Problem and Background

Sleep is common and may be ubiquitous in all major taxa of the animal kingdom, but it is poorly understood. There are multiple hypotheses for the roles of sleep, including information processing hypotheses. Human studies of a variety of low-level perceptual and motor learning tasks indicate that sleep helps to remediate memory loss that is observed in the first day following task learning. Recent evidence extends these observations to a higher-level speech perceptual learning task that requires generalized learning (Fenn, Nusbaum, Margoliash, unpublished observations). The human studies demonstrate information consolidation during sleep but give little insight into neural mechanism.

Animal models have demonstrated evidence of 'replay' during sleep, which may be a central component of the sleep consolidation mechanism. Recently, it was observed that during sleep, robustus archistriatalis (RA) neurons of the zebra finch (*Taeniopygia guttata*) song system rehearse song patterns spontaneously and respond to playback of the bird's own song (Dave & Margoliash, 2000). There is of yet no conclusive evidence that song learning in juveniles or song maintenance in adult birds requires or benefits from sleep. However, recent observations of song development in zebra finches indicates that juvenile birds start changing singing patterns the day following exposure to new vocal material from tutors - i.e. after a period of sleep (Tchernichovski et al. 2001), and there is evidence that the structure of the bursting patterns of RA neurons in adult birds are particularly labile during sleep (Rauske and Margoliash, unpublished).

Investigation of the possible role of sleep in song learning or maintenance is hampered by the limited knowledge of sleep states in passerine birds. Previous studies have not reported different phases of sleep in the zebra finch (Nick & Konishi, 2002; Hahnloser & Fee, 2002). In contrast, studies in other birds, including passerine birds (Szymczak et al., 1993), have reported REM sleep in this Class and other features of sleep similar to that reported in mammals. We therefore investigated staging of sleep in zebra finches.

Experimental Technique

We combined electrophysiological techniques with direct observation of sleeping birds. We first conducted a series of acute experiments with birds anesthetized with urethane (20%, circa 90 μl over 1 hr). Optimal recordings, as judged by amplitude and reliability of signals, were obtained using thick platinum electrodes touching the dura mater. EEGs were obtained recording differentially between the platinum electrodes while using an additional electrode as a ground reference.

After the acute experiments, we prepared birds for chronic recordings. Birds were implanted with 3 mm long L-shaped platinum electrodes. Electrodes were secured at with dental acrylic and wired to a head connector. During recordings, signals from the electrodes passed though a cable to an overhead mercury commutator, allowing the bird relative freedom of movement within the cage. This method is preferable to restraining the animal, because restrained animals experience stress, and stress is known to modify the sleep architecture. Recordings commenced no earlier than 3 days following surgery. In some cases, the circadian cycle was shifted to permit the experimenter to record sleeping birds during normal waking hours; in other cases recordings were conducted in birds whose circadian cycle had not been altered. Signals were sampled at 1 kHz (or in some cases, 20 kHz and then downsampled), and filtered 1-100 Hz.

During recordings, birds were bathed in infrared (IR) light and monitored with an IR camera. Mirrors facilitated detection of eye, head, and body movements. We were particularly interested in observing unilateral eye opening, which may be correlated with unihemispheric sleep, and tail movements, which occur in response to song playback and spontaneously (Dave and Margoliash, unpublished).

Data Analysis

Spectra of the EEG traces were computed with a multi-taper technique over 10 second increments. This technique enabled us to identify rapid transitions in the EEG signal with high resolution and estimate the variance in the spectra. We used 2.5 tapers per data increment and computed the 95% confidence interval. All analyses were conducted using Matlab.

Results

To date we have preliminary results, most securely from a single bird with additional data from 3 others. The preliminary results clearly indicate that zebra finches exhibit at least two distinct phases of sleep, which we label as rapid-eye movement (REM) and non-REM (NREM) sleep. REM was characterized by an EEG signal typically about $\pm$ 3μV. NREM was characterized by an EEG signal with distinctly higher and broader range (2-5x) of amplitudes. REM may have lower power in the delta range (1-4 Hz) than NREM, although we have yet to perform a systematic analysis. There may also be brief, higher amplitude transients during REM. Both birds on normal circadian patterns and those with shifted sleep schedules displayed REM (and NREM) sleep.

REM episodes were brief in duration (usually less than 10 seconds) at the beginning of the night. REM density was much greater later at night, as is commonly reported in other species. It is not yet clear whether individual REM episodes later at night are also longer in duration.

REM occurred reliably in conjunction with eye and often head movements. The eye movements were on the order of one saccade per second and occurred throughout the period of REM. The head movements were not as reliable, but tended to follow the direction of the eye movements when present. Head movements were not the result of displacement of the head by the weight of the attached cable during REM neck muscle atonia because the head movements were observed in conjunction with eye movements in intact, un-tethered animals. Tail movements may have also accompanied REM, although the frequency and reliability of these has yet to be determined. During NREM, birds breathed slowly and regularly, and any eye and head movements were synchronized to breathing.

We also observed numerous instances when one eye was open and the other was closed. These were apparently reliably associated with differential bilateral patterns of EEG activity, and may be evidence for unihemispheric sleep in zebra finches.