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Original Article

ORIGINAL ARTICLE

Bird-like propagating brain activity in anesthetized Nile crocodiles

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Abstract

Study Objectives: The changes in electroencephalogram (EEG) activity that characterize sleep and its sub-states—slow-wave sleep (SWS) and rapid eye movement (REM) sleep—are similar in mammals and birds. SWS is characterized by EEG slow waves resulting from the synchronous alternation of neuronal membrane potentials between hyperpolarized down-states with neuronal quiescence and depolarized up-states associated with action potentials. By contrast, studies of non-avian reptiles report the presence of high-voltage sharp waves (HShW) during sleep. How HShW relate to EEG phenomena occurring during mammalian and avian sleep is unclear. We investigated the spatiotemporal patterns of electrophysiological phenomena in Nile crocodiles (*Crocodylus niloticus*) anesthetized with isoflurane to determine whether they share similar spatiotemporal patterns to mammalian and avian slow waves.

Methods: Recordings of anesthetized crocodiles were made using 64-channel penetrating arrays with electrodes arranged in an 8 × 8 equally spaced grid. The arrays were placed in the dorsal ventricular ridge (DVR), a region implicated in the genesis of HShW. Various aspects of the spatiotemporal distribution of recorded signals were investigated.

Results: Recorded signals revealed the presence of HShW resembling those reported in earlier studies of naturally sleeping reptiles. HShW propagated in complex and variable patterns across the DVR.

Conclusions: We demonstrate that HShW within the DVR propagate in complex patterns similar to those observed for avian slow waves recorded from homologous brain regions. Consequently, sleep with HShW may represent an ancestral form of SWS, characterized by up-states occurring less often and for a shorter duration than in mammals and birds.

Statement of Significance

The presence of similar electrophysiological sleep states in birds and mammals leads to questions about their evolutionary origins. When did such states arise? Were these states inherited from a common ancestor or did they evolve independently? Most studies of non-avian reptiles report the presence of high-voltage sharp waves (HShW) during sleep. We provide the first description of the spatiotemporal patterns of HShW in crocodilians under isoflurane anesthesia. Interestingly, HShW propagated in complex patterns similar to the patterns of slow wave propagation previously described in homologous regions of the avian brain. This suggests that non-avian reptilian HShW may represent similar electrophysiological phenomena to mammalian and avian slow waves.

Key words: *Crocodylus niloticus*; dorsal ventricular ridge; evolution; high-voltage sharp waves; slow waves; traveling; propagating; isoflurane

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Introduction

Mammalian and avian sleep consists of two sub-states, slow-wave sleep (SWS) and rapid eye movement (REM) sleep. SWS—also known as non-REM sleep—is characterized by high amplitude slow waves (0.5–4.0 Hz) in the electroencephalogram (EEG) [1, 2]. In mammals, EEG slow waves reflect the synchronous slow alternation of neuronal membrane potentials between a hyperpolarized down-state, in which neurons are inactive, and a depolarized up-state with action potentials. Although far less studied, similar cellular processes are thought to account for the presence of avian EEG slow waves [3, 4]. In both mammals and birds, the intensity of SWS, as indicated by the level of EEG slow wave activity (SWA; typically around 0.5–4.5 Hz power), increases following extended periods of wakefulness [5–9]. REM sleep is characterized by low-voltage fast EEG activity similar to that occurring during wakefulness. REM sleep is also associated with reduced or complete loss of skeletal muscle tone, myoclonic twitches, including rapid eye movements, reduced thermoregulatory responses, and irregular respiratory and heart rates [10]. In both taxonomic groups, the time spent in REM sleep also increases following sleep deprivation [7, 11, 12]. REM sleep decreases over early ontogeny in altricial birds following hatching and in altricial mammals postpartum [13–15]. In precocial mammals, the decrease in REM sleep occurs primarily in utero [16–18]. Collectively, this indicates that from a phenomenological and regulatory standpoint, mammals and birds exhibit remarkably similar sleep states.

The presence of SWS and REM sleep in such distantly related groups raises questions about the evolutionary origins of these states. Mammals and birds are members of two clades (Synapsida and Sauropsida, respectively) that last shared a common amniote ancestor 300 million years ago [19, 20]. Consequently, SWS and REM sleep might have been inherited from a common amniote ancestor in whom both states were already present [21]. Alternatively, these sleep states might have evolved convergently in mammals and birds [22, 23]. As birds are a derived type of reptile (Dinosauria), these alternative scenarios can be tested by examining sleep in their closest living reptilian relatives—the crocodilians.

Unfortunately, the electrophysiological correlates of sleep in crocodilians and other non-avian reptiles are subject to debate. This is due to contradictory results across studies, in some cases even in the same species, as well as uncertainty over how to interpret EEG patterns that do not unequivocally compare to those found in mammals and birds [24]. For example, a common, albeit not universal, electrophysiological correlate of sleep behavior reported in crocodilians [25] and other non-avian reptiles, is what have been termed high voltage sharp waves (HShW) [21, 24–27]. HShW are mono- or polyphasic potentials typically with an extra-cellular amplitude of up to 300 μV lasting 50–150 ms that emerge intermittently and irregularly from a lower amplitude EEG pattern, primarily during periods of behavioral sleep. The incidence of HShW increases following sleep deprivation, suggesting that they reflect homeostatically regulated sleep processes [28–30].

It has been suggested that HShW are homologous to sharp wave ripple (SWR) complexes occurring in the mammalian hippocampus during SWS due to their similar form and irregular pattern of occurrence [21, 27]. Mammalian SWR complexes are an emergent property of the hippocampal CA1/CA3 circuitry [31]. CA3 neurons initiate intermittent highly synchronous bursts of activity that cause high amplitude (e.g. 2 mV), sharp wave field potentials in the dendritic layer and 100–200 Hz "ripples" in the pyramidal layer of CA1 [31]. Interestingly, HShW in pogona lizards (*Pogona vitticeps*) are accompanied by fast activity that has been equated to the ripple component of mammalian SWR complex [21]. In addition to these similarities in waveform, the incidence of both HShW and SWR increase following sleep deprivation [28, 29] and respond similarly to various pharmacological agents [32–38].

Despite the similarities between reptilian HShW and mammalian SWR, several lines of evidence question whether they reflect homologous phenomena. First, the pharmacologic agents that similarly influence the incidence of mammalian hippocampal SWR and reptilian HShW [35, 39–41] may have similar effects on SWS-related slow waves (e.g. [40]). Consequently, these manipulations do not necessarily rule out the possibility that reptilian HShW more closely reflect mammalian/avian slow waves than they do mammalian hippocampal SWRs. Second, mammalian SWRs have only been observed in the hippocampus, subiculum, and adjacent entorhinal cortex [31], whereas HShW occur throughout much of the reptilian brain. Specifically, HShW occur in the reptilian hippocampus (i.e. medial cortex [21, 36–38]), dorsal cortex [25, 26, 28, 29, 36–38, 42], and dorsal ventricular ridge (DVR) [21, 37], the latter being a large structure found in reptiles and birds thought to be developmentally homologous to portions of the mammalian neocortex and pallial amygdala [43]. In addition to reptilian HShW having a more widespread distribution in the reptilian brain than SWR have in the mammalian brain, HShW have not been reported in the avian hippocampus or DVR during SWS [4, 7, 44–50]; rather both structures exhibit slow waves typical of SWS. The apparent absence of HShW in birds, a derived type of reptile, further questions whether reptilian HShW are homologous to mammalian hippocampal SWR. Instead, the presence of HShW in the reptilian hippocampus, dorsal cortex, and DVR during sleep behavior and the presence of slow waves in the sleeping avian hippocampus, dorsal cortex (i.e. hyperpallium), and DVR suggests that reptilian HShW are a precursor waveform to avian slow waves.

One aspect of HShW that has not been examined previously is their fine-scale spatio-temporal properties. In mammals and birds, slow waves propagate across the brain. Mammalian slow waves typically propagate from rostral locations in the prefrontal cortex in a anteroposterior direction during SWS, although individual slow waves can originate from virtually any cortical region [51–62]. Avian slow waves occurring under isoflurane [4] or spontaneous SWS [63] generally exhibit more complex propagation patterns, likely as a result of their nuclear brain organization [4]. Interestingly, propagating slow waves have been observed in the avian DVR [4], a structure that, as previously mentioned, exhibits HShW in sleeping reptiles [21]. Herein, we investigate the spatiotemporal properties of HShW within the DVR using high-density, electrode arrays in Nile crocodiles (*Crocodylus niloticus*) lightly anesthetized with isoflurane [1, 64].

Methods

Holding and care

Six juvenile (0.5–1.5-year-old) Nile crocodiles, weighing 469 ± 168 g (mean ± *SD*) and with a snout-to-vent length of 25.5 ± 4.4 cm were used in this study. All crocodiles were housed

and cared for in accordance with the *Mindestanforderungen an die artgerechte Haltung von Krokodilen in privaten Terrarien und zoologischen Einrichtungen* and in accordance with German law. Additionally, all housing and care procedures, as well as, experimental protocols were approved by the "Regierung von Oberbayern."

Surgical instrumentation and recording procedure

Recordings were carried out using the following procedure. Anesthesia was induced using isoflurane (5.0% vaporized in O_2 delivered at a rate of 250 ml/min). Once an adequate plane of anesthesia was established each crocodile was intubated and mechanically ventilated 2–4 times per minute (maintenance: 2.0%–2.5% isoflurane). Meloxicam (0.2 mg/kg; intramuscular injection) was administered for systemic analgesia. The crocodile was then mounted into a stereotaxic frame resting on a heating pad. Core body temperature was measured with a cloacal probe and maintained at 30°C, per veterinary instruction. Twenty minutes after the analgesic injection the skull was exposed and a small window (2–3 mm^2) was opened in the cranium and underlying dura.

For our recordings, we used 64-channel silicon multielectrode arrays with iridium electrode sites (NeuroNexus Technologies, Ann Arbor, Michigan; A8x8–5mm–200-200-177 and A8x8–5mm–200-200-413). The electrodes on the arrays were arranged in an 8 x 8 grid with equal spacing of 200 μm between electrode rows and columns. Arrays with two different electrode sizes were used (177 μm^3 and 413 μm^3). To begin, the array was positioned just above the exposed brain surface, and the absence of small movements caused by respiration was verified under high magnification (40–60×). The array was then slowly inserted into the brain. Isoflurane was reduced to a lower level (0.5%–1.0%) for the recordings. Recordings started 15 minutes post-insertion and lasted 10–15 minutes. Video was recorded during all experiments and with real-time temperature readout and respiration visible.

Recordings were referenced to a silver wire placed under the skin above the probe insertion site. Signals were buffered using headstage preamplifiers (10× gain; MPA32I; Multi Channel Systems, Reutlingen, Germany) and amplified with a multichannel amplifier 250× gain; bandpass filters 0.1 to 5000 Hz; PGA64; MultiChannel Systems, Austin, Texas). The resulting signals were digitized with a sampling frequency of 14 kHz and resolution of 16-bits (NI9205; National Instruments). Digitized signals were stored in HDF5 file format [65].

At the end of each recording session, the crocodile was euthanized by increasing the isoflurane level to 5.0% for 20 minutes and subsequently severing the spinal cord. The brain was then removed and frozen at –80°C for later confirmation of electrode placement.

Data filtering and analysis

Analyses were carried out using the methods outlined in an earlier study employing the same techniques [4]. Recorded signals were filtered for high-frequency signals (0.5–5 kHz) containing action potentials and low-frequency signals (0.1–350 Hz) containing local field potentials (LFP). The LFP analyses were based on bandpass (0.5–35 Hz) filtered signals. LFP were characterized by the presence of HShW occurring relatively infrequently (1.0–48.4 per minute), as previously described in other non-avian reptiles. Analyses were carried out on identified peaks in the LFP signal, which were defined as an episode with a duration of greater than 20 ms in which the electrical potential crossed a fixed threshold of –80 μV on three or more electrode sites. In recordings containing very few HShW, we manually verified that the –80 μV threshold identified most peaks. By choosing a higher threshold value we were able to include all HShW for the analysis, but exclude smaller variations in the signals. For a detailed description of the calculation of cross-correlation coefficient and LFP time lag between sites, as well as, spatial mean trajectory calculation refer to the methods section of this earlier study [4].

Histology

The position of the array was verified histologically using a DiI fluorescent dye painted on the shanks prior to insertion [66]. Each brain was cut in 25 μm sections using a cryostat and sections were mounted on glass slides. After sectioning, electrode placement was verified by tracing back the fluorescence left by the array using fluorescent microscopy.

Results

In all six crocodiles, most of the electrode sites were in the DVR. In four of the crocodiles the shallowest electrode sites were in the dorsal cortex overlying the DVR (Supplementary Figures S1, S2, S4, and S5). For unclear reasons, we only observed distinct differences in HShW amplitude between structures in one of these individuals (crocodile 4 in Figure 3, and Supplementary Figure S4). Simultaneous recordings from medial cortex, dorsal cortex, and DVR were obtained from one crocodile (Supplementary Figure S7). Because the available data from the medial and dorsal cortices was limited, we concentrated on propagation patterns in the DVR in our analyses. Multiunit activity was not detected in any of our recordings.

HShW occurred mono-, bi-, and polyphasically with amplitudes in some cases exceeding 1,000 μV (Figure 1A and B; Supplementary Figures S1–S5). Generally, the HShW occurred with higher amplitude at deeper, more lateral recording sites (Figure 1B, C, and F; Supplementary Figures S1–S6F), which were histologically verified to be located within the DVR (Figure 1H for histology). The rate of HShW occurrence was 0.8- 66.2/min between individuals recorded under similar anesthesia levels. The signals were usually also present, albeit with a lower amplitude, at shallower recording sites (Supplementary Figure S1, B and C).

When plotted over short time scales, HShW occurred at slightly offset times across recording sites, suggesting propagation through the brain (Figure 1, C and D). To illustrate this further, videos were generated compiling still frames across recordings in which each pixel corresponds to a recording site with color indicating signal voltage (Figure 1D and Figure 2; Supplementary Videos S1–S5 and S13). The HShW shown in Figure 1 occurred earlier and with a higher mean power in the ventrolateral recording sites (Figure 1, D–G; Supplementary Video S1).

To explore systematic patterns in between-electrode delays in LFP peak occurrence, we calculated the mean of the

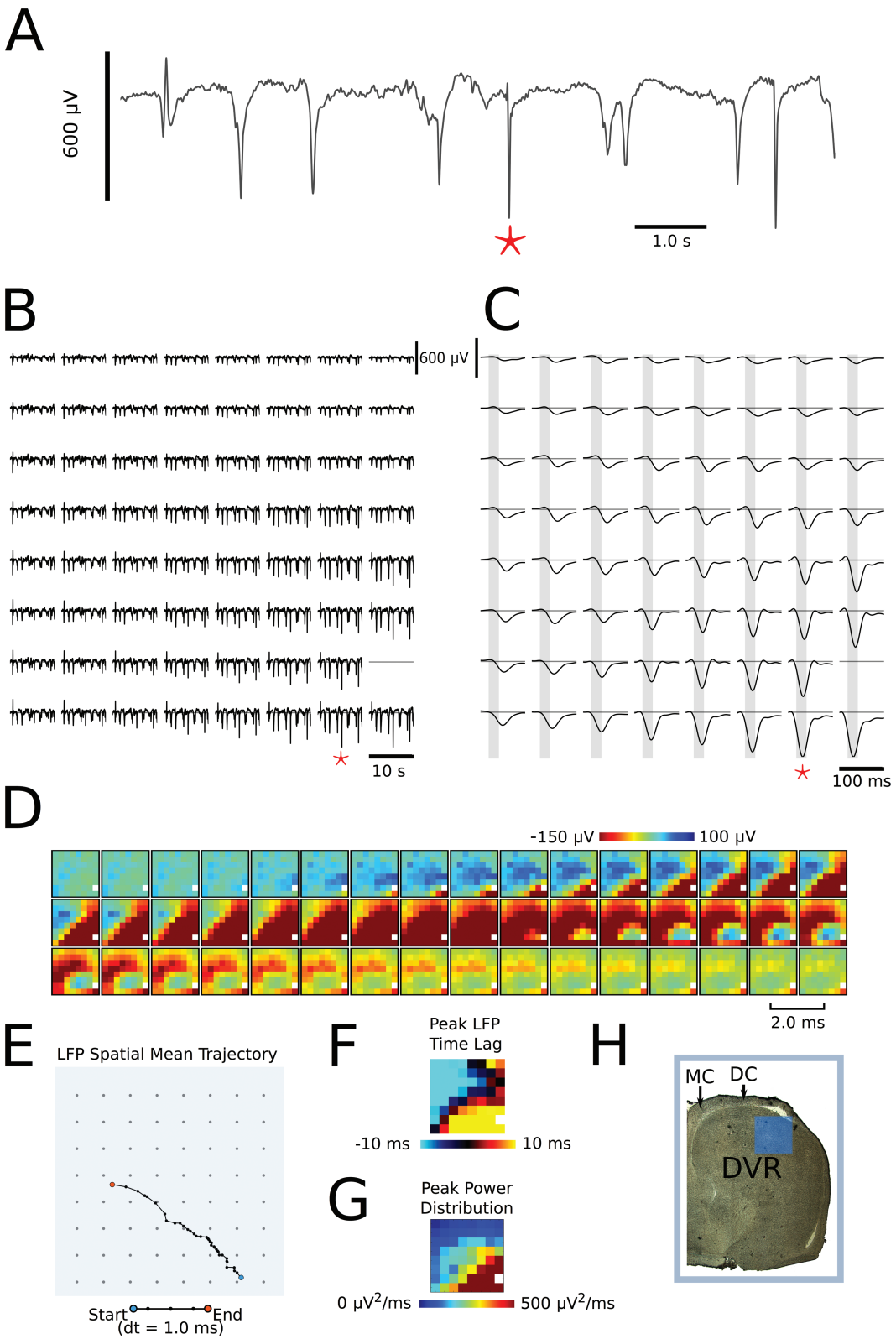

Figure 1. Characteristics of HShW occurring in the DVR for crocodile 4. (A) This is an enlarged view of a 10-second period filtered for LFP for electrode site 62 (column 8, row 4). (B) Same 10-second period from part (A) filtered for LFP for all 64 recording sites (arranged in an 8 × 8 grid). (C) A temporally expanded view of a single HShW. The grey boxes delineate the same time period across each channel to more clearly illustrate that the HShW is occurring at slightly offset times across the recording sites. (D) Plots showing the LFP voltage distribution across time for the same HShW, with voltage at an electrode site coded in color. (E) Trajectory of the spatial mean of the same selected event. (F) The mean LFP time lag of the selected peak. (G) Average LFP power distribution across the recording array for the same HShW. (H) Coronal section of the crocodile brain indicating recording array position. Array position is marked with a blue square. Scale bars and units are provided in each section of the figure, red stars mark the HShW depicted in this figure.

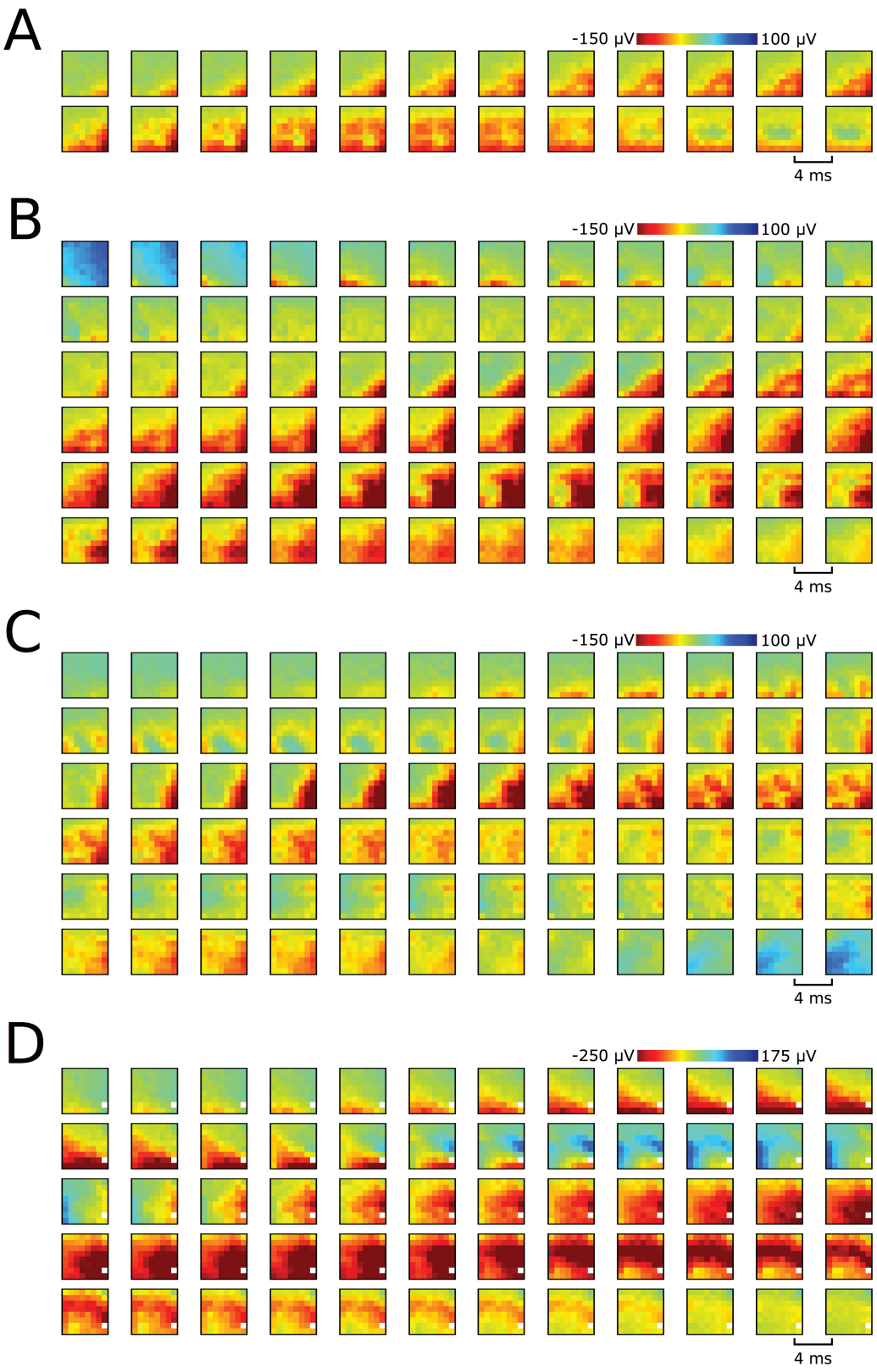

Figure 2. HShW selected to illustrate complex propagation patterns. Each plot shows a sequential series of frames with each pixel representing the voltage at an electrode site. Peak boundaries were determined using –80 µV as a detection threshold. The frames are separated by 4 ms. Events (A)–(C) were selected from crocodile 1 and (D) from crocodile 3. Events (A–C) contain recording sites in the dorsal cortex (top two rows of recording sites) and the dorsal ventricular ridge (DVR; bottom six rows of recording sites). Events from D are from a deeper recording from the DVR.

cross-correlation coefficients and LFP time lag between recording sites across all identified HShW. A clear pattern of propagation was observed in 4 of 6 crocodiles. Plots of mean LFP time lag in which a pattern was apparent revealed a propensity for signals to occur first at deep lateral recordings sites within the DVR and to occur later in more dorsomedial (i.e. superficial and nearer the midline) sites (Figure 3).

Although an overall propagation pattern is significant in only four of six crocodiles, HShW exhibiting similar, complex propagation patterns were present in recordings from all crocodiles (Figure 2; Supplementary Figures S1–S7; Supplementary Videos S2–S12 and S14). Individual HShW emerged from a variety of different directions, including both lateral (Figure 2A and C; Supplementary Videos S2 and S4) and medial (Figure 2B and D; Supplementary Videos S3 and S5) recording sites in all crocodiles (e.g. Figure 2, A–D; Supplementary Videos S2–S5). Additionally, as previously noted, the patterns with which HShW propagate across the recording array are both variable and complex, including arcs and rings of activity (Figure 2A, C, and D; Supplementary Videos S2, S4, and S5).

Plots of the movement of the spatial mean further demonstrate that on an individual level, signals have the potential to originate from a variety of regions and follow variable trajectories across recording sites (Figure 4A). However, when a larger group of trajectories are randomly selected from an individual ($N = 200$ in Figure 4B; Supplementary Figure S8, A and B), an overall pattern of propagation emerges. HShW emerge most often in

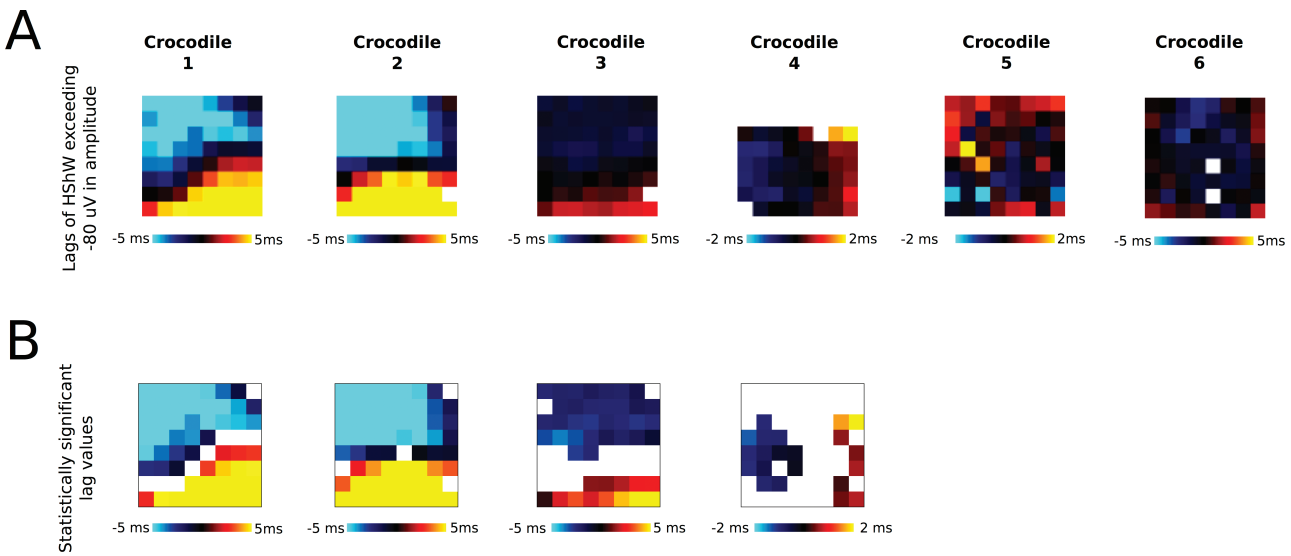

Figure 3. Mean time lags of LFP for all identified HShW for individual crocodiles. (A) Mean time lag between recording sites for all identified HShW (voltage threshold = −80 μV). (B) Mean LFP time lag between recording sites for HShW in which signals are highly correlated with statistically significant time lag differences (cross-correlation coefficient value ≥ 0.75; $p \geq 0.05$). There is an overall tendency for HShW to occur earlier in ventrolateral recording sites and later in more medial recording sites. This tendency is even stronger in individuals with statistically significant time lag differences.

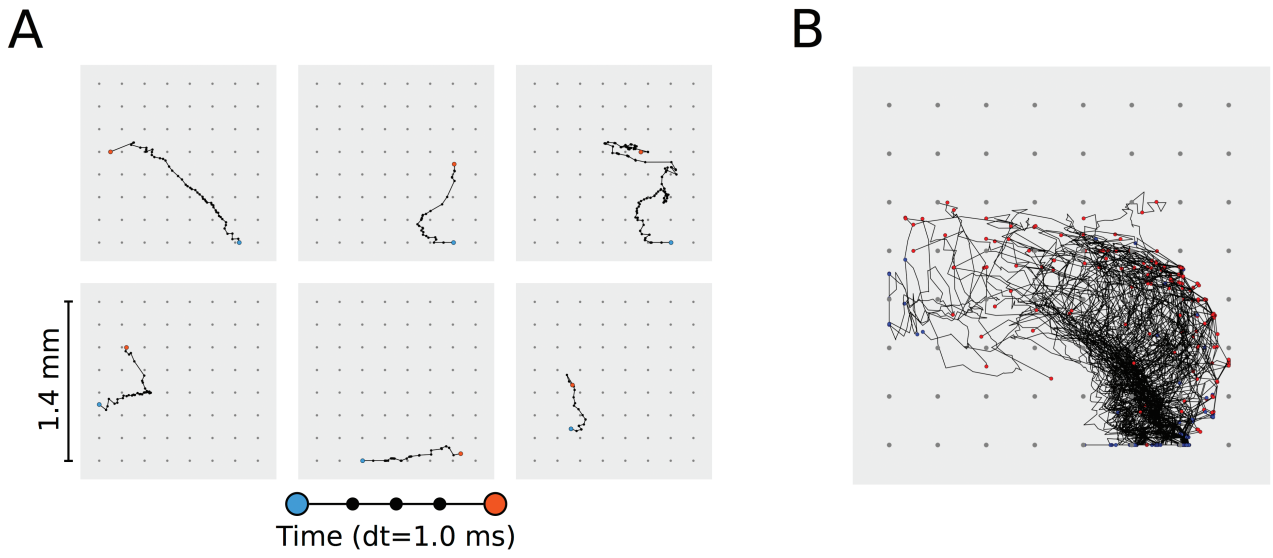

Figure 4. Trajectory plots of the HShW spatial mean across time. All trajectories are from crocodile 3. (A) Plots of the movement of the spatial mean as a product of time for individual HShW signals show the diversity of propagation patterns. (B) Plots of the movement of the spatial mean over time for 200 randomly selected HShW signals.

the ventrolateral quarter of the recording array and move in a dorsomedial direction.

Conclusions

Our recordings of anesthetized juvenile Nile crocodiles showed HShW similar to those reported in earlier studies of naturally sleeping reptiles [21, 25, 26, 28, 29, 67–76]. LFP exhibited mono-, bi-, and polyphasic HShW with amplitudes exceeding 1000 μV. Like naturally occurring slow waves in sleeping mammals [51–62] and slow waves induced by isoflurane in birds [4], HShW also propagated across the DVR in crocodiles. Interestingly, rather than exhibiting a stereotyped spatiotemporal pattern, HShW were highly variable. HShW propagated following complex patterns following a variety of different trajectories across recording sites, including arcs and rings of activity. Interestingly, as in mammals [51] and birds [4], despite this variability, there was an overall propagation pattern in most crocodiles, with HShW usually occurring earlier in ventrolateral sites and later in dorsomedial sites of the DVR.

Limitations

Unexpectedly, we did not observe any multi-unit activity (MUA) during HShW or at any other time in the recordings. By contrast in our previous recordings of zebra finches (*Taeniopygia guttata*), using the same electrodes, amplifiers, sampling rate, and type of anesthesia, MUA was closely associated with propagating negative waves in the LFPs [4]. Consequently, the absence of MUA in the crocodile recordings does not appear to reflect a technical problem with our set-up. Interestingly, in naturally sleeping pogona lizards, MUA was clearly associated with HShW occurring in the DVR [21]. Although the reasons for these discrepancies remain unclear, the higher density of neurons in the avian brain may have increased the probability of detecting MUA in finches [77]. Perhaps further study using arrays more sensitive to units (i.e. tetrodes) would be more likely to detect MUA in the non-avian reptilian brain.

The incidence of HShW varied across crocodiles and recordings. This variability likely reflects, in part, inter-individual variability in responsiveness to the anesthetic. As isoflurane at low doses induces slow waves similar to those occurring during natural SWS in rats [78], we kept the concentration of isoflurane at a low level. However, as isoflurane at higher doses suppresses slow waves in mammals [78] and birds (Rattenborg, unpublished data), it is possible that the same isoflurane dose suppressed HShW in some crocodiles. A systematic experiment, including measurements of isoflurane levels in the blood, is needed to assess the influence that this anesthetic has on the incidence of HShW in crocodiles. Temperature has also been demonstrated to have a profound effect on HShW amplitude and occurrence in reptiles [75]. A study investigating the effects of body temperature upon HShW propagation patterns will be important for our understanding of natural sleep states in non-avian reptiles.

Various anesthetics induce brain rhythms similar to those present during natural sleep. EEG patterns under isoflurane anesthesia in mammals and birds are characterized by the presence of high-amplitude, low-frequency slow waves similar to natural SWS [1, 3, 4, 78]. Isoflurane anesthesia activates sleep-promoting neurons in the ventrolateral preoptic nucleus of the hypothalamus, neurons that also fire preferentially during natural sleep [79]. In addition, slow waves occurring under isoflurane anesthesia relieve the homeostatic pressure for sleep following extended periods of wakefulness in a manner similar to natural SWS [78]. Taken together, this suggests that isoflurane induced slow waves share similar functional and regulatory mechanisms to slow waves during naturally occurring SWS. Nevertheless, it would be of interest to replicate this study using other anesthetics. It would also be of particular interest to study the spatiotemporal distribution of HShW in naturally sleeping crocodiles to see how those compare to waves occurring under anesthesia.

Evolution of slow waves

The propagation patterns of HShW in the crocodile DVR were similar to those of slow waves recorded in the avian DVR under similar conditions. Given that SWRs also propagate within the mammalian hippocampus [80, 81], propagation alone does not reveal whether reptilian HShW are more closely related to mammalian SWRs or slow waves found in mammals and birds. Nonetheless, when combined with other lines of evidence, the latter scenario seems more likely. In contrast to mammalian SWRs, which are confined to the hippocampus, reptilian HShW occur in the dorsal cortex and DVR, regions thought to be homologous to the neocortex and pallial amygdala [43]. Moreover, slow waves, but not SWRs, have been found in the avian hippocampus during SWS [82]. Instead, the presence of HShW in the reptilian hippocampus, dorsal cortex, and DVR and the presence of slow waves in the avian hippocampus, dorsal cortex (i.e. hyperpallium), and DVR, as well as the fact that both non-avian reptilian HShW and avian slow waves propagate in complex patterns in the DVR, suggest that reptilian HShW are a precursor waveform to avian slow waves.

According to this scenario, reptilian HShW may involve the same neuronal processes responsible for the slow oscillation in membrane potentials that gives rise to mammalian/avian slow waves. However, in contrast to mammals and birds, reptiles may lack the cellular or network properties needed to frequently initiate and synchronize up-states, and to sustain them once they occur [83, 84]. As a result, in contrast to up-states occurring in mammals and birds, reptilian up-states appear to occur less frequently and for a shorter duration resulting in the sharp characteristics of the HShW when compared to mammalian/avian slow waves. Moreover, the “ripples” reported in pogona lizards during HShW may reflect the increase in neuronal activity that typically occurs during the up-state of slow oscillations [85, 86]. If HShW and slow waves represent homologous waveforms, then sleep with HShW might have been the ancestral form of sleep present in the stem amniote ancestor to mammals, reptiles, and birds.

The evolutionary transition from HShW to slow waves might have been linked to evolutionary changes in physiology and/or brain organization. One possibility is that this occurred in conjunction with the shift from exothermic to endothermic physiological states. Temperature is known to have large effects on electrophysiological phenomena [87–92]. Lower temperatures have been shown to place constraints on the cellular processes involved in the maintenance of neuronal transmembrane potentials resulting in a decrease in EEG amplitude and frequency in mammals [93, 94]. However, recent research suggests that lower temperature is unlikely to explain the presence of

HShW in reptiles. In mice, cortical cooling actually increased the time spent in slow oscillation up-states [95]. Consequently, differences in temperature may not fully explain the differences in sleep-related neurophysiology observed between birds, mammals, and non-avian reptiles.

Another possibility is that neuronal density and/or connectivity plays a role in the differences in neurophysiology [83]. Corticocortical connections in the mammalian neocortex have been implicated in the synchronization of the slow oscillation across large neocortical regions [83, 96–99]. Comparatively lower corticocortical connections and neuronal density in the reptilian brain, when compared to the brains of birds and mammals [77, 100, 101], might therefore reduce the propensity of the network to initiate and sustain up-states resulting in HShW, rather than slow waves.

In addition to neuronal density and connectivity, the density of astrocytes may also influence the characteristics of sleep-related neurophysiology. Recent studies have demonstrated that astrocytes play a role in the genesis and characteristics of slow oscillations in the mammalian brain [102–104]. Of particular interest, mice in which astrocytes have been inhibited initiate up-states less often and exhibit shorter up-states, resulting in activity similar in form to reptilian HShW [102]. As the density of astrocytes in the brains of non-avian reptiles is lower than in birds and mammals [105, 106], astrocyte density could explain why non-avian reptiles exhibit HShW, rather than slow waves.

In addition to potentially explaining the differences between reptilian HShW and avian/mammalian slow waves, variation in any of the above-mentioned mechanistic explanations could also explain the variation in the incidence and duration of HShW observed across non-avian reptiles. For example, within squamates, the incidence of HShW during sleep ranges from ~1 HShW/min in the desert iguana (*Dipsosaurus dorsalis* [107]) to ~60–180 HShW/min in the bearded dragon (*Pogona vitticeps* [21]). HShW in pogona lizards also seem to have a longer duration (i.e. more slow wave-like pattern) than those reported for other reptiles [21, 27]. As such, this species might be expected to have higher neuronal density, higher neuronal interconnectivity, and/or higher astrocyte density than other reptiles. Interestingly, among the non-avian reptiles studied, the evidence for REM sleep is strongest in pogona lizards [21], raising the possibility that traits responsible for slow waves might also play a role in the genesis of REM sleep in mammals, birds, and some non-avian reptiles.

Summary and functional implications

Understanding how sleep-related electrophysiological traits of non-avian reptiles relate to brain activity observed in birds and mammals is fundamental to understanding the evolutionary origins of SWS and REM sleep states. Insight into the evolutionary process necessary for the development of these patterns of brain activity may also shed light upon the functional roles of these states. Our results show that HShW occurring in the crocodile DVR exhibit complex propagation patterns qualitatively similar to those observed for slow waves in the avian DVR recorded under similar conditions [4]. Although complex propagation alone is not diagnostic of slow waves (mammalian SWRs also propagate within the hippocampus) [80], the occurrence of HShW and slow waves in the DVR of closely related taxonomic groups suggests that HShW reflect neurophysiological phenomena closely related to slow waves, rather than morphologically similar SWRs which emerge from the unique cytoarchitecture, and perhaps function [82], of the mammalian hippocampus [31]. In this respect, HShW and avian/mammalian slow waves may reflect homologous phenomena. Morphological differences between intermittently occurring HShW and regularly occurring slow waves may simply arise from avian/mammalian neurons initiating up-states more often, and remaining in up-states longer once they occur.

Collectively, this scenario suggests that although the capacity for neurons to alternate between up- and down-states is an ancestral trait present in the common amniote ancestor to Synapsida and Sauropsida, mammals and birds independently evolved as yet unknown traits that increase the capacity to initiate and sustain up-states during sleep [84]. Although the traits responsible for the greater investment in up-states remain unclear, candidates include increased neuronal density and interconnectivity, as well as an increased concentration of astrocytes. The functional implications of spending more time in up-states remain unclear but might include increased sleep-dependent information processing which is thought to be mediated by memory reactivation occurring during up-states of the slow oscillation [108–110]. Further study is needed to confirm the presence of this ostensible evolutionary pattern and evaluate its full functional consequences.

Supplementary material

Supplementary material is available at *SLEEP* online.

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Notes

Conflict of interest statement. None declared.

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