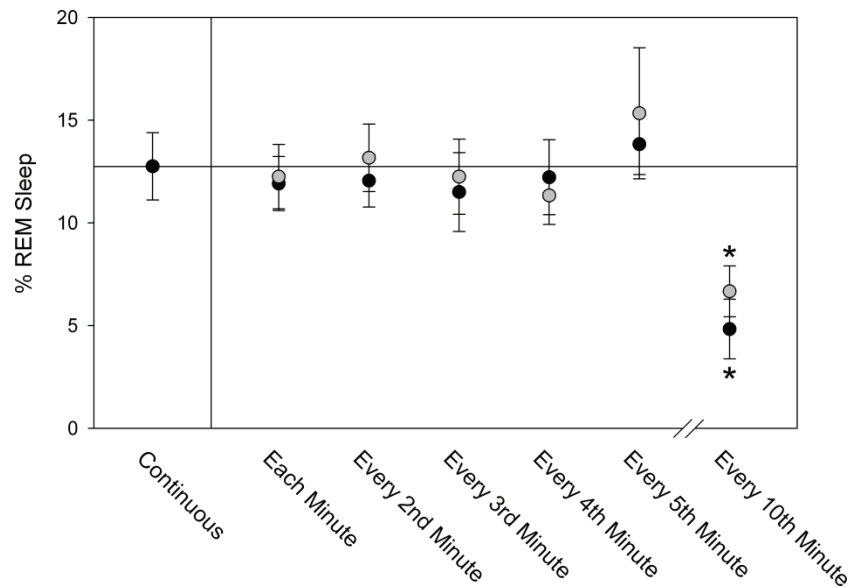


Electronic Supplementary Material

Lesku *et al.* Local sleep homeostasis in the avian brain: convergence of sleep function in mammals and birds? *Proceedings of the Royal Society B: Biological Sciences*.

Avian REM sleep differs from mammalian REM sleep most conspicuously by its short duration (usually < 8 s) and large number of episodes per 24 hr day (hundreds). Each short episode of EEG activation must be confirmed as REM sleep (opposed to a brief period of alert wakefulness) using video recordings looking for behavioral signs of muscle atonia; the electromyogram is not a reliable indicator of REM sleep in birds. This procedure is a time-consuming process, and any sampling performed to reduce the number of possible REM sleep epochs would only reduce the time needed to accurately score avian REM sleep. Accordingly, some sleep studies in birds have sampled REM sleep by scoring only the first epoch of each minute (Rattenborg *et al.* 1999; 2004). Although this sampling regime was validated by those researchers (N. C. Rattenborg, *personal communication*), the verification was not published.

Here, we provide results from a simulation to determine the validity of various sampling intervals for REM sleep using the continuously-scored EEG recordings of Martinez-Gonzalez and colleagues (2008; N = 5 pigeons). REM sleep occupied $12.75 \pm 1.64\%$ (mean \pm S.E.) of the 12 hr baseline night when scored continuously (Supplementary Figure 1). When scored at the top of each minute, the percentage of the baseline night devoted to REM sleep remained statistically unchanged relative to continuous scoring ($P > 0.24$; Supplementary Figure 1). There was likewise no significant difference when sampling at the top of every second, third, fourth or fifth minutes (all $P > 0.20$; Supplementary Figure 1). Sampling at the top of every tenth minute, however, resulted in a significantly inaccurate estimate of the amount of REM sleep ($P < 0.02$; Supplementary Figure 1). These basic results were repeatable using a +30 s offset to each sampling interval (Supplementary Figure 1). Scoring at the top of every fifth minute is as good as continuous scoring, but we nevertheless scored the top of each minute to maintain consistency with some previous sleep studies in birds (Rattenborg *et al.* 1999; 2004).



Supplementary Figure 1. Accuracy of estimating the percentage of a 12 hr night devoted to REM sleep (%REM sleep) when scored continuously (left-most black circle) by sampling the first epoch at the top of each minute, or every second, third, fourth, fifth or tenth minute (black circles). To determine the robustness of these estimations, further simulations were performed with a +30 s offset (grey circles). Only when sampling every tenth minute did the %REM sleep estimate differ significantly from continuous scoring (* $P < 0.02$). Data are presented as mean \pm S.E.

References

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