Research report

The effects of early post-hatching changes of imprinting object on the pattern of monocular/unihemispheric sleep of domestic chicks

Daniela Bobbo a, Giorgio Vallortigara b,c, Gian Gastone Mascetti a,∗

a Department of General Psychology, University of Padova, Via Venezia 8, 35131 Padova, Italy
b Department of Psychology, University of Trieste, Via S. Anastasio 12, 34123 Trieste, Italy
c BRAIN Center for Neuroscience, University of Trieste, Via S. Anastasio 12, 34123 Trieste, Italy

Received 14 October 2005; received in revised form 16 January 2006; accepted 24 January 2006
Available online 17 April 2006

Abstract

The pattern of monocular/unihemispheric sleep (Mo–Un sleep) was studied behaviourally in male and female chicks after early post-hatching changes of the imprinting object. Chicks were reared with an imprinting object on day 1 post-hatching which was removed or changed on day 2. On day 1, time spent in binocular sleep (both eyes closed) was similar in male and female chicks, though the number of episodes was lower in females than in males. There was no eye-closure bias in the pattern of Mo–Un sleep (one eye shut and the other open) in chicks of both sexes. On day 2, chicks subjected to the removal of imprinting object showed less time and number of episodes of binocular sleep than control chicks and chicks subjected to changes of imprinting object. There was no eye-closure bias in control chicks whilst a significant bias for more right Mo–Un sleep was recorded in chicks after removal and changes of imprinting object of both sexes. It is suggested that the removal or changes of imprinting object would cause a decrease of binocular sleep and trigger processes associated to secondary imprinting involving the left hemisphere. The bias for more right Mo–Un sleep (right eye-closure) could be the by-product of consolidation processes of secondary imprinting memories in the left hemisphere and/or of more left eye-opening as a result of periodical awakening of right hemisphere to control the environment after a stressful condition such as the removal or change of imprinting object.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Sleep; Unihemispheric sleep; Imprinting; Avian brain; Gallus gallus

1. Introduction

Domestic chicks (Gallus gallus) show a remarkable later-
alization of brain functions [see: 20,28]. Overall, the left hemi-
sphere seems to be mainly involved in visual learning and stimu-
lus categorization whilst the right hemisphere seems to be mainly
involved in spatial analysis and response to novelty [20,30,31].

Domestic chicks also exhibit a particular behavioural and elec-
trophysiological state called monocular-unihemispheric sleep
(Mo–Un sleep) [4,11,14,15,18,24]. During normal sleep, chicks
exhibit brief and transient periods in which one eye is opened
while the other remains shut. Electrophysiological recordings
have shown that the hemisphere contra-lateral of the opened eye
showed an EEG composed with fast waves typical of wakeful-
ness state whilst in the hemisphere contra-lateral of the closed
eye the EEG pattern is composed of slow waves typical of sleep
[4].

Chicks exhibit an age-dependent pattern of Mo–Un sleep
(Mo–Un sleep) during the first 2 weeks post-hatching [11,13]
which seems to be associated with the age-dependent shifts in
bias in the control of behaviour exerted by one or the other
hemisphere [19] and strongly affected by rearing conditions
and sex [11,13]. During the first week post-hatching, female
chicks reared with an imprinting object (a red ball) showed a
pattern of Mo–Un sleep with either right or left eye-closure
occurring with approximately the same frequency with the
exception of day 5 in which right eye-closure predominated. A
significant bias towards a more left Mo–Un sleep (left-eye clo-
sure/right unihemispheric sleep) was reported during the second
week [11]. Female chicks reared without an imprinting object
showed predominant left Mo–Un sleep (left-eye closure/right
unihemispheric sleep) during both weeks, except on day 8 in
which there was an equal amount of right and left eye-closure. Comparing the two rearing conditions, it was apparent that during the first week imprinted female chicks showed more right Mo–Un sleep (right-eye closure/left unihemispheric sleep) compared to chicks reared without the imprinting object. This effect was tentatively associated with consolidation of imprinting memories in the left hemisphere [see: 8]. A change of the imprinting object (i.e., in colour) or its removal at day 8 post-hatching caused a striking shift towards a predominant right Mo–Un sleep [11]. This effect was tentatively associated with a process of consolidation of secondary imprinting in the left hemisphere (right-eye closure) and a right hemisphere involvement (left eye opening) in response to novelty with regards to the change in colour of the imprinting object [26]. The latter process alone (left-eye opening in response to novelty) could account for the effect of removal of the imprinting object [26].

A different pattern of eye-closure was showed by male chicks [13]. During the first week post-hatching, both imprinted and non-imprinted male chicks showed a pattern of Mo–Un sleep with either right or left eye-closure occurring with approximately the same frequency. Moreover, the pattern of eye-closure was not affected by the presence or the absence of imprinting object. During the second week, imprinted and non-imprinted chicks showed opposite patterns. There was a tendency towards a predominant left Mo–Un sleep in imprinted males (a pattern similar to that found in imprint females) and a tendency for more right Mo–Un sleep in non-imprinted males (opposite to the pattern found in non-imprinted females). Apparently, the deprivation of a proper visual stimulus for imprinting has different behavioural outcomes (and, likely, neural consequences) in males and female chicks. Since, sex differences in behaviour of young chicks are well known [29] it is likely that the two sexes adopted different strategies to face the difficulties of forming stable social attachments. Males showed increased exploratory behaviour (mediated by the right hemisphere) and females showed increased fear and strong social reinstatement tendencies (mediated by the left hemisphere). Finally, males did not show any significant eye-closure bias after the removal of the imprinting object at day 8, whilst there was a mild bias towards more right Mo–Un sleep after a change in colour of the object [13]. Presumably, males were little affected by changes of imprinting object and thus less affected than females because they have lower tendencies to establish social attachments [25,27].

The effect caused by the removal or the change of the imprinting object on chick’s pattern of binocular sleep and Mo–Un sleep was further investigated in this study. The difference with previous studies resides in the fact that the removal or change of imprinting object was performed at day 2 post-hatching when the imprinting process is on the way of being implemented or it has been shortly consolidated. The questions we addressed were: are chick’s binocular and Mo–Un sleep patterns affected by the removal or the change of imprinting object performed early post-hatching? Do the removal and change of imprinting object affect similarly sleep patterns of male and female chicks?

2. Experiment

The pattern of binocular and Mo–Un sleep was studied in male and female chicks during the first 2 days post-hatching in two experimental conditions: (a) with an imprinting object during days 1 and 2; (b) after the removal or a change of colour in the imprinting object immediately the first session of recording of sleep of day 2. In an episode of binocular sleep, chick slept with both eyes closed adopting one of the classical body postures of sleep: SS (standing sleep), BF (bill forward), BOG (bill on the ground) and HOG (head on the ground) [11]. In addition, chicks showed a bilateral/bihemispheric slow wave EEG pattern [14,15]. In an episode of Mo–Un sleep, chick adopted one of the body postures of sleep (BF and BOG more frequently) and one eye remained closed whilst the other was opened [11,14]. In addition, the hemisphere contra-lateral of the opened eye showed an EEG composed with fast waves typical of wakefulness state whilst in the hemisphere contra-lateral of the closed eye the EEG pattern is composed of slow waves typical of sleep [4,14].

3. Materials and methods

3.1. Subjects

The subjects were 52 Hybro domestic chicks (Gallus gallus) (a local hybrid variety derived from the White Leghorn breed): 26 males and 26 females. Chicks arrived in the laboratory from a commercial hatchery in the early morning of day 1 post-hatching.

3.2. Apparatus

Chicks were immediately housed singly into glass cages (40 cm × 40 cm × 30 cm) with semi-transparent cloths along the inner walls that served as one-way screen. Each cage was illuminated continuously from above and contained two identical small transparent glass containers (5 cm in diameter, 5.5 cm in height), one for food and the other for water both available ad libitum throughout the period of observations. Room temperature was maintained at 28 ± 1 °C.

3.3. Procedure

There were two rearing conditions for both male and female chicks: (A) Control condition (CO-chicks, n = 14; eight males and six females) and females: a red plastic ball (4 cm × 3 cm) was suspended freely in the middle of the cage at about head height during days 1 and 2. Previous studies have shown that this stimulus is very effective in producing social attachment in chicks [26]. (B) Experimental conditions (n = 38: 20 males and 18 females) the red plastic ball was present during day 1 but it was removed from the cage or it was changed in colour at the early morning of day 2. Three experimental conditions were devised at day 2: (1) the removal of the red ball (RE-chicks, n = 12: six males and six females); (2) the red ball was replaced by a yellow ball of the same size and shape (YE-chicks, n = 14: eight males and six females); (3) a yellow ball (same size and shape) was added to the red ball (R × Y-chicks, n = 12: eight males and six females).

We used direct observations for the scoring of sleep in each session, which was performed by two experimenters who alternated in observing the pairs of chicks located in cages within a single room. The room was completely darkened, but the glass cages were illuminated singly from above. The experimenters could move all around the cages to find the best position for observing eye closure on the basis of the posture of the animal. Small mirrors mounted on rods allowed the experimenters to approach the animal and to check for eye closure or opening without disturbing it when the position or the posture of the animal made the observation from the outside difficult. There were 3 h of observation during the morning and 3 h observation during the afternoon. For each day, at least
two different experimenters alternated in the task of observing the animals. The
observers, who were unaware of the results obtained by each other, recorded
the number and the duration of the episodes of binocular and of Mo–Un sleep.
Observations were carried out from days 1 and 2 inclusive.

3.4. Data analyses

Percentage of time spent and percentage of the number of episodes of binocu-
lar sleep were analysed by ANOVAs with “sex” and “rearing” as between-subjects
factors and “age” as a within-subjects factor. For monocular sleep, a “laterality
index” was calculated for time spent and for number of episodes of right or left
Mo–Un sleep using the formula: \((\text{time (number of episodes) spent with the left
eye closed} – \text{time (number of episodes) spent with the right eye closed})/\text{(time
(number of episodes) spent with the left eye closed + time (number of episodes)
spent with the right eye closed)}) \times 100.\)

Both laterality indices were analysed by ANOVAs with “sex” and “rearing
condition” as between-subjects factors and “age” as a within-subjects factor.
Significant departures from chance level (0%), which indicated significant bias
towards right- or left-eye closure, were estimated by one-sample two-tailed \(t\)-
tests.

4. Results

4.1. Binocular sleep

The percentage of the time spent and the mean number of
episodes of binocular sleep in female and male chicks dur-
ing day 1 post-hatching are shown in Fig. 1A and B, respec-
tively. The ANOVA showed that the effect of sex was not
statistically significant with regards to the time spent in binocu-
lar sleep \((F(1,50) = 0.805, p = 0.373)\), whereas it was significant
for the number of episodes of binocular sleep \((F(1,50) = 9.838,
p = 0.002)\), with males showing an higher number of episodes
than females.

The ANOVA on the percentage of time spent in binocu-
lar sleep on day 2, revealed that the main effect of rearing
was significant \((F(3,44) = 2.945, p = 0.043)\) whilst sex and inter-
action sex \(\times\) rearing were both not significant \((F(3,44) = 0.135,\)
\(p = 0.714; F(3,44) = 1.341, p = 0.273,\) respectively). Therefore,
data for males and females were lumped together in Fig. 2. Post
hoc analysis (Fisher’s protected LSD) revealed a significant dif-
ference between: RE-chicks and R + Y-chicks \((p = 0.006)\) and
CO-chicks and R + Y-chicks \((p = 0.049)\).

The percentage of episodes of binocular sleep during day 2 is
shown in Fig. 3A and B for female and male chicks, respectively.
The ANOVA revealed significant effects of sex \((F(1,44) = 11.285,\)
\(p = 0.001)\), rearing \((F(3,44) = 4.813, p = 0.005)\) and sex \(\times\) rearing
interaction \((F(3,44) = 4.674, p = 0.006)\). In females, post hoc
analysis (Fisher’s protected LSD) revealed a significant differ-
ence between: CO-chicks and RE-chicks \((p = 0.002)\), RE-chicks
and R + Y-chicks \((p = 0.042)\) and RE-chicks and YE-chicks
\((p = 0.011)\). In males, post hoc analysis (Fisher’s protected LSD)
revealed a significant difference between: CO-chicks and RE-
chicks \((p = 0.009)\), CO-chicks and R + Y-chicks \((p = 0.021)\), RE-
chicks and YE-chicks \((p = 0.012)\) and YE-chicks and R + Y-
chicks \((p = 0.028)\).

Overall, on day 1 the time spent in binocular sleep was similar
in male and females, whilst the number of episodes was lower in
males than in females. On day 2, RE-chicks of both sexes spent
relatively less time in binocular sleep than CO-chicks and R + Y-
chicks. The amount of episodes was lower in female RE-chicks
than in CO-chicks, R + Y-chicks and YE-chicks.

After removal of the imprinting object, females spent less
time in binocular sleep than females of the other groups. In
males, RE-chicks and R + Y-chicks had a lower number of
episodes than males of the other groups. In
males, RE-chicks and R + Y-chicks had a lower number of
episodes than CO-chicks and YE-chicks. Thus, males after both the removal of imprinting object and the addition of a yellow ball showed a decrease in the number of episodes of binocular sleep with respect to control males and males in which the red ball was substituted by a yellow ball.

4.2. Monocular sleep (Mo–Un sleep)

The laterality index for the time and the number of episodes with either right or left eye-closure on day 1 are shown in Fig. 4A and B, respectively. The ANOVA on the time spent and on the number of episodes revealed that the sex factor was not significant (respectively, \(F_{1,50} = 1.284, p = 0.262; F_{1,50} = 1.222, p = 0.274\)). One sample t-tests revealed a significant bias towards a left eye-closure: time (\(t_{51} = 2.190, p = 0.033\)); episodes (\(t_{51} = 2.375, p = 0.021\)).

The laterality index for the time and the number of episodes on day 2, are shown in Fig. 5A and B, respectively. The ANOVA on time revealed a significant main effect of rearing (\(F_{3,44} = 3.934, p = 0.014\); sex and sex x rearing interaction were not significant (\(F_{1,44} = 0.027, p = 0.870; F_{3,44} = 0.092, p = 0.964\), respectively). One sample t-test revealed significant bias for more right eye-closure in YE-chicks (\(t_{14} = 3.274, p = 0.001\)), RE-chicks (\(t_{14} = 12.270, p = 0.001\)) and R + Y-chicks (\(t_{14} = 3.985, p = 0.002\)). No eye-closure bias was found in CO-chicks.

Overall, on day 1 a slight but significant bias towards more left Mo–Un sleep was found in females, whilst males showed a similar amount of left and right Mo–Un sleep. On day 2, no eye-closure bias was apparent in CO-chicks, but a significant bias for more right Mo–Un sleep was observed in experimental male and female chicks after either the removal or a change of the imprinting object.

5. Discussion

Time spent in binocular sleep on day 1 post-hatching was similar in male and female chicks whilst the number of episodes was significantly lower in females than in males (about 10%
gested that such bias might be connected to the establishment of a process of secondary imprinting [5], that is to say, to a renewed need for the left hemisphere to fixate in memory the characteristics of a novel imprinting object. A novel object (a yellow ball) beside the imprinting object (red ball) as well as a change in colour of the imprinting object (from red to yellow) may have also triggered a process of secondary imprinting. It is has been reported that memory trace of imprinting is initially stored in the left IMHV (intermediate part of Hyperstratum Ventrale), an associative forebrain structure [7], as a form of enriching simple representation of imprinting object. Furthermore, imprinting is accompanied by a learning-related increase in NMDA-type receptor binding levels in the left IMHV whilst this binding levels remained unchanged in right IMHV [10]. Then, it may be assumed that the memory trace of secondary imprinting would be stored in the left IMHV of experimental chicks and that the increase of right Mo–Un sleep might be associated with consolidation of that memory trace in the left hemisphere. This assumption is in agreement with the current hypotheses that sleep is necessary for the consolidation of memories acquired during wakefulness [6,21,22]. An increase of paradoxical sleep following imprinting has been described in chicks was reported [23] and although no data are available regarding the pattern of sleeping of the two hemispheres, the association with imprinting makes the occurrence of lateralization very probable. On the other hand, the removal of imprinting object was relatively more effective in triggering the bias shift towards more right Mo–Un sleep. Possibly, RE-chicks developed a secondary imprinting process towards some features present in the environment such as the glass containers or the cage walls [3,13].

Another interesting aspect is that the bias for more right Mo–Un sleep would be associated with a bias for more left eye-opening (right hemisphere awakening) which may serve to periodically monitoring the environment after potentially important and stressful episodes, such as the removal and changes of imprinting object. It is known that the right hemisphere has a special competence in spatial analysis and in response to novelty [16,26].

At this point, an important question merits to be considered: since the amount of Mo–Un sleep is about 1% of total time spent sleeping, would the increase right Mo–Un sleep have a biological meaning? In previous studies [4,11–13] the time spent in Mo–Un sleep ranged steadily around 1–2% of sleep time. However, the unilateral closure of right or left eye during sleep does not occur randomly but it is closely correlated with the shifts of hemispheric dominance during chick’s development [11,13], it is modulated by specific experiences during wakefulness such as presence, absence and changes of imprinting object [11,13], the light stimulation in “ovo” [4,12] and learning experiences during wakefulness (Masotti, unpublished data). Therefore, Mo–Un sleep seems to be a function correlated with chick’s behavioural experiences and with the hemisphere that was in control of behaviour during the previous wakefulness. In addition, it has been proposed that Mo–Un sleep would be involved in processes of local cerebral recovery and particularly in the hemispheric recovery [2,9,32]. In birds, it has been reported that one eye-opening during sleep would have a vigilance and anti-predatory functions [17,18]. Likely, 1–2% of
Mo–Un sleep should be enough time for accomplishing those features.

In previous studies on Mo–Un sleep in chicks [11,13] changes in the imprinting object were performed at the beginning of second week. The removal of imprinting object on day 8 caused a bias shift towards right Mo–Un sleep in both males and females [11,13], whilst the Mo–Un sleep pattern was symmetrically altered by the change in the imprinting object in males [13] and after the addition of a novel object to the original imprinting object in females [11]. Therefore, the removal of the imprinting object after day 2 (present experiments) and day 8 [11] seems to produce similar eye-closure and eye-opening bias shifts, whilst changes in the imprinting object did not. Probably, changes of imprinting object performed later post-hatching would have a lower effect because other stimuli become of interest to the chicks, such as objects that are not related with imprinting and other features present in the environment. It has been reported that chicks on the second week start to move about exploring the environment and to abandon strict physical contact with the mother hen and siblings [1]. This can account reasonably well for similarities and differences in the shifts in bias in Mo–Un sleep at different ages.

Acknowledgment

This research was supported by Grant COFIN-PRIN no. 200411375.004 of the Italian Ministry of University and Research (MIUR).

References