English Summary:

In the animals studied to date, sleep behavior is omnipresent. From the smallest insect to the largest mammal, from jellyfish, which lack a central nervous system, to mammals with their highly interconnected neocortex, all animals studied to date engage in this state of behavioral shutdown. In spite of the widespread presence of sleep it still remains a state of relative mystery. Sleep has been implicated in numerous processes, such as memory consolidation, energy conservation, adaptive immobility, immune system function, and clearing of neuronal waste. Indeed, sleep deprivation has substantial detrimental effects and can result in death. All of this suggests sleep is an important state that serves a highly conserved function essential for animal life. In spite of this, the fundamental process or processes leading to sleep's universal presence remain unidentified.

Sleep in both birds and mammals can be subdivided into two distinct states, slow wave sleep (SWS) and rapid eye movement (REM) sleep. In both phylogenetic groups SWS and REM sleep are characterized by similar electrophysiological and behavioral traits. These states also appear to share similar regulatory mechanisms in birds and mammals. The evolutionary steps leading to the presence of such similar states in two phylogenetic groups separated by hundreds of millions of years of evolution remains a mystery. In order to elucidate these evolutionary steps one must investigate how other closely related phylogentic groups sleep.

Although the two sleep sub-states share a number of similar attributes in both birds and mammals, many aspects of avian sleep remain unexamined. Mammals are capable of modulating sleep in response to ecologically relevant changes in their environment (Lesku et al., 2008; Gravett et al., 2017a; Gravett et al., 2017b). Specifically, mammals suppress REM sleep in response to ecologically risky scenarios. In chapter 4, I provide the first study to my knowledge describing a similar ability to modulate REM sleep in birds. In this chapter, I demonstrate that

pigeons (*Columba livia*) suppress REM sleep when forced to sleep on a perch site near to the ground. The similarity of the avian and mammalian response to an ecologically risky situation suggests that the regulatory mechanisms underlying this modulation may be the same in both phylogenetic groups.

Sleep in Monotremes and Palaeognathic birds

The class Mammalia is composed of three extant clades; the eutherian mammals, marsupials, and monotremes. Monotremes are a mammalian group retaining many primitive traits, such as egg laying. In contrast to the eutherian mammals and marsupials, which engage in SWS and REM sleep as described above, studies investigating sleep in several monotremes observed a unique combination of behavioral and electrophysiological patterns. In studies of the platypus and echidna, individuals were observed to engage in a mixed-REM sleep state characterized by the presence of EEG slow waves occurring in the forebrain in conjunction with REM-related phasic movements or an increase in neuronal discharge variability in the brainstem, a pattern associated with REM sleep in eutherian mammals. This suggests that perhaps REM sleep emerged first in the brainstem in mammals and later became associated with forebrain activation.

The class Aves is composed of two extant clades; the Neognathic and Palaeognathic birds. Neognathic birds are mostly small and capable of flight, whereas most Palaeognathic birds are large and flightless. Neognathic birds engage in SWS and REM sleep as described above, whereas the only electrophysiological study of a Palaeognathic bird to date, the ostriches, identified mixed REM sleep periods similar to those occurring in monotremes, characterized by the presence of slow waves in the forebrain EEG occurring in conjunction with behavioral signs of REM sleep (closure of the eyes, REMs, and falling of the head resulting from reduction of neck muscle tone; Lesku et al., 2011). In chapter 3 of this thesis, I recorded EEG patterns in naturally sleeping elegant crested tinamous (*Eudromia elegans*), another member of the Palaeognathic birds. Tinamous engaged in SWS and REM sleep as found in Neognathic birds, suggesting the presence of a mixed-REM sleep state may not be widespread within the Palaeognathic birds. Tinamous retain several ancestral traits, including a small body size and the ability to fly. Because tinamous have retained other ancestral traits, making them more representative of the common ancestor to Palaeognathic and Neognathic birds, it is possible that REM sleep associated with EEG activation may also be an ancestral trait present in the common ancestor to both extant bird clades. Further studies of other Palaeognathic birds are necessary to understand whether or not the mixed state observed in the ostriches is present in other members of this group.

Sleep in non-avian reptiles

Birds are a derived type of reptile. Within the reptiles, crocodilians are the closest extant non-avian reptilian relative to the birds. The presence of similar sleep states to those present in birds and mammals in the non-avian reptiles would indicate that these states were likely also present in the common ancestor to the birds and non-avian reptiles, and potentially the common ancestor to amniotes. The absence of similar states in the non-avian reptiles would indicate that SWS and REM sleep evolved in birds after their divergence the non-avian reptiles, and that SWS and REM sleep evolved independently in birds and mammals. Sleep studies in the non-avian reptiles have observed varied and often contradictory results. A majority of studies have reported the presence of intermittent deflections in the EEG, termed high voltage sharp waves (HShW), during behavioral behaviorally defined sleep (reviewed in Libourel and Herrel, 2016). However, how HShW relate to electrophysiological phenomena in birds and mammals remains unclear. Homology between HShW and mammalian hippocampal sharp waves (SWs) has been suggested. However, in contrast to mammalian SWs which are restricted to the hippocampus, reptilian HShWs have a more widespread distribution. A recent study implicated the dorsal ventricular ridge (DVR), a nucleated forebrain structure also present in the birds, in the generation of HShW (Shein-Idelson et al., 2016). The DVR has also been associated with slow wave generation in birds (Beckers et al., 2014). The similar distribution of HShW and avian slow waves suggests homology between these two electrophysiological phenomena may be more likely.

In chapter 2, I demonstrate that HShW propagate following complex and varied spatiotemporal patterns in Nile crocodiles (*Crocodylus niloticus*). The propagation patterns observed in this study resemble those described in birds. Propagation alone does not guarantee homology, however, it is a further attribute suggestive of a homology between these two signals. If HShW are homologous to slow waves, then this sleep state may be homologous in some respects to mammalian and avian SWS. The difference in wave morphology (i.e. slow verses sharp), may arise from reptilian neurons spending less in the depolarized up-states that give rise to slow waves in birds and mammals.

Anesthesia induced brain states

Numerous anesthetics induce brain rhythms similar to those observed during natural SWS in mammals. The ability to induce sleep-like brain states is of great importance to scientists wishing to study these states using electrophysiological recording methods (i.e., intracellular recordings of membrane potentials) not feasible in freely moving animals. Although phenomenological similarities have been suggested between anesthetic induced states and natural sleep in birds, this the specific similarities and differences in EEG have not been systematically quantified. In chapter 5, I compared spectral properties of isoflurane and urethane induced EEG patterns to those occurring during natural SWS in pigeons (*Columba livia*). Electrophysiological phenomena occurring during isoflurane and urethane showed similar spectral properties to those occurring during natural SWS, with most power being concentrated below 2 Hz under both anesthetics. In addition, EEG patterns under both anesthetics showed a significantly higher concentration of power in most frequency bins below 45Hz compared to SWS. Both isoflurane and urethane-induced brain rhythms showed similar spectral concentration at higher frequencies above 55 Hz as compared to naturally occurring SWS. Isoflurane, as well as many other anesthetics have been shown to act on natural sleep promoting pathways in mammals. The observed spectral properties of electrophysiological phenomena occurring during anesthesia-induced states and those occurring during natural SWS in pigeons in this study suggests that while both anesthetics are likely acting on natural sleep-promoting pathways, they may be specifically activate pathways generating lower frequency components of slow wave sleep.

Conclusions

The work described in this thesis aimed to characterize aspects of sleep across several phylogenetic groups. By doing this I hoped to gain a better understanding of how the various electrophysiological manifestations of sleep across phylogenetic groups relate to one another. Ultimately, I hope that this type of research will shed light on fundamental aspects of the mechanisms underlying these states, as well as, any essential functions being performed during these states. The studies presented in this thesis all give support to commonly accepted theory that SWS is not present in its current form in the common ancestor to birds and mammals. Rather an ancestral form of SWS characterized by shorter up-states occurring less often was likely present in the common ancestor to birds and mammals, and SWS as present in the birds and mammals likely evolved independently in each phylogenetic group with the evolution of longer up-states.