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Article

Dynamics of a Memory Trace: Effects of Sleep on Consolidation

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Summary

Background: There is evidence that sleep is important for memory consolidation, but the underlying neuronal changes are not well understood. We studied the effect of sleep modulation on memory and on neuronal activity in a memory system of the domestic chick brain after the learning process of imprinting. Neurons in this system become, through imprinting, selectively responsive to a training (imprinting) stimulus and so possess the properties of a memory trace.
Results: The proportion of neurons responsive to the training stimulus reaches a maximum the day after training. We demonstrate that sleep is necessary for this maximum to be achieved, that sleep stabilizes the initially unstable, selective responses of neurons to the imprinting stimulus, and that for sleep to be effective, it must occur during a particular period of time after training. During this period, there is a time-dependent increase in EEG activity in the 5–6 Hz band, that is, in the lower range of the theta bandwidth. The effects of sleep disturbance on consolidation cannot be attributed to fatigue or to stress.
Conclusions: We establish that long-term trace consolidation requires sleep within a restricted period shortly after learning. Undisturbed sleep is necessary for the stabilization of long-term memory, measured at the behavioral and neuronal levels, and of long-term but not short-term neuronal responsiveness to the training stimulus.

Introduction

After learning, memories undergo a process of stabilization or consolidation ([see [1]). In mammals [2, 3] and birds [4, 5], this process is influenced by periods of sleep. Recent studies have demonstrated that sleep affects developmental synaptic plasticity in the visual cortex of kittens [6], but the impact of sleep on the physiological and cellular mechanisms underlying memory is predominantly unknown [2]. For addressing this issue, it is necessary to track the formation of a particular memory "trace" and to determine whether sleep is involved in its stabilization.

There is good evidence of such a trace for the recognition memory of imprinting in the domestic chick. A wide body of converging evidence suggests that information acquired through this learning process is stored in a region of the brain known as the intermediate and medial mesopallium (IMM, formerly known as intermediate and medial hyperstriatum ventrale, IMHV [7, 8]). The medial mesopallium may also serve as a memory store in passive avoidance learning [9] and auditory imprinting [10]. In addition, in songbirds, recent evidence has implicated the caudal part of the medial mesopallium as a store for the tutor's (usually the father's) song that the nestling hears and months later reproduces ([11], for review, see [12]). Collectively, these findings suggest that the medial mesopallium has a generic role in memory storage functions within the avian brain.

In respect of imprinting, young visually naive domestic chicks quickly learn the characteristics of certain visually conspicuous objects (imprinting stimuli) on being exposed to them ("training"). The chicks subsequently approach and direct complex social behaviors to the imprinted stimulus rather than to other, novel stimuli (see e.g., [13]). Imprinting leads to a substantial increase in the proportion of neurons in IMM, but not in the hippocampus, that respond to the imprinting stimulus [14–16]. Some IMM neurons respond selectively to this stimulus (IS neurons) and so exhibit the postulated properties of the memory trace [17]. In a previous study using behaving chicks, the development of increased responsiveness to the imprinting stimulus was investigated [18]. Recordings were made of IMM neurons before, during, and after (up to $\sim$21.5 hr) the end of training. The number of IS neurons doubled after 1 hr of training and remained at approximately this level after an additional 1 hr of training. By the last recording session of the experiment ($\sim$21.5 hr after training), the number of IS neurons had increased to a maximum and was approximately treble the proportion found before training. The proportion of neurons that responded to a visual stimulus not seen during training did not vary significantly across time.

Some insight into how this posttraining increase might have been achieved came from tracking a number of individual neurons across time [18]. Approximately two-thirds of the IS neurons recorded after the first hour of training ceased to respond selectively to the imprinting stimulus after the second hour of training. Their number was taken up, almost exactly, by neurons that had previously failed to respond selectively to this stimulus. Neurons were not tracked beyond this recording period, so it was not possible to determine how the final increase occurred. However, the final increase could be predicted by adding the number of IS neurons recruited during the second hour of training to the number of IS neurons recorded after the first hour of training. It was hypothesized that the IS neurons that had ceased to respond selectively to the imprinting stimulus recovered this responsiveness as a result of sleep preceding the final session of recording.

The present study was designed to test this hypothesis by extending the period over which the activity of individual

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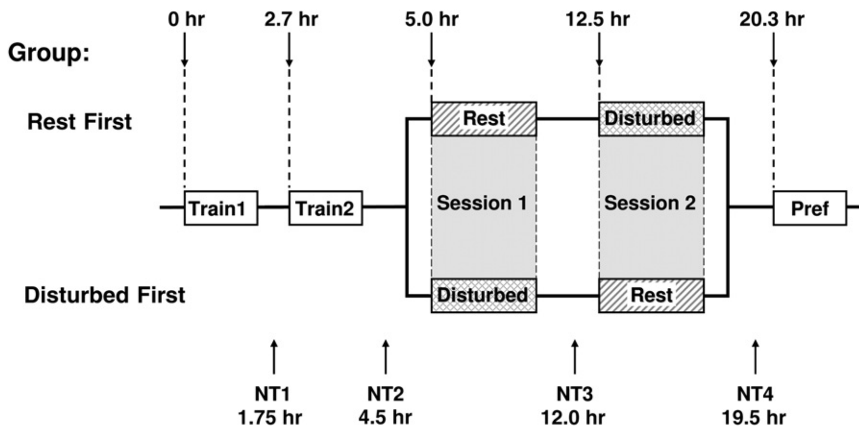

Figure 1. Experimental Protocol; see Supplemental Experimental Procedures

Zero hours is the time at which training began. Upward-pointing arrows indicate the approximate mean time of the midpoints of the neuronal tests NT1 through NT4. Downward-pointing arrows indicate the approximate start times for the procedures indicated in the boxes. The following labels are defined: Train, training session; NT, neuronal test; and Pref, preference test. "Rest" and "Disturbed" refer to treatment conditions; "Session" refers to the first 6 hr (session 1) or second 6 hr (session 2) of treatment.

neurons could be tracked and by determining whether sleep after training affects the stabilization of IMM neuronal responses to the imprinting stimulus.

Results

A total of 101 neurons ("overall neuronal population") were recorded from two groups of five chicks, i.e., a total of ten chicks. Each group was given two training periods followed by two treatment sessions. In the first 6 hr session, shortly after the end of training, one group (Rest First) was allowed undisturbed rest shortly after the end of this training, whereas the other group (Disturbed First) had their period of rest during this session disturbed to prevent prolonged periods of sleep (see session 1, Figure 1). Subsequently, in the second 6 hr session, the Disturbed First group was allowed a period of undisturbed rest, whereas the Rest First group was disturbed (see session 2, Figure 1). Neuronal responsiveness was measured at four times: shortly after the first (NT1) and second (NT2) periods of training and after the first (NT3) and second (NT4) sessions (Figure 1). Thirty-seven neurons were recorded from the Rest First group, and 64 neurons were recorded from the Disturbed First group.

Of the 101 recorded neurons, 62 (61%) responded selectively to the imprinting stimulus and so were classed as imprinting stimulus neurons (IS neurons) during at least one neuronal responsiveness test (Rest First group, 25 neurons; Disturbed First group, 37 neurons; Figure S1 available online). IS neurons responded to the compound training stimulus, a rotating red box together with the sound of a maternal call (Tcomp), and/or to the visual component of Tcomp (Tvis) but not to an alternative visual stimulus (Avis). The visual training stimulus (Tvis) and the alternative visual stimulus (Avis) are used in behavioral tests of chicks' visual preferences [19] (Figure 1). An example of the activity of an IS neuron is given in Figure S1. The activity of 65 individual neurons could be followed through all the four tests of neuronal responsiveness (NT1-4; "tracked neurons"; Rest First group, 21 neurons; Disturbed First group, 44 neurons; see Table 1). Tracked waveforms of two such neurons may be seen in Figure S2). Of the 65 tracked neurons, 50 were classed as IS neurons during $\geq$1 test (Rest First group, 18 neurons; Disturbed First group, 32 neurons).

Overall Neuronal Population

Selective Responsiveness to the Imprinting Stimulus: Effects of Group, i.e., Rest First, Disturbed First

The numbers of IS neurons and of neurons that failed to respond selectively to the imprinting stimulus (nonimprinting stimulus, NIS, neurons) are given in Table 1. The mean percentages of IS neurons in each neuronal test are plotted in Figure 2A. The distributions of these percentages in the Rest First and Disturbed First groups differed significantly (group × neuronal test interaction binominal error model [BEM] $\chi^2_3 = 12.30$, $p = 0.006$; see Figure 2A). The difference between the two groups is attributable to the difference in the percentages observed at NT4, the final neuronal test. The mean percentage of IS neurons at this test in the Rest First group was greater than double that in the Disturbed First group (BEM $\chi^2_1 = 12.88$, $p < 0.001$; see Figure 2A and Table 1). In contrast, in each of the other neuronal tests (NT1, NT2, and NT3), the mean percentages of IS neurons were similar in the two groups (Figure 2A). In none of these three neuronal tests was there a significant difference between the two groups. Within each group, responsiveness at NT4 was compared to that at NT1 (the test after the first period of training). In the Rest First group, there was a significantly greater percentage of IS neurons at NT4 than at NT1 (BEM $\chi^2_1 = 10.17$, $p = 0.001$). In contrast, in the Disturbed First group, the percentage of IS neurons at NT4 was slightly, though not significantly, lower than that at NT1 (Figure 2A). Thus, when tested at NT4 ~15.5 hr after the end of training, the group allowed undisturbed rest shortly after (~1.3 hr; Rest First group) the end of training had a higher percentage of IS neurons than the group not allowed undisturbed rest until later (~9 hr after the end of training; Disturbed First group; Figure 2A). Moreover, these experiments establish that by this final test (NT4), the proportions of IS neurons had increased significantly for the group that rested shortly after training (Rest First group); no such increase occurred in the group in which rest was delayed (Disturbed First group).

In the Rest First group, the percentage of IS neurons increased approximately linearly with time to a maximum at NT4 (linear regression, BEM $\chi^2_1 = 8.84$, $p = 0.003$; Figure 2A). In the Disturbed First group, the percentage increased linearly with time until test NT3 (BEM $\chi^2_1 = 7.18$, $p = 0.007$) and thereafter declined significantly (comparison between NT3 and NT4, BEM $\chi^2_1 = 11.05$ $p < 0.001$; Figure 2A).

What factors might account for the differences in selective responsiveness to the imprinting stimulus at NT4? Rest per se does not lead to increased responsiveness: in the Disturbed First group, responsiveness declined after a 6 hr rest period (Figure 2A, broken line, NT3 to NT4). Disturbance per se does not lead to decreased responsiveness; in fact, our results would suggest the contrary. Both groups of chicks experienced a 6 hr period during which rest was disturbed (Figure 1, "Disturbed"). In both groups, selective responsiveness

Table 1. Number of Neurons Recorded within Each of the Four Neuronal tests

	Group	Classification of Neuron	Neuronal Test NT1	NT2	NT3	NT4
Overall Neuronal Population						
IS and NIS Neurons at Each Test						
	Rest First					
		IS	8	10	14	17
		NIS	23	17	16	12
		Total	31	27	30	29
	Disturbed First					
		IS	17	17	26	12
		NIS	39	39	31	41
		Total	56	56	57	53
Avis Neurons Responding at Each Test						
	Rest First					
		Avis neuron	2	2	1	1
		Total	31	27	30	29
	Disturbed First					
		Avis neuron	5	7	8	2
		Total	56	56	57	53
Tracked Neurons						
IS and NIS Neurons at Each Test						
	Rest First					
		IS	6	9	10	15
		NIS	15	12	11	6
		Total	21	21	21	21
	Disturbed First					
		IS	15	16	24	10
		NIS	29	28	20	34
		Total	44	44	44	44
Avis Neurons Responding at Each Test						
	Rest First					
		Avis neuron	1	2	1	0
		Total	21	21	21	21
	Disturbed First					
		Avis neuron	4	7	7	1
		Total	44	44	44	44

Shown are numbers of neurons set out according to their classification (IS, NIS, and Avis), together with the total number of neurons recorded within the neuronal test. Data are given according to group. Overall neuronal population: The number of IS neurons at each neuronal test are shown, together with the number of NIS neurons; numbers of Avis neurons responding at each neuronal test are also shown. Tracked neurons: Numbers of IS neurons at each neuronal test are shown, together with the number of NIS neurons; the number of Avis neurons responding at each neuronal test are also shown. For further discussion, see text.

to the imprinting stimulus increased (Figure 2A, Rest First group, continuous line, NT3 to NT4; Disturbed First group, broken line, NT2 to NT3). These findings rule out the possibility that the decline in selective responsiveness to the IS at NT4 in the Disturbed First group is due to fatigue. Instead the results, taken together, imply that selective responsiveness to the imprinting stimulus at NT4, ~19.5 hr after the start of training, is affected by the order in which the two treatments (rest and disturbed) occurred.

Selective Responsiveness to the Alternative Visual Stimulus

Of the 101 neurons recorded, 20 neurons (five in the Rest First group and 15 in the Disturbed First group) were responsive to the alternative visual stimulus (Avis) during at least one of the neuronal test sessions, without responding to any of the other stimuli during the same session. The responsiveness of the neurons toward Avis was similar between the groups and between the neuronal tests (Figure 2B, Table 1).

Tracked Neurons

It was possible to track 65 neurons through all four neuronal tests. The overall pattern of responses among these neurons was similar to that of the whole population of recorded neurons (Figures 2C and 2D and "Tracked Neurons" section of Table 1; cf. Figures 2A and 2B and "Overall Neuronal Population" section of Table 1). See Supplemental Experimental Procedures for details of statistical analysis.

Testing Hypotheses Concerning the Origin of Neurons Responsive at NT4

The previous study [18] raised several questions about the pattern of responsiveness of individually tracked IS neurons across time. We address these questions here and also inquire whether the patterns observed differ between Rest First and Disturbed First groups.

Do neurons that respond selectively to the imprinting stimulus after 1 hr or 2 hr of training continue to respond in this way at all subsequent neuronal tests? In both groups of chicks, the responses of many of these IS neurons varied across the neuronal tests (Figure 3). In particular, and as in the previous study [18], almost two-thirds of the 21 IS neurons recorded after the first 1 hr period of training ceased to respond selectively to the imprinting stimulus after the second hour of training (13/21; 62%). Their number, as in that study [18], was taken up by neurons that had previously failed to respond selectively to the stimulus.

Do all neurons that respond selectively to the imprinting stimulus at NT1 or NT2 also respond selectively to it at NT4? IS neurons are tracked in Figure 3. In the Rest First group, there were 13 IS neurons at NT1 (Figure 3A, six neurons) or NT2 (Figure 3A, seven neurons). Ten of these remained IS neurons at the last recording session, NT4. The observed proportion (10/13) is not significantly different from the proportion expected (13/13) if all neurons responding selectively to the IS at NT1 or NT2 also responded at NT4.

This result was not found for the Disturbed First group. Twenty-five IS neurons were recorded at NT1 (Figure 3B, 15 neurons) or NT2 (Figure 3B, 10 neurons); only six of these remained IS neurons at NT4. The observed proportion (6/25) is significantly different from the expected proportion (25/25). The two ratios for the two treatment groups, Rest First (10/13) and Disturbed First (6/25), are significantly different (BEM $\chi^2_1 = 10.13$, $p = 0.001$). Hence, the different order of treatments affects the probability of IS neurons recorded at NT1 or NT2 being represented in the population of IS neurons recorded at NT4.

Between both groups, a total of 12 neurons first became IS neurons ~8 hr or ~15.5 hr after the end of training (at NT3 or NT4; Figure 3). Of these 12 neurons, six neurons had previously responded to the imprinting stimulus, but they also responded to the alternative visual stimulus. Accordingly, they had then been classified as NIS neurons. They became IS neurons at NT3 because their responses had become selective. The remaining six neurons had not previously responded to the imprinting stimulus.

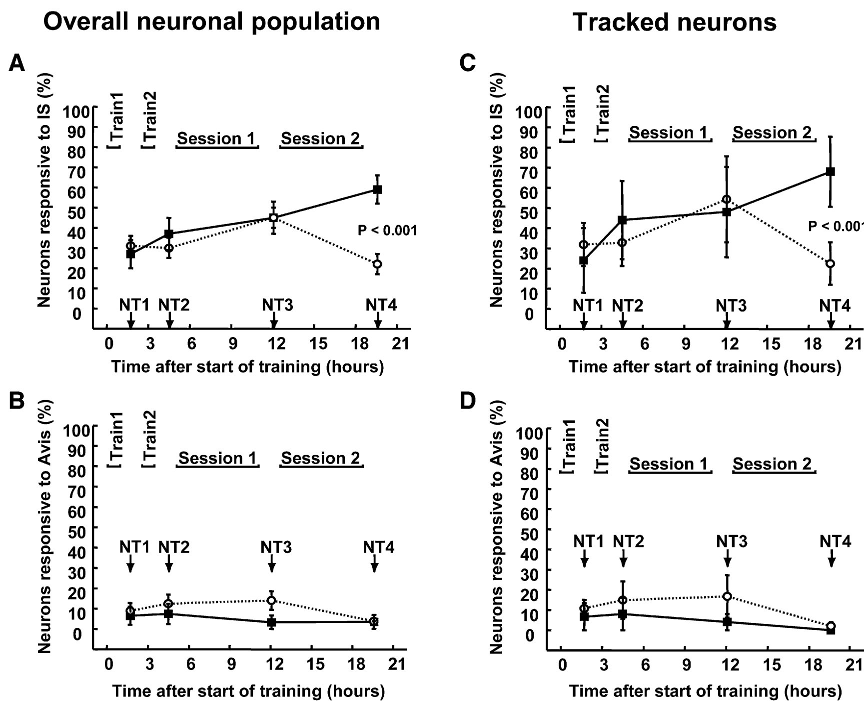

Figure 2. Neuronal Responsiveness in the Rest First and Disturbed First Groups

Neuronal responsiveness to the imprinting stimulus (IS) is similar in the Rest First and Disturbed First groups until test NT4, when IS-responsiveness is significantly greater in the Rest First group. There were five chicks in each group. Mean percentage (±SEM) of neurons in each group responding selectively to a stimulus are shown for the stated neuronal test NT1–NT4 (numbers of neurons are given in Table 1). The approximate mean times of the midpoints of the neuronal tests are indicated by arrows. The times of the training periods (Train1 and Train2) and of the treatment sessions are indicated by the horizontal bars. Filled black squares with a continuous line running through them represent the Rest First group; unfilled circles with a broken line running through them represent the Disturbed First group. Under "Overall Neuronal Population," (A) shows neurons selectively responsive to the imprinting stimulus (IS neurons). (B) shows neurons selectively responsive to the alternative visual stimulus (Avis neurons). Under Tracked neurons, (C) shows IS neurons. (D) shows Avis neurons. Group means in (A) and (C) respectively are significantly different only at NT4.

Electroencephalographic Results

EEG was measured during the sessions. When the percentage of energy in individual frequency bands was subjected to an ANOVA, a significant difference between the rest and disturbed conditions was found in all except the 0–4 Hz and 25–32 Hz bands (Figure 4A). In the 5–8 Hz (theta) band, there was a significantly greater percentage of energy in the rest than in the disturbed condition. When this band was subdivided into 5–6 Hz and 7–8 Hz bands (Figure 4B), only in the lower theta 5–6 Hz band was there a significant effect of experimental condition: There was a significantly higher percentage (11%) of energy in the rest than in the disturbed condition. There was a similar trend in the 0–4 Hz band. This increase was not statistically significant, but it was not significantly different from the increase observed in the 5–6 Hz band ($F_{1,191} = 0.04$, $p = 0.84$). Indeed, when the data from the 0–4 Hz and 5–6 Hz bands are combined, there remains a significant difference between the rest and disturbed treatments ($F_{1,16} = 5.78$, $p = 0.029$). In birds, there is an increase in the 1–6 Hz band during slow-wave sleep [20]. The rest condition contained a significantly lower percentage of energy than the disturbed condition in the 9–16 Hz and 17–24 Hz frequency bands (by 13% and 24%, respectively). In the highest frequency band measured, there was no significant difference. Thus, rest, compared with the disturbed condition, is associated with increased energy in the 5–6 Hz band and a decrease in some of the higher-frequency bands.

For the 5–6 Hz (theta) band, there was a significant interaction between group and session ($F_{1,16} = 14.90$, $p = 0.001$; see Figure 5A). The significant interaction term is partly attributable to a time-dependent difference in the level of energy in the 5–6 Hz low-theta band of the EEG of the Rest First group compared with that in the Disturbed First group during session 1. Thus, over a 3 hr period, in the second, third, and fourth hours of session 1, there was significantly more energy in the Rest First than the Disturbed First group. The difference between the groups was thus delayed and was greatest (by 41%) in the third hour of the 6 hr session (Figure 5B). In addition, the significant interaction term is partly attributable to the change in energy in the 5–6 Hz band between sessions 1 and 2: In the Rest First group, energy declined significantly ($t = 2.28$, 16 df, $p = 0.037$), whereas in the Disturbed First group, energy increased significantly ($t = 3.17$, 16 df, $p = 0.006$). Hence, energy in the 5–6 Hz band is higher during the rest than in the disturbed treatment in both groups of chicks. There were no significant differences between hemispheres.

Vocalizations

The ratio of distress calls to other vocalizations was subjected to an ANOVA according to the same design as that for each frequency band of the EEG. The ratios for the disturbed treatment were not significantly different from those for the rest treatment ($F_{1,8} = 4.43$, $p = 0.07$); the ratios for group (Rest First and Disturbed First) were not significantly different ($F_{1,8} = 1.54$, $p = 0.25$) nor was there a significant effect of time within treatment session ($F_{5,80} = 0.64$, $p = 0.67$). Thus, there was no evidence for different levels of stress between groups or treatments.

Preference Test

In order to measure strength of learning, we tested the birds behaviorally for their preference for the training stimulus over the alternative stimulus. In this preference test (Figure 1, Pref), performed after the end of the last neuronal test, the mean preference score of the chicks in the Rest First group was significantly higher than that of the chicks in the Disturbed First group ($F_{1,8} = 6.37$; $p = 0.04$). The mean preference score of chicks in the Rest First group was 84.8% ± 10.1%. This score is significantly greater than 50% (no preference score; $t = 3.44$, 8 df, $p = 0.04$). The mean preference score of the Disturbed First group of chicks was 56.2% ± 6.20%. This score is not significantly different from 50% ($t = 1.00$, 8 df, $p = 0.37$). These scores indicate that the chicks in the Rest First group preferred the visual imprinting stimulus to the alternative stimulus, whereas the Disturbed First group had no preference for either

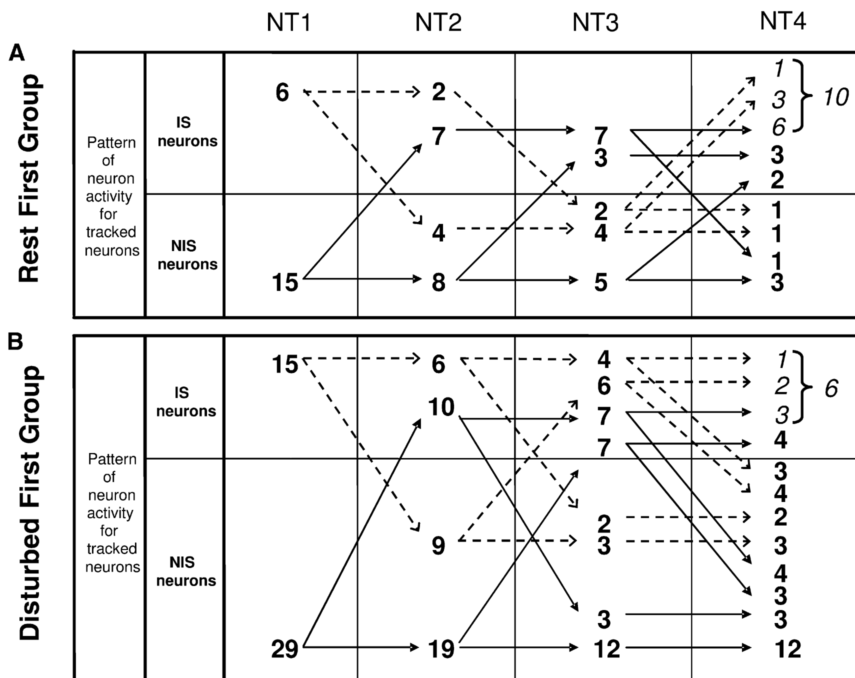

Figure 3. Numbers of Tracked Neurons in the Four Neuronal Tests that Either Were, or Were Not, Specifically Responsive to the Imprinting Stimulus

In the Rest First group, but not the Disturbed First group, a majority of neurons that respond specifically to the imprinting stimulus at test NT1 or NT2 are similarly responsive at test NT4. Individual neurons tracked across neuronal tests NT1 through NT4. The dotted arrow path tracks neurons that responded specifically to the imprinting stimulus (IS neurons) at NT1. The solid arrow path tracks neurons that were not IS neurons (NIS neurons) at NT1. Italicized numbers of IS neurons at NT4 were IS neurons at NT1 or NT2. (A) shows the Rest First group. (B) shows the Disturbed First group.

stimulus. These results imply that the Rest First group had a memory for the imprinting stimulus; the Disturbed First group of chicks failed to show evidence of memory retention.

Discussion

The present experiments demonstrate that the long-term consolidation of a memory for an imprinting stimulus, measured at both neural and behavioral levels, depends critically on the opportunities given to chicks to rest and sleep after training. Undisturbed rest shortly after the end of training is effective; such rest is ineffective if delayed for ~9 hr after the end of training. For the short-term effects of imprinting on selective neuronal responsiveness to the imprinting stimulus, early undisturbed rest is not necessary: The percentage of IS neurons in both Rest First and Disturbed First groups of chicks continued to increase to the end of the first session. Thus, this increase occurred whether or not rest was disturbed during the first 6 hr session after the end of training. The mean preference scores of the Rest First and Disturbed First groups of chicks indicate that retention of the memory of the imprinting stimulus was dependent on the temporal sequence of rest and disturbance in the same way as was neuronal responsiveness in the IMM.

There were no effects of the experimental treatment for neurons that responded only to the alternative visual stimulus, Avis (Figures 2B and 2D). These findings demonstrate that training with the imprinting stimulus does not exert its effects through a nonspecific increase in responsiveness of IMM neurons to sensory stimulation. Furthermore, the absence of a change in the percentage of neurons responding selectively to the Avis across neuronal tests shows that the stimulus exposure during these tests does not affect responsiveness to the stimulus (during these tests the chicks were exposed to the Avis for short periods). The percentage of neurons responding to the Avis at NT1 did not differ from that at NT4 (Figures 2B and 2D). In previous experiments [14, 15, 18], chicks were imprinted by being trained on either the red box or the blue cylinder in a counterbalanced design. Training with either of these two stimuli led, on the day after training, to a 200%–300% increase in the proportion of neurons in the IMM that responded specifically to the training stimulus. This increase in responsiveness was not significantly different between the two training stimuli used. Responsiveness to stimuli that had not been used for training, and to the auditory stimulus used during training [14, 15], was unchanged. In the present study, the number of IS neurons recorded at NT4 in the Rest First group was approximately double the number recorded at NT1 (see Table 1). This increase is at the lower end of the increase observed in previous studies [14, 15, 18]. However, in those studies, the baseline for comparison was the number of IS neurons in untrained chicks. In the present study, the baseline was higher because it was the number of IS neurons recorded after 1 hr of training. Together, the results of all the studies demonstrate that training with a particular stimulus selectively increases neuronal responsiveness to that stimulus.

In the group of chicks in which long-term consolidation occurred (Rest First), the period of undisturbed rest began ~1.3 hr and ended ~7.3 hr after the end of training. In an earlier study [18], in which consolidation also occurred, the period of undisturbed rest began ~5 hr after the end of training. Taken together, the two sets of results suggest that consolidation occurs if the period of undisturbed rest begins between ~1.3 and 5 hr after the end of training and imply that ~2.3 hr (i.e., 7.3–5) were available in the earlier study for sleep to have its effect on consolidation.

The EEG during sleep in adult chickens and in day-old chicks, as in mammals, is characterized by a phase ("slow-wave sleep," SWS), in which high-amplitude slow waves predominate, and a phase, "paradoxical sleep" is characterized by low-voltage fast waves [21, 22]. During SWS in birds, there is an increase in the 1–6 Hz band [20]. In the present experiment, the rest treatment was associated with an increase in energy in the 0–6 Hz band, predominantly due to an increase in the 5–6 Hz (low-theta) band (see Figures 4A and 4B). The present findings suggest that slow-wave sleep is implicated in the consolidation process. The EEG during rest varied across time. There was an increase in power in the 5–6 Hz band over a 3 hr period during the second, third, and fourth hours of rest only in session 1, when chicks were allowed to sleep undisturbed soon after training (Figure 5B). No such time-dependent changes were found during session 2 (Figure 5C). Bobbo et al. [23] found

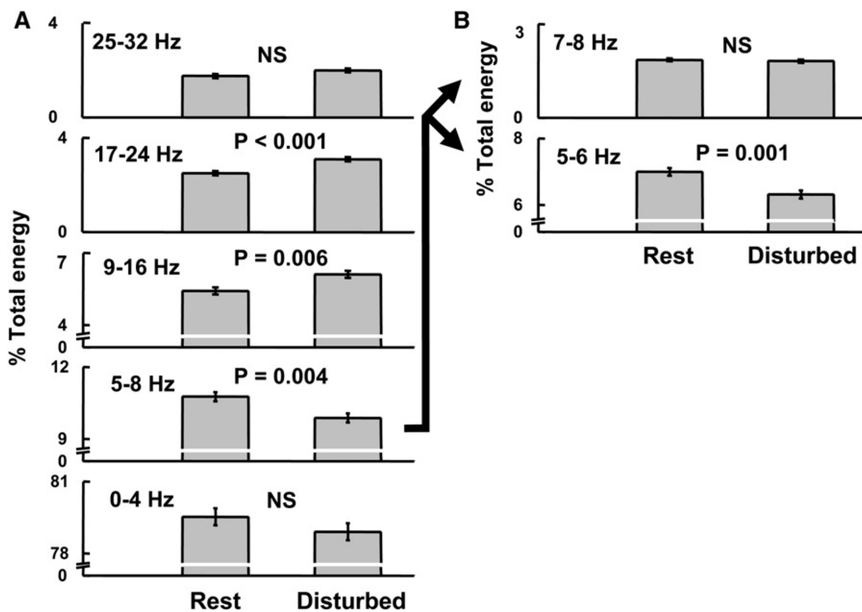

Figure 4. Mean Percentage, ± SEM, of Energy in Stated EEG Frequency Bands during the Rest and Disturbed Treatments

The EEG during the rest treatment is characterized by a higher percentage of energy at 5–6 Hz, and less energy in the range 9–24 Hz, relative to the disturbed treatment. The levels of significance are for comparison of these two treatments. (A) shows the total percentage of energy in each frequency band. (B) shows the subdivision of the 5–8 Hz band into 5–6 Hz and 7–8 Hz components. The rest and disturbed treatments differed with respect to 5–6 Hz but not 7–8 Hz.

that, over a 6 hr observation period, two-day-old chicks exhibit behavioral signs of sleep for ~2.5 hr. If chicks in the present study exhibited behavioral sleep for at least as long as this, then this sleep is likely to have been disrupted by the rotations of the wheel on five occasions, on average, during the disturbed treatment.

It has been suggested that oscillations in the theta frequency range are necessary for memory encoding (for reviews, see [24, 25]). The present findings strongly suggest that SWS soon after the end of training is involved in the stabilization of IMM neuronal responses to the imprinting stimulus and hence in long-term memory.

In the Rest First group, responsiveness to the IS at NT4 had risen to approximately double that at NT2 (Figure 2). The question arises as to whether this approximate doubling requires sleep during session 1 or disturbance during session 2. We conclude that sleep during session 1 is responsible, for the following reasons: (1) In a previous study [18], when chicks were allowed to sleep undisturbed during the period corresponding to session 2 of the present study, neuronal responsiveness was at a maximum after this time. Hence, disturbance during this time is not necessary for IS responsiveness to be high at NT4. (2) Significant encephalographic differences between the Rest First and Disturbed First groups were found during session 1 but not session 2 (Figure 5).

It is possible that consolidation depends on levels of stress rather than the pattern of sleep during the first 6 hr session. The ratio of "distress calls" to other calls increases when chicks are stressed [26]. In the present study, there were no significant differences between the ratios of "distress calls" for the disturbed and rest treatments. In particular, during the first 6 hr session, the mean ratio for chicks that were disturbed (Disturbed First group) was 1.43 ± 0.42 (SEM); the mean ratio for chicks that were undisturbed (Rest First group) was 1.42 ± 0.36. This similarity may be because disturbance was slight (two rotations of the running wheel per hour). The data from vocalizations thus provide no evidence that stress played a role in consolidation.

It is possible that when chicks were disturbed by the wheel being rotated during a period of disturbed rest, they became fatigued. If this were so, might fatigue lead to a decrease in the percentage of IS neurons in IMM? Between recording tests NT2 and NT3, the wheel was rotated in the Disturbed First group (session 1); yet, the percentage of IS neurons increased over the two neuronal tests. The increase was similar in magnitude to that found in the Rest First group of chicks allowed to sleep undisturbed during this session. Similarly, between recording tests NT3 and NT4, the wheel was rotated in the Rest First group (i.e., disturbed treatment; session 2). Among this group too, the percentage of IS neurons increased. However, the percentage of IS neurons fell across the neuronal tests in the Disturbed First group of chicks that were allowed to sleep during session 2. These results demonstrate that fatigue cannot explain the different time courses of selective IS responsiveness in the two treatment groups. Instead, the effects of undisturbed sleep on IS responsiveness depended on when that sleep occurred after the end of training.

Previously [18], it had been found that the proportion of neurons that responded selectively to the imprinting stimulus varied with time after training (see Introduction above). It was hypothesized that the IS neurons recorded ~21.5 hr after training comprised all those neurons that had been IS neurons after the first or second hour of training, even though in the intervening interval, some of these neurons had ceased responding selectively to the IS. It was also hypothesized that sleep played an important role in the recovery process. In that study, it had not proved possible to track the activity of individual neurons through to the end of the experiment, and the hypotheses could not be tested. The evidence presented in this study is consistent with both hypotheses. In respect of the first hypothesis, it was found that, in the Rest First group, IS neurons recorded ~19.5 hr after the start of training (at NT4) chiefly comprise (10/13) those neurons that were previously selectively responsive to the imprinting stimulus immediately after each of the training periods. This was not the case for the Disturbed First group, in which only a minority of IS neurons recorded at NT4 had been IS neurons at NT1 or NT2. Thus, sleep occurring shortly after training leads to the stabilization of selective responses to the imprinting stimulus observed at NT4. However, because spike activity, the expression of response, is stochastic, not all IS neurons may respond to the IS at NT4. What mechanisms might underlie the loss by some IS neurons of their IS responsiveness? One possibility is that synapses previously activated by the imprinting stimulus, Tvis and/or Tcomp, cease to be activated by it. That is, they

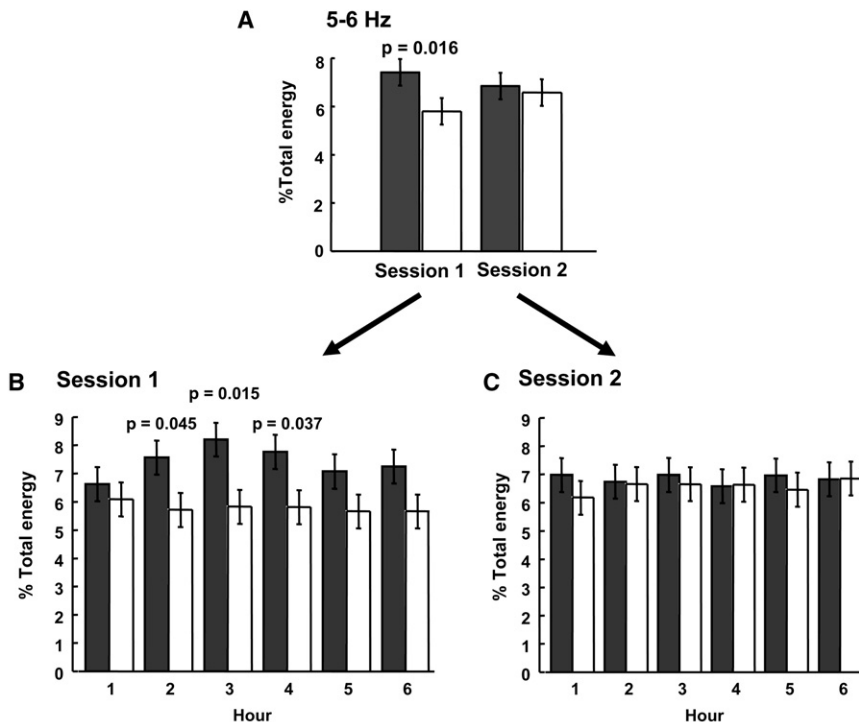

Figure 5. Mean Percentage, ± SEM, of Energy in the 5–6 Hz EEG Frequency Band

The Rest First group, receiving the rest treatment during session 1, exhibited a time-dependent increase in low-frequency theta activity relative to the Disturbed First group, which received the disturbed treatment during session 1. No such difference between Rest First and Disturbed First was observed during session 2. (A) shows the mean percentage (±SEM) of energy in the 5–6 Hz EEG frequency band during the two sessions. There was a significant interaction between group and session ($F_{1,16} = 14.90$, $p = 0.001$). In the Rest First group, energy declined significantly between session 1 and session 2 ($t = 2.28$, 16 df, $p = 0.037$), whereas in the Disturbed First group, energy increased significantly between session 1 and session 2 ($t = 3.17$, 16 df, $p = 0.006$). (B) and (C) show data from session 1 and session 2 respectively, subdivided into 1 h blocks. The black bars represent the Rest First group, and the white bars represent the Disturbed First group.

become silent synapses (see [27]). These synapses might become unsilenced (see [28, 29]) as a consequence of sleep shortly after training.

A minority of IS neurons recorded at ∼12 or 19.5 hr (NT3 or NT4) had not previously been selectively responsive to the IS: They acquired their response specificity for the IS well after the end of training. How might this change in responsiveness have occurred? Among both groups of chicks, a total of 12 neurons had this property. Six of these neurons had earlier responded nonselectively to the imprinting stimulus: They responded to this stimulus and also to the alternative visual stimulus, Avis. The neurons became IS neurons by losing their responsiveness to Avis. During training, the synaptic pathways to the recorded neuron transmitting signals evoked by the IS will have been active; the pathway for the Avis will have been inactive. It is possible, therefore, that the loss of responsiveness to this stimulus is a consequence of this asymmetric activity, the active synapses depressing or eliminating the inactive synapses ("activity-dependent depression" [30, 31]). If so, the effects of this process are not expressed until several hours after the end of the asymmetric activation of the recorded neuron during training. The remaining six neurons had not previously responded to the IS. A possible mechanism for this late responsiveness relates to the synaptic changes in IMM that underlie memory (for review, see [7]). These changes involve an increase in length of the postsynaptic density of spine synapses [32, 33] and an upregulation of NMDA receptors [34, 35]. In some neurons, such as those that come to respond selectively to the imprinting stimulus soon after training [18], the upregulation of receptors may be controlled locally, in the dendritic spine (see [36]) and so occur quite quickly (cf. AMPA receptors, see [37] for review). In other neurons, perhaps because of a limited capacity for protein synthesis by local mechanisms in the dendritic spine (see [38]), the synaptic changes may involve an upregulation of nuclear gene expression and so occur over a more extended period of time [35, 39, 40].

If the short-term learning-related increase in IS neuron numbers depends on the insertion of receptors into the postsynaptic densities of the synapses activated by the imprinting stimulus, then this process is not affected by sleep or disturbance of sleep occurring shortly after training: IS numbers continued to increase in both Rest First and Disturbed First groups from NT1 to NT3, irrespective of the treatment received during session 1. However, the maintenance of this responsiveness up to NT4 is dependent on the early occurrence of sleep. Therefore, it is possible that the stabilization of these receptors in the postsynaptic membrane is dependent on sleep. The effects of sleep, and the timing of sleep onset, on the dynamics of receptor movement and in the process of stabilizing receptors in the postsynaptic membrane may be fruitful areas for future exploration.

The IMM probably corresponds to part of the mammalian neocortex [41], receiving sensory inputs from other forebrain areas as well as a projection from the hippocampal region [33, 42]. There is increasing evidence that in mammals, including humans, certain forms of memory involve the hippocampal region and the neocortex (see for review [43]) and that sleep is involved in long-term storage in the neocortex [44]. Thus, the mechanisms of information storage in the IMM may be a useful model for investigating such storage in the mammalian neocortex.

Supplemental Data

Additional Discussion, Experimental Procedures, and two figures are available at http://www.current-biology.com/cgi/content/full/18/6/393/DC1/.

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