress (Welch and Welch, 1969), the dopamine depletion observed in Experiment 1 as a function of defeat can be readily accounted for by a stress hypothesis.

Support for this suggestion has been provided by those studies which have examined the effect of defeat or fighting on specific neurotransmitters. Eleftheriou and Church (1968) found that in rats, stress caused by the induction of defeat served to alter norepinephrine and serotonin levels as well as change other physiological factors (Eleftheriou et al., 1968). Although Eleftheriou and Church did not examine the effect of defeat on dopamine concentration, the results of Experiment 1 (dopamine depletion) are in agreement with other studies which have examined the effect of fighting but not fight outcome on dopamine level. Welch and Welch (1969) demonstrated that fighting in groups of animals led to a depletion of dopamine (and norepinephrine) in the brain stem of mice. It was

concluded that neurotransmitter depletion was a function of severe stress induced by fighting.

However, the results of Experiment 1 also showed that exposure to a high aggressive stimulus animal restrained by a partition, can deplete dopamine concentration to the same extent as defeat. This finding shows that fighting and defeat are not the only conditions under which stress and subsequent dopamine depletion can occur. Several studies have provided evidence for this conclusion. For example, Bliss and Zwanziger (1966) reported that fighting lowers brain norepinephrine in mice. However, a similar depletion in norepinephrine can be accomplished by exposing subject to other forms of stress producing situations. These include the use of unavoidable electric shock (Weiss, Glazer, Pohorecky, Brick and Miller, 1975) and sleep deprivation (Bliss, 1968). In addition, Welch and Welch (1968) showed that allowing mice to observe a fight while protected by a wire cage would also produce norepinephrine depletion. This test situation is similar to the present experiment in which SDC subjects were allowed to observe, but were protected from attack by a highly aggressive stimulus animal engaging in threat behaviours. Such situations are presumably stressful since it places the test subject in a potentially harmful situation in which there is a threat of injury. In accordance with Weiss et al., (1975), concerning stress induced norepinephrine changes,

the present study suggests that dopamine changes are likewise modifiable by purely psychological stimuli without resort to physical trauma. As suggested by Stolk et al. (1974), such a finding has important implications for the study of stress and psychosomatic disorders. However, such observations must be verified through a more systematic analysis of neurochemical content in the Siamese fighting fish.

The stress interpretation is also capable of accounting for the absence of any change in the dopamine levels of subjects which had experienced victory, if one assumes that winning a fight does not induce an approach - avoidance conflict and subsequent stress. The behavioural evidence presented, however, argues against the notion that fighting does not represent at least some degree of conflict between approaching and avoiding. Furthermore, other studies have shown that dominant subjects (rats) do experience stress as indicated by physiological changes. For example, Taylor and Costanzo (1975) allowed pairs of rats to engage in dominance encounters on a repeated basis until a dominant emerged in each pair. When these subjects were tested for stress, as measured by reticuloendothelial system activity and adrenal weights, it was found that dominant rats exhibited more stress than did isolated controls. In addition, a similar finding has been reported by Louch and Higginbotham (1967), who allowed

groups of animals to fight until a dominance hierarchy had formed. These animals were then tested for stress effects as measured by plasma corticosterone levels. It was found that both dominants and submissives exhibited stress effects, although dominant subjects were indicative of a lesser degree of stress. Since approach and avoidance tendencies were presumably also elicited in these fight situations (Louch and Higginbotham, 1967; Taylor and Costanzo, 1975), the presence of at least some physiological stress effects among dominants as well as submissives is not unexpected.

Given these observations, the question arises concerning why stress effects were not observed in test subjects which emerged as victors during Experiment 2. There are several plausible explanations for this finding. One suggestion is that physiological stress effects only become evident if the intensity of conflict exceeds a specific level. In Louch et al.'s and Taylor et al.'s studies, animals were either exposed to each other for long durations or were repeatedly paired. In contrast, the victorious test subjects of Experiment 2 had only one encounter with an opponent who was also characterized by low aggressiveness. Such fights may have represented "easy victories" for test subjects. Under these conditions the degree of conflict experienced by dominant test subjects may have been minimal and below the level necessary to elicit a

physiological stress reaction. To some extent, this conclusion is supported by the observation that the change in display latency (index of conflict) as a function of winning, was considerably less (Fig. 6) than as a function of losing (Fig. 3). If test subjects had been repeatedly exposed to fights with more aggressive stimulus animals and for longer durations, then it is conceivable that dopamine changes may have occurred as a function of winning. This suggestion, however, remains to be tested.

Absence of norepinephrine and serotonin. In addition to the need for additional pharmacological and behavioural research concerning stress, the apparent absence of norepinephrine and serotonin also suggests a need for further research into the biochemistry of Siamese fighting fish. This is because both of these neurotransmitters have been found in two other fish species (C. auratus and A. calva) by Bogdanski, Bonomi and Brodie (1963). The lack of evidence for these neurotransmitters in Betta splendens, may have been because the concentration of both serotonin and norepinephrine were below the level which the fluorometric assay procedure could detect. Although the increased sensitivity of the fluorometric procedures of Experiment 2 attempted to resolve this issue it is possible that other more sensitive techniques or the use of larger tissue samples through the pooling of

brains may still indicate the presence of these transmitters in the Siamese fighting fish. In any event, this particular issue can only be resolved by additional tests using different bio-assay techniques. Since norepinephrine and serotonin have been linked to aggressive behaviour in other animals, such continued neurochemical tests may also show a similar relationship in fish.

The effect of neurochemistry on behaviour. Since neurochemical changes are clearly evident in the present study, with the exception of norepinephrine and serotonin, it is of importance to ask whether changes in neurotransmitter level, i.e., dopamine, may have in some way influenced behavioural changes. In reply to this question, Experiments 1 and 2 demonstrated no evidence of a significant relationship between dopamine concentration and changes in behaviour. In Experiment 1, D and SDC subjects showed differences in aggressive behaviour during testing, but showed no evidence of a significant difference in dopamine concentration. In Experiment 2, victory served to increase aggressiveness in V subjects, although no evidence of a change was found in dopamine level. Similarly, in Experiments 1 and 2, dopamine levels and behavioural scores for individual subjects showed no significant correlation. This finding would suggest that there may be no direct cause and effect relationship between changes in

dopamine concentration and aggressive behaviour in the present study.

The absence of a significant correspondance between behaviour and neurochemistry is surprising since several studies have indicated that changes in neurotransmitter concentration may be directly related to changes in behaviour (Everett and Borcharding, 1970; Lycke, Modigh and Roos, 1969; Sheard, 1970). These studies have attempted to demonstrate a relationship between behaviour and neurochemistry through the administration of drugs (e.g., α -methylparatyrosine) which alter the concentration of specific brain neurotransmitters (e.g., dopamine) and subsequent behaviour. However, it is often unclear whether the behavioural changes were due to changes in neurotransmitter concentration or some other unspecified mechanism which may have been influenced by the injection of the drug. Should this be the case, changes in neurochemistry may accompany changes in behaviour, but they may not necessarily account for such changes (Eleftheriou and Church, 1968). A similar conclusion may also apply to the neurochemical and behavioural findings of the present study since no direct causal relationship was found between both measures.

Biological Implications of Fight Outcome

The preceding discussion has shown that fight

outcome can induce both neurochemical and behavioural changes. Although the biological implications of neurochemical changes in the Siamese fighting fish must await further investigation, it is apparent that changes in display latency may have important implications for the social structure of this species. Unfortunately, there has been no complete ethological description of the social behaviour of the Siamese fighting fish against which to gauge the effects of defeat and victory under natural conditions. This in part, has been corrected by Goldstein (1975) who examined the social behaviour of Betta in a large tank designed to provide a semi - natural environment. The results of this investigation showed that the Siamese fighting fish is not only aggressive, but also territorial. However, the territorial area is not geographically fixed, but rather, moves with the subject. To some extent, such territory may be characterized as a large personal space which is fiercely defended (Hediger, 1955; Brawn, 1961; Gibson, 1968). Should another male conspecific intrude upon this territorial space, one of two consequences will usually occur. One of the subjects will either withdraw from aggressive contact if given an opportunity, or a fight will ensue in order to establish dominance.

Under such conditions of dominance formation, changes in aggressiveness as a function of prior fight experience may influence which of the combatants emerges

as a winner or loser. Several studies have, in fact, suggested that prior fight experience (winning or losing) represents one of the most important factors in determining an animal's performance in a subsequent fight (Frey and Miller, 1968; Meliska et al., 1975).

Factors which may influence fight outcome. However, it should be noted that other factors exclusive of fight experience have also been shown to be of importance in determining a winner or loser. Two of these factors involve size of the opponent, and prior territorial residence. The effect of subject size in determining hierarchical rank has been studied in a number of teleost species including Platypoecilus maculatus (Braddock, 1945), Leponis megalotis (Huck and Gunning, 1967), and Moliensia latipinna (Baird, 1968). The results of these studies show that larger fish generally tend to defeat smaller opponents. However, this conclusion is by no means absolute since de Boer and Heuts (1973), using the Jewel fish, demonstrated that size of the opponents did not have any significant effect on fight outcome. Similarly, Ewing and Evans (1973), using Aphyosemion bivittatum, reported that size is not an important criterion in this species, since the 3rd smallest fish in their experiment became the most dominant. Size also does not appear to be a crucial variable in mammals since Uhrich (1937) found no strong correlation between weight and hierarchical

status in mice. This effect is also seen in the Siamese fighting fish since Meliska et al. (1975) observed no correlation between subject size and dominance or submission as a function of aggressive encounters.

The ambivalence of these findings concerning the effects of size on dominance relationships suggests that size is either not an important variable in fight outcome, or that outcome can be influenced by other factors such as prior residency. For example, numerous investigators have suggested that territorial possession, or familiarity, gives the subject an advantage in winning a fight with an intruder (Braddock, 1949; Greenberg, 1947; Noble and Curtis, 1939). This effect, in turn, can contribute more to the outcome of a fight than does size, since Baird (1968) observed that smaller M. latippina males successfully defended their areas, even against larger intruding males. Since subjects appear to be more aggressive in their own territory, it is possible that the latency to attack may also be decreased as a result.

In any event, the interaction of prior residency and size effects with the prior experience of winning or losing on aggressiveness is a complex one. As yet, there is no substantive evidence to indicate which factor or groups of factors is the most important in determining aggressiveness (e.g., display latency) and the winner or loser of fights. However, Frey and

Miller (1972) suggested that prior experience may be of greater importance in influencing aggressiveness than size or prior residency. This is because Frey and Miller noted that the effect of prior experience (winning or losing) contributed more to the dominance or submission of the Blue Gourami (Trichogaster trichopterus) than did any other factor.

A similar conclusion has also been reached by Meliska et al. (1975) in reference to the Siamese fighting fish. Since size differences were not correlated with fight outcome, and since residency was equated between subjects, they suggest that prior experience may be the most important predictor of winning or losing a subsequent fight. "It is likely that a Betta's social background, especially its history of wins or losses to an opponent, influences its later behaviour toward another animal." (p. 137). More precisely stated, it is changes in aggressive behaviour such as display latency as a function of fighting, which may cause the combatant to win or lose in a subsequent encounter.

Adaptive significance. In such situations, a decrease in threat display latency as a function of prior victory may be of adaptive value since it signifies an increased readiness to engage in aggressive behaviour. Should the increased aggressiveness of the opponent be perceived through such a decrease in display latency, it may serve to decide the winner or loser of an

encounter without recourse to a fight. Under these conditions, display behaviour is a highly adaptive method of social interaction since it eliminates the need for actual fighting and possible death on the part of one of the antagonists. Hence, species preservation is maintained by reducing aggressive contact to ritualized threat encounters which determine the winner or loser. In this manner, the display is a method of intra - species communication with regard to behavioural intent. The display serves a behavioural signalling function (Tinbergen, 1953) and determines to a great extent, the course of action that will be followed by the opposing conspecific, i.e., fight or flight.

The increase in display latency of defeated subjects may similarly serve a signalling function in indicating that the subject is less ready than naive or victorious subjects to engage in aggressive combat. Furthermore, a delay in the appearance of threat behaviour would suggest that the attack components of combat (Appendix D) might also be delayed should the encounter proceed beyond the threat phase. This would in all probability mean that defeated subjects may have less opportunity to "land the first blow". For this reason, the slowness of previously defeated test subjects to engage in fight behaviour can only contribute to their defeat especially when confronted with an opponent who shows a tendency to display and

attack first.

The consequences of winning or losing may also have important implications for the species in general. In a natural environment, defeat would cause subjects to lose rights to territorial formation and mate selection. As a consequence, this would tend to ensure that only those animals which are superior in aggressiveness would mate and reproduce. Preservation of the aggressive nature of Betta splendens through selective mating would be of value in ensuring the survival and propagation of the species. In part, changes in display latency (aggressiveness) as a function of prior experience may also contribute to such an effect.

Conclusions

The preceding study investigated the effects of defeat and victory on the subsequent aggressiveness of male Siamese fighting fish as measured by display latency. In addition, an attempt was also made to determine if such treatment effects would influence concentrations of norepinephrine, dopamine and serotonin in the neural tissue of these subjects. The results of these investigations have led to several conclusions.

1. It is apparent that the aggressiveness of Betta splendens can be modified by exposing test subjects to a form of prior social conditioning in which an encounter is either won or lost.

2. Such changes in aggressiveness can be accounted for by various explanations including conditioning, fear induction, response interference, conflicting approach - avoidance tendencies and physiological stress effects. The present study favours an approach which combines various aspects of each of these interpretations. For example, a conditioning approach suggests that subjects may learn that a victorious subject is to be feared, whereas a loser is not to be feared. The induction of fear as a function of losing can interfere with the response of attacking during testing resulting in increased display latencies. Conversely, the absence of fear as a function of winning combined with the reinforcing effects of combat may lead to a decrease in display latency. However, the arguments of the present study extend this conclusion by suggesting that learning and fear may alter the extent to which test subjects will approach (attack) or avoid (flee) aggression eliciting stimuli. It is the balance of both approach and avoidance tendencies which may determine the latency within which an aggressive display will occur. In this manner, the latency to engage in display may serve as a behavioural index of the conflict between approach and avoidance.

3. Furthermore, the presence of physiological changes (dopamine) were considered to be associated with the conflicting tendencies of approach and avoidance.

Although serotonin and norepinephrine were not found in the present study, dopamine changes were possibly related to stress effects. Dopamine changes were also seen following social exposure to high aggressive stimulus animals. However, the relationship between these neurochemical changes and behavioural changes found in the same animals was not clear because no correlation was found between dopamine levels and latency scores in individual subjects.

4. The results which show that defeat decreases aggressiveness and victory increases aggressiveness, support similar findings observed in other mammalian and avian species. The present study, however, also shows that changes in the aggressiveness of Siamese fighting fish cannot readily be attributed to sample bias, lack of stimulus constancy, fatigue or social exposure to fight combatants.

FOOTNOTES

1. This dissertation was completed during the tenure of a National Research Council Postgraduate Scholarship at Carleton University, Ottawa, Ontario, Canada.
2. In order to permit the experimenter to accurately record the duration of fights from the insertion of the test subject until its removal, the 4 fights per day were run consecutively. However, the order in which the 4 high aggressive subjects defeated test subjects was counterbalanced over days using Latin Square orders. No systematic bias could therefore occur between groups as a function of the order in which fights occurred per day.

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Appendix J

Summary of the analysis of variance for the effects of victory on dopamine concentration.

SOURCE	SS	DF	MS	F	P less than
EXPERIMENTAL CONDITIONS					
DAYS	2.6227	2	1.3113	.17	.844
	37.987	3	12.662	1.64	.196
EXPERIMENTAL CONDITIONS X DAYS	22.765	6	3.7941	.49	.809
WITHIN CELLS	277.13	36	7.6982		

$p < .002$ ***
 $p < .01$ **
 $p < .05$ *

Appendix B

Summary of the analysis of covariance for the effects of defeat on the latency to display.

SOURCE	SS	DF	MS	F	P less than
WITHIN CELLS	1.264	35	.036		
REGRESSION	.829	1	.829	22.955	.001 ***
EQUALITY OF REG. (NUM)	.339	11	.031		
EQUALITY OF REG. (DEN)	.925	24	.039		
EQUALITY OF REGRESSION (F TEST)				.799	.640
EXPERIMENTAL CONDITIONS	.382	2	.191	5.290	.010 **
DAYS	.040	3	.013	.365	.781
EXPERIMENTAL CONDITIONS X DAYS	.077	6	.013	.355	.902

P < .001 ***
P < .01 **
P < .05 *

APPENDIX C

Means and Standard Deviations for Latency
and Dopamine Data of Experiment 1

		LATENCY		DOPAMINE	
(Reciprocal adjusted data)					
Expt. Cond.	Days	Means	S. D.	Means	S. D.
D	1	.190	.081	.357	.282
D	2	.229	.096	.300	.213
D	4	.341	.073	.357	.183
D	8	.332	.096	.337	.197
SDC	1	.371	.131	.250	.261
SDC	2	.501	.379	.257	.144
SDC	4	.490	.310	.257	.311
SDC	8	.422	.203	.422	.289
DC	1	.491	.287	.507	.182
DC	2	.495	.331	.490	.626
DC	4	.451	.263	.972	.472
DC	8	.472	.330	.542	.442
Homogeneity of variance		C = .2055 *		C = .2651 *	

$p > .05 *$

APPENDIX D

Sequential Fighting Behaviour of Male Siamese Fighting Fish

Behaviour class	Type	Description
THREAT DISPLAYS	Gill cover erection	characterized by extension of gill covers at right angles to the body, frontal display occurs within 4-6 cm of the opponent
	Fin erection	simultaneous to gill cover erection, dorsal, ventral, anal and caudal fins become fully erect and spread to greatest extent.
	Lateral display	both combatants then turn parallel to each other in head to head or head to tail positions, gill cover erection is usually not present.
	Tail beating	vigorous tail beating movements now develop, directing pressure waves against the lateral surface of the conspecific.
ATTACK BEHAVIOURS	Biting	subsequent to tail beating subjects turn and attempt to bite the fins or lateral side of the conspecific body producing a tattering of fins effect.
	Mouth-locking	subjects may then engage in a mutual mouthlocking, which involves a vigorous tugging and twisting about the long axis of the body while slowly sinking to the bottom.
SUBMISSIVE POSTURES	Fleeing and/or vertical orientation	defeated subjects typically flee the encounter and remain motionless in a corner and/or assume a vertical head up orientation near the surface. dominant subjects retain their fight coloration.

Appendix E

Summary of the analysis of covariance for the effects of defeat on dopamine concentration.

SOURCE	SS	DF	MS	F	P less than
WITHIN CELLS	3.763	32	.118		
REGRESSION	.030	1	.030	.255	.623
EQUALITY OF REG. (NUM)	1.405	11	.128		
EQUALITY OF REG. (DEN)	2.357	21	.112		
EQUALITY OF REGRESSION (F TEST)				1.138	.382
EXPERIMENTAL CONDITIONS	.949	2	.474	4.036	.027 *
DAYS	.192	3	.064	.544	.659
EXPERIMENTAL CONDITIONS X DAYS	.515	6	.086	.730	.631

P < .001 ***

P < .01 **

P < .05 *

Appendix F

Summary of the analysis of variance for the effects of defeat on dopamine concentration.

SOURCE	SS	DF	MS	F	P less than
EXPERIMENTAL CONDITIONS					
DAYS	.96336	2	.48168	4.19	.024 *
	.21608	3	.072026	.63	.603
EXPERIMENTAL CONDITIONS X DAYS	.45926	6	.076544	.67	.677
WITHIN CELLS	3.7923	33	.11492		

$P < .001$ ***

$P < .01$ **

$P < .05$ *

Appendix G

Summary of the analysis of covariance for the effects of victory on the latency to display.

SOURCE	SS	DF	MS	F	P less than
WITHIN CELLS	.592	35	.017		
REGRESSION	.248	1	.248	14.667	.001 ***
EQUALITY OF REG. (NUM)	.107	11	.010		
EQUALITY OF REG. (DEN)	.485	24	.020		
EQUALITY OF REGRESSION (F TEST)				.484	.895
EXPERIMENTAL CONDITIONS	.115	2	.057	3.395	.044 *
DAYS	.070	3	.023	1.387	.262
EXPERIMENTAL CONDITIONS X DAYS	.186	6	.031	1.832	.121

P < .001 ***

P < .01 **

P < .05 *

APPENDIX H

Means and Standard Deviations for Latency
and Dopamine Data of Experiment 2

Expt. Cond.	Days	LATENCY (Reciprocal adjusted data)		DOPAMINE	
		Means	S. D.	Means	S. D.
V	1	.598	.314	1.080	0.443
V	2	.486	.132	1.417	1.820
V	4	.348	.144	4.802	4.182
V	8	.377	.158	3.262	2.533
SVC	1	.388	.086	2.095	0.947
SVC	2	.346	.168	3.292	3.590
SVC	4	.285	.054	4.255	4.082
SVC	8	.418	.055	2.035	0.100
VC	1	.363	.116	3.590	3.594
VC	2	.258	.085	1.870	1.529
VC	4	.427	.216	4.142	3.948
VC	8	.311	.096	3.250	1.915
Homogeneity of variance		C = .3520 *		C = .1893 *	

p > .05.*

Appendix I

Summary of the analysis of covariance for the effects of victory on dopamine concentration.

SOURCE	SS	DF	MS	F	P less than
WITHIN CELLS					
REGRESSION	268.553	35	7.673		
EQUALITY OF REG. (NUM)	8.579	1	8.579	1.118	.298
EQUALITY OF REG. (DEN)	93.793	11	8.527		
EQUALITY OF REGRESSION (F TEST)	174.760	24	7.282		
EXPERIMENTAL CONDITIONS	2.460	2	1.230	1.171	.356
DAYS	34.613	3	11.538	.160	.853
EXPERIMENTAL CONDITIONS X DAYS	21.530	6	3.588	1.504	.230
				.468	.828

P < .001 ***
P < .01 **
P < .05 *

Appendix J

Summary of the analysis of variance for the effects of victory on dopamine concentration.

SOURCE	SS	DF	MS	F	P less than
EXPERIMENTAL CONDITIONS	2.6227	2	1.3113	.17	.844
DAYS	37.987	3	12.662	1.64	.196
EXPERIMENTAL CONDITIONS X DAYS	22.765	6	3.7941	.49	.809
WITHIN CELLS	277.13	36	7.6982		

$p < .001$ ***
 $p < .01$ **
 $p < .05$ *

END

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FIN