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Classical conditioning increases reproductive success in Japanese quail, *Coturnix japonica*

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We examined the adaptive significance of learning by determining whether classical conditioning increases reproductive success. Male and female quail received conditioning trials in which a small light (conditioned stimulus or CS) signalled access to a copulation partner. After this learning experience, pairs of subjects received a single 2-min (experiment 1) or 5-min (experiment 2) copulation test during which both the female and the male, only the female, only the male, or neither bird received the conditioned stimulus signalling access to a copulation partner. Signalling the copulatory episode for both the female and the male significantly increased the percentage of fertilized eggs that were produced and increased the efficiency of the copulatory behaviours that occurred. Presenting the CS to just one or the other sexual partner had no effect. These findings demonstrate that classical conditioning can enhance reproductive success, but the effect requires that both the male and the female be able to anticipate a sexual encounter.

Learning has been demonstrated in a wide range of vertebrate species including numerous fish, birds and mammals. Given its prevalence in the animal kingdom, it is reasonable to assume that 'learning is a phenotypic trait that has evolved' (Plotkin 1988, page 135; see also Rozin & Schull 1988). However, direct demonstrations of the adaptive significance of learning are rare in the literature. Charlseworth (1995, page 187), for example, noted that 'there are still no convincing empirical data to support the general hypothesis that cognition and learning processes specifically make a necessary contribution to any species' survival and reproductive success'. The present study was conducted to document the contribution of a particular form of learning, classical conditioning, to fertility and reproductive success in Japanese quail.

Classical conditioning is a common form of learning that involves behavioural adjustments to an impending biologically significant event or unconditioned stimulus (US), such as being bitten by a predator. Predatory attack elicits strong unconditioned responses automatically or reflexively. These defensive responses provide some protection for the prey. However, more effective defence might be possible if the prey could respond in anticipation of the predatory attack. Classical conditioning involves such learning, namely learning to anticipate an unconditioned stimulus based on an antecedent cue or conditioned stimulus (CS).

Historically, research on classical conditioning has focused on the mechanisms involved in learning to associate a CS with an US. However, Pavlov (1927) himself hypothesized that classical conditioning is of adaptive significance, stating that the conditioned response of an organism is 'directed towards the preservation of its existence' (page 8). Functional rather than mechanistic perspectives on classical conditioning are also increasingly prominent in more recent theoretical discussions (e.g. Hollis 1990, 1997; Fanselow 1997; Domjan et al. 2000; Timberlake 2001). Encouraged by these speculations, investigators have initiated empirical studies to determine how classical conditioning facilitates the interaction of an organism with an unconditioned stimulus.

In an early study, Hollis (1984) conditioned male blue gourami, *Trichogaster trichopterus*, to anticipate a territorial intruder. During conditioning trials, a brief red light (the CS) was presented just before visual exposure to an intruder (the US). For a control group, the CS and US presentations were explicitly unpaired. Following training, the barrier between the subjects and the intruder was removed, permitting direct aggressive interactions. Conditioned subjects that received this test encounter following exposure to the CS showed more vigorous defensive

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responses, delivering more bites and tail beatings, than the subjects in the control group. In a subsequent study, Hollis et al. (1995) demonstrated that this enhanced aggression carried over to subsequent encounters, even if those were not signalled by the CS. Thus, the conditioning effect was long lasting.

Suggestive evidence of the adaptive significance of classical conditioning has also been obtained in studies of sexual conditioning where a CS signals access to a sexual partner. Exposure to a sexually conditioned CS has been found to decrease the latency of copulatory interactions in male rats (Zamble et al. 1985) and quail (Domjan et al. 1986), stimulate the secretion of luteinizing hormone and testosterone in male rats (Graham & Desjardins 1980), increase sperm output in male quail (Domjan et al. 1998), and provide an advantage in sexual competition (Gutiérrez & Domjan 1996). However, none of these studies provided direct assessments of the ultimate consequence of reproduction, namely numbers of offspring produced.

The first demonstration that classical conditioning can increase reproductive fitness was provided by Hollis et al. (1997), who showed that sexual interactions that were signalled by a CS presented to a male blue gourami yielded significantly more offspring than sexual interactions in a control group that previously had the CS unpaired with a sexual US. Interestingly, the sexually conditioned stimulus also improved how the male responded to the female. There was less biting, more nest building, shorter latencies to spawn, and greater numbers of clasps. Increased fertility induced by exposure to a sexually conditioned stimulus was also recently reported in studies with Japanese quail by Adkins-Regan & MacKillop (2003). However, behavioural changes induced by the CS were not described in that report.

In the experiment by Hollis et al. (1997) only the male received the sexually conditioned CS prior to the test copulation. Adkins-Regan & MacKillop (2003) conducted separate experiments in which either the male or the female was signalled by presentation of the CS. If the ability to anticipate a sexual encounter facilitates behavioural interactions and increases the number of offspring that are produced, it is reasonable to hypothesize that this benefit would accrue to both the male and the female. Thus, signalling both of the participants in a sexual interaction should produce a greater effect than signalling just one or the other sexual partner. The present experiments were conducted to examine this prediction and to document behavioural changes that are associated with CS induced increases in fertility in Japanese quail. The choice of species was encouraged by the considerable body of evidence that is already available on sexual conditioning in Japanese quail (Domjan et al. 2004).

EXPERIMENT 1

Experiment 1 examined the number of fertile eggs that were produced as a result of a single copulatory episode involving a male and a female Japanese quail when the copulatory episode was signalled for only the male, only the female, or both the male and the female.

Method

Subjects

Thirty-six male and 36 female Japanese quail were obtained from the breeding colony at the University of Texas at Austin. At 30 days of age, males were individually housed and females were housed three to four per cage. The subjects were 4–8 months old at the time of the experiment. Lights were on from 0600 to 2200 hours to maintain the subjects in reproductive condition.

Males were selected based on a pretest for sexual behaviour. During the pretest, a sexually experienced female was placed in the male's home cage for 5 min to determine whether the male would complete the entire copulatory sequence of grab, mount and cloacal contact responses (Wilson & Bermant 1972). Females were selected for the study only if they were reliable egg layers. Males were randomly assigned to females to compose experimental pairs. Once a male/female pair was established, the subjects remained in that pair until the end of the experiment.

Apparatus

Metal 'home' cages (22.5 × 14.0 × 33.0 cm deep) were located in the main colony room. Twelve experimental chambers (71 × 122 × 122 cm deep) were located in a separate room and had plywood walls and ceilings, and a wire-mesh floor. The front of each chamber was also made of wire mesh to permit visual access for the experimenter. Each chamber was divided in half (front to back) by a plywood wall that created two equal-sized compartments, one for the male and one for the female of each pair of subjects. The middle of this wall had an opening (23 × 22 cm) that was normally blocked by a vertically sliding plywood door. Food and water were available ad libitum in both the home cages and the experimental chambers. A small green candelabra light (7 W) placed 11 cm to the right and 2.5 cm above the door separating the male and female compartments served as the CS. The CS light on the male and the female side of the door could be controlled independently.

Procedure

The experiment was conducted in three replications, with 12 male/female pairs in each. Conditioning consisted of 15 consecutive trials, one trial per day. Subjects were housed in the experimental chamber for the duration of the conditioning phase. Each trial consisted of a 30-s CS presentation for both the male and female of a pair of subjects, after which the door separating them was raised. The birds were then allowed 5 min to interact. If after 2 min neither the male nor the female crossed over to the other's compartment, the male was pushed through to the female's side. (All males crossed over on their own within four conditioning trials.) The CS stayed on for the duration of the 5-min US period. At the completion of the US period, the centre door was lowered, the CS was turned off, and the subjects were returned to their respective compartments until the next conditioning trial.