6. Effect of repetitive visual stimuli on behaviour and plasma corticosterone of European starlings

6.1. Summary

Fluorescent lighting typically flickers at either 100 Hz (Europe) or 120 Hz (U.S.A.). Such lighting is known to cause adverse psychological and physiological effects in some humans. Less is known about whether other vertebrates suffer such effects. Previous research, showing high critical flicker fusion frequencies in birds, suggests that birds may be more at risk of adverse effects from conventional fluorescent lighting than humans are. Repetitive spatial patterns with particular spatial frequencies have also been demonstrated to cause unpleasant sensations in humans. I investigated whether birds react to the temporal frequency of fluorescent lamps and the spatial frequency of the visual surround in the manner predicted by the human literature. I assessed the effect of a given visual environment on European starlings, *Sturnus vulgaris*, by investigating their preferences, behaviour and plasma corticosterone. From the human literature, I predicted that high frequency lighting (HF, >30 kHz) and relatively low spatial frequencies (LSF, 0.1 cycle cm\(^{-1}\)) would be less aversive than low frequency lighting (LF, 100 Hz) and relatively high spatial frequencies (HSF, 2.5 cycle cm\(^{-1}\)). Birds had strong preferences for both temporal and spatial frequencies. These preferences did not always fit with predictions, although there was some evidence that LF lighting was more arousing and perhaps more stressful than HF, as basal corticosterone levels were higher in LF. There was no overall significant effect of my chosen spatial frequencies on corticosterone, although in line with predictions there was a non-significant trend (\(P=0.072\)) for the rate of rise of plasma corticosterone after capture to be higher in HSF than LSF. Although there are clearly effects of, and interactions between, the frequency of the light and the visual
surroundings on the behaviour and physiology of starlings, the pattern of results is not straightforward. It does nevertheless suggest physiological effects that may be of consequence when interpreting the responses of animals caged in surroundings lit with fluorescent light. Further research into the effect of repetitive visual stimuli on birds, and other laboratory animals would therefore be of interest.

6.2. Introduction

6.2.1. Flickering light: a welfare concern

Some species may be better able to detect motion and change within visual scenes than humans (for review, see D’Eath 1998). Consequently, there has been concern that images presented to animals in experiments via flickering media such as televisions may appear unrealistic to certain species (D'Eath 1998; Fleishman and Endler 2000). Also, the rate of flicker of conventional 100 Hz fluorescent lighting systems may appear stroboscopic to them. Apart from the risk that these factors may confound the interpretation of animal behaviour during experiments, flicker within visual scenes may also pose a risk to animal welfare (Sherwin et al. 1999; Maddocks et al. 2001c) if flickering lights have similar effects on birds as they do on some humans. It is also possible that birds suffer anomalous perceptual effects from looking at cage bars, as repetitive visual stimulation of certain spatial frequencies has been shown to lead to a variety of adverse perceptual and physiological effects in humans (Wilkins et al. 1984).

6.2.2. Effect of flicker on humans

In humans, the most serious effects from flickering light tend to occur only at flicker rates under 50 Hz, where it elicits a number of adverse reactions ranging from unpleasant emotions to eyestrain, headaches and seizures (Mundy-Castle 1953a;
Wilkins et al. 1984). Although humans generally cannot perceive flash frequencies above 60 Hz as flickering (D’Eath 1998), the flicker from conventional low frequency (LF, 100 Hz) lighting still appears to be a mild perceptual nuisance, as it increases high velocity eye movements (Kennedy and Murray 1991) and reduces performance on visual search tasks (Kuller and Laike 1998) in comparison with high frequency (HF, >30 kHz) lighting. Even though LF lights are above the typical human perceptual threshold, they can cause headache and eyestrain (Wilkins et al. 1989), increase heart rate and anxiety (Hazell and Wilkins 1990) and panic attacks (Watts and Wilkins 1989), although seizures from LF fluorescent light are unlikely (Wilkins 1995). Such adverse reactions are most pronounced in people suffering from conditions such as headache, migraine and photosensitive epilepsy (Mundy-Castle 1953b; Golla and Winter 1959; Wilkins et al. 1979, 1984; Wilkins 1995). However, it is clear that many ‘normal’ people without these conditions find LF fluorescent light mildly aversive (Wilkins 1995). For example office workers have been found to express unconscious preferences for HF over conventional LF lighting, and to have significantly more headaches under LF than HF lighting (Wilkins et al. 1989). That said, there seem to be considerable individual differences and these relationships only appear when a large group of subjects is tested.

**6.2.3. Effect of repetitive spatial stimulation on humans**

Perception and physiology may be affected by the spatial periodicity of objects within the visual scene, as well as by the flicker rate of their illumination. In humans, repetitive visual patterns of particular spatial characteristics can cause unpleasant perceptual effects from illusions of colour, shape and motion (Wilkins et al. 1980, 1984; Watts and Wilkins 1989). Certain visual patterns may also trigger headaches (Nulty et al. 1997), migraines (Marcus and Soso 1989), anxiety (Watts and Wilkins 1989; Hazell and Wilkins 1990) or seizures (Wilkins et al. 1980) in susceptible people. Moving patterns, for example the movement of windscreen wipers across a car windscreen or steps up an escalator, may have a similar trigger effect in some individuals. It has been postulated that these adverse effects occur as an unnatural
level of precisely repetitive sharp edges, or flickering illumination, can lead to excess excitation within the nervous system and generate abnormal EEG rhythms (see Mundy-Castle 1953b). This excessive excitation may break down cortical inhibition mechanisms within the nervous system, ultimately producing an array of unpleasant symptoms (Wilkins 1995).

The degree of unpleasantness a given pattern causes depends upon its shape, spatial frequency, contrast, size and position in the visual field (Wilkins et al. 1979, 1980; Wilkins 1995). The most visually provocative stimuli appear to be elongate or striped patterns of particular frequencies, which have long line contours with high modulation in luminance (Wilkins 1995). Wilkins et al. (1984) found that 35% of normal subjects get eye-ache, headache, tiredness or dizziness when looking at gratings of between 1 and 16 cycle deg$^{-1}$, with the problem being worst at 4 cycle deg$^{-1}$. A ‘cycle’ consists of a single pattern element, for example in a black and white striped grating, the width of a cycle equates to the width of one white plus one black bar. The ‘spatial frequency’ of the grating describes the proportion of the visual field one cycle stimulates at a given viewing distance, and is measured in cycles per degree of visual angle (Wilkins 1995). The narrower the bars in the grating, and the further the observer is from the grating, the higher the spatial frequency becomes.

### 6.2.4. Effect of repetitive visual stimulation on birds

As there is great similarity between the neurophysiology between humans and other vertebrates, it seems likely that non-human animals could suffer similar adverse reactions from repetitive visual stimulation, even though they may not have any conscious perception of stress or distress as humans do. In particular, birds are thought to have higher sensitivity to flicker than humans (D’Eath 1998) and are regularly kept under fluorescent lamps (which typically provide repetitive visual stimulation over time) in cages (the bars of which provide repetitive visual stimulation over space). Hence, it is of interest to investigate whether they find such environments aversive or stressful.
The effect of different environmental spatial frequencies on birds has not been investigated. However, there has been some research on how they are affected by LF light. Although available evidence suggests that birds may be slightly more sensitive to flicker from lights than humans are, there is some doubt as to whether sensitivity is high enough for the flicker from conventional 100 Hz lighting to be perceptible to birds (see Jarvis et al. 2002). That said, the evidence from the human literature suggests that even frequencies above those which are consciously perceptible may still be resolved by the nervous system (see Wilkins 1995). Previous research shows that both laying hens and domestic turkeys do not find LF fluorescent lighting aversive as compared to incandescent lighting, and may even prefer it (Widowski et al. 1992; Lewis and Morris 1998; Sherwin 1999). However, these experiments manipulated intensity and chromaticity as well as the frequency of the light, by virtue of the different irradiance spectra of the two types of lamp under comparison. Since fluorescent lamps emit more ultraviolet than incandescent lamps do, and have a spectral emission more closely approximating daylight, it has been suggested that the birds preferred the fluorescent lighting for its chromaticity rather than its flicker rate (Lewis and Morris 1998). Also, it is possible that domestic poultry, which are generally ground living and not fast flyers, have poorer motion perception than species that can fly fast. Species which fly in closely packed flocks may require particularly fast rates of visual information update to avoid collisions, and may react quite differently from poultry to LF light. One such candidate species is the European starling, *Sturnus vulgaris*, which is a fast flying diurnal passerine that frequently travels in flocks. Maddocks et al. (2001c) found a trend for wild starlings to have higher basal corticosterone when first exposed to LF versus HF light, where differences existed. However, this effect only occurred in the experimental blocks where the birds had been in captivity for some time, suggesting that the effect of LF lighting may only be detectable once the initial stress of capture has subsided. Starlings that have been in captivity for 9 months prefer HF to LF light when first given a choice between them (Chapter 5), although subsequent direct exposure experiments using recently caught wild starlings showed that there was no measurable difference in behaviour or corticosterone in response to HF or LF light after maintenance for two weeks in either condition (Chapter 5). This suggests that any
effect of housing under LF may be transient, which is consistent with previous research in humans showing that continued exposure to a flickering stimulus results in decreased sensitivity to that stimulus (Mundy-Castle 1953b; Brundrett 1974). However, it is also possible that recently caught captive starlings experience so much general stress from adjusting to captivity or to a change of housing conditions that any effect of treatment was obscured in the direct exposure experiments of both Maddocks et al. (2001c) and Chapter 5.

There is some evidence that in humans the flicker of a light source and the spatial frequency of the visual surround may interact. People who suffer from television-induced epilepsy experience epileptiform EEG abnormalities particularly when they are sitting close enough to the screen for the 25 Hz line interlace pattern to have a sufficiently low spatial frequency (Wilkins 1995), although that said, the light intensity and volume emitted by the television may also have some effect, as these become higher the nearer one sits to the screen. As birds are normally kept in cages or aviaries, the bars of which form a highly repetitive visual pattern, there may be some kind of interaction between the effect of viewing cage bar patterns and the type of light source they are viewed under, with cage bar gratings being more visually provocative if viewed under LF lighting than HF lighting.

I investigated whether rhythmic sensory stimulation, of both a temporal and spatial nature, affects the behaviour and plasma corticosterone of captive birds, using European starlings as a model species. I assessed the effects of HF (>30 kHz) versus LF (100 Hz) fluorescent light in visual surrounds consisting of gratings that were either of relatively high spatial frequency (HSF: 2.5 cycle cm\(^{-1}\)) or a lower spatial frequency (LSF: 0.1 cycle cm\(^{-1}\)). I chose these particular frequencies of lighting as they are the frequencies of conventional fluorescent lighting available in Europe, and hence the frequencies of lighting likely to be experienced by captive birds housed under artificial illumination. Also, as birds have been shown to prefer LF fluorescent to incandescent lighting (Widowski et al. 1992; Lewis and Morris 1998; Sherwin 1999), it was of interest to investigate whether they prefer LF to HF light sources that differ only in their flicker rate. In humans, the most aversive spatial frequencies are
those to which observers are most sensitive at threshold contrast (Wilkins 1995). I did not have data on the contrast sensitivity function of starlings, and there was also no previous research showing whether or not birds find particular spatial frequencies aversive. Consequently, as a starting point I took a comparative approach and chose to use frequencies that humans would either find aversive or not within the range of viewing distances available within my apparatus. At a viewing distance of 57 cm one cycle per cm equals one cycle deg$^{-1}$, and spatial frequency increases proportionately as viewing distance increases. Therefore, in my cages, which were almost 1 m long, I presented a maximum spatial frequency of nearly 5 cycle deg$^{-1}$ in my HSF treatment, which is in the range that would cause maximum physiological effect in humans. In my LSF treatment, the highest spatial frequency it was possible to view would be around 0.2 cycle deg$^{-1}$, which is outside the range of spatial frequencies known to have physiological effects in humans (see Wilkins 1995).

I evaluated the relative aversiveness of different frequency combinations to birds using preference tests, and assessed the degree of stress each environmental condition appeared to engender by keeping the birds in a given condition for three days and monitoring behaviour and plasma corticosterone. Corticosterone is a hormone that is known to rise when birds are under stress (Axelrod and Reisine 1984) or in situations requiring high metabolic demand (Reul et al. 2001). This may be a highly adaptive coping mechanism (Buchanan 2000) because, since corticosterone increases activity (Breuner et al. 1998) and prepares the body for situations requiring increased metabolic demand (Reul et al. 2001), it may promote escape from adverse conditions. However, prolonged elevation of corticosterone has harmful physiological effects (Wingfield et al. 1992), and therefore corticosterone levels are frequently used as an indicator of stressful conditions (e.g. Astheimer et al. 1992; Wingfield et al. 1992; Wingfield and Ramenofsky 1997; Marra and Holberton 1998; Silverin 1998; Kitaysky et al. 1999). Both the basal level of corticosterone and its rate of rise following an event such as capture, handling and restraint can be used to assess ‘stress’ as measured by the degree of arousal of and reactivity of the hypothalamic-pituitary-adrenal (HPA) axis (Wingfield et al. 1982; Wingfield 1994; Marra and Holberton 1998).
Based on the available evidence in the human literature, I predicted that maintenance under HF (>30 kHz) light would be both preferable and less stressful or arousing, than housing under LF (100 Hz) light, and that HSF visual surrounds (2.5 cycle cm⁻¹, see Fig. 1.4., p.65) would be more aversive and stressful than LSF surrounds (0.1 cycle cm⁻¹). As fluctuating light may interact with any spatial periodicity in the surfaces which it illuminates (Wilkins 1995), I also predicted that there would be an interaction between the frequency of the lighting and the spatial frequency of the environment, with LF in conjunction with HSF being more aversive than HF with LSF.

6.3. Materials and methods

6.3.1. Experiment 1: preference tests

The preferences of 12 groups of European starlings were ascertained for both HF versus LF lights in conjunction with either HSF versus LSF visual surrounds, using binary choice chambers. 132 starlings were caught as juveniles in a decoy trap under English Nature licence in July 2000, and the experiment was carried out between February and March 2001, when the starlings were about 9 months old. The birds were maintained in mixed sex groups of 12 birds. Seventy-two birds were housed in outdoor aviaries (1.8 x 0.9 by 1.8 m high, 12 birds per aviary), while 60 were housed in indoor cages (1.3 x 0.65 by 1.2 m high, 12 birds per cage) under artificial lighting. The artificial light consisted of Durotest ‘Truelite’ lamps (for irradiance spectra, see Hunt et al. 2001b, Fig. 3C) and were powered by high frequency ballasts (> 30 kHz, Crompton Lighting, Doncaster, U.K.) to ensure that there was no perceptible flicker (see D'Eath 1998; Fleishman and Endler 2000; Maddocks et al. 2001c). The photoperiod of the indoor lighting was altered weekly to match the natural photoperiod at the time throughout the experiment.

Birds were always tested in mixed sex groups of four birds (two male, two female), as the preferences of groups of a social species may be more valid than the preference of
an isolated individual (Greenwood et al. 2002, Chapter 3). Each group consisted of individuals that lived together in the same cage and were familiar with each other. This gave rise to the constraint that the housing conditions for some of the groups differed, with three groups being drawn from the outdoor aviaries and nine from the indoor cages.

The birds’ preferences were tested in two sets of binary choice chambers, that each consisted of two aluminium compartments (each 0.76 x 0.61 by 0.46 m high), which were connected by a 40 cm long, 20 cm diameter, tunnel (see Fig. 3.1. in Chapter 3, p.96). Each compartment was surrounded by lightproof material to enable the light environment on each side to be individually manipulated, and was individually illuminated from above by an 18 W, 60 cm Durotest ‘Truelite’ fluorescent lamp.

In a trial, a group of starlings was placed in a binary choice chamber in which they had a choice of LF (100 Hz) or HF (> 30 kHz) lighting. Both of these lighting conditions were experienced in conjunction with a horizontally striped visual surround consisting of either a high spatial frequency (HSF; 2.5 cycle cm\(^{-1}\)) or a lower spatial frequency (LSF; 0.1 cycle cm\(^{-1}\)) black and white grating.

There were six different possible pairings of different treatments that were presented to the birds in the two chambers of the apparatus:

1) HF+ HSF vs HF+LSF (all lights HF but spatial frequency varies)
2) LF+ HSF vs LF +LSF (all lights LF but spatial frequency varies)
3) HF+HSF vs LF+ HSF (lights vary, all patterns HSF)
4) HF+LSF vs LF+LSF (lights vary, all patterns LSF)
5) HF+LSF vs LF+HSF (lights and spatial frequency vary)
6) HF+HSF vs LF+LSF (lights and spatial frequency vary)

An independent subjects design was not possible as my research group’s English Nature licence restricted the number of birds I could use. Instead, all groups of birds experienced all six possible combinations of treatments according to a balanced
repeated measures design. The order in which each group experienced each treatment was counterbalanced using two 6 x 6 Latin squares to control for any possible order effects.

Each compartment in the choice chamber had three solid walls and had a gate at each end. The three solid walls were covered by removable panels that displayed black and white horizontal gratings that were either LSF or HSF as appropriate. The fourth wall was a door through which birds could be admitted to the apparatus and was not covered by a grating. Horizontal gratings were chosen rather than vertical gratings, as this enabled me to maximise the length of lines within the gratings since the choice chamber compartments were longer than they were high. I chose to do this as the longer the length of the line contours within the pattern, the more unpleasant humans perceive such patterns to be, with the degree of discomfort being unaffected by the orientation of the pattern (Wilkins 1995). These gratings were created in Paintshop Pro™ and printed onto white paper using an inkjet printer (Epson Stylus Photo 870, 1440 d.p.i.). The gratings were laminated for ease of cleaning, and glued to the removable panels. Both chamber compartments contained food, water and perches.

All birds were habituated to the binary choice chambers under HF lighting without any patterns on the walls for one hour in the week prior to starting experimental trials. During trials, I used two identical sets of apparatus simultaneously to maximise the rate of data acquisition. At the start of a trial, I split the group of birds equally between the two compartments in each apparatus, and recorded the number of birds in each compartment via remote video at 2 min. intervals for 2 h.

6.3.2. Parameters of HSF and LSF visual surround

The gratings were square-wave with a duty cycle of 50% (i.e. the black bars were the same width as the white bars). The discrete stripes within the HSF grating should have been perceptible to the birds in any part of the apparatus. It is possible to calculate whether or not a given frequency of grating should be perceptible to any animal,
provided that the equatorial diameter of the eye and the width of a typical retinal cone cell are known (T. Troscianko, University of Bristol, pers. comm.). I assumed that objects finer than the retinal mosaic could not be resolved, i.e. that starlings do not possess hyperacuity. If, however, starlings possess hyperacuity then the upper limit of spatial resolution will also depend on the degree of scattering within the ocular media as well as cone size and equatorial diameter.

I used the following method to compare the likely maximum acuity of starlings as compared to humans. I made the assumption that two cones are needed to resolve a stripe in the grating, and that therefore the size of the cones would be a limiting factor in the resolution of spatial frequency. I therefore calculated the likely maximum acuity limit of the starling using the following equation: \((d/D) \times (180/\pi)\), where \(d\) is the average diameter of a cone cell in \(\mu m\), \(D\) is the diameter the eye, and \(180/\pi\) represents the angular subtense of the cone (the amount of the visual field sampled by that cone in degrees). The average equatorial diameter of the starling eye is 10.05 mm (Martin 1986), and the average diameter of starling cones ranges from a minimum of 1.6 \(\mu m\) for the ultraviolet cones, to a maximum of 3.1 \(\mu m\) for the long wavelength sensitive cones and 2.5 \(\mu m\) for the double cones (Hart et al. 1998). It is not known which cone types are involved in avian perception of brightness. I therefore estimated that starlings could potentially resolve spatial frequencies somewhere between 54.8 cycle deg\(^{-1}\) (assuming the ultraviolet cone is involved in brightness perception) and 28.3 cycle deg\(^{-1}\) (assuming only long wave sensitive cones are used in brightness perception). Current evidence suggests that longer wavelengths are likely to be dominant in avian perception of brightness via the action of double cones, since both avian perception of motion (Campenhausen and Kirschfeld 1998) and visual texture (Osorio et al. 1999a) appears to involve perception of longer wavelengths. I therefore estimated the potential visibility of my gratings using the latter (max acuity 35 cycle deg\(^{-1}\)). From this calculation, it would appear that starlings have poorer acuity than humans, who have a maximum acuity of 60 cycle deg\(^{-1}\). However, the starlings should easily have been able to perceive the maximum spatial frequency I presented (nearly 5 cycle deg\(^{-1}\) in the HSF treatment).
6.3.3. Characteristics of HF and LF lighting

In the LF treatment, the lamp was powered by a ballast running at 100 Hz (Fitzgerald Lighting Ltd, Bodmin, U.K.), and in the HF treatment the ballast ran at >30 kHz (Tridonic, Basingstoke, U.K.). Truelite lamps approach 100% modulation (Wilkins and Clark 1990), which ensured that the least conservative possible degree of flicker was used, and that there would be little modulation in chromaticity throughout the flicker cycle. Measurements were taken with an Ocean Optics S2000 spectroradiometer with a cosine-corrected detector to quantify the quantal flux in each part of the avian visible spectrum (approximately 320-700 nm) from both the HF and LF lights. Five lamps of each treatment were measured, measuring each at 5 different positions along its length at a fixed distance of 25 cm from the lamp. There was no difference in the overall irradiance or spectral distribution of HF or LF lights (see section 5.3.5., p. 142).

6.3.4. Experiment 2: direct exposure

6.3.4.1. Design

A direct exposure experiment was carried out in January and February 2001 when the birds were about 8 months old, using European starlings that had been caught as juveniles under English Nature licence in July 2000. Prior to the experiment the birds were maintained in outdoor aviaries (1.8 x 0.9 by 1.8 m high, 12 birds per aviary) under natural light.

Twenty-four pairs of birds were brought indoors and kept in cages of different temporal and spatial frequency for three days. Their behaviour was recorded on days 1 and 2, and blood samples were taken on day 3 so that levels of plasma corticosterone could be measured. As in the preference tests, the lighting in each cage was either HF or LF in combination with walls that were either HSF or LSF according to a balanced
two-factor design. This gave four possible treatments:

1. HF+LSF
2. HF+HSF
3. LF+LSF
4. LF+HSF

In this experiment, each pair only experienced one of the four treatments (N=6 pairs per treatment). The experiment was run in six experimental blocks, with one pair of birds experiencing each treatment in each block, as a previous direct exposure experiment using starlings had found high variability between different experimental runs (Maddocks et al. 2001c). Prior to starting the experiment, each pair of birds was acclimatised to indoor maintenance by keeping them for three days in standard experimental cages (25 x 95 x 35 cm high) under HF light, without any gratings on the walls.

There were two experimental rooms of similar size, one of which was illuminated by HF and the other by LF lighting. Which room each treatment was in was counterbalanced across blocks. In each room, there were four 1.8 m long fluorescent lamps (Durotest ‘Truelite’) which were mounted vertically on the wall. In one room, these lamps were powered by HF ballasts (> 30 kHz, Hevlar, Helsinki, Finland) and in the other, by LF ballasts (100 Hz, Crompton Lighting, U.K.). Each room contained two experimental cages (25 x 95 x 35 cm high), which had their vertical walls lined with either HSF or LSF horizontal gratings that were identical to those used in the preference tests. One cage in each room was lined with HSF panels, and one with LSF panels. Both cages were at ground level at the same distance from the lights, and contained food, water and perches. In each block, birds were moved from their habituation cages on day 1 into experimental cages at 0930h. The birds were maintained under these conditions for three days, and were left undisturbed with the exception of behavioural observations via remote video at 24 and 48h, after which food and water in the cage was replenished.
6.3.4.2. Behaviour

Behaviour was recorded for 45 min. using overhead video cameras at 24h and 48h. The behaviour of a focal bird from each pair for each observation session was subsequently observed from the video footage, and the frequencies of various behaviours (see Table 6.1.) recorded using Etholog (Ottoni, E.B., San Paulo, Brazil). I log-transformed the raw data to satisfy the assumptions of parametric statistics, and then pooled the data for the whole experiment and carried out principal components analysis (PCA; Chatfield and Collins, 1995) prior to further analysis. This reduced 9 original variables into three orthogonal principal components (PCs, see Table 6.2.). Each PC is a mathematical transformation of the raw data consisting of a weighted linear sum of the original data (Chatfield and Collins 1995). This transformation multiplies the raw data by PC coefficients (weights), which can be either positive or negative, to give a PC score for each data point. The PC scores for each extracted PC were then analysed using MANOVA on Minitab (Minitab 1998). ‘Cage’ was the unit of analysis as the behaviour of birds within a pair is not truly independent. The data for each pair were therefore treated as repeated measures, even though different birds were observed from each experimental cage on different days.

Table 6.1. Description of all observed behaviours

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hop</td>
<td>Hopping on floor, or jumping from perch to perch</td>
</tr>
<tr>
<td>Head out</td>
<td>Sticking head out through cage bars so is not looking at walls</td>
</tr>
<tr>
<td>Somersault</td>
<td>Jumping upwards and flipping over backwards</td>
</tr>
<tr>
<td>Feed</td>
<td>Frequency of pecks directed at food in the feeder</td>
</tr>
<tr>
<td>Drink</td>
<td>Frequency of 1 sec intervals in which bird spends drinking</td>
</tr>
<tr>
<td>Preen</td>
<td>Running beak through feathers</td>
</tr>
<tr>
<td>Bath</td>
<td>Sitting in water bowl and flapping wings</td>
</tr>
<tr>
<td>Fight</td>
<td>Pecking member of other pair sharply, or receiving such pecks</td>
</tr>
</tbody>
</table>
Table 6.2. Coefficients relating the first three principal components to the original behavioural variables. Numbers in bold show the two highest values for each PC, which correspond to the behaviours that influence that PC most strongly.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hop</td>
<td>-0.510</td>
<td>-0.194</td>
<td>-0.078</td>
</tr>
<tr>
<td>Head out</td>
<td>-0.188</td>
<td>0.286</td>
<td><strong>0.639</strong></td>
</tr>
<tr>
<td>Somersault</td>
<td><strong>-0.542</strong></td>
<td>-0.073</td>
<td>0.050</td>
</tr>
<tr>
<td>Feed</td>
<td>0.154</td>
<td><strong>-0.649</strong></td>
<td>0.268</td>
</tr>
<tr>
<td>Drink</td>
<td>0.195</td>
<td>0.095</td>
<td><strong>0.665</strong></td>
</tr>
<tr>
<td>Preen</td>
<td>0.442</td>
<td>-0.013</td>
<td>-0.215</td>
</tr>
<tr>
<td>Bath</td>
<td>0.352</td>
<td>0.301</td>
<td>0.014</td>
</tr>
<tr>
<td>Fight</td>
<td>0.174</td>
<td><strong>-0.595</strong></td>
<td>0.152</td>
</tr>
</tbody>
</table>

6.3.4.3. Blood sampling and radioimmunoassay

Blood samples were taken from both birds in each pair on day 3, so that the basal level of plasma corticosterone, and its rate of rise post capture and restraint could be ascertained. Two experimenters (myself and Verity J. Greenwood) entered one of the experimental rooms simultaneously at 0930h and from each cage caught one bird from each pair. A series of blood samples was then taken from each of these birds by puncture of the alar vein using a 25-gauge needle. Samples (0.1 ml) were taken as soon as possible after capture (always within 1 min.) to obtain basal corticosterone, and subsequently at 10, 30 and 60 min. later to obtain both the maximum level of corticosterone attained and its rate of rise in the blood. Birds were individually restrained in bird bags between samples. Two birds from the other experimental room were also caught and blood sampled in a similar manner, with the timings of capture and blood sampling staggered to be three minutes after the first birds. The second bird in each pair was left to settle for 2h, and at 1130h the procedure above was repeated so that corticosterone for the second member of each pair in each room could be
obtained. Which treatment was blood-sampled first, and whether each experimenter bled either the first or the second member of each pair of birds, was counterbalanced within and across experimental blocks. After the procedure, birds were returned to their normal housing conditions. I did not observe any adverse effects resulting from this procedure. I centrifuged the blood samples, separated the plasma from the compacted red cells and stored the plasma samples at -20°C (for details of these procedures, see Appendix).

Corticosterone concentrations in the plasma samples were measured by radioimmunoassay (Wingfield et al. 1992) using a similar procedure to that described by Maddocks et al. (2001b, see Appendix for explanation of radioimmunoassay technique). 2000 cpm of tritiated corticosterone ([1,2,6,7-3H]-corticosterone label, Amersham, U.K.) was added to 20 µl aliquots of each plasma sample so that the recovery efficiency of the subsequent extraction of corticosterone in diethyl ether could be estimated. The evaporated extracts were reconstituted in 550 µl of assay diluent, 100 µl aliquots of which were subsequently added to 750 µl scintillant (UltimaGold, Packard, Groningen, The Netherlands). The reconstituted extracts were counted in a scintillation counter to calculate percent recovery. Duplicate 200 µl aliquots of each extract (each containing 7.3 µl of extracted plasma) were assayed using an anticorticosterone antiserum (code B21-42, Endocrine Sciences, Tarzana, California, U.S.A.) and [1,2,6,7-3H]-corticosterone label (Amersham, U.K.). The obtained corticosterone concentrations were corrected for recovery efficiency (percentage recoveries varied between 72 and 94%) and expressed in ng/ml. The assay was run with a bound:free ratio of 0.62, 50% binding was 1.06 ng/ml (106 pg per tube) and the detection limit (for 7.3 µl aliquots of extracted plasma) was 1.09 ng/ml.
6.4. Results

6.4.1. Experiment 1: preference tests

The data from each trial was combined into means for the four 0.5h timeblocks within the 2h of each trial. This enabled analysis of whether the birds’ preferences changed during the trial as they became acclimatised to the available visual environments. I calculated the average proportion of birds in each of the two treatments in each timeblock, and used General Linear Models (GLMs), using Minitab 13.30, to establish whether there was any significant preference for a given treatment and whether there was any effect of timeblock. GLM output gives a test for whether the constant term in the regression model is different from zero. I used the significance of this constant term as a test for whether the proportion of birds in each treatment differed. To achieve this, I subtracted 0.5 from the raw proportionate data so that I could use the significance of the constant term as a test for deviations from 0.5, the proportion of birds predicted to be in a chamber if choice is random. All the residuals were normal, so transformation of the data was not necessary.

All treatments had significant effects, except treatment 5 (HF+LSF vs LF+HSF). With the exception of treatment 5, there was no treatment*timeblock interaction (see Table 6.3.). In treatment 5, there was such an interaction, but without a consistent trend across timeblocks (See Table 6.3.; mean proportion of birds choosing HF+LSF, first timeblock: 0.53; second: 0.45; third: 0.48; fourth: 0.50).

If the lighting condition was identical in both chambers, birds preferred LSF to HSF visual surrounds regardless of whether the lighting was HF or LF (see treatments 1 and 2 respectively, Table 6.3.). If the spatial frequency of the surround was identical in both chambers, birds preferred HF lighting if the walls were HSF, but LF lighting if the walls were LSF (see treatments 3 and 4 respectively, Table 6.3.). They also preferred LF lighting with LSF walls to HF with HSF walls (see treatment 6, Table...
6.3). Birds had no significant preference for LF+HSF versus HF+LSF (see treatment 5, Table 6.3.).

**Table 6.3.** Preference test results: effects of treatments 1-6, using test of whether constant term in GLM differs from 0.5. All treatments had significant effects except treatment 5. The mean proportion of birds in each treatment is shown. None of these effects changed over the four timeblocks within the two hour trial.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect of Treatment:</th>
<th>Preferred Treatment:</th>
<th>Less Preferred Treatment:</th>
<th>Effect of timeblock:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HF+HSF vs HF+ LSF</td>
<td>$T=3.16$</td>
<td>HF+LSF</td>
<td>HF+HSF</td>
<td>$F_{3,33}=0.74$</td>
</tr>
<tr>
<td>1. HF+HSF vs HF+ LSF</td>
<td>$P=0.003$</td>
<td>Mean: 0.56</td>
<td>Mean: 0.44</td>
<td>$P=0.536$</td>
</tr>
<tr>
<td>2. LF+ HSF vs LF +LSF</td>
<td>$T=4.01$</td>
<td>LF+LSF</td>
<td>LF+HSF</td>
<td>$F_{3,33}=1.16$</td>
</tr>
<tr>
<td>2. LF+ HSF vs LF +LSF</td>
<td>$P&lt;0.001$</td>
<td>Mean: 0.58</td>
<td>Mean: 0.42</td>
<td>$P=0.339$</td>
</tr>
<tr>
<td>3. HF+HSF vs LF+ HSF</td>
<td>$T=2.35$</td>
<td>HF+HSF</td>
<td>LF+HSF</td>
<td>$F_{3,33}=0.40$</td>
</tr>
<tr>
<td>3. HF+HSF vs LF+ HSF</td>
<td>$P=0.025$</td>
<td>Mean: 0.55</td>
<td>Mean: 0.45</td>
<td>$P=0.753$</td>
</tr>
<tr>
<td>4. HF+LSF vs LF+LSF</td>
<td>$T=4.52$</td>
<td>LF+LSF</td>
<td>HF+LSF</td>
<td>$F_{3,33}=0.78$</td>
</tr>
<tr>
<td>4. HF+LSF vs LF+LSF</td>
<td>$P&lt;0.001$</td>
<td>Mean: 0.60</td>
<td>Mean: 0.40</td>
<td>$P=0.515$</td>
</tr>
<tr>
<td>5. HF+LSF vs LF+HSF</td>
<td>$T=0.47$</td>
<td>LF+HSF</td>
<td>HF+LSF</td>
<td>$F_{3,33}=0.55$</td>
</tr>
<tr>
<td>5. HF+LSF vs LF+HSF</td>
<td>$P=0.664$</td>
<td>Mean: 0.51</td>
<td>Mean: 0.49</td>
<td>$P=0.652$</td>
</tr>
<tr>
<td>6. HF+HSF vs LF+LSF</td>
<td>$T=4.23$</td>
<td>LF+LSF</td>
<td>HF+HSF</td>
<td>$F_{3,33}=2.41$</td>
</tr>
<tr>
<td>6. HF+HSF vs LF+LSF</td>
<td>$P&lt;0.001$</td>
<td>Mean: 0.61</td>
<td>Mean: 0.39</td>
<td>$P=0.085$</td>
</tr>
</tbody>
</table>
6.4.2. Experiment 2: direct exposure

6.4.2.1. Behaviour

The first three components from PCA (see Table 6.2.) explained 60% of the variation in the 8 behaviours (see Table 6.1.). Further principal components (PCs) were not analysed as they had eigenvalues less than 1.0. These PCs were analysed using repeated measures ANOVA on Minitab (Minitab 1998), with block being treated as the unit of repeated measurement. Following the methods of Armitage and Berry (1985, p.238), non-significant interactions involving block were pooled with the error mean square to gain degrees of freedom. For this reason, the error d.f. differ between tests, even though the same number of birds are involved.

The frequency of the lighting significantly affected PC1 and PC3, but not PC2 (PC1: $F_{1,30}=4.49$, $P=0.043$; PC2: $F_{1,20}=0.05$, $P=0.834$; PC3: $F_{1,20}=12.87$, $P=0.016$). PC1 is related to overall activity, being negatively associated with hopping and somersaulting, and PC3 is positively associated with drinking and putting the head outside of the cage (see Table 6.2. in conjunction with Table 6.1.). Birds in LF light hopped and somersaulted less often, but drank and put their head outside of the cage more often (see Fig. 6.1a,b in conjunction with Table 6.2.) than birds in HF light. There was a significant main effect of observation day, unrelated to treatment, on PC1 ($F_{1,30}=26.24$, $P<0.001$), but not on the other two PCs (both $P>0.262$). PC1 scores decreased on the second observation day, which corresponds to an increase in hopping and somersaulting (see Fig. 6.1a and Table 6.2.). There were no significant light*observation day interactions (all $P>0.115$).
**Figure 6.1.** Mean scores ± SE for PC1 and PC3, split by lighting treatment and observation day, shown in Fig. 6.1a,b respectively. Fig. 6.1a shows that PC1 scores were higher in LF than HF, and decreased in both treatments from the first to the second observation day. Fig. 6.1b shows that PC3 scores were higher in LF on both observation days.

The spatial frequency of the cage wall patterns did not significantly affect any of the three PCs overall (all $P>0.228$). The absence of an overall significant effect of spatial
frequency on PC1 may have been due to an inconsistency in the effect of treatment within block 5. In all the other blocks, PC1 was consistently higher in HSF. However in block 5 this situation reverses, with PC1 scores being higher in LSF (see Fig. 6.2.). There were no light*spatial frequency interactions on PC1, PC2 or PC3 (all \( P>0.442 \)). There was a non-significant trend towards a spatial frequency*order interaction for PC3 (\( F_{1,20}=3.21, P=0.088 \)), but not for the other PCs (both \( P>0.647 \)). PC3 scores were higher in LSF than HSF on the first day (\( X \pm SE, \) LSF: +0.254±0.318; HSF: -0.449±0.235) but not on the second (\( X \pm SE, \) LSF: +0.147±0.310; HSF: +0.048±0.409). This means that birds in LSF drank and put their heads outside of the cage more often than birds in HSF on day 1, but not on day 2.

**Figure 6.2.** Effect of spatial frequency on mean PC1 scores, showing standard error bars. There was no significant treatment effect, although there was a significant treatment*block interaction. PC1 scores were higher in HSF in all blocks except block 5.
6.4.2.2. Plasma corticosterone

The starlings showed a typical rise in corticosterone concentration in response to capture and restraint (see Fig. 6.3.). Plasma corticosterone concentrations were analysed using repeated measures ANOVA. Prior to analysis, I optimised the normality of the data with transformations where necessary. Basal corticosterone was higher in birds kept under LF than those kept under HF light (Box-Cox transformed data: $F_{1,35}=4.67$, $P=0.038$, see Fig. 6.4.), but was not affected by spatial frequency ($F_{1,35}=0.41$, $P=0.527$, see Fig. 6.5.). There was no significant effect of block on basal corticosterone ($F_{1,35}=1.05$, $P=0.405$). However, there was a significant effect of time order of sampling, with birds being blood sampled at 0930h having higher basal corticosterone than those sampled at 1130h ($F_{1,35}=32.69$, $P<0.001$, see Fig. 6.6.). There was no effect of lighting, spatial frequency, block or order on the maximum level of corticosterone attained (all $P >0.105$). Residuals were normal, so no transformations were required. I also analysed the rate of rise of corticosterone in the blood, which I defined as the corticosterone concentration taken at 10 min. minus the corticosterone concentration taken at 1 min. Rate was unaffected by the frequency of the lighting (square-root transformed data; $F_{1,15}=0.15$, $P=0.715$); however, there was a non significant trend for rate to be higher for birds kept in HSF than LSF ($F_{1,15}=5.19$, $P=0.072$; compare 1 and 10 min. values in Fig. 6.5.). There was also a non significant trend for rate to be higher in birds blood sampled at 1130h than at 0930h if they had been kept in HSF (spatial frequency*order interaction, $F_{1,15}=3.11$, $P=0.098$).
Figure 6.3. Mean plasma corticosterone concentrations rose from baseline levels at 1 min. in response to capture and restraint in all four treatments. The bars are standard errors.

![Graph showing corticosterone concentrations over time for different treatments]

Figure 6.4. Mean plasma corticosterone response for birds kept under HF and LF light, showing standard error bars. Basal levels are higher in LF.

![Graph showing corticosterone concentrations over time for HF and LF]
**Figure 6.5.** Mean plasma corticosterone response for birds kept under HSF and LSF, showing standard error bars. Basal levels in both treatments are similar. There is a non-significant trend for corticosterone to rise faster in response to capture and restraint in HSF.

**Figure 6.6.** Mean plasma corticosterone response is higher for birds sampled at 0930h than at 1130h. Data are pooled across treatments; the bars are standard errors.
6.5. Discussion

In summary, these results provide evidence that European starlings have strong preferences for different temporal and spatial frequencies in the visual surround. I found that these factors affected their activity levels and plasma corticosterone. It is well established that birds are sensitive to the temporal properties of visual stimuli (D'Eath 1998; Fleishman and Endler 2000), but previously no consideration has been given to the effect of spatial periodicity of stimuli on bird behaviour or physiology. Interestingly, the effect of different flicker rates of fluorescent lamps on birds appears to be modulated by the spatial frequency of the environment the birds are in. The preference test results suggest that the relationship between these variables is not simple, and also suggest that birds may experience these frequencies differently from humans, as their pattern of preference for the different visual environments did not always fit with my predictions based upon the human literature.

I investigated whether birds react differently to high frequency (HF, >30 kHz) and low frequency (LF, 100 Hz) fluorescent lighting, in conjunction with either relatively high spatial frequencies (HSF, 2.5 cycle cm\(^{-1}\)) or relatively low spatial frequencies (LSF, 0.1 cycle cm\(^{-1}\)). I predicted that in the preference tests, birds would prefer HF to LF light, and LSF to HSF. I also predicted that there would be an interaction effect, with LF in conjunction with HSF being more aversive than any other treatment combination. These predictions were based on assumptions that the spatial frequency to which most bird neurons respond to is similar to that which causes maximal physiological arousal in humans.

Previous work has shown that in the absence of significant spatial periodicity in the environment, starlings consistently prefer HF to LF light (see Chapter 5). However, I found that starlings’ preferences for a particular flicker rate of lighting appear to depend on the spatial frequency of the environment that the lamp illuminates. Furthermore, although the birds generally displayed strong preferences, their pattern of preference did not always fit with my predictions, nor was it entirely internally
consistent. In line with predictions, if there was no choice available between the light environments, birds preferred LSF to HSF. Also as predicted, if there was no choice between available spatial frequencies, birds preferred HF to LF if the environment in both compartments was HSF. However, if the environment in both compartments was LSF, the preference reversed, with birds significantly preferring LF to HF. In fact, birds always chose LF+LSF when this option was available to them, as birds preferred LF+LSF to LF+HSF, although in the latter case this is what I anticipated. Interestingly, the starlings had no significant preference for HF+LSF over LF+HSF, which I predicted would have been a choice between the least aversive and the most aversive environment. There is no immediately obvious explanation for this pattern of results. However, I only investigated preferences of the birds for these environments over a short period, and in the case of the HF+LSF versus LF+HSF treatment, the absence of aversion may not indicate an absence of treatment effects. It may be that there is an optimum level of visual stimulation, if such stimulation is pleasurable or exciting. There is some evidence in humans that repetitive visual stimulation may be perceived as exciting, as there are case studies of patients with epilepsy who deliberately give themselves seizures by sitting up close to a television, or by waving their fingers past their eyes to create a flickering grating effect. Perhaps LF+HSF is very exciting but unbearable for more than a short period, whereas HF+LSF is relatively unstimulating. If this is the case, the observed lack of preference in treatment 5 may have resulted from the birds moving continually back and forth between the two environments. However, the overall pattern of results is inconsistent, and does not suggest that the birds were trying either to maximise or minimise their level of visual stimulation overall. That said, birds may have different sensitivities to spatial frequencies than humans, which may explain why the pattern of preferences the birds showed did not fit with my predictions. It is possible that the range of spatial frequencies that causes maximum physiological arousal in starlings is different from that in humans. This situation could occur if starlings have either dissimilar contrast sensitivity functions to humans, or if their eye movement patterns are dissimilar, as the range of possible eye movements affects the range of spatio-temporal interactions likely to affect the visual system.
In the direct exposure experiment, I found that the behaviour of European starlings is sensitive to the flicker frequency of fluorescent lighting, as birds housed under LF consistently somersaulted and hopped less, but drank and looked outside of the cage more, under LF than HF light. Birds in HF moved more than birds in LF to a greater degree on the first day of observation than on the second, which indicates the effect may decline as the birds habituate to their environment. It is not certain how the difference in behaviour between treatments relates to their disposition with regard to potential stress. Increased activity may indicate increased arousal, which could either indicate a positive subjective state such as excitement or a negative subjective state such as fear or anxiety. However, as there was a general trend for activity to increase by the second day in both treatments, higher activity may show that the birds are more settled, which would suggest that birds brought into LF light are more stressed than their counterparts housed in HF light.

There was no overall significant main treatment effects of my chosen spatial frequencies on behaviour as there was significant inter-block variability, with the fifth experimental block having opposite effects on PC1 than those seen in all other five blocks. Initial activity levels appear to be marginally lower in HSF, as there was a non-significant trend ($P=0.088$) for birds in LSF to drink more and put their heads outside of the cage more on day 1 than birds in HSF, but not on day 2. These results, taken together with the preference test results, indicate that there is some interaction effect between the temporal frequency of the light and the spatial frequency of the surround on behaviour, but the effects of lighting appear to be more powerful than those of my chosen spatial frequencies.

There was a significant effect of order of sampling on basal corticosterone, with birds being blood sampled at 0930h consistently having higher levels than those sampled at 1130h, regardless of experimental treatment. This may be a time of day effect, or perhaps because the birds sampled at 0930h may have been more aroused from the general background noise of the technician staff beginning work at this time. As predicted, basal corticosterone was higher in birds kept under LF than those kept under HF light, but was unaffected by spatial frequency. As glucocorticoids such as
corticosterone are involved in the physiological cascade responsible for mobilising glucose stores in preparation for physical activity (Reul et al. 2001), any rise in corticosterone may simply reflect increased metabolic activity as a result of exercise. However, as the birds were consistently less active in LF than HF, the rise in corticosterone seen in LF suggests that any increase in metabolic activity was not triggered by simple changes in the birds’ activity levels. It is not clear whether this increased level of arousal is harmful, as increased physiological arousal is seen in response to positive as well as to negative mental states (Toates 1995; Reul et al. 2001). However, since the mechanism by which LF lights are proposed to have harmful effects on humans is via causing excess physiological excitation (Wilkins et al. 1984), it is plausible that the increased physiological arousal caused by LF lighting may have some negative effect on the starlings. There was no effect of lighting or spatial frequency on the maximum corticosterone levels attained by the birds during capture and restraint. However, there was a non-significant trend for the rate of rise of corticosterone to be higher for birds kept in HSF than LSF ($P=0.072$), and also a non-significant trend for rate to be higher in birds blood sampled at 1130h than at 0930h if they had been kept in HSF ($P=0.098$). Both of the latter are consistent with idea that HSF may be more arousing or stressful than LSF. Although neither trend was significant and there were no main effects of spatial frequency, these trends, in conjunction with the subtle effects on behaviour and the strong preferences birds have for spatial frequencies, suggest that spatial frequencies in the visual surround do affect bird physiology and behaviour. The spatial frequencies I chose to use were selected for the effect that they would have on a human, and it is possible that the most aversive spatial frequencies for a bird are not within my chosen range. Hence, further preference tests to investigate which spatial frequencies birds find most aversive would be of interest.

As my approach was comparative, I chose to use stimuli consisting of gratings that were comparable to those used in human vision research. As the effect of spatial frequencies on birds was previously untested, I aimed to be non-conservative and use gratings with high modulation and a 50% duty cycle as these have maximum effect on humans. However, cage bars typically do not have 100% modulation and have
differing duty cycles. Therefore it would also be of interest to investigate bird preferences for both modulation and duty cycle of gratings, as well as their spatial frequency. Although vertical and horizontal gratings appear to affect humans similarly (Wilkins 1995), it would also be of interest to establish whether this is the case for birds, as cage bars are predominantly vertically orientated.

In summary, the preference of birds for the flicker rate of their light environment over a short period is affected by the spatial frequency of the visual surround, although the pattern of results suggests the relationship between these variables is not simple. The flicker rate of the lighting had a more powerful effect on the behaviour and plasma corticosterone concentrations of the birds than did my chosen spatial frequencies. This does not mean that spatial frequency is of lesser importance, as I may not have selected frequencies within the range that are maximally aversive to birds. Although the preference tests results were not always internally consistent, it is clear from the direct exposure experiment that birds housed under HF are more active and have lower corticosterone than birds housed under LF, indicating that LF is the more arousing and perhaps the more stressful condition. This is at odds with previous work (Maddocks et al. 2001c, and also my own findings in Chapter 5), which found that LF light does not measurably affect the behaviour or corticosterone concentrations in starlings. The starlings in the present experiments were more accustomed to captivity than those of Maddocks et al. (2001c) and the starlings I used in the direct exposure experiments in Chapter 5, which suggests that effects of the flicker of lighting are only measurable once the initial stress of adjusting to captivity has subsided. Alternatively, it may be that LF lighting is only stressful when the visual environment is striped. Further direct exposure experiments in environments with little spatial periodicity, using starlings that have been kept in captivity for some time, would distinguish these alternatives. However, it is difficult to be certain that the effects of LF on physiology and behaviour indicate that LF is aversive and should be avoided in bird husbandry, as birds did not always avoid it when they had a choice. That said, humans do not necessarily avoid LF light, even if it makes them feel unwell, as they fail to attribute their symptoms to the light (Wilkins et al. 1989). I conclude that although the welfare implications are not clear cut, both temporal and spatial frequencies in the visual
environment clearly have effects on the preferences, behaviour and physiology of birds. The effects of repetitive visual stimulation on the behavioural and physiological responses of birds and other animals seems worthy of further research, as the practice of housing laboratory animals in cages under 100 Hz fluorescent lights may have consequences for both the welfare of the animals, and the interpretation of behavioural and physiological data derived from animals kept in these conditions. This may be particularly relevant to neurophysiological studies, as if 100 Hz flicker causes an increase in physiological arousal via altering the responsiveness of visual neurons as Wilkins et al. (1984) propose, then the flicker rate of the ambient lighting may greatly affect the activity of the visual pathway, and subsequently the activity of other parts of the nervous system.