

SHORT COMMUNICATION

Unihemispheric sleep in crocodylians?

Michael L. Kelly¹, Richard A. Peters¹, Ryan K. Tisdale² and John A. Lesku^{1,*}

ABSTRACT

Reduced vigilance is the conspicuous cost of sleep in most animals. To mitigate against this cost, some birds and aquatic mammals have evolved the ability to sleep with one-half of their brain at a time, a phenomenon known as unihemispheric sleep. During unihemispheric sleep the eye neurologically connected to the 'awake' hemisphere remains open while the other eye is closed. Such unilateral eye closure (UEC) has been observed across avian and non-avian reptiles, but has received little attention in the latter. Here, we explored the use of UEC in juvenile saltwater crocodiles (1) under baseline conditions, and in the presence of (2) other young crocodiles and (3) a human. Crocodiles increased the amount of UEC in response to the human, and preferentially oriented their open eye towards both stimuli. These results are consistent with observations on unihemispherically sleeping cetaceans and birds, and could have implications for our understanding of the evolution of unihemispheric sleep.

KEY WORDS: Anti-predator, Archosaur, Interhemispheric asymmetry, Laterality, Vigilance

INTRODUCTION

Many animals face a trade-off between the reduced vigilance associated with sleep and its neurophysiological benefits (Lima et al., 2005). Some animals, such as birds and aquatic mammals (cetaceans, manatees, otarid seals and walruses) have resolved this conflict with the evolution of unihemispheric sleep (Rattenborg et al., 2000; Lyamin et al., 2008). During unihemispheric sleep, brain activity measured by an electroencephalogram shows a pattern characteristic of sleep in one hemisphere, while the other hemisphere shows brain waves more closely resembling wake-related activity. This between-hemisphere difference in brain state is reflected at the level of the eye by unilateral eye closure (UEC), with the eye neurologically connected to the 'awake' hemisphere being open. In this way, UEC can be a behavioural correlate of unihemispheric sleep. Unihemispherically sleeping birds and aquatic mammals use the open eye to maintain vigilance on the local environment. Notably, ducks arranged in a row sleep largely bihemispherically when safely flanked by others, yet switch to sleeping unihemispherically when on the edge of the group, and orient their open eye away from the others as if watching for potential threats (Rattenborg et al., 1999). Similarly, cetaceans use the open eye adaptively, but instead orient it towards conspecifics, purportedly to maintain pod cohesion (Lyamin et al., 2005, 2008).

Although the electrophysiological correlates of UEC have gone unstudied in non-avian reptiles (Flanigan et al., 1973; Warner and Huggins, 1978), UEC per se appears to be widespread across reptiles, having been observed in numerous lizards, turtles and

tortoises, and caiman (reviewed in Rattenborg et al., 2000). As with birds and cetaceans, UEC in reptiles is used for vigilance. Western fence lizards (*Sceloporus occidentalis*) increase their use of UEC in the presence of a predator and orient their open eye towards the last known location of that threat (Mathews et al., 2006). Importantly, given that crocodylians are more closely related to birds than they are to other reptiles, an examination into the plasticity of UEC in crocodylians might provide insight into its evolutionary history. Here, we explored the use of UEC by juvenile saltwater crocodiles (*Crocodylus porosus* Schneider 1801). Briefly, in 24 h sessions, we measured the amount of UEC and orientation of the open eye in response to baseline conditions and the presence of another young crocodile. Then, in an acute experiment, we presented the crocodiles with a human stimulus to see whether they would likewise orient their open eye during UEC towards this salient presentation.

RESULTS AND DISCUSSION

Twenty-four hour recordings

Baseline

A crocodile was moved from the holding tank to an experimental enclosure for study (see Materials and methods for details). The experimental tank was divided in half with a Plexiglas partition; opaque plastic visually isolated the focal crocodile from the other animals. In the experimental tank, each crocodile was housed individually and filmed for 24 h. Bilateral eye closure was the most common eye state, typically occupying more than half of each hourly time bin, followed by bilateral eye opening and UEC (Fig. 1A). To our knowledge, this is the first report of UEC in saltwater crocodiles. Taken together with observations of UEC in caiman (Rattenborg et al., 2000) and Nile crocodiles (*Crocodylus niloticus*; R.K.T., unpublished observation), UEC may be widespread across extant crocodylians. Consistent with a vigilance function for UEC, UEC and bilateral eye opening were more common during the day, and bilateral eye closure was more common at night (Fig. 1A). The amount of UEC was highest early in the day and decreased thereafter, which may have been a lingering response to handling at 07:00 h, as handling can induce a stress response in crocodiles (Franklin et al., 2003). Plasticity in the use of UEC is to be expected given that, in birds, its use can depend upon the presence of stressful (potentially dangerous) stimuli (Rattenborg et al., 1999). Interestingly, animals spent more time with their right eye open during periods of UEC than their left (Fig. 1A). The avian visual system is lateralized, with the right eye/left hemisphere being more responsive to large changes in the environment than the left eye/right hemisphere (reviewed in Rogers, 2008). Given the close evolutionary relatedness between birds and crocodylians, this may also be true for crocodiles, and warrants further study.

Crocodyle presentation

The experiment above was repeated except that a crocodile was introduced into the other half of the tank and the black plastic visually isolating the focal animal was removed. In this way, we could assess whether young crocodiles engaged in UEC are more likely to orient

¹La Trobe University, School of Life Sciences, Melbourne, Australia. ²Max Planck Institute for Ornithology, Avian Sleep Group, Seewiesen, Germany.

*Author for correspondence (j.lesku@latrobe.edu.au)

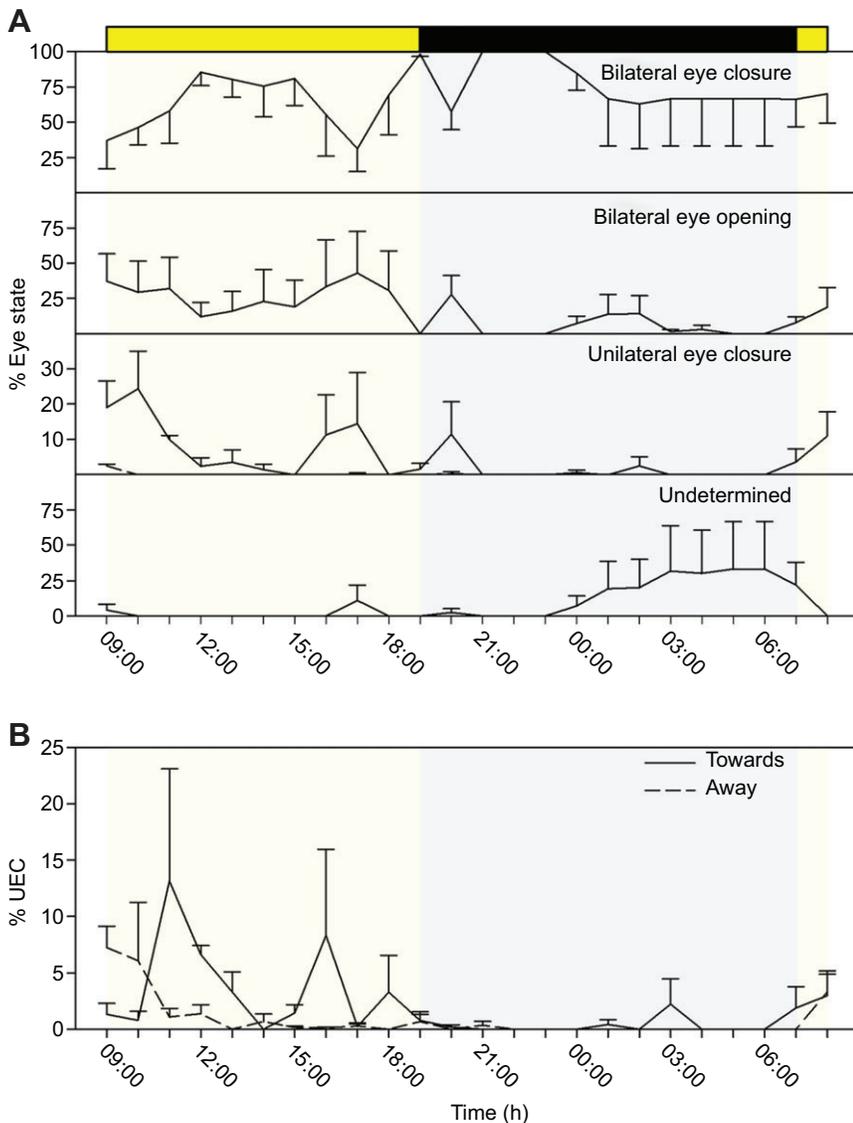


Fig. 1. Eye state data arranged into hourly time bins. (A) Percentage of time in each eye state during the baseline recording, notably revealing that saltwater crocodiles engage in unilateral eye closure (UEC). The animals preferred to open their right eye during UEC (solid line) over their left (dashed line). Eye state could not be determined reliably when an animal was underwater. (B) During UEC in the crocodile presentation recording, crocodiles oriented their open eye preferentially towards (rather than away from) conspecifics. The photoperiod is shown by the vertical bars at the top of the plot and shading within. Values are presented as means \pm s.e.m., plotted at the end of each hour.

their open eye towards conspecifics. First, we note that all daytime patterns in eye states identified above were observed during this recording. Counter to expectations, the crocodiles did not significantly increase the amount of UEC in response to the presentation of other crocodiles ($t=0.79$, d.f.=8, $P=0.454$). During the crocodile presentation, however, animals showed a significant preference to direct the open eye during UEC towards conspecifics ($t=19.47$, d.f.=2, $P=0.003$; Fig. 1B). This result is consistent with a vigilance function for UEC, and work on cetaceans that use UEC as a means to maintain visual contact with others of the same species (Lyamin et al., 2005, 2008). Such a function could well have ecological relevance in crocodiles, as hatchling saltwater crocodiles form crèches in the wild as an anti-predator adaptation. Animals best able to maintain group cohesion may have a greater chance at survival.

Acute human presentation

In a separate experiment, we presented the crocodiles with a human stimulus for 10 min and looked at the time course of eye states before, during and after the presentation, and the orientation of the open eye during UEC. The amount of UEC was strongly influenced by the presence of a human (Fig. 2A). Pairwise contrasts revealed a significantly greater proportion of time in UEC during the 10 min

exposure to the human compared with the two preceding 10 min time blocks (20 min before: $t=2.54$, d.f.=8, $P=0.033$; 10 min before: $t=2.34$, d.f.=8, $P=0.047$). The proportion of time engaged in UEC declined in the two time blocks post-stimulus, but this decrease did not initially reach significance (10 min after: $t=-2.02$, d.f.=8, $P=0.078$; 20 min after: $t=-2.32$, d.f.=8, $P=0.049$). The increase in the amount of UEC during the stimulus presentation arose from longer UEC bouts, increasing from 15 s on average immediately before the presentation to a sustained episode 25 \times as long (Fig. 2B). Although analyses of the 24 h recordings revealed that the animals most often opened their right eye during episodes of UEC, evidence for this side-bias was less apparent during the acute presentation of a human (Fig. 2C). Consistent with a vigilance function for UEC, the open eye was always directed towards the human, an effect that persisted even after the human had left the room (Fig. 2D).

Are crocodiles sleeping unihemispherically during UEC?

Our data show that (1) juvenile saltwater crocodiles engage in UEC, (2) we can elicit the behaviour in response to visual stimuli and (3) the animals preferentially orient their open eye towards the salient stimulus. These results are broadly consistent with those of Mathews et al. (2006) on UEC in fence lizards, and with

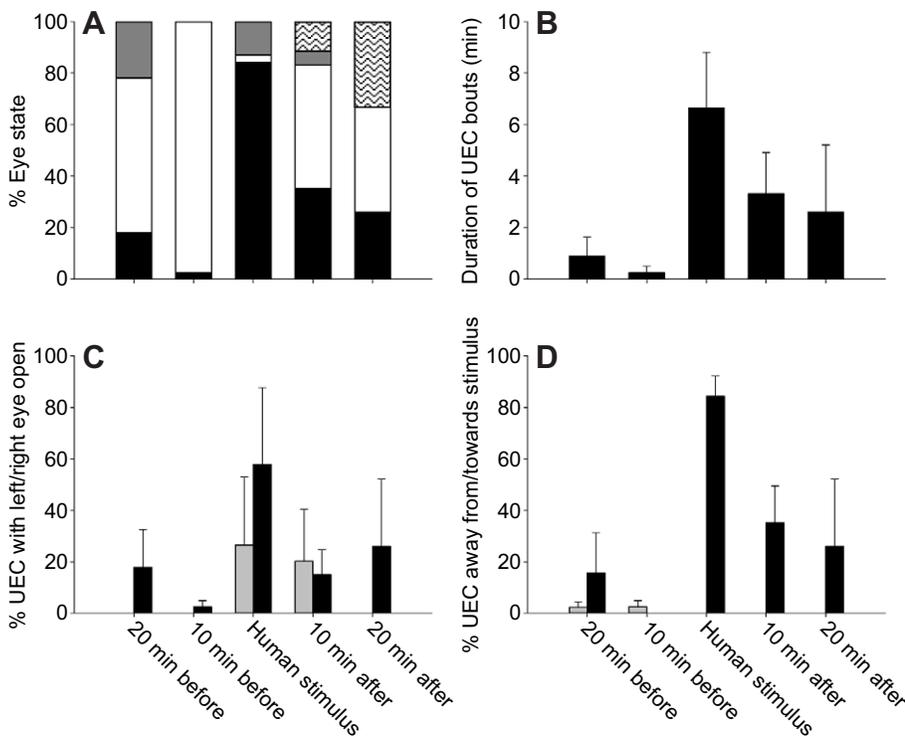


Fig. 2. Response of crocodiles to an acute visual presentation. (A) Percentage of time that crocodiles exhibited each eye state across the five 10 min time bins before, during and after the visual stimulus, showing the large increase in UEC (black) during the presentation, which came at the expense of bilateral eye closure (white); bilateral eye opening (grey) and underwater (waves) were unaffected by treatment. (B) The increase in the amount of UEC arose from longer episodes of UEC. (C) Crocodiles could open either their left (grey) or right (black) eye during UEC. (D) Before the visual stimulus, the crocodiles oriented their open eye during UEC both away from (grey) and towards (black) the direction in which the stimulus would be presented. During and after the presentation, the crocodiles oriented their open eye only towards the location of the stimulus. For B–D, values are presented as means+s.e.m.

observations from unihemispherically sleeping birds and aquatic mammals (Rattenborg et al., 2000; Lyamin et al., 2008). Consequently, these data might suggest that crocodylians engage in unihemispheric sleep. If true, then unihemispheric sleep is likely to have been present in the most recent common ancestor to archosaurs (crocodylians and dinosaurs, including birds), and given the phylogenetic breadth of UEC across reptiles (Rattenborg et al., 2000), may have evolved even earlier, perhaps in the ancestor to Sauropsids (avian and non-avian reptiles) or in the stem amniote with a subsequent loss of the state in the ancestor to mammals. Alternatively, and equally interesting, is the possibility that UEC first evolved in early Sauropsids for a purpose beyond that of sleeping unihemispherically, perhaps related to focusing visual acuity and attention in a lateralized brain (Rogers, 2008) and/or eliminating superfluous parts of a visual field in animals with laterally placed eyes (Mathews et al., 2006). Birds then evolved the ability to enter sleep with the hemisphere associated with the closed eye as a derived feature of sleep. If true, then unihemispheric sleep may have first evolved in the archosaur lineage with the appearance of birds by elaborating upon a pre-existing behaviour inherited from a common ancestor with non-avian reptiles. Distinguishing between these two ideas will require electrophysiological recordings from both brain hemispheres of crocodiles engaged in UEC, and would do much to enlighten our understanding of the evolutionary history of unihemispheric sleep.

MATERIALS AND METHODS

Three young saltwater crocodiles (40–50 cm total length) were purchased from a commercial breeder (Koorana Crocodile Farm, QLD, Australia) and housed in a large (290×80×70 cm high) aquarium. The tank was filled with fresh water 30 cm deep, maintained at 30°C by submerged water heaters. Semi-submerged docks allowed access to shallow water (8 cm deep); large terracotta pots (54 cm diameter) provided access to dry land. Mercury vapour heat lamps (160 W) were fitted above three of the four sites for basking opportunities (40°C at the level of the crocodile). Room air temperature was maintained at 26°C with 60% humidity. Animals were kept

on a 12 h light/dark photoperiod with lights on at 07:00 h. Each animal was identified by a unique mark of nail polish on their dorsal surface. Crocodiles were fed three times per week on a diet of fresh chicken mince dusted in calcium powder. All methods were approved by the La Trobe University Animal Ethics Committee (AEC14-04).

During an experimental trial, at 07:00 h, each crocodile was moved individually to an adjacent aquarium (122×80×70 cm high), divided in half (i.e. 61×80 cm), and equipped with a similar arrangement of dry land and shallow and deep water, for 1 day. The perimeter of the experimental tank was lined with eight video cameras with infrared capabilities, including one camera positioned 1 m above the tank. All cameras were connected to a computer in a neighbouring room. For the baseline recording, black opaque plastic visually isolated the focal animal from the other half of the tank and the crocodiles in the larger housing aquarium. For the crocodile presentation recording, a crocodile was added to the other half of the experimental tank, and the black plastic was removed, such that the focal animal could be in visual contact with other crocodiles. Young crocodylians form groups (crèches) in the wild and it was thought that the animals might use the open eye during UEC to maintain visual contact with nearby conspecifics, as per work on young cetaceans (Lyamin et al., 2005). Next, to assess whether we could elicit UEC in response to a different visual stimulus, we presented the animals with a human. After a habituation period of 1 h, the human entered the laboratory and stood silently for 10 min facing one side of the tank in line of sight of the animal. The side of the tank where the human stood was kept constant throughout the study. The holding and experimental tanks, as well as the room door, were fixed in place; however, the animals (both focal and presentation) were free to move within their enclosures, such that the position of the focal animal relative to its conspecifics was not necessarily always the same. After 10 min, the human left the room. Eye states were scored in 10 min blocks: (i) 20 min and (ii) 10 min before the presentation, (iii) during the presentation, and (iv) 10 min and (v) 20 min after the presentation. Each animal was returned to the housing aquarium for at least 2 weeks between treatments.

Video recordings were examined and eye state was scored every second as bilateral eye opening, UEC or bilateral eye closure; eye state could not be reliably determined when the animal was underwater. For UEC, we recorded which eye was open and whether it was oriented towards or away from the stimulus. The amount of each eye state was tallied and expressed as a proportion of the total number of seconds in that time bin (e.g. hourly or 10 min). Eye state data were analysed with the `glmMPQL` function from the

MASS package (Venables and Ripley, 2002) in the R statistical environment (R Development Core Team, 2014). In three separate models, we compared the proportion of time that the crocodile engaged in UEC during the baseline and crocodile presentations, the proportion of time during the crocodile presentation that the open eye during UEC was directed toward conspecifics, and the proportion of UEC during each 10 min time bin during the human presentation. Crocodile identity was used as a random effect in all models to account for repeated observations of the same animal. We examined paired contrasts in each case, as an overall F -statistic is not provided by glmmPQL. For the human presentation, we considered contrasts between the 10 min presentation time bin and each of the other time blocks.

Competing interests

The authors declare no competing or financial interests.

Author contributions

All authors conceived the study and contributed to writing the manuscript; M.L.K. collected the data; M.L.K., R.A.P. and J.A.L. analysed the data.

Funding

This study was supported by La Trobe University and the Australian Research Council [DE140101075 to J.A.L.].

References

- Flanigan, W. F., Wilcox, R. H. and Rechtschaffen, A. (1973). The EEG and behavioral continuum of the crocodilian, *Caiman sclerops*. *Electroencephalogr. Clin. Neurophysiol.* **34**, 521–538.

- Franklin, C. E., Davis, B. M., Peucker, S. K. J., Stephenson, H., Mayer, R., Whittier, J., Lever, J. and Grigg, G. C. (2003). Comparison of stress induced by manual restraint and immobilisation in the estuarine crocodile, *Crocodylus porosus*. *J. Exp. Zool. A* **298A**, 86–92.
- Lima, S. L., Rattenborg, N. C., Lesku, J. A. and Amlaner, C. J. (2005). Sleeping under the risk of predation. *Anim. Behav.* **70**, 723–736.
- Lyamin, O., Pryaslova, J., Lance, V. and Siegel, J. (2005). Animal behaviour: Continuous activity in cetaceans after birth. *Nature* **435**, 1177.
- Lyamin, O. I., Manger, P. R., Ridgway, S. H., Mukhametov, L. M. and Siegel, J. M. (2008). Cetacean sleep: an unusual form of mammalian sleep. *Neurosci. Biobehav. Rev.* **32**, 1451–1484.
- Mathews, C. G., Lesku, J. A., Lima, S. L. and Amlaner, C. J. (2006). Asynchronous eye closure as an anti-predator behavior in the western fence lizard (*Sceloporus occidentalis*). *Ethology* **112**, 286–292.
- R Development Core Team. (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.
- Rattenborg, N. C., Lima, S. L. and Amlaner, C. J. (1999). Half-awake to the risk of predation. *Nature* **397**, 397–398.
- Rattenborg, N. C., Amlaner, C. J. and Lima, S. L. (2000). Behavioral, neurophysiological and evolutionary perspectives on unihemispheric sleep. *Neurosci. Biobehav. Rev.* **24**, 817–842.
- Rogers, L. J. (2008). Development and function of lateralization in the avian brain. *Brain Res. Bull.* **76**, 235–244.
- Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics with S*, 4th edn. New York: Springer.
- Warner, B. F. and Huggins, S. E. (1978). An electroencephalographic study of sleep in young caimans in a colony. *Comp. Biochem. Physiol. A Physiol.* **59**, 139–144.