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# "Let There be Light!" pigeon eggs are regularly exposed to light during breeding

Jens-Uwe F. Buschmann *, Martina Manns, Onur Güntürkün

*Biopsychologie, Ruhr-Universität Bochum, Germany*

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**Abstract**

Light stimulation before hatching initiates the emergence of avian visual lateralisation. Since several studies show that birds benefit from being lateralised, we can conjecture that their clutch is being exposed to light during breeding. We tested this assumption in pigeons with a semi-natural setup where the animals were systematically recorded using a movement detection system throughout their breeding period. The results show that pigeon pairs perform their relieves in a regular way by abandoning their clutch for a mean of about 55 s at approximately every 43 min. Thus, the developing visual pathways are repetitively stimulated by light for cumulatively over 3 h before the breeding period ends. It becomes apparent that both the duration as well as the repetitions of light stimulation play a crucial role in the onset of visual asymmetry.


*Keywords:* Visual lateralisation; Birds; Tectofugal system

## 1. Introduction

Functional cerebral asymmetries are a universal phenomenon (Rogers and Andrew, 2002). Theories trying to explain the ontogenetic events leading to adult lateralised brain patterns in humans and other animals can be broadly labelled either genetic or epigenetic. While genetic theories propose that the intergenerational transmission of cerebral asymmetries is directly controlled by genetic factors (McManus, 2002), epigenetic theories assume that the initial neural pattern is largely symmetric (Previc, 1991). However, it develops into an asymmetric form by lateralised factors interfering during ontogeny. Avian visual lateralisation is currently the best example available for an explanation of the development of asymmetrical brain functions that stresses the interaction of genetic and epigenetic factors.

Prior to hatching, embryos of most avian species keep their head turned to the right such that the left eye is occluded by the body and the right eye is close to the translucent shell and thus exposed to light stimulation (Kuo, 1932). Light exposure of pigeon eggs induce the establishment of visual lateralisation with a superiority of the right eye in object discriminations, while dark incubation prevents the emergence of this asymmetry (Rogers, 1982; Skiba et al., 2002). The same result is achieved by right-sided monocular deprivation after hatching (Manns and Güntürkün, 1999b). Thus, light incubated pigeons show a population asymmetry (all or at least most individuals are biased into the same direction) in diverse visual discrimination tasks (see Güntürkün, 2002, for review). The 'loss' of asymmetry in dark-incubated pigeons could be due to two phenomena. The first is the replacement of population asymmetry by individual asymmetry. In this case each pigeon is lateralised but about half of them have a right eye bias while the other half is skewed to the other side. The second possibility is that no individual animal shows a substantial left–right difference. A detailed analysis showed that indeed, dark incubation produces the second alternative, i.e. these pigeons show no individual bias to the right or the left eye in object discriminations (Skiba et al., 2002).

The situation in chicks appears to be different to some extent, where light-incubation seems to align different forms of asymmetrical control to form a coherent pattern of population asymmetry (food discrimination: Rogers, 1982, 1990; imprinting:

* Corresponding author at: FBI Science, Markstr. 265, 44799 Bochum, Germany. Tel.: +49 234 5892323; fax: +49 17633 22886352.
*E-mail address:* ju.buschmann@gmx.de (J.-U.F. Buschmann).

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Johnston and Rogers, 1998; social recognition Vallortigara et al., 2001; monocular sleep: Mascetti and Vallortigara, 2001). Dark-incubated chicks, however, show either no clear-cut asymmetry (Rogers, 1982) or show other patterns of asymmetry (Johnston and Rogers, 1998; Mascetti and Vallortigara, 2001; Vallortigara et al., 2001). Thus, asymmetry of light stimulation as a secondary consequence of head turning is the key event leading to visual lateralisation at the population level in birds.

Behavioural lateralisation is associated with morphological left–right differences in the ascending visual systems (Rogers, 1996; Güntürkün, 1997a). Chicks exhibit transient left–right differences in the thalamofugal pathway which transfers retinal information via the lateral geniculate nucleus of the thalamus (GLd) onto the telencephalic Wulst. In response to biased visual input, the left thalamus gives rise to more projections to the right Wulst than the right GLd to the left Wulst (Deng and Rogers, 1999). In pigeons, visual lateralisation is related to lifelong morphological asymmetries in the tectofugal pathway, transferring visual information via the mesencephalic optic tectum and the diencephalic nucleus rotundus to the forebrain. Apart from tectal (Güntürkün, 1997b; Manns and Güntürkün, 1999b; Skiba et al., 2002) and rotundal (Manns and Güntürkün, 1999a) cell size differences, the tectorotundal projection is asymmetrically organised with more tectal fibres ascending from the right tectum to the left rotundus than vice versa (Güntürkün et al., 1998). Additionally, top-down projections from the Wulst modulate tectofugal processing in an asymmetrical way (Folta et al., 2004).

Lateralised animals benefit from being left–right different since birds with higher asymmetries were shown to be significantly more successful in discriminating grain from grit (Güntürkün et al., 2000). This means that a rise in asymmetry results in a concomitant rise of foraging efficiency. Lateralised chicks benefit from asymmetry also because they are, in contrast to their non-lateralised companions, able to perform the dual task of food search and predator evasion (Rogers et al., 2004). Hence, asymmetry pays.

If visual asymmetry is valuable and triggered by pre-hatch light stimulation, we expect breeding parents to stand up and leave their clutch often enough to enable light exposure to the eggs. Since this critical prediction has to our knowledge never been studied under natural or semi-natural conditions, it is the focus of the presented study. In view of the fact that visual asymmetry in pigeons is morphologically manifested in the tectofugal visual system and since retinal fibres reach the optic tectum on the 14th day of incubation, starting to make synaptic contacts with E15 (Manns and Güntürkün, 1997), specific attention will be given to the behaviour of the parental pairs between the 14th and the 17th day of incubation, the last number being the time of hatch. Since both parental animals participate in breeding, a very short duration of light exposure is to be expected during relieves. Moreover, these periods could be extremely small, if both adults simply swap breeding positions instantly. Such a very short time span would also prevent cooling of the eggs as well as the clutch being freely exposed to predators. For our hypothesis to be correct, however, we would expect that pigeons leave their clutch for slightly longer periods to enable the establishment of visual asymmetry.

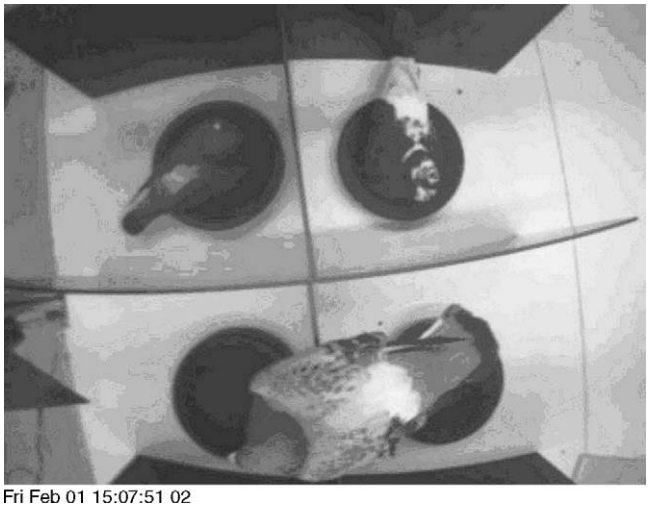


Fig. 1. Screenshot of the breeding area with two pigeons breeding and one pigeon resting above.

## 2. Methods

### 2.1. Subjects

Eight pairs of sexually mature homing pigeons (*Columba livia*) obtained from local breeders were used for this study. Their socialisation took place inside a group enclosure (2 m × 2 m × 2.5 m) with transparent walls, a wire mesh ceiling and a solid wooden ground with an exchangeable plastic coating. Water and food was present ad libitum; cleaning of the enclosure took place every second day. Every access to the enclosure was registered. Four breeding bowls filled with wood shavings or paper were placed in between plastic separators (1.5 m × 0.75 m × 0.4 m) resulting in eight compartments. A metal square of 1 m × 1 m × 1 m was hanging from the ceiling to provide space for the pigeons to sit on. A camera was mounted above this metal square, observing the breeding area (see Fig. 1). A second camera recorded the scene from outside the enclosure. The room was illuminated for 12 h a day (switch time was 7 a.m. and 7 p.m.) by daylight fluorescent lamps mounted at the ceiling of the room. Illumination at the level of the eggs was approx. 250 lux. Two pairs of pigeons were always kept in this enclosure for breeding (each during spring, summer, fall and winter) and observed by time-lapse recording. After having raised their clutch, they were replaced by the next two pairs until five breeding pairs were recorded.

### 2.2. Apparatus

B/W video images were sampled using a Fast Movie Machine capture card. “Gotcha! 3.0” (by Prescient Systems) was used to detect motion within the observed compartments (Fig. 1). Hence, every movement was recorded and tagged with a time stamp.

After recording, the video frames were analysed with the software INVAS (Buschmann and Dambach, 1997) for periods of transitions showing a changeover of breeding between the parental animals of the nest. The duration of this relieve deter-

mined the period of light exposure to the eggs, where the start was defined as the first frame with a completely exposed egg and the end time as the subsequent coverage of at least 50% of the egg by one of the breeding birds. The advent of eggs and hatching could be seen during the first cover up after the egg was laid or the offspring hatched. Finally, the times during disturbances due to water/food change or cleaning were excluded from the measured time periods and the number of relieves.

The analysed time period commenced with the appearance of the second egg and continued until the hatch of the first offspring at day 17 with regards to the second egg. However, we excluded day 17 in reference to this second egg from quantitative analysis, since they often included a mixture of care for the first chick and a still ongoing breeding period of the second egg. Time periods of light exposure ($t_{le}$), the number of relieves ($n_{re}$) and the quotient 'time of exposure' ($t_{expo} = (t_{le}/n_{re})$) were the basis of our descriptive statistics. Generally, non-parametric tests were used.

## 3. Results

The analysis is based on a complete breeding period of five out of eight pairs; three pairs crushed their eggs or abandoned their clutch. Most eggs (11 out of 15) were laid during darkness (between 7 p.m. and 7 a.m.), especially the first of the two eggs (6 out of 8). There seems to be an equal share of breeding between the female and the male. However, individuals could not always be distinguished with certainty in the observation video.

While the time of light exposure of the eggs varied from day to day and differed to some extent between the pigeon pairs (Fig. 2), the long-term mean exposure time of the nest was

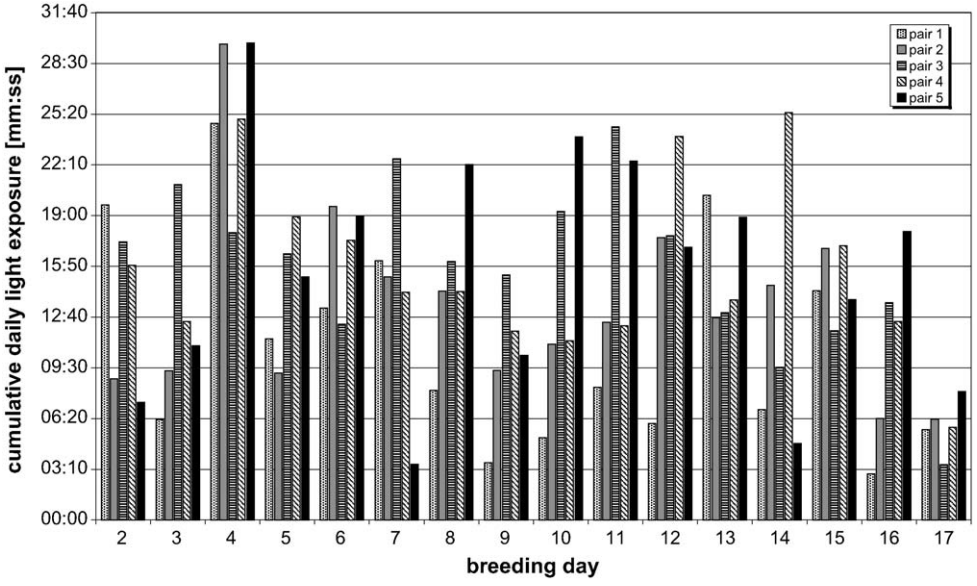


Fig. 2. Daily light exposure of the eggs for each of the five pigeon pairs.

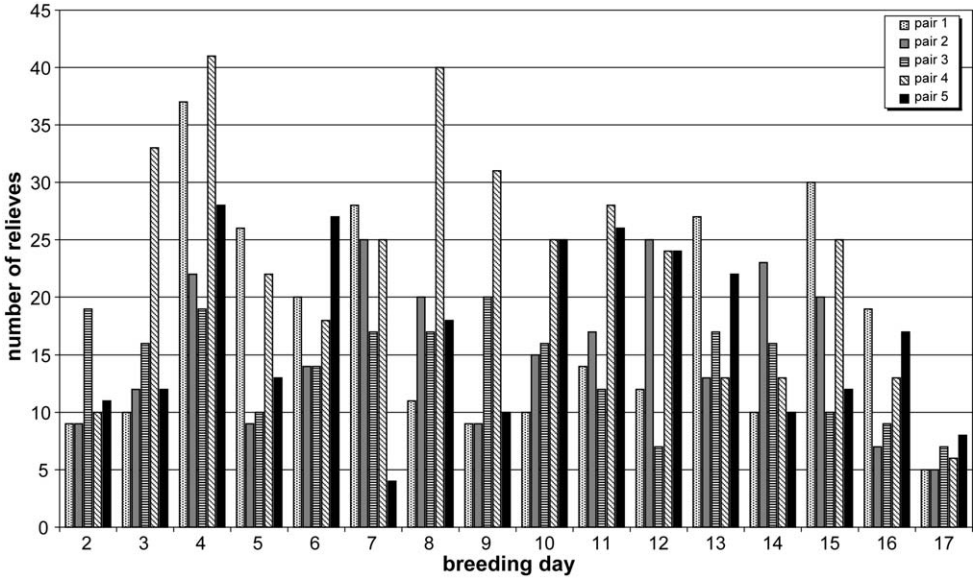


Fig. 3. Number of relieves for each day of the breeding period.

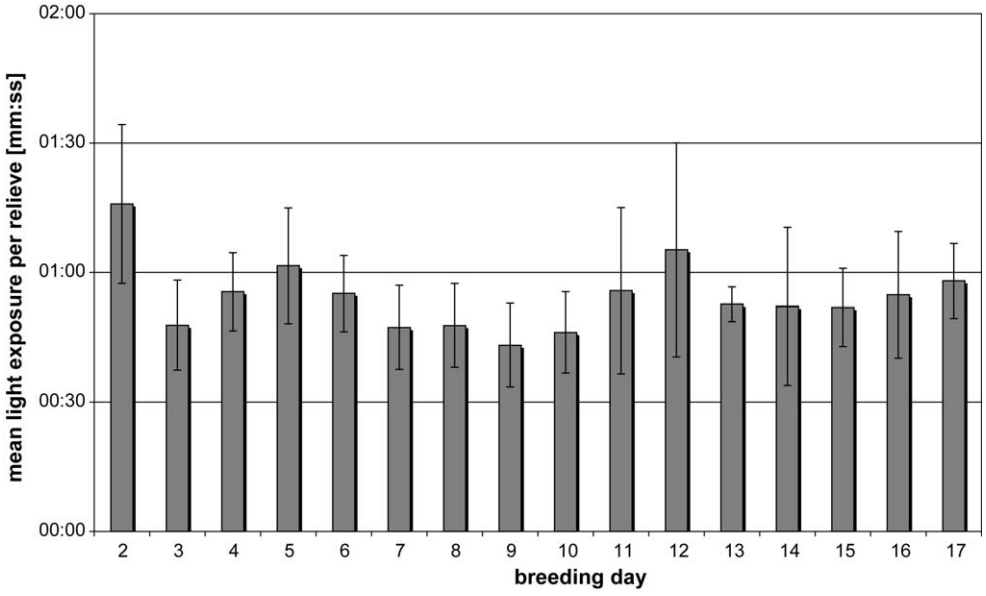


Fig. 4. Average light exposure per relieve during the breeding period.

approximately the same for all five pairs and ranged between 10:47 and 15:45 min per day with a mean of 14:11 min. The overall exposure time during the observed 16 breeding days varied between 2:53 and 4:12 h with an overall mean of 3:47 h. The homogeneous course of light exposure times also increased on day 3, however, the number of relieves are increased (Fig. 3).

If light exposure time is divided by the number of relieves, a rather constant light stimulation period of 55 s (±5 s) per relieve becomes visible (Fig. 4). On average, pigeons had a relieve every 43 min.

An univariate analysis with repeated measurements across time for the breeding days 2–17 shows no significant results for the variables $t_{le}$, $n_{re}$, and $t_{expo}$. The non-parametric Kruskal–Wallis test by ranks reveals an $H$ of (14, $n = 75$) = 4.44 with $p = .992$ for the variable $t_{le}$ (overall light exposure during a day), an $H$ of (14, $n = 75$) = 17.35 with $p = .238$ for the variable $n_{re}$ (number of relieves during the day) and an $H$ of (14, $n = 75$) = 6.71 with $p = .946$ for $t_{expo}$ (light exposure time). All three variables were tested for auto-correlation in order to find out if the light exposure or number of relieves of a particular day had an influence on the values of consecutive days. As the variation analysis tests already indicated, no auto-correlation was found for at least six lags. Hence, the time series of sequential breeding days showed a rather homogenous breeding behaviour with about the same amount of daily light exposure to the clutch.

## 4. Discussion

The present study shows that pigeons organise breeding relieves such that their clutch is light exposed for about a minute during each changeover. Relieves seem to take place as a quite stereotyped behaviour with a rather fixed duration. Daily variances in light exposure are therefore primarily a function of the variances in the number of relieves. With about 15 relieves per day, total light exposure time during a 17-day long breeding period is about 4 h. Cumulative light exposure for the critical tectofugal period (days 14–17) is close to 1 h.

Pre-hatching light stimulation initiates and/or modulates visual lateralisation in birds (Rogers, 1982, 1990; Skiba et al., 2002), but it is unclear how much light input is actually needed to induce an asymmetry of brain function. According to Rogers (1982), 2 h of light exposure within the last days before a hatch was sufficient in dark-incubated chicken eggs to establish visual lateralisation. Because this study did not aim to define the minimum amount of required light stimulation, shorter periods were not tested. As will be argued below, it is very likely that time periods even considerably lower than that observed in the present semi-natural study suffice to alter neural systems.

Since functional lateralisation is accompanied by anatomical left–right differences in the ascending visual pathways, it is very likely that asymmetric photic stimulation primarily affects maturation of these systems. In fact, the differentiation of the retinotectal system is an activity-dependent process which is characterised by a highly dynamic phase of dendritic and axonal arbor growth, retraction and stabilisation (Prior et al., 2004). During this period, neurons react very quickly to changes of the afferent input. In response to stimulation, intracellular signalling cascades are activated within minutes. These activity-dependent signalling pathways induce neural gene transcription by modulating the function of transcriptional activators and repressors which finally lead to structural changes of the nerve cells (West and Grace, 2002). Accordingly, 2–4 h of light stimulation are sufficient to increase dendritic growth rates (Sin et al., 2002), or to enhance translation of the neurotrophic factor BDNF (Tropea et al., 2001) while blocking synaptic activity for 1 h enhances tectal cell death (Galliresta et al., 1993) and reduces growth of tectal dendrites or promotes arborisation of retinal ganglion cell axons (O'Rourke et al., 1994; Rajan et al., 1999; Cohen-Cory, 1999). Increased axonal arbor dynamics are also achieved within 2 h by tectal BDNF application (Cohen-Cory and Fraser, 1995;

Alsina et al., 2001). However light can effect neuronal morphology faster since an increase in dendritic arbor growth within the optic tectum of tadpoles becomes already obvious during the first hour of visual stimulation (Sin et al., 2002). Moreover, the enhanced growth rates are maintained during a subsequent dark rearing period (Sin et al., 2002). Hence, light pulses to which the pigeon embryos of the present study were regularly exposed during relieves very likely continued to have an effect even after the parental bird covered the eggs again. Accordingly, it is conceivable that the length of light exposure is even less important for the onset of brain asymmetry than the number of relieves. Thus, it is plausible that the frequent relieves with their rather constant length could be a mean to repetitively induce asymmetrical morphological alterations in the developing visual system.

Trophic light effects are not infinite since the growth promoting effects onto tectal dendrites cannot be further enhanced by longer stimulation than 4 h (Sin et al., 2002). Consequently, there is possibly an optimal amount of light stimulation that reflects a balance between the necessity of retinal activation for visual development and the protection of the clutch that is achieved by the parental bird sitting on the eggs. Hence, short and repetitive periods of visual stimulation can be optimal to induce subtle imbalances between left- and right-hemispheric visual circuits, which are then stabilised during later phases of development to ultimately trigger the development of a behavioural lateralisation.

Retinal fibres reach the tectum at E14 and start making synaptic contacts from E15 on (Manns and Güntürkün, 1997). Under the present conditions, this ontogenetic period would enable the pre-hatch retinotectal system to be light exposed for about 1 h in a total of 36 bouts. Since pigeons raised under these conditions usually show a normal visual asymmetry, it is evident that these large numbers of very short light pulses are sufficient to provoke anatomical asymmetries. Since a recent study showed the visual Wulst to exert asymmetrical control over the tectofugal system (Folta et al., 2004), it is likely, that asymmetrical light input induces asymmetries both in the tectofugal and in the thalamofugal system.

Little is known about the developmental speed of the thalamofugal pathway. When retinal fibres reach the optic tectum, thalamic projection areas are already innervated (McLoon and Lund, 1982; O'Leary et al., 1983) and the thalamo-hyperpallial projection is already established at hatching (Wu and Karten, 1998), although activity levels within the Wulst only rise at a later point of time (Rogers and Bell, 1989). Thus, repetitive pre-hatch light stimulation probably modulates the thalamofugal system at least as long as the tectofugal one.

Pre-hatch light stimulation asymmetry seems to be the conditio sine qua non to induce visual lateralisation of object discrimination in pigeons (Skiba et al., 2002), though this is not essential for other forms of visually guided behaviour: dark-incubated chicks have functional asymmetries in imprinting (Johnston and Rogers, 1998) and display biochemical left–right differences in the frontal forebrain (Johnston et al., 1995). These asymmetries, however, can be altered by a lateralised light input (Johnston et al., 1997; Johnston and Rogers, 1999). Similarly, asymmetries of social recognition and monocular sleep patterns can be modified and aligned to coherent patterns of population asymmetry but also exist in dark-incubated chicks (Mascetti and Vallortigara, 2001; Vallortigara et al., 2001). Consequently, for various forms of visually guided behaviour, a lateralised and repetitive light input seems to be critical to either induce or to modify neural left–right differences.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.beproc.2006.03.012

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