The difference between acoustic delays observed was used to calculate the location of the acoustic window. In an example shown in figure 2, with the positions of sources S1, S2, and S3 differing by 60°, point W with coordinates x = 84 mm and y = 196 mm (relative to the reference point O) is defined by the requirement that d(1,2) = 110 μs (165 mm) and d(2,3) = 140 μs (210 mm). A total of 47 measurements was available for the calculation of the acoustic window coordinates. Of special interest is the longitudinal Y coordinate along the body axis because it is this parameter that is likely to indicate whether the acoustic window is located on the lower jaw or near the bulla and the auditory meatus. Taken together, these data are presented in figure 3 A showing the distribution of the observed distances between the reference point (melon tip) and the acoustic window. The mean distance and the standard deviation were 191 +/ −21 mm, with more than 95% of the measurements (45 out of the 47 available) exceeding 165 mm.

To compare these results with the basic head and cranial measurements, figure 3 B presents the side-view of the experimental dolphin's head and figure 3 C shows the side-view of the partially dissected head of the dead animal with the intact melon and exposed lower jaw, basal and occipital parts of the skull. A site 19 cm from the melon tip can be seen lying near the auditory meatus and the bulla, and outside the lower jaw.

Thus the data presented herein suggest that the acoustic window is located close to the auditory meatus and the bulla rather than on the lower jaw.

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Response of the starling circadian system to transitions between light and darkness

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Summary. The effects of exposure to sudden transitions from dark to light (D/L) and light to dark (L/D) were determined on the free running circadian feeding rhythm of European starlings (*Sturnus vulgaris*). D/L transitions (step-up) produced phase advances throughout the circadian cycle. In contrast L/D transitions (step-down) produced both advances and delays. The L/D transition phase-response curve has a contour and shape similar to the phase-response curves previously obtained in birds with light pulses.
Key words. Light-dark transitions; light pulse; phase-response curves; *Sturnus vulgaris*.

Recent views about how light/dark cycles synchronize circadian rhythm have originated primarily from studies of how brief pulses of light affect the timing of circadian rhythms [2]. Light pulses provided in 24-h-cycles reset the phase of the circadian clock (either by advancing or delaying) so that the rhythms under their control express a 24-h periodicity. Effects of light pulses and their phase-dependent phase shifts have been documented in detail in many species [3]. In contrast the effects of other forms of lighting information such as 'step-up' and 'step-down' (D/L and L/D transitions, respectively) have received a much lesser attention. Just as pulses of light and/or darkness do, D/L and L/D transitions also produce shifts in the phase of circadian rhythms depending upon the time of presentation within the cycle [4]. Such phase-dependent phase shifts, produced by simple transitions like D/L and L/D, have been recognized in organisms ranging from plants to mammals [5]. Despite these observations few data are available that systematically analyse the phase shifts produced by these light-on and light-off steps. The purpose of the present study is to investigate how these discrete D/L and L/D transitions phase shift circadian

rhythms of feeding in the European starling *Sturnus vulgaris*.

Materials and methods

20 male European starlings were kept individually in cages (60 × 50 × 50 cm) in a temperature controlled (21°–24 °C) chamber. Each cage contained a feeder with food pellets. Access to the food was through a hole (2 cm). Feeding starlings interrupted an infra-red light beam aligned with a photocell mounted across the feeding hole. Feeding activity data were collected continuously on an Esterline-Angus event recorder. Food and water were available ad libitum. Fluorescent tubes mounted above the cages provided continuous illumination. The effect of transitions between L and D on the phase of the circadian feeding rhythm was examined by changing the bright light (200 lx) and dim light (0.2 lx) conditions at intervals of about 10 days. The change in the light intensity was abrupt. Free running periods and phase shifts in feeding rhythms produced by these transitions were calculated by the linear regression method of Dann and Pittendrigh[6].

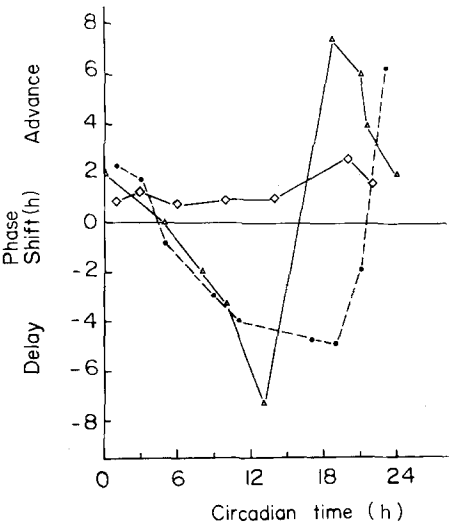

Figure 2. Phase response curve for D/L (◇—◇) L/D (▲—▲) transitions. Starlings were exposed to these single transitions at different phases spaced out 3 h apart. The magnitude of phase shift is plotted as a function of the circadian time (CT). CT = O denotes the onset of feeding of starlings. ●---● denotes 6-h light pulse phase response curve for sparrows[7].

Results and discussion

Figure 1 illustrates an ideal case of the effect of phase shifts on the feeding rhythm evoked by D/L and L/D transitions. D/L transitions evoked only advance phase shifts during the entire circadian cycle. In contrast L/D transitions produced both advances and delays in circadian phases of feeding rhythms, depending on the time in the cycle. L/D transitions consistently produced phase advances between 0 and 4 CT (circadian time) and between 16 and 24 CT. These transitions produced phase delays during the rest of the phases.

Phase shifts in response to D/L and L/D transitions have been observed in a variety of organisms[7]. For instance in tropical bats and temperate finches, the phase shifts produced by D/L and L/D transitions were found to be opposite in sign[3,4,7] (functionally important for entrainment). Furthermore, in some day-active species[3] and in a tropical bat[4] D/L transitions produced only phase advances and L/D transitions produced only phase delays; thus the sign of the phase shift was unidirectional[3]. In contrast L/D transitions in starlings effected both advance and delay phase shifts. Similar findings have been reported in the diurnal ground squirrel *Ammospermophilus*[7]. The direction and the relative magnitude of the phase shift obtained from L/D transitions in starlings resemble closely the direction and magnitude of the phase shifts that have been reported for 6-h light pulses in birds[8] (fig. 2).

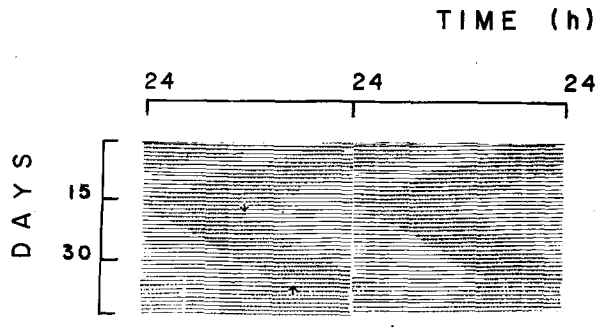

Figure 1. Effect of L/D transitions on feeding activity of *Sturnus*. ↓ indicates the phase at which L/D transition occurred, causing delay phase shift. ↑ indicates the phase at which D/L transition occurred causing an advance phase shift. L:200 lx (23.46 ± 0.38 h SD); D:0.2 lx (24.77 ± 0.31 h SD)

It is possible that light pulses phase shift circadian rhythms discretely once with D/L and later with L/D transitions[9]. Further, at least in pulses of shorter duration, no parametric modulations of period length (τ) are to be expected. If this is so then phase shifts may often be a residual expression of the dominance of the effects of L/D transitions (step-down component). The data suggested that the phase shifts produced by D/L and L/D transitions may be an important part of the process of how the circadian system determines its response to light pulses. How these photic stimuli (step-up and step-down) are physiologically communicated to the putative circadian clock(s) in starlings remains to be determined.

In addition to producing phase shifts D/L and L/D transitions altered the free running circadian periods. It is well known that in most day-active species, like the diurnal starling, the free running circadian period is shortened more in constant light than in constant darkness. This dependence of circadian period length on light intensity has been summarised in Aschoff's rule[10]. In starlings τ depends on light intensity in a manner consistent with Aschoff's rule.

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Changes in cAMP concentration in the rat preoptic area during synchronized and desynchronized sleep [1]

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Summary. cAMP concentration was found to be significantly lower during desynchronized sleep than during synchronized sleep in the preoptic area of rats kept at normal laboratory temperature. No significant changes in cerebral cortex cAMP concentration were observed in the same experimental conditions.
Key words. cAMP; sleep; preoptic area.

Sleep is a behavioral state characterized by two contrasting sets of functional events, occurring in cycles of ultradian periodicity. A reductionist classification of sleep is based on electroencephalographic (EEG) criteria, namely: synchronized (high amplitude, low frequency EEG waves) sleep (SS) and desynchronized (low amplitude, high frequency EEG waves) sleep (DS). SS is a stage of behavioral quiescence in which homeostasis is maintained at a lower level of energy expenditure than in wakefulness. DS is characterized by an impairment of the hypothalamic-preoptic control of homeostatic regulation [3]. In this respect, SS and DS are two opposite functional states. This dichotomy may be underlied by specific changes in cellular activity, as suggested by the finding that the responsivity of hypothalamic-preoptic neurons to direct thermal stimulation is strongly depressed during DS [4]. The possibility that there are specific cellular processes related to sleep in the hypothalamic-preoptic region is also suggested by recent biochemical results from this laboratory [5]. These findings have shown that the concentration of a second messenger (cAMP) changes in this region in accordance with the modification of the sleep cycle induced by a broad variation of ambient temperature (Ta). In particular, cAMP concentration decreases during sleep deprivation and increases during sleep recovery. Furthermore, both conditions are characterized by the disappearance of the nucleotide circadian rhythm (light-minimum and dark-maximum) observed in control conditions [6].

The present study was performed in order to assess whether the functional states corresponding to SS and DS, respectively, might also be characterized by changes in the concentration of cAMP in the preoptic area.

Materials and methods

45 male Sprague-Dawley rats (300 g) housed in normal laboratory conditions (Ta 22 ± 0.5 °C, food and water ad libitum, 12:12 h light-dark schedule (LD); L: 07.00-19.00 h) were used. Animals were implanted with surface electrodes for EEG recording under general anesthesia (50 mg/kg ketamine-HCl and 1 mg/kg flunitrazepam, i.p.). Recording sessions were started 3-5 days after surgery, and sacrifice was carried out between 13.00 and 18.00 h. Animals were acclimatized to the experimental condition during the first L hours of the recording day and during this period the sleep pattern was monitored. They were assigned to each experimental session according to a randomized block experimental design and sacrificed in liquid nitrogen (at least 30 s from the onset of SS and DS) by opening the cage floor by remote control. The removal of samples from the preoptic area and the cerebral cortex, taken as a control, was performed as previously described [7]. The samples were homogenized in 150 µl of 5% ice-cold trichloracetic acid (TCA) and centrifuged at 10,000 g for 10 min at 2 °C. 100 µl of 1 M HCl was added to 100 µl of supernatant which, following the extraction of TCA with aqueous diethyl ether, was lyophilized and stored at − 80 °C. The sediments were resuspended in 150 µl of 1 M NaOH and total protein concentration was determined on 40-µl aliquots using a modified Bradford's protein assay [8], with rabbit gamma-globulins as standard (Sigma, UK). cAMP was determined by means of a competitive radiobinding assay using a commercial kit (Amersham, UK). The statistical analysis of the results was carried out by means of covariance analysis (regression of pmol of cAMP on mg of protein).