CHAPTER 6

Sleep and Learning in Birds: Rats! There's More to Sleep

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Why should a neuroscientist study sleep in birds? Considering mammals, there is a broad literature describing neuronal and genetic mechanisms of sleep regulation, evidence in rodents and humans supporting a role for sleep in synaptic homeostasis, a well-developed rodent model of spatial processing involving hippocampal sleep reactivations, a visual system model for developmental effects of sleep in cats, and extensive behavioral, imaging, and polysomnographic evidence of sleep consolidation in humans. Why study birds, let alone flies or worms: What is missing?

SLEEP RESEARCH FROM THE ETHOLOGICAL PERSPECTIVE

There are many reasons beyond certain manifest technical advantages to study sleep in birds, but here we identify four conceptual issues. Because sleep has a complex physiology that broadly influences behavior, understanding how the physiology of sleep influences a specific behavior requires an appropriate animal model of that behavior. With regard to sleep and learning, the focus of mammalian animal research has been more on neurophysiological mechanisms of plasticity, and less on the behavioral consequences of sleep processes. Conversely, though our understanding of the neurophysiology of bird sleep is still in its infancy, some important results have already been achieved by connecting strongly with ethologically grounded behaviors. For example, recent behavioral and neurophysiological studies have connected developmental sensorimotor vocal learning to sleep mechanisms, with surprising conclusions that reshape our thinking about offline components of vocal learning, and skill learning in general. The well-established similarities between aspects of song learning and language acquisition make direct predictions on human behavior, which have gained some support in recent studies. This conclusion emphasizes a broader one, that since sleep is manifest broadly in the animal kingdom, it can profitably

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be studied in relation to specific behavioral adaptations in a broad range of animal species. Conversely, if the goal is to inform human behavior, comparative studies bear the burden to assess similarity both in behavioral and physiological traits in relation to humans, ultimately evaluated in terms of evolutionary processes. All comparative work bears such a burden, including work in mammals, but whereas there are mammalian systems for studying sleep consolidation effects or neuronal plasticity as is observed in humans, the two approaches have yet to be combined in a single system.

A second conceptual issue concerns the relation between the memory systems thought to be engaged in human studies of sleep consolidation compared to animal studies. The most consistent evidence of sleep consolidation in humans has been for nondeclarative memory tasks. Though the effects of sleep are increasingly being studied for other types of memory, there is a disconnect between studies of human memory and rodent studies that are largely focused on patterns of hippocampal place cell activity. Moreover, the pattern of performance changes observed across waking and sleep in adult humans has only been compellingly replicated recently, in an adult songbird (European starling) model. If the functional distinction of memory systems is important in assessing learning and memory behaviors, and if identifying similar patterns of performance in humans and animals facilitates understanding the mechanisms of human behavior, this currently recommends the starling model. We develop this concept and line of inquiry in a separate section that follows.

A third issue is more comparative and phylogenetic. There has been a longstanding misconception regarding the organization of bird pallium and its relation to (mammalian) neocortex. The avian cortex is not a layered structure but is organized into regions or fields, often separated by fiber tracts (Fig. 6.1, *left panel*). Early anatomists mistakenly assumed that the entire avian pallium was a hypertrophied striatum (Jarvis et al., 2005). A recent broad reevaluation of these relations considering anatomical, physiological, behavioral, and molecular data has identified homologies between bird cortex and neocortex, facilitating the placement of bird studies in a meaningful mammalian context (Reiner et al., 2004). For example, numerous hodological characteristics and histological markers identify a limited, circumscribed region of the avian pallium as the proper avian striatum (Fig. 6.1, *right panel*). In other regions of the avian cortex a series of canonical sensory pathways mimic the canonical neocortical circuit. Each avian pathway includes a granule cell layer receiving ascending input from a different dorsal thalamic nucleus, projections from that layer (possibly through intermediate local

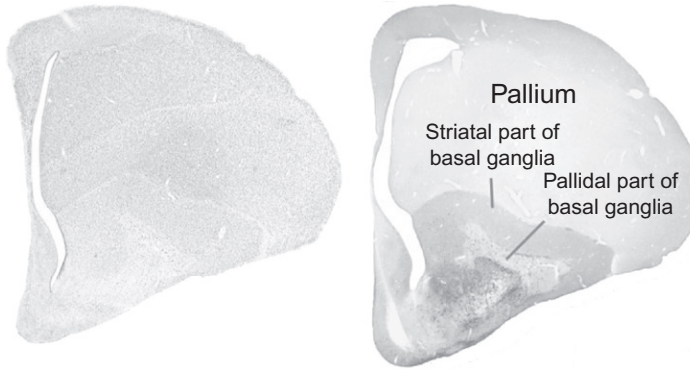

Figure 6.1 ***The avian telencephalon is dominated by a cortex (pallium), not striatum.*** *Left panel:* A sagittal section through pigeon telencephalon, showing regions separated by fiber tracts. Not having neocortical laminar organization, avian telencephalon was incorrectly thought to represent a massively enlarged striatum, not cortex. *Right panel:* Pigeon telencephalon labeled with choline acetyltransferase, one of a very large panel of chemical and molecular markers indicating that avian striatum is restricted to the ventral region of the telencephalon. *Up*, dorsal; *left*, medial. *Adapted from Reiner et al. (2004).*

connections) to secondary neurons (akin to neocortical layer 2/3 neurons), which in turn project to neurons that project out of the cortex (akin to neocortical layer 5 neurons). Neocortical layer-specific molecular markers are selectively expressed in corresponding structures of the avian cortex (Dugas-Ford, 2009). The avian forebrain also has a full complement of ascending modulatory systems arising from brainstem, midbrain, and basal forebrain, as is seen in mammalian cortex (Ball & Balthazart, 2010). Remarkably, recent data have demonstrated radial, columnar-like organization in the avian auditory cortex (Wang, Brzozowska-Prechtl, & Karten, 2010).

Collectively, these observations compellingly support the hypothesis that cortical cells and circuits in birds and mammals share a common schema and evolutionary history (Karten, 1997). The central conclusion of this profound hypothesis is that the basic pattern of forebrain connectivity shared between birds and mammals arises from cells and circuits sharing a common ancestor. This helps to explain why all vertebrate systems share common principles of functional organization (Ulinski, 1984). This conceptualization of the avian cortex helps to interpret physiological/functional data collected in avian auditory cortex that was difficult to reconcile with the old hierarchical organization scheme. It additionally challenges uniqueness claims for neocortex and brings equivalent claims regarding mammalian sleep under scrutiny.

Of relevance to the value of avian sleep studies are recent observations showing that sleep in songbirds shares many features with mammalian sleep (see below). Not all aspects of human sleep physiology have been observed in birds (nor are all aspects of mammalian nonhuman (e.g., rodent) sleep prominent in humans). Whether the similarity between songbird and mammalian sleep arises from deep homology shared between birds and mammals remains an open question given the current understanding of sleep in basal birds, basal mammals, and in reptiles. Nevertheless the homology between avian and mammalian cortical structures coupled with a similar sleep structure make songbirds an attractive model system for sleep research. Furthermore, the exceptionally rich song production system of songbirds along with their well-developed auditory system allows the work to be placed in a comparative and ethological context supported by rigorous experimental observation.

A final conceptual issue is that these converging lines of evidence suggest deep homologies linking birds and mammals. Presumably these arise from shared molecular mechanisms for pattern formation along the neuroaxis, mechanisms that are broadly expressed in invertebrates as well as all vertebrates, which are somehow differentially expressed in higher vertebrates. Understanding how the expression of these mechanisms leads to the formation of an elaborated pallium and sleep structure is a fundamental question for sleep research (Rattenborg, 2006). Restricting the study of sleep to mammals limits the phylogenetic scope of the work, yet the deepest mysteries of sleep—why do most if not all animals sleep (Cirelli & Tononi, 2008; but see Siegel, 2008), and what is the relation between an elaborated pallium and an elaborated sleep structure—are best addressed with a broad evolutionary perspective.

THE ORGANIZATION OF SLEEP IN BIRDS

Sleep in birds, as in mammals, is associated with species-specific behavioral postures and may be triggered by environmental releasers, features of innate behavior well known in ethology. Beyond such overt behaviors, birds also exhibit complex EEG patterns that are associated with different stages of sleep. Birds produce slow wave activity associated with slow wave sleep (SWS) as well as rapid eye movements (REM) with occasional correlated head movements associated with REM sleep. The weight of evidence is that both SWS and REM sleep are absent in reptiles and fish, suggesting that birds are the only nonmammalian species expressing both SWS

and REM. Sleep homeostasis, which is commonly observed in mammals, is also observed in birds (Jones, Vyazovskiy, Cirelli, Tononi, & Benca, 2008). Likewise, a tendency for REM sleep to increase throughout the night has been observed in a number of bird species (Low, Shank, Sejnowski, & Margoliash, 2008; Szymczak, 1987; Tobler & Borbely, 1988).

The sleep structure of numerous avian species has been characterized with EEG recordings (see Campbell & Tobler, 1984; Rattenborg et al., 2002). Sleep in most avian species has been characterized by non-REM (NREM) activity, with REM sleep observed in brief and infrequent periods. The songbirds (passerine, or perching birds) appear to be outliers to this overall pattern. The several songbird species studied showed more REM than observed in nonpasserine species, systematic variation of SWS and REM sleep, and other features that are distinct from the typical avian pattern (e.g., Szymczak et al., 1996; Jones et al., 2008). In a striking example, an ultradian pattern was observed in adult male zebra finches that was characterized by SWS density falling from 50% at the onset of sleep to 25% by the end of the night. Concurrently, the number and duration of REM bouts systematically increased so that REM accounted for 30% of sleep and individual REM bouts averaged 15 seconds by the end of the night. This pattern is more complex than that seen for nonpasserine species and is more similar to mammalian sleep than has previously been observed for birds.

It is valuable to consider these observations through the lens of phylogeny. The pattern of sleep-related cortical activity common among mammals is not observed in basal mammals, which nevertheless exhibit a similar pattern of sleep-related brainstem activity. Thus, one hypothesis is that increased encephalization within the mammalian lineage was associated with the emergence of sleep-related cortical activity. A similar process may have occurred in the avian lineage. Approximately 5,000 of the more than 9,000 avian species are passerines. Of these, almost 4,000 are oscine passerines, so-called true songbirds. There are additionally some 350 species of parrots (psittacines). Parrots and songbirds have been proposed to be sister groups in a recent molecular phylogenetic study of birds. It remains to be seen if the more complex cortical patterns of sleep recently observed in a few songbird species obtain more generally in oscines, in passerines, or even in psittacines. If complex cortical patterns of sleep are associated with increased encephalization, then we would expect this to emerge in the lineage including songbirds and parrots, which include the most cognitively advanced avian species. Thus the principle distinction between avian and mammalian sleep might simply be related to the timing

of increased encephalization within each lineage, which occurred earlier in mammals than in birds. This also hints at a conserved feature of sleep (perhaps brainstem mechanisms) that might be shared with reptiles, albeit a study in turtles failed to identify brainstem activity associated with REM sleep (Eiland, Lyamin, & Siegel, 2001). Given the emerging understanding of avian and mammalian forebrain homology, this motivates continued study of reptilian sleep.

TOWARDS AN ANIMAL MODEL: SLEEP-DEPENDENT MEMORY CONSOLIDATION IN HUMANS

Here, we take up the challenge for comparative research investigating the role of sleep in memory consolidation. First we describe a substantial literature that helps to define sleep-dependent memory consolidation in humans. These observations, which have helped shape our efforts to develop a corresponding animal model system, are described in the following section.

Memory consolidation describes a process in which a newly acquired memory is transformed from a labile state, where it may be susceptible to interference or decay, to a more stable and strengthened form. Sleep is widely believed to play a fundamental role in the consolidation of memories (Diekelmann & Born, 2010; Walker & Stickgold, 2006), a position that is broadly supported by behavioral studies of human memory. The standard approach for uncovering behavioral evidence of sleep consolidation has been to train participants on a memory task in the morning or evening. The participants are then retested after a 12 hr retention interval that consists entirely of wakefulness or that includes a normal night of sleep in order to determine whether performance after sleeping retention is better than after waking retention. Using this approach, sleep consolidation has been reported for a variety of memory tasks, including associative memory (Ellenbogen, Hulbert, Jiang, & Stickgold, 2009; Ellenbogen, Hulbert, Stickgold, Dinges, & Thompson-Schill, 2006), emotional memory (Hu, Stylos-Allan, & Walker, 2006; Payne, Stickgold, Swanberg, & Kensinger, 2008), prospective memory (Scullin & McDaniel, 2010), and episodic memory (Racsmany, Conway, & Demeter, 2010).

Though studies relying on 12 hr retention intervals can indicate the differential effects of waking and sleeping retention on the consolidation of a newly formed memory, interpretation of the results is confounded by circadian factors because training and testing for the two conditions occur at different times of day. Consequently, performance changes could reflect

circadian influences on memory rather than an underlying consolidation process if the ability to acquire or perform a task is different in the morning and evening. Accordingly, behavioral studies of sleep consolidation often include conditions with 24 hr retention intervals in addition to the 12 hr retention periods. In these conditions, participants are trained and tested in the morning or evening and retested 24 hrs later, ensuring that performance cannot be attributed to the circadian time. Human studies have provided extensive behavioral evidence of sleep-dependent consolidation for a broad range of memory tasks with this more complete experimental design. For example, sleep has been shown to benefit motor-sequence learning (Brawn, Fenn, Nusbaum, & Margoliash, 2010; Korman et al., 2007; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002), sensorimotor learning (Brawn, Fenn, Nusbaum, & Margoliash, 2008; Robertson, Pascual-Leone, & Press, 2004), visual texture discrimination learning (Gais, Plihal, Wagner, & Born, 2000), the perceptual learning of synthetic speech (Fenn, Nusbaum, & Margoliash, 2003), spatial navigation memory (Ferrara et al., 2008), spatial associative learning (Talamini, Nieuwenhuis, Takashima, & Jensen, 2008), and relational memory (Ellenbogen, Hu, Payne, Titone, & Walker, 2007).

Variants of this approach have further confirmed a role for sleep in memory consolidation. In the nap paradigm, participants receive task training in the morning and are retested later in the day after a retention period that either includes or does not include a nap. Naps have been shown to benefit memory in a manner similar to a full night of sleep (e.g., Korman et al., 2007; Mednick, Nakayama, & Stickgold, 2003; Nishida & Walker, 2007; Tucker et al., 2006). These studies represent a powerful approach for investigating sleep consolidation. On the one hand, nap studies avoid circadian confounds because the time-of-training and time-of-testing are identical for the nap and no-nap conditions. On the other hand, nap studies provide evidence against a purely time-dependent mechanism of consolidation. According to the time-dependent hypothesis, memory consolidation merely requires the passage of an appropriate amount of time. Any memory benefits that appear after a night of sleep would result from a time-dependent mechanism that may act more efficiently across many hours of sleep due to a lack of interfering experiences. However, nap studies establish that memory benefits can arise after a short retention period as long as it includes sleep, indicating that the occurrence of sleep rather than the passage of time is the critical factor.

Finally, sleep deprivation studies have provided additional support for a sleep-dependent rather than time-dependent consolidation mechanism. In the sleep deprivation paradigm, participants are trained on a task and

then retested at least two days later. Participants in a deprivation condition are not allowed to sleep on the first night after training but are allowed a full night of recovery sleep on the second night before being retested the following day. This recovery night of sleep ensures that any performance impairments during the retest cannot be attributed to the many confounds that would result from being tested while in a sleep-deprived state. If a strictly time-dependent process governed consolidation, memory benefits should appear even after sleep deprivation because the passage of time, not sleep, would be responsible for the consolidation. Yet, deprivation studies have demonstrated that the expected memory benefits do not appear when participants are deprived of sleep on the first night after training (e.g., Fischer, Nitschke, Melchert, Erdmann, & Born, 2005; Gais, Lucas, & Born, 2006; Stickgold, James, & Hobson, 2000), suggesting a necessary role for sleep.

Overall, there is compelling human behavioral evidence that sleep is important for the consolidation of newly acquired memories. While the exact effects of waking and sleeping retention on task performance depend on the type of memory probed and the experimental procedures used, many human studies highlight a pattern of consolidation defined by performance deterioration across waking retention periods prior to sleep followed by performance enhancement and stabilization after sleep.

THE HIPPOCAMPAL SYSTEM AND SLEEP-DEPENDENT MEMORY CONSOLIDATION: LIMITED EVIDENCE OF BEHAVIORAL CONSEQUENCES

Human studies have presented convincing behavioral evidence of sleep-dependent consolidation but are ultimately limited at uncovering the underlying neural mechanisms, which will require appropriate animal models. To date, most animal studies of sleep consolidation have explored the "standard model" of memory consolidation. According to the standard model, the hippocampal formation receives input from neocortical regions involved in the initial encoding of an experience, which binds the information into a coherent memory trace. During sleep, coordinated reactivation of the memory trace is thought to transfer the memory from a hippocampal to a neocortical representation (Frankland & Bontempi, 2005; Rattenborg, Martinez-Gonzalez, Roth, & Pravosudov, 2011). This model is supported by studies showing that the firing patterns expressed in hippocampal neurons of rats moving along a track are reactivated during subsequent sleep (e.g., Kudrimoti, Barnes, & McNaughton,

1999; Lee & Wilson, 2002; Wilson & McNaughton, 1994) and coincide with reactivations in the visual cortex (Ji & Wilson, 2007). Furthermore, sleep reactivation of waking neural activity has been identified in the medial prefrontal cortex after rats have learned a rule (Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009) or been trained to run to a sequence of locations (Euston, Tatsuno, & McNaughton, 2007). These hippocampal and cortical sleep reactivations have been interpreted as evidence of a hippocampus-to-cortex memory transfer that underlies memory consolidation and the associated memory benefits of sleep (Marshall & Born, 2007; O'Neill, Pleydell-Bouverie, Dupret, & Csicsvari, 2010; Rasch & Born, 2007). There are elegant studies relating hippocampal physiological activity to brain rhythms, mechanisms of plasticity, and behavioral state (see Poe, 2010).

Memory consolidation, however, cannot be inferred from neural events alone (Hennevin, Huetz, & Edeline, 2007). Reactivation of waking neural activity during subsequent sleep is not by itself evidence of a memory consolidation process. Learning may alter brain activity during subsequent sleep, but neural plasticity is only tenuously linked to the consolidation of a newly formed memory trace in studies that do not verify that sleep reactivations confer any memory benefit on the animal.

Recent studies have begun to address to this concern by incorporating behavioral measures of spatial tasks into studies of sleep reactivation. For example, in one study, an increase in hippocampal sharp wave ripples, which are associated with hippocampal reactivations, was correlated with performance on a place-reward association task (Ramadan, Eschenko, & Sara, 2009). In two other studies, electrical stimulation was used to disrupt sharp wave ripples during post-training sleep. These stimulations produced performance impairments compared to control conditions on a place-reward association task (Girardeau, Benchenane, Wiener, Buzsaki, & Zugaro, 2009) and a spatial navigation task (Ego-Stengel & Wilson, 2010). Given that the performance benefits only became apparent after several days of training and testing, however, this makes it difficult to determine the respective influences of sleep, waking time, and repeated training/testing. While these studies have forged a stronger link between sleep reactivation and memory than prior work, the relatively weak behavioral effects in these animal models are in stark contrast to human studies that reveal clear memory benefits after a single period of sleep.

We emphasize that our point is not that studies need to achieve some arbitrary threshold to be "acknowledged" as being "relevant" to learning. Our point is that without a strong and direct linkage to a learning phenomenon,

it is difficult to relate changes in behavior with changes in neural activity; hence the mechanisms of learning remain unresolved. We amplify on these points by beginning to develop an example in the following section.

EUROPEAN STARLINGS AND SLEEP-DEPENDENT MEMORY CONSOLIDATION: BEHAVIOR

Though human and animal studies of sleep-dependent consolidation have been mutually beneficial, a fundamental discrepancy between the two lines of research has remained. Human studies have provided compelling behavioral evidence of sleep-dependent consolidation while being limited at uncovering the underlying mechanisms that account for the sleep-dependent performance benefits. Animal studies have provided neural data that could be the basis of memory consolidation but with limited evidence that adult animals receive any memory benefit from the proposed mechanisms. It remains unresolved whether adult animals express sleep-dependent memory benefits similar to those observed in humans.

To address this question, we developed a paradigm using European starlings (*Sturnus vulgaris*) that was modeled after the standard behavioral approach of human sleep-memory studies. Starlings are a species of songbird with complex vocalizations that consist of long sequences of temporally discrete motifs, with each motif itself (≈1 s duration) being a complex sequence of syllables (Adret-Hausberger & Jenkins, 1988; Eens, 1997). Wild starlings maintain large repertoires of mostly unique motifs (Chaiken, Bohner, & Marler, 1993), and can learn to identify individuals by associating the production of certain motifs with specific individuals (Gentner & Hulse, 2000; Gentner, Hulse, Bentley, & Ball, 2000). Starlings are also advantageous in that they can be trained using operant techniques to classify auditory stimuli through differential reinforcement of responses to different stimuli. In the Go/No-Go paradigm, starlings are rewarded with food access when they respond to the "Go" stimulus but are given a lights-out punishment when they respond to the "No-Go" stimulus. Over the course of training, starlings learn to respond to the Go stimulus and withhold response from the No-Go stimulus, thus demonstrating the ability to learn and maintain auditory classifications. Thus, this approach takes advantage of natural song recognition behavior along with the ease in which starlings can perform operant learning tasks in the laboratory (Gentner, 2004). This results in an attractive model system for studying auditory perceptual learning and associative memory.

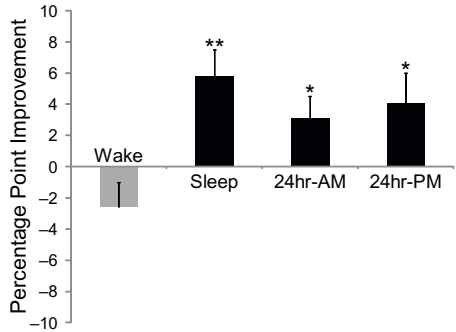

Figure 6.2 ***Auditory classification performance improvement in starlings.*** Starlings were trained to classify two 5 s segments of novel starling song and retested after a retention period that consisted of wakefulness (gray bar) or that included a night of sleep (black bars). Performance improvement scores were calculated as the difference between the post-retention and post-training test scores. Data are means ± SEM (*$p < 0.05$; **$p < 0.01$). *Data are from Brawn, Nusbaum et al. (2010).*

To determine whether starlings express behavioral evidence of sleep-dependent consolidation, 24 starlings each completed six experimental conditions in which they learned to classify pairs of 5 s segments of novel starling song. In each condition, starlings were given a 2 hr training session during which they could complete up to 200 trials followed by a 50 trial post-training test. The starlings were then given a 50 trial post-retention test after an interval that consisted of a full day awake or that included a night of sleep. In the "wake" condition, in which starlings were trained in the morning and retested the same evening, classification performance decreased nonsignificantly from the post-training test to the post-retention test. By comparison, the "sleep" condition, in which starlings were trained in the evening and retested the following morning, exhibited a significant performance improvement. Likewise, the "24-hr AM" and "24-hr PM" conditions, both of which included a night of sleep during the retention interval, expressed significant performance improvements. These results were then replicated in two conditions that were retested after both waking and sleeping retention. In the "AM-PM-AM" condition, classification performance declined nonsignificantly across wakefulness and then improved significantly after sleep. In the "PM-AM-PM" condition, performance improved significantly after sleep followed by a nonsignificant change across the day. The results demonstrate that sleep produces a pattern of memory benefits in starlings that is similar to that observed in humans (Fig. 6.2) (Brawn, Nusbaum, & Margoliash, 2010).

We note that to establish the sleep-dependent memory consolidation behavior in starlings (and in humans), it has been necessary to simplify the learning task so that there are significant performance increases at the end of training that forms a baseline for assessing any subsequent changes in performance. How these sleep-dependent learning processes interact with more cognitively complex learning processes that presumably recruit greater forebrain participation remains an open question.

One critical difference between the starling results and human studies is that human task performance often deteriorates significantly before sleep (Brawn et al., 2008; Brawn, Fenn, et al., 2010; Ellenbogen et al., 2009; Ellenbogen et al., 2006; Fenn et al., 2003; Payne et al., 2008), whereas the performance decline across waking retention failed to reach significance in the starlings. This difference could be attributed to interference from the richer waking experience in humans because daytime behavior in human studies is rarely controlled. In contrast, each starling only encountered a very familiar baseline stimulus set when it was not engaged in training or testing sessions, thus reducing potential interference. Because real-world learning experiences often involve the acquisition of similar skills or information that could interfere with each other, investigating memory processing under conditions of interference may prove to be a more informative approach for understanding how memories are consolidated.

To test this hypothesis, we extended the auditory classification paradigm to explore the interaction between interference and consolidation across waking and sleeping retention by training starlings on two similar, therefore putatively interfering, classification tasks (T. Brawn, unpublished data). Starlings each completed seven experimental conditions that followed an A-B-A (interference) or A-A (control) design. The interference training (additional training on new song stimuli) occurred immediately after completing task A ("Early Interference") or 4 hours later ("Late Interference"). As in the prior experiments, starlings were maintained on a simple baseline task to preclude other sources of interference. Thus we had strong experimental control of the amount and timing of interfering exposure. We observed that interference training caused significant declines in performance when tested on the first evening. This was observed for performance on both tasks, indicating that learning task B resulted in retroactive effects (learning B affects performance on A) and proactive effects (learning A affects performance on B). Despite these interference-induced impairments, performance on both tasks was significantly improved when the starlings were retested on the following day.

These studies establish a new paradigm to investigate memory consolidation. They demonstrate that acquiring new material in the presence of other, interfering material actually magnifies the memory benefit of sleep. This is consistent with the effects of associative interference on declarative memory in humans (Ellenbogen et al., 2009; Ellenbogen et al., 2006). These observations demonstrate that sleep consolidation separately enhances memory of interfering experiences, facilitating opportunistic daytime learning. Our ability to separately manipulate the two memories, potential differences in the time course for manifestation of proactive and retroactive interference, and the distinction between the labile memory representation on the first day and the consolidated memory after a period of sleep, should facilitate our search for neuronal correlates of these behaviors.

FUNCTIONAL ORGANIZATION OF THE STARLING AUDITORY SYSTEM

The auditory classification paradigm described above has provided compelling evidence that sleep produces behavioral memory benefits in starlings that are consistent with the patterns of performance changes across waking and sleep in humans. Though it is not yet known how sleep acts to benefit memory in starlings, the extensive knowledge of the songbird auditory system helps to guide such studies.

Hierarchical organization. We focus on the forebrain, which is the central locus of learning complex and species-specific vocalizations (Simpson & Vicario, 1990), and has descending influence on midbrain and brainstem responses (Dick, Lee, Nusbaum, & Price, 2011; Strait, Chan, Ashley, & Kraus, 2012; Suga & Ma, 2003). In the main avian ascending auditory pathways, nucleus ovoidalis (Ov) of the thalamus receives input from nucleus mesencephalicus lateralis pars dorsalis (MLd) of the midbrain (Karten, 1967) (see Fig. 6.3 for a schematic of the auditory system connections). Neurons in different subdivisions of Ov project to different subdivisions of field L (Karten, 1968), a pallial structure that has traditionally been considered analogous to the mammalian primary auditory cortex. Of the five subdivisions of field L (Fortune & Margoliash, 1992), L2a and L2b receive the majority of thalamic input from the core region of Ov, with a shell region of Ov projecting to subdivisions L1 and L3. Thus Ov core and shell represent different functional pathways (Durand, Tepper, & Cheng, 1992; Wild, Karten, & Frost, 1993). The field L subdivisions are highly interconnected and project to separate higher order structures in

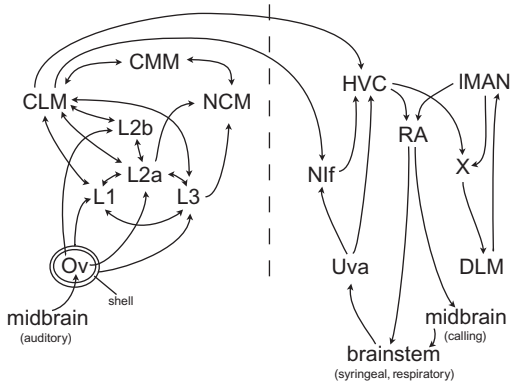

Figure 6.3 ***Connections of the auditory system and song system in a passerine bird.*** *Left side*: Auditory connections showing input from the thalamus (Ov) to subdivisions of Field L in the telencephalon and beyond to secondary auditory areas. There are multiple ascending pathways, roughly separated into one leading to NCM and anther leading to CM (CMM and CLM). *Right side*: Song system connections including a motor pathway (HVC and RA), a basal ganglia pathway (X, DLM, and lMAN), and ascending and descending connections from the motor pathway. Abbreviations: CLM, caudal lateral mesopallium; CMM, caudal medial mesopallium; DLM, nucleus dorsolateralis anterior, pars medialis of the thalamus; HVC, acronym is the proper name; lMAN, lateral magnocellular nucleus of anterior nidopallium; L1/2a/2b/3, subdivisions of field L; NCM, caudal medial nidopallium; NIf, nucleus interface of the nidopallium; Ov, nucleus ovoidalis; RA, robust nucleus of the arcopallium; Uva, nucleus uvaeformis of the thalamus; X, area X.

distinct pathways (Vates, Broome, Mello, & Nottebohm, 1996; Wild et al., 1993) (see Fig. 6.3).

A traditional view of the avian secondary auditory areas emphasizes a hierarchical organization, but this is under active revision. There are unidirectional field L projections to the caudomedial nidopallium (NCM) and reciprocal connections between field L and the caudolateral mesopallium (CLM). In the traditional view, these lead to the caudomedial mesopallium (CMM) at the top of the hierarchy, which has reciprocal connections with NCM and CLM but no direct connections with field L (Vates et al., 1996). Recent anatomical studies in auditory regions of chick brain slices, however, have suggested a revision of this view. Radial patterns of organization were identified with connections between CM (CMM and CLM) and field L that are reminiscent of neocortical columnar organization and connectivity (Wang et al., 2010). This anatomical work places field L3 as an output structure, whose neurons have properties similar to those of neocortical layer 5 neurons.

Changes in neuronal response properties along the auditory pathway have also been interpreted to reflect the traditional hierarchical scheme. An increase in nonlinearity and selectivity for complex acoustic features along the ascending auditory pathway has been noted comparing neuronal responses in the various subdivisions of field L and in CLM and CMM (Muller & Leppelsack, 1985; Sen, Theunissen, & Doupe, 2001; Theunissen et al., 2004). The most compelling physiological distinctions observed, however, have been restricted to comparisons of neurons in L2a/b with neurons in L1, L3, and secondary areas. In contrast, a recent survey of six auditory regions in the starling (L1, L3, CMM, CLM, and NCM) identified L3 as having more selective neurons than any other region in addition to a broader distribution of neurons tolerant to within-class distinction than any other region (C. D. Meliza, unpublished results). Selectivity for a class and tolerance for differences within a class are both features of object recognition. This result provides a physiological context for the recent anatomical findings.

Neurophysiology of song perceptual learning. The responses in the higher order auditory areas NCM, CMM, and CLM show differential effects of learning (Jeanne, Thompson, Sharpee, & Gentner, 2011; Thompson & Gentner, 2010). These are likely to be sites of physiological mechanisms that drive sleep-dependent memory consolidation. Recordings from CMM in operantly trained starlings seem particularly promising. Neurons in CMM rapidly learn to distinguish between different classes of song stimuli in an operant conditioning paradigm similar to that described above for sleep-dependent memory consolidation. Training starlings to classify starling songs using a Go/No-Go (GNG) or Two-Alternative Choice (2AC) task enhances the neural representation of the trained songs within CMM such that CMM neurons exhibit a significant preference, as quantified by a response strength index, for the trained songs compared to novel songs, as well as a preference for positively reinforced songs (Go stimuli) over negatively reinforced songs (No-Go stimuli) (Gentner & Margoliash, 2003). This indicates that CMM neurons are strongly influenced by the behavioral saliency of the song in addition to its spectrotemporal characteristics. Though some neurons respond to a large proportion of motifs in the training songs, CMM neurons are generally selective for a limited number of trained motifs, suggesting that a population of CMM neurons tuned to behaviorally salient motifs mediates song recognition and classification. Significantly, the enhanced response to training stimuli was broadly represented across the population of CMM neurons.

Our initial study of CMM had examined responses at a single point in time, immediately after birds had achieved asymptotic behavior on the GNG or 2AC training (Gentner & Margoliash, 2003). More recently we have investigated the time course of the classification learning (D. Zaraza, unpublished results). In this new study, starlings were extensively trained on a GNG task to classify a pair of starling songs, remaining on task for at least 10 days of asymptotic performance. Thereafter, awake-restrained starlings participated in multiple single-unit recording sessions, each conducted at the conclusion of a day's operant training session. Unexpectedly, the population of neurons did not show the strong, rate-driven differential responses between the overtrained and unfamiliar songs, which contrasts with the results described above. There was a residual trace of the training in the neuronal response, however, detected in the statistically significant preference of a subpopulation of neurons for familiar motifs over unfamiliar motifs.

In the design of the new study (D. Zaraza, unpublished results), starlings were then transferred to new training stimuli. Birds rapidly learned the new stimuli (in a single session) because they were already familiar with the GNG task. This is very similar to the rapid learning starlings exhibited in the sleep-memory experiments described above. It contrasts with our original study where starlings learned a single task that was more complicated (more songs to discriminate), and the initial task of learning the apparatus contributed to the time course of learning (Gentner & Margoliash, 2003).

We observed that after transferring to a second stimulus, CMM neurons showed the broad population-wide response to the new stimulus, as observed in the prior experiment (Gentner & Margoliash, 2003). Interestingly, the response to the old stimulus that birds were no longer training on also suddenly induced the broad population-wide response. Both facets of the population-wide response (to new and old stimuli) declined over multiple days of recordings, with the response to the new stimulus maintained only in a smaller population of neurons selective for familiar motifs. We speculate that if acquiring multiple memories on the same day induces a population-wide response for each of those memories, the interaction between those responses might be related to the behavioral interference between the memories that we have observed.

A hypothesis on sleep perceptual learning. The correlation between changes in classification performance and changes in CMM activity as starlings rapidly transitioned from chance to asymptotic performance highlights the connection between neural activity in CMM and classification memory. There appear to be two distinct processes associated with perceptual learning. Both

selective responses to familiar motifs in limited numbers of neurons and population-wide increases in average responses to songs emerge after learning, but apparently only the population-wide response declines slowly over days.

These preliminary results have many features of memory consolidation that are consistent with a role of sleep. We hypothesize that the population-wide response (first process) helps "tune" or strengthen the motif selective response (second process). The first process is elicited suddenly after the onset of new training, induces responses broadly across the CMM population, induces the response for stimuli that had previously been learned, and decays over days. This suggests that the information is present in a broad network and can be recruited by autoassociative mechanisms. If so, this opens the possibility that the information might also be recruited in sleep replay, which requires correlated activity over populations of neurons. We speculate that an interaction between the two processes occurs during sleep, resulting in changes in the distribution of responses within CMM and across the auditory hierarchy. An important first step in testing these hypotheses will be to assess if changes in CMM neuronal responses over days are time-dependent or sleep-dependent.

Our hypothesis is based on preliminary results from neuronal recordings combined with established results from behavioral experiments. It is the structure of the behavioral results and the confidence in those results that guides our neurophysiological analyses. This helps to emphasize the principal point we are presenting in this chapter, the importance of behavioral studies for a mechanistic understanding of sleep memory consolidation.

AN INTRODUCTION TO BIRDSONG PRODUCTION LEARNING AND THE ROLE OF SLEEP

The majority of sleep-dependent memory consolidation studies in humans have been conducted in adults. Yet the most intensive periods of learning are experienced during development. Sleep is linked to developmental learning through many processes and finds support in the requirement of infants for extensive sleep (Tarullo, Balsam, & Fifer, 2011), and additional confirmation arising from perceptual learning experiments in infants (Gomez, Bootzin, & Nadel, 2006; Hupbach, Gomez, Bootzin, & Nadel, 2009).

Animal studies in birds provide further evidence linking sleep and memory consolidation. Here, we focus on adult song maintenance and developmental learning of bird song. Song learning involves perceptual and procedural (i.e., nondeclarative) learning, but this does not exclude the possibility that

declarative memory also contributes to song learning. For example, adult birds use song for individual recognition (Falls, 1982; Stoddard, 1996), and this behavior could conceivably rely in part on declarative memories.

Developmental song learning is extensively studied and thoroughly reviewed (Konishi, 1978; Kroodsma & Miller, 1982; Margoliash, 2002; Marler, 1997), so we provide only a brief description, emphasizing aspects related to sleep-dependent processes. In the classical description, a juvenile songbird still in the altricial phase forms a sensory memory of the songs produced by its father or other adult conspecifics. This represents the sensory phase of song learning, and the memory of song is often conceived of as an acquired sensory "template" used to guide subsequent vocal learning. The sensorimotor phase of song learning begins with subsong, akin to human babbling, which is dominated by relatively long and variable sequences of relatively unstructured sounds. In this phase, the bird produces low amplitude vocalizations with increased spectrotemporal complexity and greater variability in duration and spectral content than innate calls. Subsong transitions to more structured singing where individual syllables and sequences of syllables can be recognized, albeit with variation in both. This plastic singing continues to be modified until a bird crystalizes a song pattern, typically at the time of sexual maturity. In zebra finches, the subject of many developmental studies, the crystallized song starts with introductory notes which are followed by a fixed sequence of syllables called a motif. Unlike the starling song described above, a zebra finch learns only one motif, and a song bout consists of singing that motif one or more times.

This impoverished description of song learning gains vitality when elaborated by the biological richness afforded in the natural history of 4,000 species of oscine passerines. A wealth of studies in numerous species, however, suggests a constant feature. Apparently, every one of these species requires auditory feedback for song learning (Kroodsma, 1982; Kroodsma & Baylis, 1982). In many species, including zebra finches, adult individuals express a reduced requirement for auditory feedback to maintain their songs. In contrast, suboscine species likely show variability in this pattern, with some species not requiring auditory feedback for developmental song learning (Kroodsma & Konishi, 1991) while others likely do (Saranathan, Hamilton, Powell, Kroodsma, & Prum, 2007).

THE BIRD SONG SYSTEM

All oscine passerines express specialized forebrain nuclei associated with song learning and production. One forebrain "motor" pathway, which consists of

projections from the association/secondary motor cortical nucleus HVC to the motor output nucleus RA, is necessary for and heavily recruited during singing. A second "anterior forebrain" pathway (AFP) receives input from HVC and ultimately projects onto RA. This includes the basal ganglia components of the song system (in Area X) (Carrillo & Doupe, 2004; Farries, Ding, & Perkel, 2005; Farries & Perkel, 2002) and represents a cortico-basal ganglia-thalamocortical pathway. AFP input to RA gives rise to variation in song production (Kao, Doupe, & Brainard, 2005; Olveczky, Andalman, & Fee, 2005; Scharff & Nottebohm, 1991; Sohrabji, Nordeen, & Nordeen, 1990). The AFP is the principle source of drive onto RA early in development, which is thought to explain the heightened variability of subsong (Aronov, Andalman, & Fee, 2008). The influence of the AFP wanes throughout development, and it has little direct influence on singing in adult birds. Nevertheless the AFP is involved in regulating the influence of auditory feedback (Brainard & Doupe, 2000), and so may contribute to sleep-related regulation of singing (Andalman & Fee, 2009). HVC receives auditory inputs from several sources that ultimately arise from higher-order auditory structures (reviewed above) as well as ascending input from the brainstem that is thought to contain feedback information (Ashmore, Renk, & Schmidt, 2008) (Fig. 6.3). RA projects to the brainstem and midbrain structures involved in syringeal and respiratory control, and these also provide feedback via the ascending input to HVC, completing the loop.

The physiology associated with song production, auditory feedback, song perception, and the development of these properties is complex and extensively analyzed (see reviews in Bolhuis & Gahr, 2006; Margoliash & Schmidt, 2010; Mooney, 2009). Here we emphasize the activity patterns of a few classes of neurons that have been the most carefully studied relative to sleep mechanisms of birdsong learning. In nucleus HVC of the motor pathway, there are two categories of projection neurons (and classes of neurons within each category): HVC neurons that project exclusively to RA (HVC-RAn) and HVC neurons that project exclusively to Area X (HVC-Xn). During singing in adult zebra finches HVC projection neurons emit precisely timed and structured bursts of a few (1–4) spikes at very high rate (burst duration 6–10 ms). Bursting is exceedingly sparse, with a given HVC-RAn emitting a single burst per motif and each HVC-Xn emitting 1–4 bursts per motif (Hahnloser, Kozhevnikov, & Fee, 2006; Kozhevnikov & Fee, 2007). In contrast, during singing the HVC interneurons (HVCi) fire tonically throughout song (Yu & Margoliash, 1996). As described below, a burst firing mode during sleep has been associated with neuronal replay.

Finally, during singing, RA projection neurons (RAn) also emit bursts characterized by a few spikes produced at high frequency. In contrast to the HVC-RAn neurons, RAn burst densely throughout many syllables of song, on average 12 bursts per motif, and they reliably emit each burst every time the bird sings the motif (Leonardo & Fee, 2005; Yu & Margoliash, 1996). Each burst occurs at the same point in time relative to the song acoustics and is thought to be associated with individual notes (components) of a syllable. Typically, each spike burst associated with a different part of a song has a different number of spikes and/or timing of spikes within the burst. The variation within bursts associated with one part of a song is far lower than the differences in bursts associated with other parts of a song. Thus, there are burst "types" that are unique, which has greatly facilitated analysis of neuronal replay during sleep.

NEURONAL REPLAY IN THE SONG SYSTEM

In neuronal rehearsal, activation across regions (and temporal relations in the activation patterns) observed offline, such as during sleep, approximates the patterns of activation observed during daytime behavior. In neuronal replay, the offline activity of neurons and populations of neurons produce veridical if approximate copies of the patterns observed during daytime behavior (Foster & Wilson, 2006; Louie & Wilson, 2001; Wilson & McNaughton, 1994). Veridical replay has been considered *sine qua non* for sleep-dependent processing. This is a limited view driven by the technical challenge of identifying offline structure in activity (typically bursting) without spike activity templates derived from online activity and by the conceptual challenge of interpreting offline activity that is not associated with an objective behavior. Neuronal activity during sleep, however, shows variability not observed during daytime activity, but this variation may carry information rather than noise—for example, activity whose patterns help regulate network plasticity by driving networks into nearby states or related dynamical paths. Furthermore, the activity during sleep may carry signals (information) about different modalities that are not represented in the daytime activity of the same neurons. This can arise via neuromodulatory mechanisms that can, for example, gate sensory information into a motor pathway. Such gating has been observed in the song system, where the responses of HVC and RA neurons to auditory stimuli are weaker or absent in awake compared to sleeping zebra finches (A. S. Dave, Yu, & Margoliash, 1998; Schmidt & Konishi, 1998). This gating also

shows circadian variation. HVC neurons of juvenile zebra finches in the plastic song stage of sensorimotor development express auditory activity both day and night. However, these neurons respond more vigorously to playback of the tutor song during the day and more vigorously to playback of the bird's own song at night (Nick & Konishi, 2005). This suggests that different types of information are processed during singing and during offline replay of singing. Veridical playback is also observed in the song system (see below), but this result helps to emphasize that sleep-dependent processing is a far more complicated and multidimensional process than simple veridical playback.

There are extensive neuromodulatory pathways and mechanisms differentially activated in the song system depending on behavioral state (Cardin & Schmidt, 2004a, 2004b; Shea, Koch, Baleckaitis, Ramirez, & Margoliash, 2010; Shea & Margoliash, 2003). These features and the neurochemistry of the song system have been reviewed elsewhere (Ball & Balthazart, 2010; Castelino & Schmidt, 2010; Shea & Margoliash, 2010). The neuromodulatory pathways in birds help regulate state changes, hence sleep states, in much the same way they do in mammals. This is an integral part of sleep-dependent memory processing but will not be reviewed here.

NEURONAL REPLAY IN ADULT SONG BIRDS

Basic features. Neuronal replay of RAn in adult zebra finches is perhaps the most compelling example of replay at a single neuron level that has been reported to date (A. S. Dave & Margoliash, 2000). To demonstrate replay, it is necessary to directly compare single or population neuronal activity during waking and sleeping. Correlated bursting and other evidence suggest that replay is observed throughout the song system (A. S. Dave & Margoliash, 2000; Hahnloser, Kozhevnikov, & Fee, 2002; Kozhevnikov & Fee, 2007; Sutter & Margoliash, 1994), and this assumption underpins some analyses of HVC activity (Hahnloser et al., 2002). But to date, replay has only formally been demonstrated for RAn in adult zebra finches. The technical challenges of maintaining recordings during singing and then while an animal sleeps are substantial.

A related feature of zebra finch song system activity during sleep is that neurons express strong and highly selective response to a single stimulus—playback of the bird's own song (BOS) (Dave & Margoliash, 2000). Neurons give stronger responses to BOS than to conspecific songs or BOS presented in reverse (which preserves the overall spectral content).

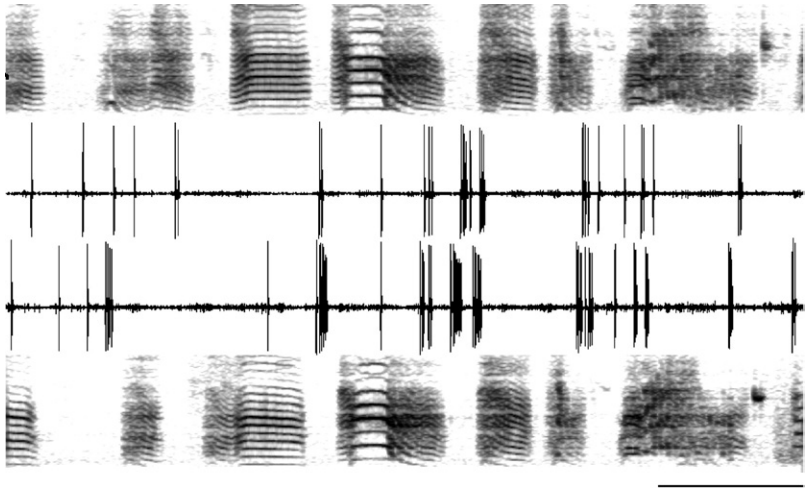

Figure 6.4 ***Neuronal replay and auditory responses in the song system.*** *Top two panels:* Sonograph of a rendition of the bird's own song and response of an RA neuron to playback of the song while the bird was sleeping. *Bottom two panels:* Activity of the same RA neuron during the day when the bird sang; the sonograph of the corresponding song is the *bottommost panel*. Note the pattern bursts are very similar for the right-hand side of the figure, where the syllables and timing of syllable sequences were the most similar. Scale bar is 200 ms. *From Dave (2001).*

The selective response to BOS arises from integration over the sequence of preceding syllables (up to many hundreds of ms) leading up to a nonlinear facilitated response expressed only for the correct sequence of syllables (Margoliash, 1983; Margoliash & Fortune, 1992). As with spontaneous replay, responses to BOS in sleeping birds release patterns of bursting that are related to the bursting observed during singing (Fig. 6.4). This indicates that information expressed in the bursting of RAn during sleep (and sleep bursting of other song system neurons) represents sensorimotor information. RAn activity during singing is premotor, preceding syllables by approximately 40 ms. RAn auditory responses show similar timing, hence the onset of a burst can precede the syllable the burst is associated with. This integration over prior syllables allows sensory and motor responses to be expressed in the same temporal framework, uniting the two.

RAn replay and its modification. The bursting pattern of an RAn during singing is precise and reliable. In a sleeping zebra finch, RAn occasionally emit individual bursts and trains of bursts. A burst was defined as continuous sequences of interspike intervals falling outside the normal (nonbursting) distribution. Considering only relatively long bursts (≥8 spikes), approximately

7% of spikes occurred in bursts and approximately 15% of bursts matched a burst that the neuron had emitted during singing (A. S. Dave & Margoliash, 2000). These estimates represent a lower bound. Note that the definition is limited to considering only longer bursts and relies on the precise structure within individual bursts. Yet RAn occasionally emit multiple bursts in sequence whose relation to the sequence of bursts during singing is compelling even if one of the bursts in the sequence differs radically from that observed during singing. Moreover, bursting in the song system tends to recruit many cells synchronously. These considerations suggest that the actual numbers of RAn bursts related to song replay may be much higher than the original estimates we reported. Recording from a population of RAn during replay may resolve this issue.

The neuronal replay phenomenon in RA indicates that rather precise information—and perhaps precise variation in information—regarding singing is represented in the discharge of single neurons and populations of neurons during sleep. But how is the information used, if at all? The fundamental prediction of the sleep-learning hypothesis is that neuronal activity patterns should change over periods of sleep, and in a nonrandom fashion. A related prediction is that the observed changes should be adaptive with regard to learning. Given the reliability of RAn activity during singing, one conceptually simple if technically challenging approach to addressing this question is to compare the activity patterns of individual RAn recorded during singing before and after a period of sleep. We have recently reported this result, observing systematic changes in the burst discharge properties of RAn over periods of sleep (Rauske, Chi, Dave, & Margoliash, 2010). Of 115 spike bursts observed in 15 neurons, 33 bursts in 10 neurons showed changes in burst structure over sleep. In contrast, changes in burst structure during singing were about an order of magnitude less common (18 changed bursts of 551 bursts recorded during daytime singing). In almost all cases, the principal change over the sleep period was a decrease in the number of spikes in the burst (Fig. 6.5), thus the changes were not the result of random fluctuation around a mean number of spikes per burst. For altered bursts only, the interspike intervals increased so that the overall burst duration remained approximately constant.

Approximately half of all neurons exhibited one or more bursts that change when comparing the burst patterns over the two periods of singing. On average 20% of all bursts in RA emitted during singing changed after a night's sleep. Although these changes in burst structure are modest, even over one night and certainly over multiple nights, the accumulated

changes would profoundly change a zebra finch's song if there was no compensatory response. Given that adult zebra finch song is highly stable, we conclude that spike loss during sleep is part of a mechanism to consistently modify song. We hypothesize this mechanism is involved in auditory

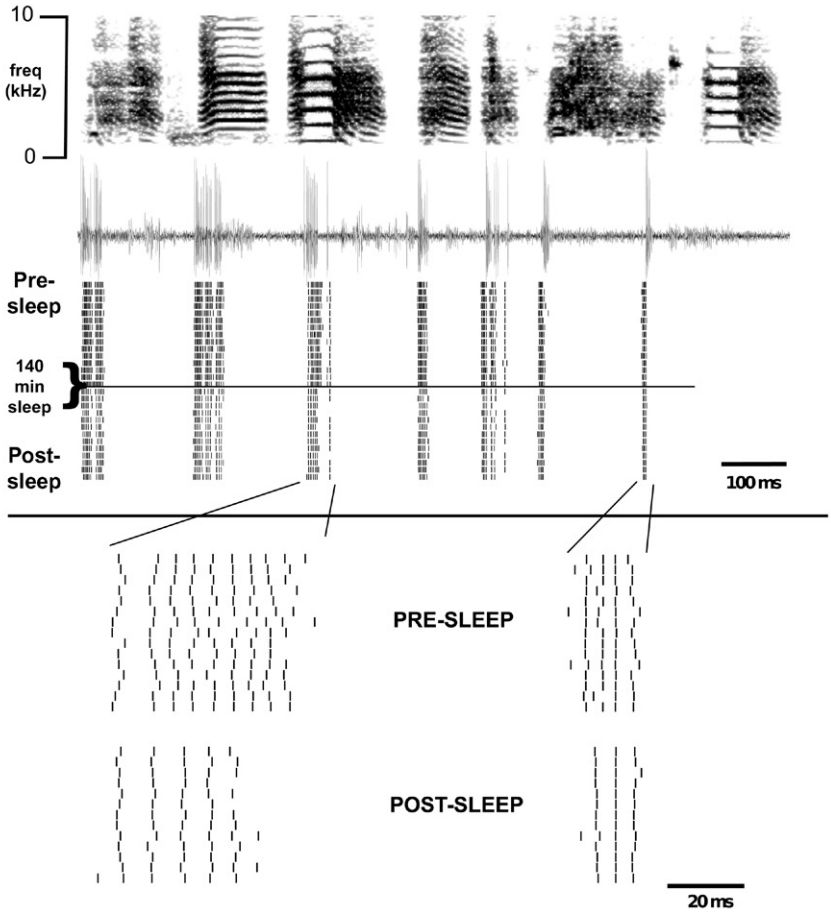

Figure 6.5 ***The structure of RA spike bursting during singing changes over a period of sleep.*** *Top panel:* Sonograph of a rendition of song the bird sang prior to sleep and a raw trace of an RA neuron's activity during that song. *Below* raster plots are shown of activity for 15 renditions of the song that were sang prior to a sleep period of 140 min and 13 renditions of the song that were sang after the sleep period. Note the timing of the burst sequences is preserved before and after sleep but there are slight differences in individual burst structure. *Bottom panel*: To help visualize the change in burst structure across sleep, two bursts are shown at higher temporal resolution. For both bursts, following sleep there are fewer spikes per burst and the interspike intervals have increased. *From Rauske (2005)*.

feedback regulation of adult song (Nordeen & Nordeen, 1992) to maintain precision in singing.

Most neurons showed changes in a small number of bursts over sleep (typically zero, one or two), but one neuron showed changes in almost every burst (Fig. 6.5). The distribution of numbers of bursts changing per neuron was approximately exponential, although larger samples of neurons are required to confirm this result. This suggests that neurons expressing changes across sleep are selected randomly at a uniform rate. In neurons with two or more bursts that changed, however, the changes tended to cluster together (i.e., they were not distributed randomly across song).

Spike loss and synaptic homeostasis. If there is a strong tendency for bursts to loose spikes over periods of sleep, how do spikes get back into the system? There is a compelling neurogenesis phenomenon associated with HVC-RAn (Goldman & Nottebohm, 1983), in which new neurons are first incorporated into HVC local circuits (Paton & Nottebohm, 1984). They then project to and synapse in RA within one to two weeks (Alvarez-Buylla, Theelen, & Nottebohm, 1988). We speculate that newly incorporated HVC-RAn synapse onto those RAn that are expressing relatively few spikes in song. These RAn are weakly driven by local circuits, perhaps because they have unused synaptic space for which the new HVC-RAn can compete. To date we have not observed an RAn during sleep that suddenly and dramatically increased the number of bursts or the number of spikes within a burst—a plausible scenario arising from our speculation. But if our speculation is accurate, the RAn that would do so would be expressing very low levels of activity prior to the change in their status. Consequently, there would be a strong bias against detecting such silent neurons in extracellular recordings. Perhaps such changes could be observed in recordings of populations of RAn.

It appears that the loss of spikes within bursts of RAn is not first expressed during singing in the morning. Rather, changes in the structure of burst patterns are observed when emitted in ongoing discharge during sleep. The modified bursts show spike–timing structure that is more similar to the pattern of the corresponding burst that will be expressed during singing after sleep than the pattern of the corresponding burst that was expressed during singing before sleep. This was observed by using a course to fine temporal pattern filtering approach, first identifying sequences of bursts and then identifying the structure of a target burst within the sequence (Z. Chi, unpublished results). It remains to be determined if the modified burst pattern observed during sleep is really novel;

for example, the neuron could be cycling through a series of burst patterns (stable points in a network dynamics). However, the observation of a modified burst pattern during sleep prior to incorporation of that modified burst into singing after sleep is suggestive of a causal relation. The strong tendency of modified bursts to emit fewer spikes also argues against the caveat. We note that this description of spike loss over sleep may represent a specific instantiation of the proposed phenomenon of synaptic homeostasis, a sleep-related mechanism to regulate hyperexcitability of neuronal networks (Tononi & Cirelli, 2003). This helps to emphasize the point that the actual effects of proposed sleep mechanisms of neuronal activity have to be evaluated in the context of the specific systems that they operate.

Caveats and conclusions. A principal weakness that remains with our studies of adult replay is that we have not directly tied it to learning (in this case, auditory–feedback mediated song maintenance). To date we have only observed changes in bursting of individual neurons over periods of sleep. Singing in adult zebra finches shows circadian variation (Glaze & Troyer, 2006) that in juveniles is associated with sleep-related song learning (Derégnaucourt, Mitra, Feher, Pytte, & Tchernichovski, 2005), but that association has not been confirmed in adults. Singing in adult zebra finches also depends on auditory feedback, but the changes in song following adult deafening develop slowly over days and weeks (Leonardo & Konishi, 1999; Nordeen & Nordeen, 1992). The loss of spike bursts observed for RAn is presumably a mechanism to remove small variations in song, given that the songs zebra finch males direct towards females have much less variation than even their quite invariant undirected songs. We have yet to confirm this important prediction, however. This awaits development of a reliable technique for recording from populations of neurons and correlating changes in sleep bursts of the recorded population with song maintenance-related changes (e.g., by manipulating auditory feedback). In this important sense, both the neuronal replay work in bird song production and in the rat hippocampal replay system share a common and significant limitation.

These concerns notwithstanding, the replay phenomenon in RA offers significant potential for studying sleep and learning that has yet to be embraced. Given that the bird's song is known, recordings from a sufficient population of RA neurons should allow determination of the exact portion of song being replayed at each moment, without direct knowledge of the burst patterns the neurons express during singing. Such analysis could be bootstrapped by examining sequential burst structure in response

to playback of the bird's own song. Such a development would be highly illuminating of many of the issues described above.

SLEEP AND SENSORIMOTOR SONG LEARNING: BEHAVIORAL EVIDENCE

We now turn to song developmental learning. There is only indirect evidence that the early perceptual learning experience involves sleep-dependent memory consolidation. When isolated birds are first exposed to the singing of an adult tutor, they sometimes respond by rapidly falling asleep, an observation first reported from the Tchernichovski lab (T. Lints, unpublished data), which we have confirmed (P. Adret, unpublished data). Certainly a relation between sleep and perceptual memory in juveniles would be consistent with studies of adults in humans and starlings.

The behavioral evidence supporting a role of sleep in the sensorimotor phase of song learning is far more secure, albeit to date this has been demonstrated only for a single species (zebra finch). To achieve this, it was necessary to develop techniques to analyze the large corpus of variable songs produced by each developing bird (Tchernichovski, Lints, Deregnaucourt, Cimenser, & Mitra, 2004; Tchernichovski, Mitra, Lints, & Nottebohm, 2001; Tchernichovski, Nottebohm, Ho, Pesaran, & Mitra, 2000). It was also necessary to develop a paradigm to bring the timing of developmental song learning under strong experimental control (Tchernichovski, Lints, Mitra, & Nottebohm, 1999; Tchernichovski & Nottebohm, 1998). This requires isolating the normally group-living juvenile birds from live tutors (adult males) to control for the variation in singing performance by tutors and social interactions between tutors and juveniles. In this approach, juvenile birds are raised by their mothers (who do not sing) until they are independent. They are then individually reared in a sound isolation acoustic chamber, which facilitates acquisition of high quality vocal recordings. Birds gain access to song through a form of instrumental conditioning, either pecking a key or pulling a string. Access to the song is limited by only rewarding a small number of pecks with song, a contingency that improves song copying (Tchernichovski et al., 1999).

A remarkable change in the singing behavior is observed under these conditions when a circa 40-day-old bird is first exposed to an adult conspecific song (Derégnaucourt et al., 2005). The young bird rapidly develops a circadian variation in singing behavior such that the songs produced early in the subjective day have less structure than songs sung later in the day. This pattern

repeats each day so that songs produced in the afternoon are more complex and better copies of the tutor song than songs from the preceding or following morning. Notably, the circadian variation in singing does not emerge on the day of tutor song exposure but on the following day after a night of sleep.

The circadian pattern is adaptive and directly related to sleep (Derégnaucourt et al., 2005). Juveniles with a greater magnitude of circadian variation are those that eventually achieve the best copies of the tutor song. Juveniles prevented from singing in the morning will produce low structure songs in the afternoon when they are allowed to sing normally. Juveniles induced to sleep briefly during the day will sing a second bout of low structure songs upon awakening, followed by songs with increasing structure. These are forms of "awake deprivation" with a positive result that is much easier to interpret than the effects of sleep deprivation, the often-cited gold standard for sleep research.

The strength of these observations notwithstanding, a series of opportunities remain to more fully characterize the role of sleep in developmental song learning. The isolation rearing paradigm delays the onset of song copying later in development than normal. By this time the juveniles have already begun to sing but as birds that are isolated from tutor song exposure. Recent observations indicate that juveniles raised with live tutors develop a similar circadian variation, helping to address this point (M. Lusignan, unpublished observations). Juvenile zebra finches can show at least two different large-scale patterns of song learning (a syllable repetition or motif-centered pattern) (Liu, Gardner, & Nottebohm, 2004) but birds raised under instrumental conditioning of song exposure only express the motif-centered pattern (Tchernichovski et al., 2001). It would be valuable to compare sleep effects for both patterns of learning. The observed circadian variation is also somewhat fragile and shows significant individual variation, increasing the difficulty in studying it. Perhaps the biggest limitation to date, however, is that such studies have yet to be extended to other species, which limits confidence in the generality of the results. A challenge of the behavioral results is to explain why sleep (normally viewed as consolidating memories) should degrade song performance. Understanding how this arises requires a mechanistic explanation, to which we now turn.

NEURONAL REPLAY AND SONG DEVELOPMENT

Neuronal replay in the adult song system is expressed through high frequency spike bursting. Examining the emergence of bursting during

development has shed additional light on neuronal replay and song learning (Shank & Margoliash, 2009). RAn were recorded on nights before and after the onset of instrumentally conditioned daily tutor song exposure. Prior to the onset of tutoring, RAn exhibited low spontaneous rates and little bursting during sleep, resulting in unimodal interspike interval (ISI) histograms. On the first night after tutor song exposure, however, RAn exhibited substantially modified ongoing discharge properties. There were more short-interval ISIs and many more sequences of short-interval ISIs organized into protobursts, which was characterized in the shape of the now bimodal ISI distributions. Note that the emergence of the sleep-related circadian pattern of singing behavior was observed on the day after the first day of tutor song exposure but prior to circadian singing patterns that begin the following morning, suggesting that the emergence of sleep bursting is part of the causal mechanism driving the circadian singing pattern.

In these experiments, most birds were exposed to only one of three tutor songs, resulting in three groups of birds. The average ISI histograms calculated for each bird were similar within each group and differed across groups. The shape of the ISI histogram is a dynamic property: in birds exposed to one tutor song for several days then switched to another, RAn recordings during sleep showed corresponding changes in ISI histograms. Given that the AFP, not HVC, provides the principal source of drive to RA in the first days after tutor song exposure when the bird is producing subsong (Aronov et al., 2008), this indicates that auditory song "template" information is expressed through the cortical–basal ganglia pathway in the song system.

It remains to be determined if there were systematic differences in the subsong of the different groups of birds, which would reflect the influence of the different tutor songs. The dynamic nature of the tutor song as represented in the ISI distributions during sleep, however, suggests that the information represented in the sleep discharges was directly related to the tutor song and not to singing. Furthermore, in the first nights after tutor song exposure, RAn do not respond to actual playback of the tutor song (or BOS). Thus, if RAn activity during sleep contributes to song development, it is probably not expressed through a replay mechanism as it is normally described.

Preliminary results indicate that RAn first exhibit auditory responses during sleep at the time the bird transitions from subsong to plastic song (M. Lusignan, unpublished results). This transition is also marked by HVC activity becoming dominant in driving RA. At this point, RAn neurons respond selectively to playback of BOS, which represents the emergence of neuronal replay as it is normally characterized. We speculate that

sensorimotor mappings between HVC-Xn and HVC-RAn achieve some critical threshold, enabling populations of HVC-RAn to fire in a structured fashion. During sleep, this structured input to RA drives bursting and conveys auditory activity from HVC to RA.

OLD AND NEW IDEAS REGARDING SLEEP IN A NEW THEORY OF SONG LEARNING

The weight of the results in juvenile birds suggests that neuronal discharge during sleep contributes to developmental song learning. The first effects of exposure to a tutor song may include representational plasticity induced during sleep. This goes beyond the traditional view of how auditory memories influence song learning (Margoliash & Schmidt, 2010). In the new conception, there are distinct plastic processes, one associated with daytime singing and the other with sleep replay (Hinton, Dayan, Frey, & Neal, 1995). The distinction observed between the forms of auditory information expressed in daytime and sleep activity of HVCn is consistent with this hypothesis (Nick & Konishi, 2005). Early in development these two plastic processes are not well coordinated, resulting in the poorly structured songs observed during morning singing.

The principal feature of system consolidation theory is that information is transferred from local to global memories through long-term interactions (Buzsaki, 1998; Squire, Cohen, & Nadel, 1984). Consistent with this idea is the suggestion that song auditory memories are transferred through multiple structures including secondary auditory nuclei outside of the traditional song system, and through the AFP. System consolidation theory also is described in terms of a single mechanism or network structure, whereas the data from the song learning studies indicate that sleep-related information in networks changes over the time course of development. Finally, song learning has long been held up as a model for speech and language acquisition in humans (Doupe & Kuhl, 1999; Marler, 1970). The complex set of results obtained in zebra finches make basic predictions as to how humans should acquire and consolidate features of language (Margoliash, 2003).

SUMMARY AND FINAL CONCLUSIONS

We began by asking why a neuroscientist should study sleep in birds. One answer was grounded in an evolutionary perspective. If one seeks an understanding of sleep behavior and its ultimate functions, this requires a

comparative approach. Complex mammalian-like sleep in some species of birds and shared organizational elements in neocortex and avian cortex represent part of a long lineage that includes shared traits across vertebrates and beyond. Examining sleep in the context of that lineage should help inform why sleep is observed so broadly in the animal kingdom. A second answer was grounded in a more ethological perspective. The study of animal behavior and its underlying physiology is facilitated by taking advantage of behavioral specializations in the target species. This neuroethological approach has guided our studies of sleep and learning in the two avian models we highlighted. The starling model we described, which relies on song recognition behavior, expresses a pattern of sleep-dependent memory benefits that is comparable to what is observed in humans. This will allow us to measure and manipulate memory consolidation while we examine its physiology in the starling auditory system. We contrasted this with the strengths and limitations in the prominent studies of sleep and learning in rodents.

Whereas the physiological analysis of sleep in relation to starling song perceptual learning is only beginning, the role of sleep for sensorimotor learning has profited by taking advantage of the intensively studied song learning and bird song system. We have observed a compelling neuronal replay phenomenon in adult zebra finches and that its emergence during development is related to significant song learning milestones. A promising avenue for future research is to investigate the role of replay in helping to shape the sleep-dependent vocal developmental trajectories. The novel hypotheses for sensorimotor learning these observations have suggested emphasize the potential for sleep research to uncover new physiological mechanisms.

We conclude by noting that sleep may have a myriad of effects in different species, in different physiological systems, and on different behaviors. Some overarching principles may apply very broadly, such as synaptic downscaling and system consolidation. Yet, there is no one thing that "sleep does." To understand the application of these principles, we need to look at specific behaviors and how those are affected by sleep. Songbirds in particular represent attractive model systems towards such goals.

REFERENCES

Adret-Hausberger, M., & Jenkins, P. F. (1988). Complex organization of the warbling song in the European starling *Sturnus vulgaris*. *Behaviour*, *107*, 138–156.

Alvarez-Buylla, A., Theelen, M., & Nottebohm, F. (1988). Birth of projection neurons in the higher vocal center of the canary forebrain before, during, and after song learning. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(22), 8722–8726.

Andalman, A. S., & Fee, M. S. (2009). A basal ganglia-forebrain circuit in the songbird biases motor output to avoid vocal errors. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(30), 12518–12523.
Aronov, D., Andalman, A. S., & Fee, M. S. (2008). A specialized forebrain circuit for vocal babbling in the juvenile songbird. *Science*, *320*(5876), 630–634.
Ashmore, R. C., Renk, J. A., & Schmidt, M. F. (2008). Bottom-up activation of the vocal motor forebrain by the respiratory brainstem. *Journal of Neuroscience*, *28*(10), 2613–2623.
Ball, G. F., & Balthazart, J. (2010). Introduction to the chemical neuroanatomy of birdsong. *Journal of Chemical Neuroanatomy*, *39*(2), 67–71.
Bolhuis, J. J., & Gahr, M. (2006). Neural mechanisms of birdsong memory. *Nature Reviews Neuroscience*, 7(5), 347–357.
Brainard, M. S., & Doupe, A. J. (2000). Alteration of auditory feedback causes both acute and lasting changes to Bengalese finch song. *Society Neuroscience Abstracts*, *26*, 269.266
Brawn, T. P., Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2008). Consolidation of sensorimotor learning during sleep. *Learning & Memory*, *15*(11), 815–819.
Brawn, T. P., Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2010). Consolidating the effects of waking and sleep on motor-sequence learning. *Journal of Neuroscience*, *30*(42), 13977–13982.
Brawn, T. P., Nusbaum, H. C., & Margoliash, D. (2010). Sleep-dependent consolidation of auditory discrimination learning in adult starlings. *Journal of Neuroscience*, *30*(2), 609–613.
Buzsaki, G. (1998). Memory consolidation during sleep: A neurophysiological perspective. *Journal of Sleep Research*, 7(Suppl. 1), 17–23.
Cardin, J. A., & Schmidt, M. F. (2004). Auditory responses in multiple sensorimotor song system nuclei are co-modulated by behavioral state. *Journal of Neurophysiology*, *91*(5), 2148–2163.
Cardin, J. A., & Schmidt, M. F. (2004). Noradrenergic inputs mediate state dependence of auditory responses in the avian song system. *Journal of Neuroscience*, *24*(35), 7745–7753.
Carrillo, G. D., & Doupe, A. J. (2004). Is the songbird Area X striatal, pallidal, or both? An anatomical study. *Journal of Comparative Neurology*, *473*(3), 415–437.
Castelino, C. B., & Schmidt, M. F. (2010). What birdsong can teach us about the central noradrenergic system. *Journal of Chemical Neuroanatomy*, *39*(2), 96–111.
Chaiken, M., Bohner, J., & Marler, P. (1993). Song acquisition in European starlings, Sturnus vulgaris: A comparison of the songs of live-tutored, tape-tutored, untutored, and wild-caught males. *Animal Behaviour*, *46*(6), 1079–1090.
Cirelli, C., & Tononi, G. (2008). Is sleep essential? *PLoS Biology*, *6*(8), e216.
Dave, A. S. (2001). *Mechanisms of sensorimotor vocal integration*. Chicago: University of Chicago.
Dave, A. S., & Margoliash, D. (2000). Song replay during sleep and computational rules for sensorimotor vocal learning. *Science*, *290*(5492), 812–816.
Dave, A. S., Yu, A. C., & Margoliash, D. (1998). Behavioral state modulation of auditory activity in a vocal motor system. *Science*, *282*(5397), 2250–2254.
Derégnaucourt, S., Mitra, P. P., Feher, O., Pytte, C., & Tchernichovski, O. (2005). How sleep affects the developmental learning of bird song. *Nature*, *433*(7027), 710–716.
Dick, F., Lee, H. L., Nusbaum, H., & Price, C. J. (2011). Auditory-motor expertise alters "speech selectivity" in professional musicians and actors. *Cerebral Cortex*, *21*(4), 938–948.
Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews Neuroscience*, *11*(2), 114–126.
Doupe, A. J., & Kuhl, P. K. (1999). Birdsong and human speech: Common themes and mechanisms. *Annual Review of Neuroscience*, *22*, 567–631.
Dugas-Ford, J. (2009). *A comparative molecular study of the amniote dorsal telencephalon*. Chicago: University of Chicago.
Durand, S. E., Tepper, J. M., & Cheng, M. F. (1992). The shell region of the nucleus ovoidalis: A subdivision of the avian auditory thalamus. *The Journal of Comparative Neurology*, *323*(4), 495–518.

Eens, M. (1997). Understanding the complex song of the European starling: An integrated ethological approach. *Advances in the Study of Behavior*, *26*, 355–434.

Ego-Stengel, V., & Wilson, M. A. (2010). Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. *Hippocampus*, *20*(1), 1–10.

Eiland, M. M., Lyamin, O. I., & Siegel, J. M. (2001). State-related discharge of neurons in the brainstem of freely moving box turtles, terrapene carolina major. *Archives Italiennes de Biologie*, *139*(1–2), 23–36.

Ellenbogen, J. M., Hu, P. T., Payne, J. D., Titone, D., & Walker, M. P. (2007). Human relational memory requires time and sleep. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(18), 7723–7728.

Ellenbogen, J. M., Hulbert, J. C., Jiang, Y., & Stickgold, R. (2009). The sleeping brain's influence on verbal memory: Boosting resistance to interference. *PLoS ONE*, *4*(1), e4117.

Ellenbogen, J. M., Hulbert, J. C., Stickgold, R., Dinges, D. F., & Thompson-Schill, S. L. (2006). Interfering with theories of sleep and memory: Sleep, declarative memory, and associative interference. *Current Biology : CB*, *16*(13), 1290–1294.

Euston, D. R., Tatsuno, M., & McNaughton, B. L. (2007). Fast-forward playback of recent memory sequences in prefrontal cortex during sleep. *Science*, *318*(5853), 1147–1150.

Falls, J. B. (1982). Individual recognition by sound in birds. In D. E. Kroodsma, & E. H. Miller (Eds.), *Acoustic communication in birds, vol. 2, song learning and its consequences* (pp. 237–278). New York: Academic Press.

Farries, M. A., Ding, L., & Perkel, D. J. (2005). Evidence for "direct" and "indirect" pathways through the song system basal ganglia. *Journal of Comparative Neurology*, *484*(1), 93–104.

Farries, M. A., & Perkel, D. J. (2002). A telencephalic nucleus essential for song learning contains neurons with physiological characteristics of both striatum and globus pallidus. *Journal of Neuroscience*, *22*(9), 3776–3787.

Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2003). Consolidation during sleep of perceptual learning of spoken language. *Nature*, *425*(6958), 614–616.

Ferrara, M., Iaria, G., Tempesta, D., Curcio, G., Moroni, F., Marzano, C., et al. (2008). Sleep to find your way: The role of sleep in the consolidation of memory for navigation in humans. *Hippocampus*, *18*(8), 844–851.

Fischer, S., Nitschke, M. F., Melchert, U. H., Erdmann, C., & Born, J. (2005). Motor memory consolidation in sleep shapes more effective neuronal representations. *The Journal of Neuroscience : The official Journal of the Society for Neuroscience*, *25*(49), 11248–11255.

Fortune, E. S., & Margoliash, D. (1992). Cytoarchitectonic organization and morphology of cells of the field L complex in male zebra finches (Taenopygia guttata). *The Journal of Comparative Neurology*, *325*(3), 388–404.

Foster, D. J., & Wilson, M. A. (2006). Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature*, *440*(7084), 680–683.

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nature Reviews Neuroscience*, *6*(2), 119–130.

Gais, S., Lucas, B., & Born, J. (2006). Sleep after learning aids memory recall. *Learning & Memory*, *13*(3), 259–262.

Gais, S., Plihal, W., Wagner, U., & Born, J. (2000). Early sleep triggers memory for early visual discrimination skills. *Nature Neuroscience*, *3*, 1335–1339.

Gentner, T. Q. (2004). Neural systems for individual song recognition in adult birds. *Annals of the New York Academy of Sciences*, *1016*, 282–302.

Gentner, T. Q., & Hulse, S. H. (2000). Perceptual classification based on the component structure of song in European starlings. *Journal of the Acoustical Society of America*, *107*(6), 3369–3381.

Gentner, T. Q., Hulse, S. H., Bentley, G. E., & Ball, G. F. (2000). Individual vocal recognition and the effect of partial lesions to HVc on discrimination, learning, and categorization of conspecific song in adult songbirds. *Journal of Neurobiology*, *42*(1), 117–133.

Gentner, T. Q., & Margoliash, D. (2003). Neuronal populations and single cells representing learned auditory objects. *Nature*, *424*(6949), 669–674.

Girardeau, G., Benchenane, K., Wiener, S. I., Buzsaki, G., & Zugaro, M. B. (2009). Selective suppression of hippocampal ripples impairs spatial memory. *Nature Neuroscience*, *12*(10), 1222–1223.

Glaze, C. M., & Troyer, T. W. (2006). Temporal structure in zebra finch song: Implications for motor coding. *Journal of Neuroscience*, *26*(3), 991–1005.

Goldman, S. A., & Nottebohm, F. (1983). Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proceedings of the National Academy of Sciences of the United States of America*, *80*(8), 2390–2394.

Gomez, R. L., Bootzin, R. R., & Nadel, L. (2006). Naps promote abstraction in language-learning infants. *Psychological Science*, *17*(8), 670–674.

Hahnloser, R. H., Kozhevnikov, A. A., & Fee, M. S. (2002). An ultra-sparse code underlies the generation of neural sequences in a songbird. *Nature*, *419*(6902), 65–70.

Hahnloser, R. H., Kozhevnikov, A. A., & Fee, M. S. (2006). Sleep-related neural activity in a premotor and a basal-ganglia pathway of the songbird. *Journal of Neurophysiology*, *96*(2), 794–812.

Hennevin, E., Huetz, C., & Edeline, J. M. (2007). Neural representations during sleep: From sensory processing to memory traces. *Neurobiol Learning & Memory*, *87*(3), 416–440.

Hinton, G. E., Dayan, P., Frey, B. J., & Neal, R. M. (1995). The "wake-sleep" algorithm for unsupervised neural networks. *Science*, *268*(5214), 1158–1161.

Hu, P., Stylos-Allan, M., & Walker, M. P. (2006). Sleep facilitates consolidation of emotional declarative memory. *Psychological Science*, *17*(10), 891–898.

Hupbach, A., Gomez, R. L., Bootzin, R. R., & Nadel, L. (2009). Nap-dependent learning in infants. *Developmental Science*, *12*(6), 1007–1012.

Jarvis, E. D., Gunturkun, O., Bruce, L., Csillag, A., Karten, H., Kuenzel, W., et al. (2005). Avian brains and a new understanding of vertebrate brain evolution. *Nature Reviews Neuroscience*, *6*(2), 151–159.

Jeanne, J. M., Thompson, J. V., Sharpee, T. O., & Gentner, T. Q. (2011). Emergence of learned categorical representations within an auditory forebrain circuit. *Journal of Neuroscience*, *31*(7), 2595–2606.

Ji, D., & Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nature Neuroscience*, *10*(1), 100–107.

Jones, S. G., Vyazovskiy, V. V., Cirelli, C., Tononi, G., & Benca, R. M. (2008). Homeostatic regulation of sleep in the white-crowned sparrow (Zonotrichia leucophrys gambelii). *BMC Neuroscience*, *9*, 47.

Kao, M. H., Doupe, A. J., & Brainard, M. S. (2005). Contributions of an avian basal ganglia-forebrain circuit to real-time modulation of song. *Nature*, *433*(7026), 638–643.

Karten, H. J. (1967). The organization of the ascending auditory pathway in the pigeon (Columba livia). I. Diencephalic projections of the inferior colliculus (nucleus mesencephali lateralis, pars dorsalis). *Brain Research*, *6*(3), 409–427.

Karten, H. J. (1968). The ascending auditory pathway in the pigeon (Columba livia). II. Telencephalic projections of the nucleus ovoidalis thalami. *Brain Research*, *11*(1), 134–153.

Karten, H. J. (1997). Evolutionary developmental biology meets the brain: The origins of mammalian cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(7), 2800–2804.

Konishi, M. (1978). Auditory environment and vocal development in birds. In R. D. Walk, & H. L. J. Pick (Eds.), *Perception and experience* (pp. 105–118). New York: Plenum.

Korman, M., Doyon, J., Doljansky, J., Carrier, J., Dagan, Y., & Karni, A. (2007). Daytime sleep condenses the time course of motor memory consolidation. *Nature Neuroscience*, *10*(9), 1206–1213.

Kozhevnikov, A. A., & Fee, M. S. (2007). Singing-related activity of identified HVC neurons in the zebra finch. *Journal of Neurophysiology*, *97*(6), 4271–4283.

Kroodsma, D. E. (1982). Learning and the ontogeny of sound signals in birds. In D. E. Kroodsma, & E. H. Miller (Eds.), *Acoustic communication in birds, vol. 2, song learning and its consequences* (pp. 1–23). New York: Academic Press.

Kroodsma, D. E., & Baylis, J. R. (1982). Appendix: A world survey of evidence for vocal learning in birds. In D. E. Kroodsma, & E. H. Miller (Eds.), *Acoustic Communication in Birds, vol. 2, song learning and its consequences* (pp. 311–337). New York: Academic Press.

Kroodsma, D. E., & Konishi, M. (1991). A suboscine bird, eastern phoebe (Sayornis-phoebe), develops normal song without auditory feedback. *Animal Behaviour*, *42*(3), 477–488.

Kroodsma, D. E., & Miller, E. H. (1982). *Acoustic communication in birds. vol. 2. song learning and its consequences*. New York: Academic Press.

Kudrimoti, H. S., Barnes, C. A., & McNaughton, B. L. (1999). Reactivation of hippocampal cell assemblies: Effects of behavioral state, experience, and EEG dynamics. *Journal of Neuroscience*, *19*(10), 4090–4101.

Lee, A. K., & Wilson, M. A. (2002). Memory of sequential experience in the hippocampus during slow wave sleep. *Neuron*, *36*(6), 1183–1194.

Leonardo, A., & Fee, M. S. (2005). Ensemble coding of vocal control in birdsong. *Journal of Neuroscience*, *25*(3), 652–661.

Leonardo, A., & Konishi, M. (1999). Decrystallization of adult birdsong by perturbation of auditory feedback. *Nature*, *399*(6735), 466–470.

Liu, W. C., Gardner, T. J., & Nottebohm, F. (2004). Juvenile zebra finches can use multiple strategies to learn the same song. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(52), 18177–18182.

Louie, K., & Wilson, M. A. (2001). Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. *Neuron*, *29*(1), 145–156.

Low, P. S., Shank, S. S., Sejnowski, T. J., & Margoliash, D. (2008). Mammalian-like features of sleep structure in zebra finches. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(26), 9081–9086.

Margoliash, D. (1983). Acoustic parameters underlying the responses of song-specific neurons in the white-crowned sparrow. *Journal of Neuroscience*, *3*(5), 1039–1057.

Margoliash, D. (2002). Evaluating theories of bird song learning: Implications for future directions. *Journal of Comparative Physiology A*, *188*(11–12), 851–866.

Margoliash, D. (2003). Offline learning and the role of autogenous speech: New suggestions from birdsong research. *Speech Communication*, *41*, 165–178.

Margoliash, D., & Fortune, E. S. (1992). Temporal and harmonic combination-sensitive neurons in the zebra finch's HVc. *Journal of Neuroscience*, *12*(11), 4309–4326.

Margoliash, D., & Schmidt, M. F. (2010). Sleep, off-line processing, and vocal learning. *Brain and Language*, *115*(1), 45–58.

Marler, P. (1970). Birdsong and speech development: Could there be parallels? *American Scientist*, *58*(6), 669–673.

Marler, P. (1997). Three models of song learning: Evidence from behavior. *Journal of Neurobiology*, *33*, 501–516.

Marshall, L., & Born, J. (2007). The contribution of sleep to hippocampus-dependent memory consolidation. *Trends in Cognitive Sciences*, *11*(10), 442–450.

Mednick, S., Nakayama, K., & Stickgold, R. (2003). Sleep-dependent learning: A nap is as good as a night. *Nature Neuroscience*, *6*(7), 697–698.

Mooney, R. (2009). Neural mechanisms for learned birdsong. *Learning & Memory*, *16*(11), 655–669.

Muller, C. M., & Leppelsack, H. J. (1985). Feature extraction and tonotopic organization in the avian auditory forebrain. *Experimental Brain Research*, *59*(3), 587–599.

Nick, T. A., & Konishi, M. (2005). Neural song preference during vocal learning in the zebra finch depends on age and state. *Journal of Neurobiology*, *62*(2), 231–242.

Nishida, M., & Walker, M. P. (2007). Daytime naps, motor memory consolidation and regionally specific sleep spindles. *PLoS ONE*, *2*(4), e341.

Nordeen, K. W., & Nordeen, E. J. (1992). Auditory feedback is necessary for the maintenance of stereotyped song in adult zebra finches. *Behavioral & Neural Biology*, *57*(1), 58–66.
O'Neill, J., Pleydell-Bouverie, B., Dupret, D., & Csicsvari, J. (2010). Play it again: Reactivation of waking experience and memory. *Trends in Neurosciences*, *33*(5), 220–229.
Olveczky, B. P., Andalman, A. S., & Fee, M. S. (2005). Vocal experimentation in the juvenile songbird requires a basal ganglia circuit. *PLoS Biology*, *3*(5), e153.
Paton, J. A., & Nottebohm, F. (1984). Neurons born in adult brain are recruited into functional circuits. *Science*, *225*, 1046–1048.
Payne, J. D., Stickgold, R., Swanberg, K., & Kensinger, E. A. (2008). Sleep preferentially enhances memory for emotional components of scenes. *Psychological Science*, *19*(8), 781–788.
Peyrache, A., Khamassi, M., Benchenane, K., Wiener, S. I., & Battaglia, F. P. (2009). Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nature Neuroscience*, *12*(7), 919–926.
Poe, G. R. (2010). Cognitive neuroscience of sleep. In G. A. Kerkhof & H. P. A. van Dongen (Eds.), *Human sleep and cognition* (Vol. 185, pp. 1–19). Oxford: Elsevier Science.
Racsmany, M., Conway, M. A., & Demeter, G. (2010). Consolidation of episodic memories during sleep: Long-term effects of retrieval practice. *Psychological Science*, *21*(1), 80–85.
Ramadan, W., Eschenko, O., & Sara, S. J. (2009). Hippocampal sharp wave/ripples during sleep for consolidation of associative memory. *PLoS ONE*, *4*(8), e6697.
Rasch, B., & Born, J. (2007). Maintaining memories by reactivation. *Current Opinion in Neurobiology*, *17*(6), 698–703.
Rattenborg, N. C. (2006). Evolution of slow-wave sleep and palliopallial connectivity in mammals and birds: A hypothesis. *Brain Research Bulletin*, *69*(1), 20–29.
Rattenborg, N. C., Martinez-Gonzalez, D., Roth, T. C., II, & Pravosudov, V. V. (2011). Hippocampal memory consolidation during sleep: A comparison of mammals and birds. *Biological Reviews of the Cambridge Philosophical Society*, *86*(3), 658–691.
Rauske, P. L. (2005). The modulation of sensorimotor activity by sleep. Chicago: University of Chicago.
Rauske, P. L., Chi, Z., Dave, A. S., & Margoliash, D. (2010). Neuronal stability and drift across periods of sleep: Premotor activity patterns in a vocal control nucleus of adult zebra finches. *Journal of Neuroscience*, *30*(7), 2783–2794.
Reiner, A., Perkel, D. J., Bruce, L. L., Butler, A. B., Csillag, A., Kuenzel, W., et al. (2004). Revised nomenclature for avian telencephalon and some related brainstem nuclei. *Journal of Comparative Neurology*, *473*(3), 377–414.
Robertson, E. M., Pascual-Leone, A., & Press, D. Z. (2004). Awareness modifies the skill-learning benefits of sleep. *Current Biology : CB*, *14*(3), 208–212.
Saranathan, V., Hamilton, D., Powell, G. V., Kroodsma, D. E., & Prum, R. O. (2007). Genetic evidence supports song learning in the three-wattled bellbird Procnias tricarunculata (Cotingidae). *Molecular Ecology*, *16*(17), 3689–3702.
Scharff, C., & Nottebohm, F. (1991). A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: Implications for vocal learning. *The Journal of Neuroscience*, *11*(9), 2896–2913.
Schmidt, M. F., & Konishi, M. (1998). Gating of auditory responses in the vocal control system of awake songbirds. *Nature Neuroscience*, *1*(6), 513–518.
Scullin, M. K., & McDaniel, M. A. (2010). Remembering to execute a goal: Sleep on it!. *Psychological Science*, *21*, 1028–1035.
Sen, K., Theunissen, F. E., & Doupe, A. J. (2001). Feature analysis of natural sounds in the songbird auditory forebrain. *Journal of Neurophysiology*, *86*(3), 1445–1458.
Shank, S. S., & Margoliash, D. (2009). Sleep and sensorimotor integration during early vocal learning in a songbird. *Nature*, *458*(7234), 73–77.

Shea, S. D., Koch, H., Baleckaitis, D., Ramirez, J. M., & Margoliash, D. (2010). Neuron-specific cholinergic modulation of a forebrain song control nucleus. *Journal of Neurophysiology*, *103*(2), 733–745.

Shea, S. D., & Margoliash, D. (2003). Basal forebrain cholinergic modulation of auditory activity in the zebra finch song system. *Neuron*, *40*(6), 1213–1226.

Shea, S. D., & Margoliash, D. (2010). Behavioral state-dependent reconfiguration of song-related network activity and cholinergic systems. *Journal of Chemical Neuroanatomy*, *39*(2), 132–140.

Siegel, J. M. (2008). Do all animals sleep? *Trends in Neurosciences*, *31*(4), 208–213.

Simpson, H. B., & Vicario, D. S. (1990). Brain pathways for learned and unlearned vocalizations differ in zebra finches. *The Journal of Neuroscience*, *10*(5), 1541–1556.

Sohrabji, F., Nordeen, E. J., & Nordeen, K. W. (1990). Selective impairment of song learning following lesions of a forebrain nucleus in the juvenile zebra finch. *Behavioral and Neural Biology*, *53*(1), 51–63.

Squire, L. R., Cohen, N. J., & Nadel, L. (1984). The medial temporal region and memory consolidation: A new hypothesis. In H. Weingartner, & E. Parker (Eds.), *Memory consolidation* (pp. 185–210). Hillsdale, NJ: Lawrence Erlbaum.

Stickgold, R., James, L., & Hobson, J. A. (2000). Visual discrimination learning requires sleep after training. *Nature Neuroscience*, *3*(12), 1237–1238.

Stoddard, P. K. (1996). Vocal recognition of neighbors by territorial passerines. In D. E. Kroodsma, & E. H. Miller (Eds.), *Ecology and evolution of acoustic communication in birds* (pp. 356–374). Cornell, Ithaca: Cromstock.

Strait, D. L., Chan, K., Ashley, R., & Kraus, N. (2012). Specialization among the specialized: Auditory brainstem function is tuned in to timbre. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*, *48*(3), 360–362.

Suga, N., & Ma, X. (2003). Multiparametric corticofugal modulation and plasticity in the auditory system. *Nature Reviews. Neuroscience*, *4*(10), 783–794.

Sutter, M. L., & Margoliash, D. (1994). Global synchronous response to autogenous song in zebra finch HVc. *Journal of Neurophysiology*, *72*(5), 2105–2123.

Szymczak, J. T. (1987). Daily distribution of sleep states in the rook *Corvus frugilegus*. *Journal of Comparative Physiology [A]*, *161*(2), 321–327.

Talamini, L. M., Nieuwenhuis, I. L., Takashima, A., & Jensen, O. (2008). Sleep directly following learning benefits consolidation of spatial associative memory. *Learning & Memory*, *15*(4), 233–237.

Tarullo, A. R., Balsam, P. D., & Fifer, W. P. (2011). Sleep and infant learning. *Infant and Child Development*, *20*(1), 35–46.

Tchernichovski, O., Lints, T., Mitra, P. P., & Nottebohm, F. (1999). Vocal imitation in zebra finches is inversely related to model abundance. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(22), 12901–12904.

Tchernichovski, O., Lints, T. J., Deregnaucourt, S., Cimenser, A., & Mitra, P. P. (2004). Studying the song development process: Rationale and methods. *Annals of the New York Academy of Sciences*, *1016*, 348–363.

Tchernichovski, O., Mitra, P. P., Lints, T., & Nottebohm, F. (2001). Dynamics of the vocal imitation process: How a zebra finch learns its song. *Science*, *291*(5513), 2564–2569.

Tchernichovski, O., & Nottebohm, F. (1998). Social inhibition of song imitation among sibling male zebra finches. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(15), 8951–8956.

Tchernichovski, O., Nottebohm, F., Ho, C. E., Pesaran, B., & Mitra, P. P. (2000). A procedure for an automated measurement of song similarity. *Animal Behaviour*, *59*(6), 1167–1176.

Theunissen, F. E., Amin, N., Shaevitz, S. S., Woolley, S. M., Fremouw, T., & Hauber, M. E. (2004). Song selectivity in the song system and in the auditory forebrain. *Annals of the New York Academy of Sciences*, *1016*, 222–245.

Thompson, J. V., & Gentner, T. Q. (2010). Song recognition learning and stimulus-specific weakening of neural responses in the avian auditory forebrain. *Journal of Neurophysiology*, *103*(4), 1785–1797.

Tobler, I., & Borbely, A. A. (1988). Sleep and EEG spectra in the pigeon (Columba livia) under baseline conditions and after sleep deprivation. *Journal of Comparative Physiology A*, *163*, 729–738.

Tononi, G., & Cirelli, C. (2003). Sleep and synaptic homeostasis: A hypothesis. *Brain Research Bulletin*, *62*(2), 143–150.

Tucker, M. A., Hirota, Y., Wamsley, E. J., Lau, H., Chaklader, A., & Fishbein, W. (2006). A daytime nap containing solely non-REM sleep enhances declarative but not procedural memory. *Neurobiology of Learning and Memory*, *86*(2), 241–247.

Ulinski, P. S. (1984). Design features in vertebrate sensory systems. *American Zoologist*, *24*, 717–731.

Vates, G. E., Broome, B. M., Mello, C. V., & Nottebohm, F. (1996). Auditory pathways of caudal telencephalon and their relation to the song system of adult male zebra finches. *Journal of Comparative Neurology*, *366*(4), 613–642.

Walker, M. P., Brakefield, T., Morgan, A., Hobson, J. A., & Stickgold, R. (2002). Practice with sleep makes perfect: Sleep-dependent motor skill learning. *Neuron*, *35*(1), 205–211.

Walker, M. P., & Stickgold, R. (2006). Sleep, memory, and plasticity. *Annual Review of Psychology*, *57*, 139–166.

Wang, Y., Brzozowska-Prechtl, A., & Karten, H. J. (2010). Laminar and columnar auditory cortex in avian brain. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(28), 12676–12681.

Wild, J. M., Karten, H. J., & Frost, B. J. (1993). Connections of the auditory forebrain in the pigeon (Columba livia). *Journal of Comparative Neurology*, *337*(1), 32–62.

Wilson, M. A., & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep [see comments]. *Science*, *265*(5172), 676–679.

Yu, A. C., & Margoliash, D. (1996). Temporal hierarchical control of singing in birds. *Science*, *273*(5283), 1871–1875.