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A Method for Simulating Signal Evolution Using Real Animals

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Abstract

We studied response biases to visual stimulation using a new experimental technique. The subjects (hens, *Gallus gallus domesticus*) were confronted with several rewarding and non-rewarding patterns on a computer screen. In contrast with standard discrimination tasks the rewarding patterns were not identical and varied in a dimension differentiating them from the non-rewarding patterns. The rewarding patterns changed in response to hens' biases in selection of patterns. The aim of the study was to examine the possibility of receivers being a driving force in signal evolution. In one of the experiments a clear-cut result was obtained. During the course of the experiment the rewarding patterns became gradually more different from the non-rewarding one, a result expected from theoretical studies of the effect of response bias in signal evolution. A second similar experiment was less conclusive, with ceiling and floor effects influencing the results.

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Introduction

Attempts at understanding signal form have mainly focused on factors extrinsic to the receiver. However, recently it has been suggested that the mechanism of recognition can be biased and thereby drive the evolution of signal form (e.g. Staddon 1975; Leimar et al. 1986; Basolo 1990; Ryan 1990; Enquist and Arak 1998). Both sense organs and the nervous system/memory are involved in recognition. Recognition bias in the nervous system or in the memory formation seems to contain phenomena of a certain generality (Enquist and Arak 1998), suggesting that one should be able to make some general predictions about selection pressures on signal form. These conclusions rest on numerous studies in ethology and, in particular, in comparative psychology about how stimuli control behaviour (Mackin-

tosh 1974; Baerends 1982; Pearce 1994). A key observation is that a test stimulus
sometimes elicits a stronger response than the familiar ones. It should be noted
however, that this is not a response to the novelty or rarity per se, but a preference
which is more long lasting. In ethology this has been referred to as super normal
stimuli, whilst psychologists have talked about a 'peak shift' in maximum respond-
ing (Mackintosh 1974; Hogan et al. 1975; Baerends 1982; Pearce 1994).

The evolutionary consequences of recognition biases (e.g. peak shift) for sig-
nal evolution have been studied theoretically (Leimar et al. 1986; Enquist and
Arak 1993, 1998; Holmgren and Enquist 1999). These studies suggest that extreme
signal characters such as exaggerated morphological traits or saturated colours as
well as symmetries can easily originate, and persist, from recognition biases. (The
results were independent of whether aposematic coloration, mimicry or sexual sig-
nals were studied.) The studies depend on the assumptions made about recognition
memory formation. The memory of the receiver was either modelled by Spence's
gradient interaction theory (Spence 1937; Leimar et al. 1986) or by artificial neural
networks (see Enquist and Arak 1998). To what extent these models apply to real
animals has been debated (e.g. Dawkins and Guilford 1995; Ghirlanda and
Enquist 1998).

In this paper we try to develop a method that can provide empirical tests of
the theoretical results mentioned above. Such a method has to fulfil two require-
ments: First, real animals should be used as receivers; secondly, artificial signals
should evolve during repeated encounters with the receivers, thus mimicking evo-
lution. The technique we suggest is to confront the animal with several rewarding
and non-rewarding stimuli in an operant task. The animal is given a choice of sev-
eral rewarding stimuli (S+) that are slight variations of each other. By relating this
variation to the non-rewarding stimulus (S–) we can make predictions about, and
test for biases in, responding. The next step is to allow S+ to evolve as a conse-
quence of the animal's choice. The two experiments presented here include such
evolution. In a sense, we are repeating our earlier theoretical studies in which we
simulated the co-evolution between a signal and a recognition mechanism in a
computer, but we now replace the model of the recognition system with a real ani-
mal. In short, the aim of the present study is firstly to develop an experimental
technique and secondly to test the hypothesis about repulsion, i.e. signals evolve to
increased difference to other existing stimuli (repulsion) due to a bias in memory
formation (see, e.g. Enquist and Arak 1998).

Methods

The experimental animals were adult hens, *Gallus gallus domesticus*. They
were given free access to water at all times (except during the sessions) and free
access to food (commercial chicken pellets) after their last daily test session. They
had access to the food for 2 h. This is a very low restriction schedule, compared to
those normally used in operant conditioning experiments, which typically involves
keeping the animals at 80–90% of their ad lib body weight. In contrast, the use of
the current schedule ensures that we are able to keep the birds at their ad lib body

weight and motivated to work for food at the same time (B. Forkman unpubl. data). The birds were housed singly in battery cages with a 12-h light/dark cycle.

The birds were tested using a computer screen with infrared beams in front of it (80 × 49 beams). An Acorn Risc computer registered whenever a beam was broken. In front of the screen was a feeding tray; food was delivered by an automatic feeder controlled by the computer. Each food delivery consisted of 0.5 g of chicken pellets (the same food that the birds received in their home cages).

Experiment 1 (Column-Bar Position)

Ten birds were trained to peck at a red column, 30 × 80 pixels (100 pixels corresponds to approximately 20 mm). The column could be in any one of seven different locations on the screen. If the bird had pecked the column it was given a reward and the location of the column was changed to one of the other six locations. If the bird pecked a location that did not contain the column there was a time-out of 10 s and the column appeared again in the same location. This training was done to diminish any tendency to peck a specific location, as opposed to pecking the column. The training continued until the birds showed a pecking response that did not differ significantly from the distribution of the locations of the column. After 3 wks of training only one bird had failed to learn the task; this bird was excluded from the rest of the study.

During the testing sessions seven columns were presented simultaneously, four of them non-rewarding (S–) and three of them rewarding (S+). All columns were red and of the same size, 80 pixels high and 30 pixels wide. On each column there was a yellow band, 5 pixels wide. The non-rewarding columns had a yellow band at a fixed distance from the bottom of them, each rewarding column also had a yellow band but the position of these could change (See Fig. 1).

For half of the birds the band of the non-rewarding columns was constant at 1 pixel from the bottom. These birds started with one of the rewarding columns having a band at 1 pixel, the second column had the band at 7 pixels and the third at 13 pixels from the bottom. The distance between these bands was always 6 pixels. The condition for the other half of the birds was the mirror image of the first
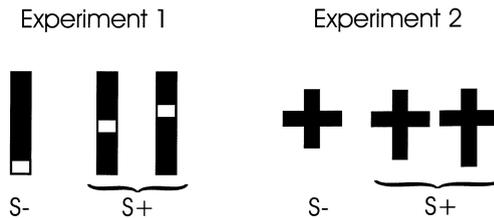
Fig. 1: The patterns used in expt 1 and expt 2. For rewarding patterns (S+) two examples are shown for each experiment. In expt 1 the position of the bar could vary and in expt 2 the length of the tail (the cross arm pointing down) varied

so that the top of the band of the non-rewarding columns was constant at 1 pixel from the top. These birds started with one of the rewarding columns having a band with a top 1 pixel from the top, the second column had the band at 7 pixels and the third at 13 pixels from the top. If the birds pecked any of the non-rewarding columns there was a time-out of 10 s, but the columns did not change places. If the birds pecked a rewarding column a buzzer sounded, the house light came on and the birds were given a reward. The house light was on for 9 s; 2 s after the house light went off the symbols were presented again.

After each rewarded trial the new position of the bands was recalculated. The position of the middle band was calculated as the mean of the position of the middle band during the last four rewarded trials plus the position of the band pecked. The positions of the higher and lower bands were then simply the position of the middle band plus six and the position of the middle band minus six.

The position of the columns was randomized, with one exception; the position last pecked was always assigned to a non-rewarding column. This prevented strong place preferences from developing.

Each session lasted 30 min, and there were two sessions per bird per day for a period of 10 d (20 sessions in total).

Experiment 2 (Cross Tail Length)

The nine birds that had completed the first task were then trained for the next task. A series of new discrimination sessions were run. In the new discrimination sessions there were two symmetrical crosses (length × width of each arm was 10 × 10 pixels), and one asymmetrical cross with the arm pointing down (the tail) twice as long as the others (i.e. 20 pixels) (see Fig. 1). All crosses were green. Each cross could occupy one of five locations.

The asymmetrical cross functioned as an S+, whereas the two symmetrical crosses were S−. Each time the bird pecked one of the symmetrical crosses there was a time-out and the crosses were shown again in the same locations. If the bird had pecked the asymmetrical cross a buzzer sounded, the house light went on and it received a reward. The crosses were assigned new, random, locations. The location that had previously contained the S+ was never used on the subsequent trial. This prevented strong place preferences from developing. Pecking outside the crosses had no effect.

The birds were trained until they had reached 70% correct responses. After 3 wks one of the birds had still not reached the criterion and was therefore excluded from further trials; this left eight birds.

In the actual experiment there were five crosses presented simultaneously, three symmetrical and two asymmetrical; the two asymmetrical crosses had tails of different lengths. When the experiment started the tails of the asymmetrical crosses were 20 pixels and 26 pixels. These lengths changed during the experiment but the difference was always 6 pixels. If the bird pecked one of the S−, there was a brief time-out (as in the previous experiment), when the crosses came back again they were in the same places as before.

If the bird pecked one of the two asymmetrical crosses a buzzer sounded, the lights went on and the birds were given a reward. Before the crosses came back on again their new lengths were calculated, and their positions randomized (with the location last pecked always containing a non-rewarding cross in the next trial).

New tail lengths were calculated along similar lines as in the previous experiment, with the new values being based on the mean from the five last rewarded trials. One cross was assigned a tail length of this mean + 3 pixels and the other a length of mean − 3 pixels. The tail of the asymmetrical crosses could never become longer than 40 pixels and never shorter than 20 pixels. The length of the arms of the symmetrical crosses was kept unchanged.

Each session lasted 30 min, and there were two sessions per bird per day for a period of 5 d (10 sessions per bird in total).

Results

Experiment 1 (Column-Bar Position)

The result of expt 1 is shown in Fig. 2. The average number of rewarded trials per session was 39. During the experiments the rewarding pattern became more and more different from the non-rewarding pattern over the 20 sessions (Spearman's rank correlation: $r_s = 0.94$, n = 21 [starting point plus number of sessions], p < 0.001). On average the position changed 1.02 units per session. All individual
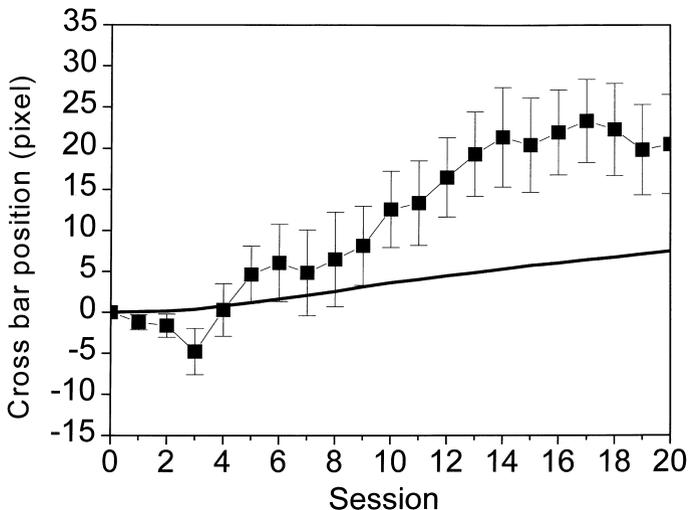
Fig. 2: Result of expt 1. The black squares show the mean bar location over all individuals in successive sessions. The thick line is the expected change given random choice of patterns

trajectories are, on average, above the starting position (one-sample t-test: t = 4.96, n = 9, p = 0.001); mean = 11.7 (SE = 4.5) compared with 0.

However, since the bar could only move within a given range we have to consider floor and ceiling effects before reaching a definite conclusion. The start position of the bar was closer to the lower limit and the non-rewarding pattern. Thus we expect the position of the bar to, on average, move away from the non-rewarding pattern even if pecking is random. We can, however, calculate the expected change due to floor/ceiling effect by simply running the program and simulating random pecking. The average position for each session based on 100 000 simulations under random choice is indicated in Fig. 2. If these values are subtracted from values obtained in the experiment we still find a significant increase in bar position over time (Spearman's rank correlation: r_s = 0.90, n = 21, p < 0.001) and all individual trajectories still lie, on average, above the mean trajectory given random pecking (one-sample t-test: t = 3.08, n = 9, p = 0.008); mean = 11.7 (SE = 4.5) compared to 4.23.

Inspection of individual curves reveals some further details (see Fig. 3 for examples). Only three curves (one illustrated in Fig. 3) show the expected gradual increase of the bar position over the sessions. Instead, most curves (n = 6) have a non-linear shape. First the bar position does not change very much then suddenly it jumps by up to 30–40 pixels in a few sessions, followed by smaller changes. In two cases there was an unexpected drastic drop in bar position in the last sessions. These results indicate that the bias in responding was not constant for a subject (defining bias as the number of responses to the most different rewarding pattern
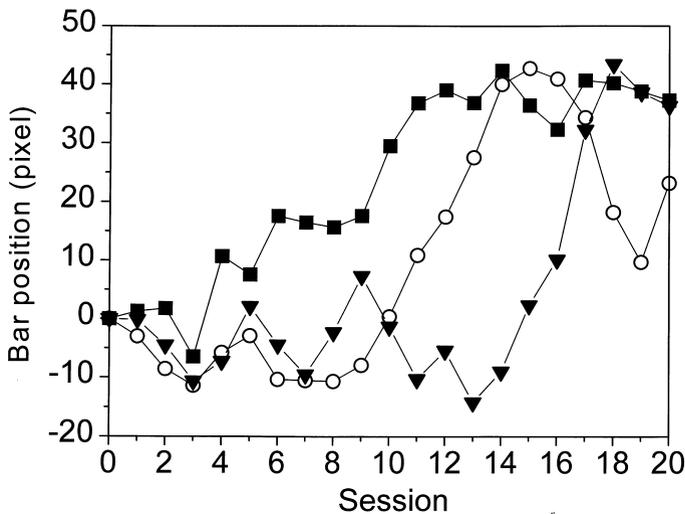
Fig. 3: Change of bar positions of three individual hens in expt 1

divided by the number of responses to the least different rewarding pattern). In sessions with a considerable change in bar position the bias was over 50%. Calculated over all sessions the average bias among subjects was 3.7% (SD = 3.8%, SE = 1.2%). Although small, this bias is significantly different from 0 (one-sample t-test: $t = 2.94$, $n = 9$, $p = 0.009$).

The percentage rewarded hits of the birds increased over testing sessions (regression coefficient = 0.63, $n = 20$, $p = 0.003$).

Experiment 2 (Cross Tail Length)

The result of the expt 2 is shown in Fig. 4. The average number of rewarded trials per session was 58. Over the 10 sessions, the rewarding pattern became more and more different from the non-rewarding pattern (Spearman's rank correlation: $r_s = 0.54$, $n = 11$ [starting point plus number of session], $p = 0.041$). On average the position changed 0.96 units per session (one-sample t-test: $t = 9.75$, $n = 8$, $p < 0.001$); mean = 31.5 (SE = 1.5) compared to 23.0 (the starting point).

This change, however, can be fully explained by floor and ceiling effects. These effects are stronger here since the range is much smaller and we start at a lower limit. The average position for each session based on 100 000 simulations under random choice is shown in Fig. 4. If these values are subtracted from values obtained in the experiment no significant increase can be detected (Spearman's rank correlation: $r_s = 0.31$, $n = 11$, ns), and individual trajectories do not, on average, lie above the average trajectory for random pecking (one-sample t-test: $t =$
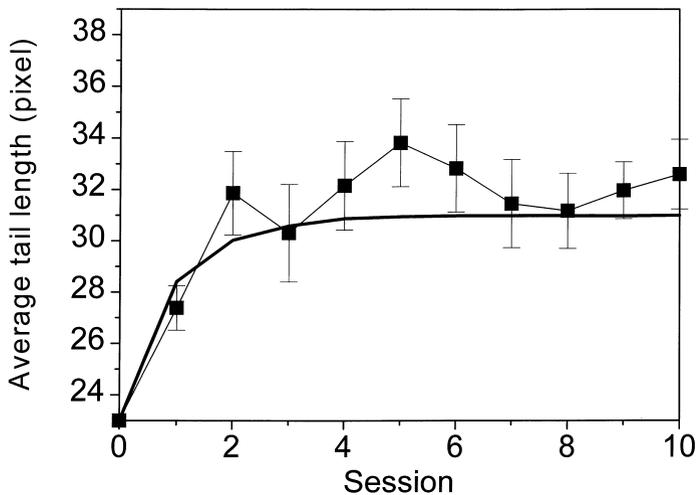
Fig. 4: Result of expt 2. The black squares show the mean location of the bar over all individuals in successive sessions. The thick line is the expected change, given random choice of patterns
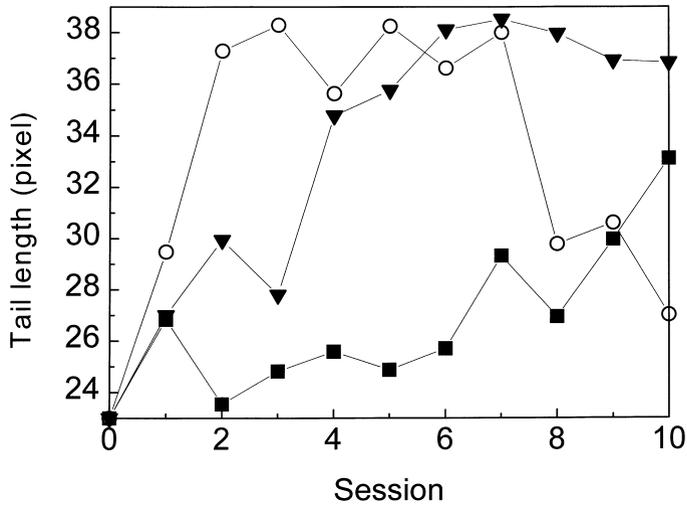
Fig. 5: Change of cross tail length of three individual hens in expt 2

1.12, n = 8, ns); mean = 31.5 (SE = 1.5) compared to 30.58 (the value of the average trajectory).

Individual curves show similar variation as in the first experiment. Sudden jumps of the tail length over a single session occur in six of the eight cases (see Fig. 5 for one example). Three curves dropped considerably in the last sessions, after having been close to the maximum value in at least four sessions (see Fig. 5 for one example). Calculated over all sessions, the average bias among the subjects is 9.3%. (SD = 15.8%, SE = 5.6%) to peck on the pattern with the longer rather than the shorter tail. This bias is not statistically significant (one-sample t-test: t = 1.65, n = 8, ns).

The percentage rewarded hits did not increase significantly over experimental sessions (regression coefficient = 0.50, p = 0.13, n = 10).

Discussion

In expt 1 the hens showed a significant tendency to select rewarding patterns that were more different from the non-rewarding pattern. This preference led, over the time of the experiment, to an increasing difference between rewarding and non-rewarding stimuli. In expt 2 the result was less clear, possibly due the strong floor and ceiling effects; however, the tendency was similar.

When receivers are faced with a discrimination problem involving a signal they will generate a 'selection pressure' on the signal to become different from

other stimuli. Although this paper focuses on the experimental technique, the patterns used in the two experiments were partly chosen on the bases of simulations carried out at our laboratory showing signal repulsion, i.e. the signal evolves to become more and more different from other stimuli (Enquist and Arak 1998). This expectation was fulfilled in expt 1 but not in expt 2.

A possible alternative explanation for the results could be that the hens had a bias for pecking the least common stimulus, rather than pecking the rewarding one. However, this seems improbable since the hens showed no decrease in the proportion of pecks to the least common stimulus, i.e. the reinforcing stimulus. In fact the reverse occurred, with the hens of expt 1 becoming significantly better with the number of trials; the same trend was found in expt 2, although not significantly so.

How can experiments of the kind described in this paper contribute to our understanding of signal evolution? While it is true that the time scale on which changes occur is very different compared with biological evolution, we believe that the same mechanisms are involved. A technical view of our experiment is to regard them as a straightforward repetition of our earlier computer simulations in which both the signal and receiver mechanisms were modelled. Here we have simply replaced the model of the receiver with a real nervous system. We think this view is very important because it can provide us with some confidence of whether the selection pressures emerging from artificial neural networks or other models of recognition are realistic and could change signals in the same directions as real nervous systems. If we had been unable to obtain the same results as in our computer simulations this would cast doubt on the realism of our simulations. However, since we got the same result in simulations and experiments it is likely that the selection pressure from the receiver would have some effect in evolution. In our experiment we observe an effect on the signal after a limited number of interactions. Similar effects ought to occur in evolution after many generations and interaction between senders and receivers.

Our experiments highlight some important considerations when designing these kinds of experiments. It is possible that the failure to detect any significant preference in expt 2 was caused by strong ceiling and floor effects, resulting from the limited range in which the pattern could vary. If one wants to develop this type of experiment further, patterns should be chosen more carefully and they must be allowed for variation over a considerable range. Furthermore, initially we had a problem with the animals developing place preferences on the screen. Routines must be added to minimize this problem.

We believe that the type of experiment we have suggested here can be developed much further both with respect to the experimental technique and biological issues. In the experiments presented here the signal evolved together with a single hen. By allowing the signal to evolve in the presence of more than one hen a more natural situation can be studied. This could also allow for more realistic mimic of real signal evolution, in particular if recognition is learned. Alternatively one could use many hens in a sequence with each hen considered as a generation in an evolutionary process.

Although motivated by an evolutionary issue the experiment may also be of interest for the mechanism of stimulus control and learning. In contrast to traditional studies of generalization and peak shift (Guttman and Kalish 1956; Mackintosh 1974; Rilling 1977; Cheng et al. 1997) our approach may be used to locate the steepest gradient in a multi-dimensional signal space, rather than following a dimension chosen by the experimenter. This method is very flexible and whereas more traditional methods usually investigate only one dimension, the current method of using a touch sensitive computer screen can easily be modified to involve several dimensions.

In conclusion, our hope is that in the absence of real evolutionary experiments, our approach should provide some confidence in the theory that the forms of signals can evolve through a recognition bias that is inherent in the nervous system.

Acknowledgements

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