Effects of UCS Habituation on the Conditioned Aggressive Display in Betta Splendens

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EFFECTS OF UCS HABITUATION
ON THE CONDITIONED AGGRESSIVE DISPLAY
IN BETTA splendens

by

Timothy G. Plaska

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
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Timothy G. Plaska
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INTRODUCTION

Learning has been broadly defined by Hilgard and Marquis (1961) in terms of a range of processes usually resulting in a more or less permanent change in behavior occurring as a result of practice. Conditioning is one phenomenon usually discussed as a learning process, and generally refers to two methodological procedures; classical conditioning, derived from the work of Pavlov, and instrumental conditioning, stemming from Bechterev and Thorndike. The basic distinction between these two types of learning has been viewed by Hilgard and Marquis in terms of the consequences of the conditioned response. In classical conditioning the sequence of events is independent of the subject's behavior, while in instrumental conditioning rewards and punishments are made to occur as a consequence of the learner's response or failure to respond.

Classical conditioning, as originally discussed in the experimental investigations of Pavlov (1927) was viewed as the establishment of new stimulus-response relationships. These new relationships occurred when a stimulus which did not ordinarily elicit a particular response was repeatedly paired with a stimulus that did ordinarily elicit the response. After a number of such pairings, a response similar to that elicited by the second stimulus generally came to be elicited by the first.

More recently Denny and Ratner (1970) also explained learning processes such as classical conditioning, which generally result in...
an increase in responding, as the building of new associations between stimuli and responses. Other processes resulting in a loss of responding, such as extinction, were explained by them as the organism's learning of competing responses, or learning not to respond. Ratner (1971) described habituation as an example of such a learning process and defined the phenomenon as a change in responding, usually a reduction, associated with repeated presentations of a stimulus. For example, Ratner and Gardner (1968) stimulated an earthworm with a pulse of light and observed the organism's rapid contraction of one to two inches on the first trial decrease to a weak contraction on the twentieth trial and finally to no response at all on the fortieth trial. Figure 1, reproduced from Ratner's analysis of habituation (1970), shows two components of the response of earthworms to vibration and illustrates how habituation across-trials involves a change in response to a stimulus.

Harris (1943), in a review of the earlier literature, operationally defined habituation to be "response decrement as a result of repeated stimulation" (p. 385). Thompson and Spencer (1966) reported that the only authority to diverge from Harris' characterization of habituation was Thorpe (1956) who regarded it as a "relatively permanent waning of a response as a result of repeated stimulation" (p. 54). Although several authors have suggested a distinction between "short-term" and "long-term" habituation processes (Sharpless and Jasper, 1956; Sokolov, 1955), Harris' definition has gained widespread acceptance as a general description of the procedure.
Figure 1

Habituation curves for two components of the response of earthworms to a vibratory stimulus (Adapted from Gardner, 1966).
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Thorpe clarified the process by separating habituation changes from changes associated with muscle fatigue and sensory adaptation by defining habituation as "the relatively persistent waning of the response as a result of repeated stimulation which is not followed by any kind of reinforcement" (p. 74). The phrase "relatively persistent waning" differentiated the process from fatigue and adaptation, both of which may occur when a stimulus repeatedly elicits a response, and "not followed by any kind of reinforcement" differentiated the process from those of classical and instrumental conditioning in which changes of responses to stimuli may also occur.

Habituation can be demonstrated by use of a relatively simple procedure. A stimulus which is said to originally result in an unconditioned response is repeatedly presented to an organism a number of times and its response to that stimulus is recorded. Habituation is said to have occurred when the presentation of the stimulus no longer leads to the response. Thorpe (1963) illustrated how experimenters have shown habituation with species of every phylum, and Thompson and Spencer (1966) summarized various parametric relationships between stimulus and training variables characterizing behavioral habituation in intact organisms. Ratner (1971) further illustrated the process as one involving (1) modification of a response from repeated presentations of a stimulus, (2) generality of this modification across all species of a phyla, (3) stable individual differences within the species when other conditions are constant, (4) a general process of behavior
modification that is not explainable in terms of fatigue or adaptation effects and may involve changes in the CNS for species in which this is possible, (5) stimuli for habituation which involve novel stimuli for an organism, and (6) many variables and processes associated with learning which are also associated with habituation.

Lorenz (1965) discussed the adaptative significance of habituation as "the elimination of the organism's response to often recurring, biologically irrelevant stimuli without impairing its reactions to other," and suggested, as did Thorpe (1963), that habituation is probably the oldest phylogenetic process for modifying an organism's behavior. Although a number of investigators (Harris, 1943; Hinde, 1960; Thorpe, 1963; Thompson and Spencer, 1966; Ratner, 1967) have assumed it to be a learning process, or more generally a process of behavior modification, others such as File (1973) suggested that empirical support for this assumption is still needed.

Kimmel and Goldstein (1967) applied the term habituation to "temporary decrements in response potential following repeated elicitation which are not due to changes in the physiological state of either the receptor (sensory adaptation) or effector (fatigue) organs," and concluded that there is something less than "temporary" about the process (i.e., there is something "learned" in habituation). Thompson and Spencer (1966) have also contended that if a common denominator definition of learning such as "change in behavior under conditions of practice" is adopted, habituation must be included as an aspect of learning.
Denny and Ratner (1971) have pointed out that since the concept of learning in general usage implies the building of associations between stimuli and responses, habituation on first examination does not seem to be a learning procedure. Eliciting a response by the repeated presentation of a single stimulus does not suggest an associative process. Furthermore habituation procedures usually lead to a decrease in some class of responses. However, other aspects of learning such as extinction also involve a decrease in some class of responses, and the fact remains that habituation procedures lead to behavior modification as a result of repeated trials, and generally such modification is at least a part of what is meant by learning.

Although early experimenters such as Harris (1943) regarded habituation as a simple form of learning, little empirical support was provided for their assumptions. File (1972) discussed how many of the initial physiological investigations of habituation highlighted the difficulties in distinguishing the process from fatigue due to the large number of stimulus presentations and the rapid repetition rates needed to obtain response decrements (Wickelgren, 1967; Sharpless and Jasper, 1956) and led to Horn's (1967) serious questioning of whether habituation was, in fact, a form of learning.

The results of several recent studies have again suggested a return to the position that habituation should be regarded as a type of learning (Miller, 1967; Kimmel and Goldstein, 1967; Bishop and Kimmel, 1969). Empirical support for this position is provided by the studies of Barret and Ray (1969), who concluded that the acquisition
of habituation involves processes similar to the acquisition of a learned response. Blanchard, Shelton and Blanchard (1970) reported evidence suggesting that habituation involves storage of information, and it is therefore unlikely that it depends on any short-term change such as fatigue. The results of other experimenters investigating the specificity of habituation also support the position of a learning process. Miller and Murray (1966) reported habituation to be specific to the stimulus frequency used in presentation trials, and File and Russell (1972) found specificity for the stimulus modality, frequency and melody. File (1973) also found habituation to be specific to both the stimulus duration and intensity. Peeke and Veno (1973) also reported that habituation was highly specific to the stimulus used, and furthermore, that it could also be influenced by changing geographic cues, such as the location of the stimulus, within the apparatus used.

Horn (1967) suggested that habituation can be accounted for by neural fatigue, and one difference between habituation and learning processes which has frequently been emphasized in the literature is that habituation is a relatively temporary change in behavior while learning is relatively permanent. Contrary to these positions are the results of a number of studies indicating that habituation may involve a relatively long-term storage of information. Ratner (1971) discussed how retention of orienting components generally persists for more than one day. Van Deventer (1967) habituated contraction responses of planaria to a tactile stimulus and reported retention of habituation for 96 hours. Gardner (1968) found similar retention
using a vibratory stimulus, and Hinde (1960) reported retention of more than a week after eliciting orienting components of a bird's reaction to predators. Denny and Leckart (1965) found that rats in a maze retained habituation of approach responses for at least a week, and File (1973) measuring the orienting response in rats interrupted by a tone presentation reported considerable retention of habituation for twelve days.

Thus, evidence exists suggesting habituation to be specific to the stimulus modality, frequency, duration, intensity, melody and geographic cues. This stimulus specificity, as well as the suggestions of long-term retention, certainly supports positions regarding habituation as a form of learning, but little is currently known concerning the relationships between habituation and other learning processes.

Practically all studies of learning involve habituation to novel stimuli, such as equipment and procedures used during initial training, and experimenters have used habituation to explain the results that arise from repeated presentations of a stimulus. For example, it has been shown that habituation trials with a stimulus retards subsequent conditioning when that stimulus is used in the procedure (Carlton and Vogel, 1967; Siegel, 1968). Ratner and Stein (1965), as well as Kimble and Pennypacker (1963), both explained differential amounts of conditioning obtained in their experiments as a result of differential amounts of pre-conditioning habituation to the unconditioned stimulus.
While the results of these studies have reflected some effects of pre-conditioning habituation to a stimulus and have provided information of both a practical and theoretical significance to others using either conditioning and habituation procedures, only one investigation (Rescorla, 1973) has examined any effects of UCS habituation following conditioning.

Rescorla (1973) reported that habituation of the UCS following acquisition of conditioning reduced the magnitude of the conditioned response on subsequent presentations of the conditioned stimulus. Rescorla further suggested that the continued potency of the UCS is important for the continued production of a conditioned response.

The purpose of this investigation was to examine the effects of similar post-conditioning UCS habituation on a conditioned aggressive response. An additional purpose of this investigation was to observe the individual components of the response for any sequential changes during conditioning, habituation, retention and re-acquisition procedures, and to investigate any similarities observed between these various processes.
METHOD

Several investigators (Peeke, 1969; Peeke et al., 1969; Assem and Molen, 1969; Gallagher et al., 1972) have demonstrated that the aggressive responses of territorial fish (Convict Cichlids and Three-Spined Stickelbacks) will habituate as a function of exposure to live conspecific stimulus fish or to various models. A similar complex unconditioned aggressive display is elicited from male Siamese Fighting Fish (*Betta splendens*) in the presence of another male Betta. Simpson (1968) described the display in detail. Adler and Hogan (1963) and Thompson and Sturm (1965) classically conditioned components of the aggressive display using a weak electric shock and a red light as conditioned stimuli. Several studies have also presented evidence suggesting habituation of the aggressive display in *Betta splendens* (Baenninger, 1966; Clayton and Hinde, 1968; Peeke and Peeke, 1970; Shapiro and Schuckman, 1971; Figler, 1972, Melvin and Ervey, 1973), but none have investigated any relationships between post-conditioning habituation and classical conditioning, nor similarities between habituation and other processes resulting in a loss of responding such as extinction.

Thompson and Sturm (1965) reported an orderly sequential acquisition of the individual components of the display during conditioning, and an additional purpose of this investigation was to observe if similar orderly sequences occurred during other learning processes.
Experiment I

Subjects

Four adult, blue-green male *Betta splendens*, selected on the basis of a vigorous aggressive display, were obtained from a local tropical fish dealer. The fish were large "show Bettas", each approximately four inches long. All fish had begun construction of a bubble nest during an initial adaptation period, which was regarded as an index of maturity as suggested by Simpson (1968). Each fish was housed in an individual rectangular shaped glass aquarium (12 by 6 by 6 inches) located within a large wooden enclosure and visually isolated from each other by wooden partitions. Each tank was filled with four inches of conditioned tap water and cleaned and refilled each week to maximize healthy living conditions. Water temperature was maintained at 80 degrees and constant illumination was provided by two 40 watt light bulbs mounted 24 inches above the tanks. Each fish was maintained on a diet of Tetra-Min, fed once a day, which was supplemented by frozen brine shrimp.

Apparatus

Each home tank also served as the subjects' test chamber and contained a three inch diameter glass tube located in the middle of the tank. Each tube served to house a small red *Betta splendens* used as the stimulus fish and was covered by a white plastic cylinder which could be raised to expose the stimulus fish during conditioning. Each
tank also contained a standard aquarium heater and a small, square-shaped plastic bottom filter (3 by 3 by 4 inches).

Conditioning trials took place in an adjacent wooden chamber isolated from the home tank area. The test chamber contained a 4 inch thick foam rubber base on which the test tank was placed to isolate the subjects' from any extraneous cues and a solonoid apparatus used to mechanically lift the stimulus tube cover. A 25 watt red light bulb was suspended 10 inches above the top of the tank and interior illumination was provided by two 40 watt light bulbs attached to the ceiling of the enclosure. All timing and stimulus presentations were controlled by appropriate electro-mechanical equipment and each session was observed using a video-tape monitor. Response frequencies and durations were recorded on an Esterline Angus 20 pen event recorder.

Procedure

Each tank was transferred to the experimental chamber at approximately the same time each day and each subject was allowed a 10 minute adaptation period before conditioning trials began. The conditioning procedure consisted of pairing an unconditioned stimulus (UCS), a stimulus which without prior training had elicited an unconditioned response (UCR), with a conditioned stimulus (CS) which had not resulted in such an unconditioned response. As a result of such a classical conditioning procedure the repeated pairing of the CS and UCS generally results in a response to the CS which resembles the unconditioned response but which is described as the conditioned
response (CR) and comes to be elicited by the originally ineffective CS.

A delayed conditioning procedure was used in the present experiment. As illustrated in Figure 2 the CS, a red light, was presented for 10 seconds before the UCS, a 20 second stimulus fish exposure was presented. The red light overlapped with the stimulus fish presentation until both stimuli were simultaneously terminated constituting one conditioning trial. Thus the UCR of aggressive display to the stimulus fish presentation, the UCS, was repeatedly elicited and paired with the originally ineffective CS of the red light. All subjects were tested daily for ten trials programmed at random intervals varying from 60 to 120 seconds with a mean intertrial interval of 90 seconds minimizing the development of temporal discrimination. The experimenter observed the subjects in the video-tape monitor and recorded the frequency and duration of the following four display components during the initial 10 second CS presentation (the interval between the onset of the CS and the onset of the UCS): (1) fin erection, defined as a sudden increase in the distance between the subject's dorsal fin and body surface, regardless of the position preceding the stimulus presentation; (2) gill extension, a sudden increase in the distance between the distal edge of the operculum and the body, regardless of how far the operculum was to start with; (3) approach, defined as any approach to within one inch of the stimulus tube or if the subject was within one inch of the tube, any orientation towards it following the stimulus onset was scored; (4) undulation, defined as the subject
Figure 2

A diagram of the pairing and the temporal relationships between the conditioned and unconditioned stimuli which constituted one conditioning trial in this investigation.
ONE TRIAL

CS

10 sec.

20 sec.

UCS
rapidly swimming with continuous symmetrical undulating movements of the tail.

Each subject was tested for two adaptation sessions of ten trials each during which the CS only was presented using the same random inter-trial interval to establish that the red light did not originally elicit any of the display components. Conditioning trials continued on this schedule for ten consecutive daily sessions.

Results and Discussion

During the initial ten sessions the frequency and duration of each subject's aggressive display to the UCS was observed to decrease until each was consistently swimming to one corner of the tank following the red light onset and displaying while oriented away from the stimulus tube. Since the interior walls of the tank were glass, the increased illumination following the red light presentation was enabling the subjects to view their own images in the reflective surface. To control for this confounding the interior walls of each tank were covered with a thin sheet of pliable white rubber and daily conditioning trials continued. As shown in Figure 3, following the introduction of the rubber sheets on session 11, the frequency of each subject's conditioned responding continued to display variable acquisition rates between the individual components. Additionally, all four fish began to lose their previously bright body color and became relatively inactive settling to the bottom of the tank during conditioning trials. Few observable responses were elicited by the stimulus fish (which was maintained in a
Figure 3

Frequency of display components during acquisition of conditioning for each subject in Experiment I.
NUMBER OF CONDITIONED RESPONSES

FIN ERECTION

GILL EXTENSION

APPROACH

UNDULATION

TRIALS (IN BLOCKS OF TEN)
separate tank not containing any rubber) whose responding remained relatively unchanged. Three of the subjects died following this modification while the fourth slowly improved and continued to be tested for the next 18 days.

Figure 4 presents the frequency of conditioned responding for subject E-2 during the daily conditioning sessions of Experiment I which was interrupted by two interpolated UCS habituation procedures. The first habituation session was conducted following session 30. This consisted of running subject E-2 for a five hour UCS habituation session during which 150 successive presentations of the UCS was made using the same schedule as during conditioning but without the red stimulus light. Data for the last 20 minutes (approximately 15 stimulus presentations) during this period showed that the experimental subject was still responding vigorously on each UCS presentation, thus, indicating that habituation had not occurred. Since this procedure resulted in virtually no observed decrement in conditioned responding to the CS during the conditioning trials of session 31, a second habituation procedure was begun. The cover of the stimulus fish's chamber was left up after the tenth conditioning trial of session 31, and remained up for a 24 hour continuous exposure session. No response components were observed during a 20 minute observation period following this session. These data suggest that habituation of the display had occurred during the continuous exposure session. Following this 20 minute observation period, the stimulus cover was lowered and each subject allowed to adapt to this stimulus change for an additional 20 minute period.
Figure 4

Frequency of display components for subject E-2 during the conditioning sessions of Experiment I, and effects of two interpolated UCS habituation procedures which are indicated by the broken lines.
Immediately following this the ten conditioning trials of session 32 were begun. This was done to test for any effects of UCS habituation on the previously acquired conditioned response to the red light. As can be seen in Figure 4 the CS of the red light continued to be presented, paired with the UCS with the same conditioning schedule as previously used, for ten conditioning trials during session 33 to investigate re-acquisition of conditioned responding.

Following the second habituation procedure a complete loss of conditioned responding for all components was noted on the first two conditioning trials of session 32. As shown in Figure 4, the continued pairing of the CS and UCS as originally done in the first conditioning phase resulted in a gradual re-acquisition of conditioned responding over the remaining eight trials of session 32, and the ten trials of session 33, 24 hours later. This loss of conditioned responding following a post-conditioning habituation procedure supports Rescorla's finding (1973) that UCS habituation attenuates a previously established conditioned response, and is in agreement with his contention that the continued potency of the UCS is important for the continued production of a conditioned response.
Experiment II

The purpose of Experiment II was to again investigate the effects of post-conditioning habituation. The deterioration of the physical condition and behavioral responses of the subjects following the introduction of the rubber material in Experiment I suggested that possibly the material was toxic to the organisms. The apparent healthy condition of the stimulus fish who was not exposed to such material further supported this assumption. Experiment II was designed to additionally test this assumption by using a plastic material known to be non-toxic to fish as a means of controlling for the effects of interior reflections.

Many investigators using Betta splendens as experimental subjects report keeping the fish in visual isolation from each other and from their own reflections to control for the effects of display to such images. Observations in our laboratory have suggested that total visual isolation such as this results in a loss of coloration and a latency in responding when a subject is subsequently presented with a live stimulus fish as in the classical conditioning experiments reported here. Experiment II was also designed to investigate this additional variable. All subjects were isolated from each other but maintained in individual glass containers which allowed them to display to their own images. All conditioning trials were conducted in a separate test tank which eliminated any visual stimuli other than the programmed stimulus fish and red light presentations while controlling for any effects of interior reflections.
Subjects

Four blue-green adult male *Betta splendens* were obtained from a local supplier and selected using the same criteria as in Experiment I. The fish were slightly smaller than those used in Experiment I and ranged in size from 2 to 3 inches long. All fish were housed in 600 ml. Pyrex beakers filled with 400 ml. of conditioned tap water, and visually isolated from each other during the entire duration of the study. Each beaker had a galvanized wire mesh cover, and was cleaned and refilled weekly. Water temperature ranged from 76 to 80 degrees in the home tanks and illumination in the enclosed wooden chamber was provided by two 40 watt light bulbs mounted 24 inches above the containers. Each fish was maintained on a diet of frozen brine shrimp fed once a day, which was occasionally supplemented with live Tubifex worms. Either of these foods was preferred by most of the fish in this study and was vigorously consumed. Many studies report maintaining *Betta splendens* on a diet of Tetra-Min, but many of the fish used in our investigations would seldom consume such dry food as vigorously or as completely as either frozen brine shrimp or live worms.

Apparatus

Conditioning trials took place in a rectangular shaped glass test tank (12 by 6 by 6 inches) located within a square wooden enclosure adjacent to the subjects' home chamber. The conditioning tank rested on a 4 inch thick base of foam rubber to attenuate
extraneous cues. Sheets of opaque white plastic but from commercially available aquarium dividers were attached to the interior walls of the tank to isolate the subjects from their own reflections during conditioning. A one inch thick layer of white aquarium gravel covered the bottom of the tank and aided in the discrimination of the individual display components. A distinctive stimulus fish, white with red tipped fins, was housed in a 4 inch diameter glass tube located in one end of the tank and was covered by a cylinder constructed of the same plastic material used to line the tank interior. A standard aquarium heater, also covered by non-reflective plastic, was attached to one corner and maintained the temperature at 80 degrees. A 25 watt red stimulus light, suspended 6 inches above the surface of the water at the opposite end of the tank, was again presented for 10 seconds before and during a 20 second stimulus fish presentation. All timing and stimulus presentations were controlled by appropriate electro-mechanical equipment. Subjects were observed in an 8 by 10 inch mirror mounted 6 inches above the test tank. All response components were easily discriminated in the mirror and frequency, as well as duration, of the individual components were recorded using an Esterline-Angus event recorder.

Procedure

Each fish was transferred to the test tank by means of a small net at approximately the same time each day, and allowed a 10 minute adaptation period before conditioning trials began. All subjects were tested daily using the same schedule as in Experiment I. The
experimenter was seated directly in front of the conditioning tank, and while obscured from the subject's view, recorded the frequency and duration of the same four display components observed in Experiment I.

Each fish was initially tested for two adaptation sessions of ten trials each, during which the CS only was presented using the same random inter-trial interval to establish that the red light did not originally elicit any of the display components. Conditioning trials continued on this schedule until each subject's unconditioned responding to the stimulus fish was observed to decrease from the previously vigorous display to virtually no response at all following the stimulus exposure in the conditioning tank. Since each subject had been observed to rapidly approach the stimulus tube on initial presentations, and all were observed to be intermittantly struck by, or trapped under the plastic cover, it was hypothesized that the decrease in responding might reflect the effects of punishment following the elicitation of the display components by the UCS. To eliminate any punishing consequences for display activity a 4 inch high wire mesh screen was introduced to divide the tank in half. This prevented the experimental subjects from approaching any closer than 1/2 inch away from the cover. Conditioning trials were then continued for 10 additional sessions.

Results and Discussion

Although each fish remained healthy during the duration of Experiment II and no loss of coloration was noted, a decrease in both
conditioned and unconditioned responding was again observed in the experimental subjects. Figures 5 and 6 present the frequencies of the display components for each subject during each block of ten trials in Experiment II. During the two adaptation sessions no subject displayed aggressively in the presence of the red light. While a differential acquisition of the individual components was observed in three of the fish, all conditioned responding returned to zero levels before the conditioning criteria was met. The conditioning criteria used was two successive sessions during which all display components were elicited by the CS on every trial. Since the data of Thompson and Sturm (1965), as well as pilot work in our laboratory, suggested that this criterion could be met, and that the display could be brought under the control of a previously neutral stimulus by classical conditioning, the data possibly could reflect the effects of punishment. The data of Melvin and Ervey (1972) which confirmed the findings of Adler and Hogan (1963) that strong punishment completely suppressed the aggressive display in Betta splendens, as well as the re-acquisition of the conditioned responding following the introduction of the screen suggested punishment as a plausible explanation for the apparent suppressions of the behavior.
Figure 5

Frequency of display components during acquisition of conditioning for subjects E-1 and E-2 during Experiment II before and after the introduction of the wire mesh barrier dividing the tank in half, as indicated by the broken lines on the graph.
NUMBER OF CONDITIONED RESPONSES

FIN ERECTION

GILL EXTENSION

APPROACH

UNDULATION

NUMBER OF CONDITIONED RESPONSES

TRIALS (IN BLOCKS OF TEN)

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Figure 6

Frequency of display components during acquisition of conditioning for subjects C-1 and C-2 during Experiment II before and after the introduction of the wire mesh barrier dividing the tank in half, as indicated by the broken lines on the graph.
Experiment III

Experiment III was a further attempt to investigate any effects of post-conditioning habituation while specifically designed to control for any punishing consequences affecting the elicitation and conditionability of the display components. Because of the possibility that the decrease in responding could simply reflect habituation, the unconditioned responding of the experimental fish during the UCS presentation was also observed during this experiment. Frequency as well as duration of both the conditioned and unconditioned display components were recorded to determine if concurrent UCS habituation was affecting the acquisition of conditioned responding.

A recent study by Peeke and Veno (1973) presented data which indicated that while the aggressive responses in territorial fish (Three-Spined Sticklebacks) habituated, the stimulus to which they habituated was highly specific. Peeke and Veno further reported a resurgance in responding when either the stimulus fish or geographic cues within the test tank were changed. If habituation of the aggressive display in Betta splendens is as highly stimulus specific as that of Three-Spined Sticklebacks, the introduction of the wire screen in Experiment II could have been the cause of a similar resurgance in responding observed in all four subjects. Incorporating the screen into the apparatus for the entire duration of Experiment III was intended to control for any effects of changing the stimulus complex present in the test tank. An additional purpose of this experiment was to determine if habituation in Betta splendens was
similarly highly stimulus specific, and to test for retention of habituation over time.

Subjects

Four additional blue-green male *Betta splendens* ranging in size from 2 to 3 inches were used. Three of the fish were naive while the fourth had been used in a previous habituation investigation during which its extremely aggressive display had habituated to a low level when exposed to a live stimulus fish for 100 daily sessions using a fixed 30 second ITI. All subjects, including the previously habituated fish, were selected on the basis of a complete aggressive display and were maintained under the same conditions as the subjects in Experiment II. Two additional fish were eliminated from this group following their failure to consistently display to the stimulus presentation during the first three conditioning sessions.

Apparatus

The equipment used in Experiment II was utilized. Two modifications were made. The wire screen used to isolate the subjects from the stimulus cover was installed before any testing or conditioning trials were begun. The red stimulus light was also lowered to 2 inches above the surface of the water to enhance the discriminability of the CS by brightly illuminating the interior of the tank with red light during the CS presentation.
Procedure

Following tests which indicated that the red stimulus light did not originally elicit any display components, all subjects were run for ten trials daily using the same presentation schedule as used in Experiments I and II. The experimenter observed and recorded the same four components of the display during both the CS and UCS presentations. Conditioning trials continued for ten daily sessions or until a subject achieved the habituation criteria. Habituation was defined as two successive sessions during which no display components were elicited by the UCS on each of the ten trials. The two fish which had previously been randomly assigned to the experimental group were used to investigate the stimulus specificity of habituation and rehabituation processes in *Betta splendens*. The stimulus fish for this group was changed from the original white fish to a slightly larger reddish-green fish for six additional daily sessions, and then to a smaller blue fish for six sessions. The two fish in the control group were concurrently tested for retention of habituation by presenting them with the original white stimulus fish, using the same random variable-interval conditioning schedule, at intervals of one, three and seven days after the tenth habituation session. During one additional session following the seventh day retention test, these fish were presented with a new stimulus fish for ten trials using the same presentation schedule to determine if any retention observed was also stimulus specific.
Results and Discussion

Figures 7, 8, 9 and 10 present the frequency of conditioned and unconditioned responding for each fish during the various phases of Experiment III. A systematic differential acquisition of the individual components, similar to that observed in Experiments I and II, was again noted during the first phase for each subject. While fin erection and approach were the two most rapidly acquired responses, no fish achieved the conditioning criteria of two successive sessions in which all four components were elicited by the CS on each trial. A consistent trend observed for all subjects was a rapid partial acquisition of conditioned responding followed by a decrease in frequency as the number of daily conditioning sessions increased. While conditioned responding was observed to some extent in the fin erection, approach, and in three subjects the gill extension components, no conditioned undulation responses were observed in any of the fish during the first phase of the experiment.

Concurrent with the decrease, and in the case of subject E-3, the relatively large amount of variability observed in conditioned responding, a consistent trend in each subject's unconditioned responding to the stimulus fish was also noted. Observations of each subject's responding to the stimulus fish revealed a differential decrease in frequency between the individual components as the number of daily sessions increased. Gill extension and undulation decreased most rapidly for each subject. While changes in fin erection and approach responses reflected more variability in three.
Figure 7

Illustrates the frequency of both conditioned and unconditioned responses for fish E-2 during the three phases of Experiment III in which a new stimulus fish was substituted for the original UCS.
NUMBER OF CONDITIONED AND UNCONDITIONED RESPONSES

FIN ERECTION

GILL EXTENSION

APPROACH

UNDULATION

TRIALS (IN BLOCKS OF TEN)

conditioned responses
unconditioned responses
Figure 8

Illustrates the frequency of both conditioned and unconditioned responses for fish E-3 during the three phases of Experiment III in which a new stimulus fish was substituted for the original UCS.
NUMBER OF CONDITIONED AND UNCONDITIONED RESPONSES

FIN ERECTION

GILL EXTENSION

APPROACH

UNDULATION

TRIALS (IN BLOCKS OF TEN)

- conditioned responses
- unconditioned responses

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Figure 9

Illustrates the frequency of both conditioned and unconditioned responding for fish C-1 during the habituation phase and the retention tests of Experiment III.
NUMBER OF CONDITIONED AND UNCONDITIONED RESPONSES

CONDITIONING DAYS POST CONDITIONING

FIN ERECTION

GILL EXTENSION

APPROACH

UNDULATION

TRIALS (IN BLOCKS OF TEN)

conditioned responses
unconditioned responses

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Figure 10

Illustrates the frequency of both conditioned and unconditioned responding for fish C-3 during the habituation phase and the retention tests of Experiment III.
CONDITIONED AND UNCONDITIONED RESPONSES

CONDITIONING DAYS POST CONDITIONING

FIN ERECTION

GILL EXTENSION

APPROACH

UNDULATION

TRIALS (IN BLOCKS OF TEN)

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of the fish, the data suggests habituation to the UCS as a possible explanation for the decrease in each subject's frequency of aggressive display to the stimulus fish during the first phase of the experiment.

Phase two and three were designed to test for habituation by repeatedly presenting fish E-2 and E-3 with a new stimulus during each phase. Fish C-1 and C-3 were tested for retention of the phenomenon following phase one by presenting them with the original stimulus at various intervals. Fish E-2 had achieved the UCS habituation criterion after six sessions in phase one which was terminated when none of the four components were elicited by the UCS on any presentation for two consecutive sessions. Phase two, during which a larger red stimulus fish was used, began on the next day. Fish E-3, while exhibiting a similar differential loss of responding to the UCS, failed to meet the habituation criteria used and was tested for ten sessions before phase two was begun.

Figures 7 and 8 also present the frequency of unconditioned responding for fish E-2 and E-3 during the three phases of the experiment. Phase two consisted of six sessions during which the only manipulation made in either apparatus or procedure was the substitution of a new stimulus fish. This procedure resulted in a differential resurgance of unconditioned responding for three components of the display for fish E-2, and all components of the display for fish E-3. With some variability this increase in unconditioned responding generally decreased for both subjects as daily sessions continued.
Phase three during which a smaller blue fish was used as the UCS began on the next day following termination of phase two and also lasted for six sessions. Again a differential resurgance of the individual components, followed by a decrease in frequency as the number of sessions increased, was noted for both subjects.

Figures 9 and 10 also show the frequency of unconditioned responding for fish C-1 and C-3 during the first phase and the following retention tests of Experiment III. Both fish were tested for retention of habituation at intervals of one, three and seven days following the last session of phase one. The original white stimulus fish was used for the retention tests. A green stimulus fish was used for one session following the seven day retention test to determine if the retention was stimulus specific. Figures 9 and 10 illustrate the differential habituation and retention of the individual response components for each fish which was followed by a recovery or resurgance of responding when the new stimulus fish was presented, indicating that habituation and the retention of the process was specific to the stimulus used.
GENERAL DISCUSSION

The results of experiment I, which one adult male "show Betta" achieved the conditioning criterion used in this study suggested the feasibility of employing a classical conditioning procedure using a live stimulus fish as the UCS. Observations made following a 24 hour continuous exposure session during which the experimental subject and the stimulus fish were allowed visual access to each other suggested that habituation of the aggressive display had occurred. Data from this initial experiment also showed a complete loss of conditioned responding when the CS used in daily conditioning sessions was re-introduced following a post-conditioning UCS habituation procedure during which the CS was never presented to the organism. The loss of conditioned responding was followed by a gradual, sequential re-acquisition of the individual display components.

The failure of any of the other subjects in Experiments I, II or III to achieve the conditioning criteria used prevented the replication of these findings, but did result in data supporting earlier studies of habituation and conditioning in Betta splendens. Additional support was also provided for the position of habituation as a learning process. A number of interesting observations and information relevant to certain critical variables associated with the use of Betta splendens as experimental subjects were also obtained.

The results of Experiments I, II and III tend to support the
findings of Adler and Hogan (1963), and Thompson and Sturm (1965), who reported that the species-specific aggressive response of *Betta splendens* can be brought under the control of a previously ineffective stimulus by classical conditioning. The results of the present study also show that while the display is composed of several individual components, they are not equally conditionable, and are acquired under conditioning procedures in an orderly sequential manner. Thompson and Sturm (1965) reported marked differences in the acquisition rates of the individual components during conditioning, and this observation was further substantiated by the results of these experiments.

While both of the previous studies dealing with the conditioning of the aggressive display have reported rapid acquisition of conditioned responses which met the criteria used, neither has reported any difficulties due to concurrent UCS habituation as observed in this investigation. One critical difference between the present study and those previously reported appears to be the use of the live fish as the UCS, and not a mirror image such as they used.

Throughout this investigation a complex interaction between the behavior of the experimental and stimulus fish was observed. The occurrence of each subject's display appeared to be directly related to the display activity of the other fish. Simpson (1968) addressed this point and reported that in a typical aggressive encounter, the display ends when one member of a pair of fish, the loser, merely ceases to display without there having been any obvious preceeding
changes in the winner's display. The winner also stops after spend­ing a few moments displaying to a decreasingly responsive partner.

After a number of such encounters were produced, for example during the daily sessions used in this investigation, it would be reason­able to assume that rapid habituation could take place. Peeke and Peeke (1970) suggested that using a mirror image creates a situation in which an animal's behavior serves as an immediate stimulus to him­self for the precise kind of behavior he has just performed. If the behavior elicited in the animal is facilitated by having his adversary perform the same act, then the mirror image situation is one of perfect positive feedback which could be expected to increase the rate of the elicited response. This increased rate of responding would serve to facilitate rapid conditioning of the response. Con­versely then, the use of a live stimulus fish in place of the mirror image might result in a more rapid habituation of the response and disrupt conditioning. Figler (1972) reported that the degree of habituation and retention of habituation in Betta splendens is re­lated to the initial eliciting strength of the stimulus, and his data supports the contention of more rapid habituation occurring with a live stimulus. When he compared an unhabituated male fish, a mirror image, a habituated male, and a cut-out model as stimuli, he found the greatest absolute amount of habituation associated with the unhabituated male stimulus. This evidence, when consid­ered with that of studies using mirror images and reporting relatively slow or incomplete habituation of the display components (Clayton and Hinde, 1968; Shapiro and Schuckman, 1971; Figler, 1972), further
supports the proposition of greater habituation resulting from the use of a live unhabituated fish as a stimulus, and suggests an explanation for the lack of conditioning obtained in this study.

Large individual differences in responding were noted for all measures between subjects, and although individual differences are a general characteristic of the habituation process (Ratner, 1970), consistent habituation trends were observed for all subjects. Peeke and Veno's demonstration that territorial fish are highly specific in terms of the stimulus to which they habituate and can discriminate morphological cues was also supported. The consistent trend of a decrease in response frequency and duration as a function of repeated trials, followed by a resurgence in responding when a new stimulus was introduced, suggested that habituation in Betta splendens is stimulus specific.

This stimulus specificity considered with the retention data rules out the possibility of fatigue or sensory adaptation as an explanation of the consistent response decrements observed and is in agreement with Kimmel and Goldstein (1967) who concluded that "there is something less than temporary about the habituation process, i.e., there is something learned." As suggested in the literature (File, 1973; Bishop and Kimmel, 1969) such stimulus specificity as well as retention and similarities between habituation and other generally accepted processes of learning suggest that habituation involves storage of information. Such storage, as observed in the present investigation, further supports the contention of habituation as a learning process and not an example of neural fatigue as Horn
Possibly the major finding of this investigation is the fact that concurrent habituation disrupts the classical conditioning procedure and the implication that more research is needed to determine if the lack of conditioning under other circumstances can be accounted for as a result of similar effects.

A point alluded to earlier in this investigation concerning the individual differences between subjects merits further consideration. There were large variations observed between the acquisition of conditioned responding throughout this investigation, but of special interest was the variability between subjects in regards to unconditioned responding. Previous studies do not address this issue directly and fail to report or discuss in detail any inconsistencies observed in innate, or species specific responses. It appears to be the case, at least with Betta splendens, that such responses are not as consistently elicited by unconditioned stimuli as the name "instinctive" or "innate" generally implies. Such variability both between and within individual subjects must certainly affect behavioral processes under investigation and should be taken into consideration before such experimental investigations are begun.

The rapid UCS habituation during conditioning sessions in this series of experiments was also an usual and unexpected finding. Classical conditioning has been demonstrated to be an extremely lawful procedure, and concurrent UCS habituation during acquisition has not been generally reported in studies of the process. The results of this investigation suggest that replication is certainly
necessary.

Unpublished findings from our laboratory have suggested that repeated habituation procedures over a number of days, using a live fish as a stimulus, results in a more rapid re-habituation process during successive sessions. All the experimental fish used throughout this study were obtained from local pet shops where they had been maintained in individual glass containers within sight of other male "Bettas". This practice may have procided each fish with an extensive history of habituation sessions before they were ever used in these studies. This contention is somewhat supported by the observation that most male *Betta splendens* in pet shops display at a relatively low rate unless the adjacent fish are changed providing a new stimulus for display.

Any replication of these experiments should definitely control for the prior learning history of the organisms used. The use of at least one group of fish isolated from other males at an early age would suggest whether the rapid UCS habituation observed in this study was a result of such experiences.
SKETCH OF CONDITIONING APPARATUS
USED IN EXPERIMENT I
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