

CLASSICAL HEART-RATE CONDITIONING AND DIFFERENTIATION OF VISUAL  
CS WITH AN APPETITIVE UCS IN PIGEONS

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ABSTRACT

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A tachycardic response was classically conditioned in thirsty pigeons using water as an unconditioned stimulus and localized lights as conditioned stimuli. This was successful in 6 out of 9 birds. In one experiment the subjects had their heads immobilized so that the positions of the stimuli within the visual field could be precisely specified. When the stimuli were shifted from the binocular to the monocular field and vice versa there was no initial transfer of a conditioned differentiation between a white and a green light.

INTRODUCTION

Using pigeons and an aversive UCS it was earlier found that it is easy to obtain a classical differentiation of a conditioned heart-rate response to two auditory CS differing in frequency (Delius and Tarpy, 1974). However, with the same method it proved difficult to demonstrate a differentiation of two visual stimuli differing in hue even though the same visual stimuli were easily discriminated by the same subjects in a food-reinforced instrumental paradigm (Delius and Emmerton, 1978). On the basis of additional instrumental conditioning evidence (LoLordo and Furrow, 1976), it was surmised that difficulty with the visual stimuli was not due to the use of the classical heart-rate conditioning paradigm as such but rather to the employment of a painful UCS. This was interpreted within the framework of learning constraints of phylogenetic origin related to the fact that, while in nature colour cues can contribute markedly towards the elaboration of efficient foraging behaviour, they can not substantially support adaptive predator-avoidance behaviour in a species like the pigeon.

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To test this hypothesis an effort was made to devise a Pavlovian heart-rate conditioning technique using an appetitive UCS. At the same time there was interest in developing a discrimination method which would allow perimetric presentation of visual stimuli. This would enable a systematic study of the suspected functional inhomogeneities of the visual field of pigeons. Their retinae evince regional morphological differentiations that must be expected to affect the transduction and processing of the visual image. Some behavioural evidence to that effect is already extant (Blough, 1979, Martin and Muntz, 1979, Bloch and Martinoya, 1982, Delius et al., 1981, Goodale and Graves, 1982).

#### METHODS

Eleven adult homing pigeons (Columba livia) of local origin were used. Under chloral hydrate-pentobarbital anaesthesia they were implanted with four chronic ECG electrodes made of hard stainless steel wire (3 strands, each 0.13 mm in diameter). The electrodes were looped under the scapulae or the pubic spines with the aid of a thick curved cannula and led externally to the back of the subject through short lengths of vinyl insulation sleeving. Here they terminated on a four-pole miniature connector. The four pigeons used in experiment II were additionally equipped with a small rectangular brass block (4x5x3 mm) with a tapped hole, cemented to the skull in a standardized position (see Mallin and Delius, 1983).

Conditioning began after a few days' recovery. Before each experimental session the animals were deprived of water for 47 hours. They were then secured in a closely fitting cloth sack with the head protruding through an opening and placed on a foam-rubber bed within a brightly lit sound-insulating chamber. With the aid of a miniature plug the left scapular and right pubic electrodes were connected to the inputs of a differential ECG amplifier, the right scapular electrode serving as an indifferent. In the first experiment the subject's head was not restrained. In the second experiment the animal's head was firmly held in a standard position by attaching the headblock to a slotted arm with a small knurled screw. A miniature fountain was placed under the pigeon's beak, such that when an electromagnetic valve opened, the pigeon was offered a drink of about 0.1 ml water coming from an elevated reservoir. Any remaining water was automatically removed

after 2 s with the aid of a suction line controlled by a separate electromagnetic valve. While the water was available, the fountain was illuminated by a clear miniature light bulb. Stimuli were provided by a two-colour (white, broadband green) illuminator with a 6 mm diameter frosted exit pupil, placed in selected positions within the visual field of the subject with an adjustable stand.

The sequence of events within a session was controlled by modular digital programming equipment. Conditioned stimuli were presented for 12 s, the CS+ always terminating simultaneously with the end of the 2 s water UCS. The sequence of the 30 CS+ and 30 CS- presented per session was determined by quasi-random sequences (Fellows, 1967). The interstimulus interval was 48 s. Heartbeats, converted to pulses by a trigger stage, were counted during 10 s baseline periods preceding each CS period and during the first 10 s of each CS period. The counts corresponding to each period were printed along with codes identifying the baseline, CS+ or CS- condition that pertained. To assess the discrimination performance the net heart-rate change between each baseline period and the following CS period was calculated. The average signed difference between the changes associated with the CS- (which were generally close to zero) and the CS+ over a session were taken as an index of discrimination (see Delius and Tarpy, 1974). During selected sessions the ECG was also recorded continuously with a pen-recorder, with marker channels identifying the various counting periods.

## RESULTS

In preliminary sessions with two pigeons it was found that the water UCS tended to generate, apart from drinking, a heart-rate increase as a UCR, particularly if the pigeons were water deprived for two days rather than one day. Furthermore the white light CS accompanying the water UCS reliably induced a CR heart-rate increase when presented alone after five conditioning sessions.

Experiment I was performed with 5 new subjects for 16 sessions. Two birds had to differentiate between a green CS+ and a white CS-, the other three learned the reverse discrimination. The animals were free to move their heads but mostly they held them so that their eye-beak plane was about 30° from the horizontal, with the beak pointing downwards. The stimuli were presented at the mid-line plane, about 30° below the eye-beak plane with reference

to the midpoint between the eyes. This position is within the estimated binocular field of vision of the pigeon (Delius et al., 1981) and about 40 mm away from the eyes. As conditioning proceeded some birds began to peck at the CS+ (autoshaping, Jenkins and Moore, 1973). Fig. 1 presents the results relating to heart-rate. Three of the 5 pigeons showed definite evidence of differentiation. During the last 9 sessions their heart-rates corresponding to the CS+ were significantly higher than those

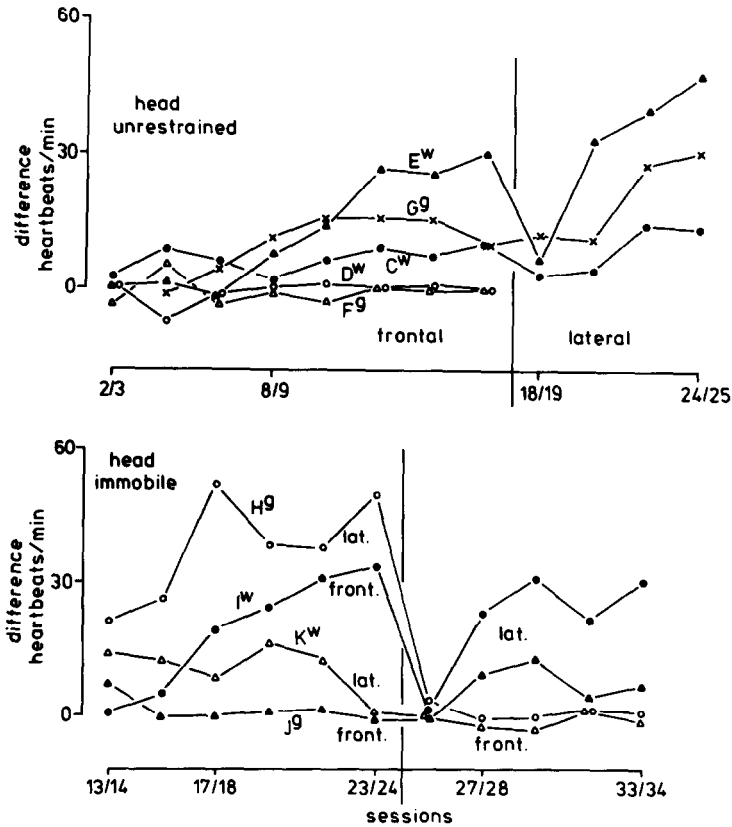


Fig. 1. Discrimination learning curves for the 5 pigeons in experiment I (top) and the 4 pigeons in experiment II (bottom). Combined means of pairs of sessions, some initial sessions have been omitted to save space. Capital letters identify the subjects; g: green CS+, w: white CS+; frontal, front.: frontal binocular stimulus presentation; lateral, lat.: lateral, monocular stimulus presentation; vertical line: stimulus position change.

corresponding to the CS- (Wilcoxon tests,  $p < 0.01$  in each case). For these 3 birds the stimuli were then shifted to a position 50 mm from the eye in the upper lateral monocular field  $100^\circ$  to the right of the midline plane and  $50^\circ$  above the eye-beak plane. There was initially a marked weakening of the differential CR but within 5 training sessions the response returned fully.

A second experiment was run with 4 new subjects that had their heads fixed in a standard position during the conditioning. Two subjects began with the stimuli presented in the frontal binocular field, the other two began with the stimuli located in the upper lateral monocular field of the right eye, both locations as specified above. For one subject in each group the green light was the CS+, for the other the white light, the converse colour being in each case the CS-. Fig. 1 shows the results. Three birds learned the differentiation (Wilcoxon tests based on the last 8 sessions, in each case  $p < 0.01$  or better) but one pigeon (frontal stimuli) did not.

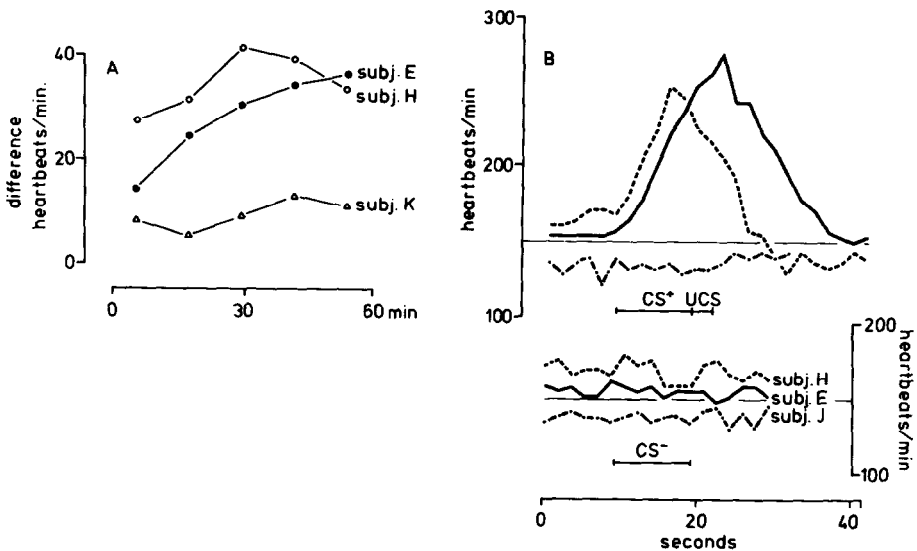


Fig. 2. A: mean differential heart-rate response as a function of the progression of blocks of trials within a session, 3 examples (see text for details). B: mean heart-rate before, during and after CS-, and CS+/UCS presentation, 3 examples (see text for details).

When the stimuli were switched from the frontal to the lateral position for one half of the birds and vice versa for the others, none of the animals showed immediate transfer. With further training sessions the two pigeons for which stimuli were shifted from frontal to lateral positions showed significant discrimination, even though one of them had not discriminated during the initial training (Wilcoxon tests, last 8 sessions, in each case  $p < 0.01$  or better). The two subjects that were switched from lateral to frontal stimulus presentation did not regain discrimination within the 10 sessions.

A finer grain description of the performance during differentiation is afforded by the course of the heart-rate differentiation within sessions. 10 post-acquisition sessions, each pertaining to 3 animals selected from experiments I and II, were divided up into 5 successive blocks, each containing 6 CS+ and 6 CS- periods, and the mean heart-rate differences obtained in each block were noted. These were averaged across the sessions separately for each subject. Overall there is a tendency for the heart-rate difference to increase within each session but the individual variability is marked (Fig. 2 A).

To assess more closely the nature of the CR, average time courses of the heart-rate around the CS+/UCS and CS- presentations were computed from the continuous ECG records obtained from 3 subjects during one session in experiments I and II, including records from one subject that did not discriminate. Fig. 2 B shows these functions. It is clear that the CS- presentations had no effect on the heart-rate. In the subjects that discriminated, the CS+/UCS combination produced a prompt and marked CR that merged with the UCR to the water presentation. We did not record the UCR to UCS presentations alone. The subject that did not discriminate obviously did not show a CR but neither did it evince a UCR. Informal observations suggested that this may often have been the case when birds did not show differentiation: the subjects either did not show a UCR right from the beginning or they habituated before a CR developed. The CR/UCR combination seemed quite resistant to habituation. This issue, however, requires more detailed investigation. It is clear, though, that the heart-rate response was only a component of a more general arousal or orienting response. This was particularly obvious in experiment I where the pigeons could exhibit motor activity. The heart-rate CR was often associated with vigorous head movements,

the UCR with frantic mandibulation and drinking movements.

#### DISCUSSION

We conclude that an appetitive conditioning of heart-rate is possible in the pigeon. Furthermore, this again confirms the plasticity that seems to characterize the mechanisms controlling the cardiac rhythm in birds (Cohen, 1980, Sieland et al., 1981). In at least a proportion of the individuals, a differentiation of colour stimuli presented no particular problems. This contrasts with the difficulty of obtaining such a discrimination with heart-rate conditioning using aversive stimuli which we observed in our earlier study (Delius and Emmerton, 1978). The present results support the notion that species-specific proclivities of phylogenetic origin may determine the associability of different stimuli. In the natural environment it is unlikely that hue can be a useful predictor of pain whereas it certainly is of water.

The poor transfer of discrimination when the stimuli were shifted from one visual field locus to another is remarkable. It supports other evidence that such intraocular transfer presents problems for pigeons (Mallin and Delius, 1983). This may be due to the local retinal differentiations and corresponding central specializations that characterize the pigeon's visual system (Emmerton, 1983). It is also possible that the birds quite specifically associated the absolute spatial position of the CS with the water UCS and that better transfer would have been obtained if the subjects, rather than the stimuli, had been shifted to a new position. Be that as it may, the conditioning procedure clearly holds promise for perimetric studies of the pigeon's vision.

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