Cyclic nature of the REM sleep-like state in the cuttlefish Sepia officinalis

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ABSTRACT
Sleep is a state of immobility characterized by three key criteria: an increased threshold of arousal, rapid reversal to an alert state and evidence of homeostatic ‘rebound sleep’ in which there is an increase in the time spent in this quiescent state following sleep deprivation. Common European cuttlefish, Sepia officinalis, show states of quiescence during which they meet the last two of these three criteria, yet also show spontaneous bursts of arm and eye movements that accompany rapid changes in chromatophore patterns in the skin. Here, we report that this rapid eye movement sleep-like (REMS-like) state is cyclic in nature. Iterations of the REMS-like state last 2.42±0.22 min (mean±s.e.m.) and alternate with 34.01±1.49 min of the quiescent sleep-like state for durations lasting 176.89±36.71 min. We found clear evidence that this REMS-like state (i) occurs in animals younger than previously reported; (ii) follows an ultradian pattern; (iii) includes intermittent dynamic chromatophore patterning, representing fragments of normal patterning seen in the waking state for a wide range of signaling and camouflage; and (iv) shows variability in the intensity of expression of these skin patterns between and within individuals. These data suggest that cephalopods, which are mollusks with an elaborate brain and complex behavior, possess a sleep-like state that resembles behaviorally the vertebrate REM sleep state, although the exact nature and mechanism of this form of sleep may differ from that of vertebrates.

KEY WORDS: Invertebrate sleep, Rapid eye movement, Cephalopod, Chromatophore pattern, Atonia, Behavior

INTRODUCTION
In mammals, birds and some reptiles (Libourel et al., 2018; Shein-Idelson et al., 2016) (but see Aulsebrook et al., 2016), sleep is characterized neurologically by the occurrence of two distinct brain states denoting slow-wave sleep and rapid eye movement sleep (REMS). Behaviorally, slow-wave sleep is quiescent, while REMS is accompanied by rapid eye movements and muscular atonia with occasional muscular twitches (Mascetti, 2016; Stickgold and Walker, 2010). Sleep follows a circadian rhythm and within individuals. These data suggest that cephalopods, which are mollusks with an elaborate brain and complex behavior, possess a sleep-like state that resembles behaviorally the vertebrate REM sleep state, although the exact nature and mechanism of this form of sleep may differ from that of vertebrates.

MATERIALS AND METHODS

Six common cuttlefish, *Sepia officinalis* (1 year old, ca. 7.5 cm mantle length), hatched from eggs collected from the English Channel and raised in captivity in the Marine Resources Center at the Marine Biological Laboratory, were transferred singly to one of four 19 l glass tanks (L×W×D: 40.64 cm×20.32 cm×24.5 cm). Visual isolation was accomplished by covering the sides of the tanks with black and white patterned cloth, and white grating was used to constrain cuttlefish to one part of the tank for better video viewing. Animals had ample room to swim (L×W×D: 24.5 cm×20.32 cm×24.5 cm). Black plastic sheeting was used to isolate the tank area and reduce human disturbance. At least 1 cm of gravel substrate was provided as this seemed to calm the animals, along with a 10 cm tall, black cone placed in front of the inflow tube to reduce water agitation and cuttlefish movement due to water flow. Animals were provided with thawed frozen shrimp every day. Tanks were supplied with flow-through seawater at a temperature of 15°C. At this temperature, animals grew slowly and did not develop secondary sex characteristics, thereby precluding sex determination of animals used in this study. Room lighting was on a 12 h light:12 h dark cycle. One overhead camera and one side-view camera (Sanyo CCD camera model VCB-3384 and Everfocus Polestar II model EQ610) were placed such that they could record activity in one tank at a rate of 30 frames s$^{-1}$ in black and white. Red lights (to which cuttlefish exhibited rapid pupil expansion indicating weak sensitivity) were in constant use to illuminate the dark period to enable video recording of body patterns from above, and eye movement and arm twitching from the side. Video was captured and saved in real time to a hard drive.

Video was reviewed at high speed (4–32 times) approximately every 24–48 h to determine whether an animal had experienced a REMS-like state. After REMS-like behavior was recorded at least once, the animal was returned to the regular holding tanks and a new individual was placed in that observation tank. The camera pair was moved to record an animal that had already spent at least 24 h in an observation tank. The number of days each animal was observed was as follows: Hippo, 28; zZ, 12; MrChips, 9; Medusa, 9; Dumbo, 7; and Clyde, 4.

Durations of REMS-like and QS states were determined from the video using the video time code for each recording. Video clips were made of the REMS-like states including a 30 s lead-in of the QS state before the start of the REMS-like state and ending approximately 5 s after the animal expelled water through the siphon and/or repositioned itself on the substrate (Fig. 2). These behaviors were performed consistently after the REMS-like state. Following these movements, animals either resumed the QS state or became active with open pupils. The pixel intensity data for this 30 s lead-in were excluded when calculating the distance matrix for the hierarchical clustering dendrogram and for the Mantel tests (see below). Overhead and side-view camera footage of REMS-like states were combined in one video using Adobe After Effects (Fig. 1; Movies 1–6) so that QS states could be confirmed (animal partially buried in substrate with pupils closed; Frank et al., 2012).

Pixel value measurements were used to assess chromatophore activity; they were performed using custom-written MATLAB code (MathWorks, Natick, MA, USA). Most animals were relatively immobile as they were slightly buried in the substrate; however, one animal, zZ, drilled during the REMS-like state because the substrate was too shallow as a result of repeated self-burying in the same spot. Videos for zZ were digitized using Adobe After Effects, using the motion-tracking feature so that the region of interest (head or mantle) was constantly at the center of view. Video clips of REMS-like states were analyzed in MATLAB. A square 5×5 pixel region was selected on the head and on the mantle of the cuttlefish (Fig. 2). The average pixel values (ranging from 0 to 255 in 8-bit gray level images) were collected for each video frame (30 frames s$^{-1}$) over the entire video clip for these two regions (Fig. 2).

The absolute pixel values were affected by the amount of ambient light in the video. Because the aim was to compare patterns of pixel value changes throughout the REMS-like state within and between individuals and across different iterations of the REMS-like state, the pixel value at the beginning of sequences was subtracted from all subsequent values so that all REMS-like iterations had a standardized start at zero (see Fig. 2). In other words, in the standardized data, positive numbers indicate brightening of pixels and negative numbers indicate darkening of pixels. The REMS-like state began with a rapid darkening of the head and mantle region. We visually determined from the videos that a darkening by approximately 10 mean pixel values coincided with the start of the REMS-like state and only used the subsequent sequence for our analysis (see Fig. 2).

The temporal structure of body patterning was compared between and within individuals using the R package *dtwclust* (Sarda-Espinosa, 2016), which calculated all pair-wise distances/dissimilarities between pixel value series for all 55 iterations of the REMS-like state. This package stretches or condenses signals, based on user-specified settings, to determine how similar (or different) signals are to one another (Fig. S1). The settings disallowing unlimited stretching and condensing of signals were determined using the R package *dtw* (Giorgino, 2009) and were as follows: step=symmetric2, window.type=slantedband and window.size=30. In *dtwclust*, the function ‘hclust’ was used to build a hierarchical dendrogram for head and mantle data with animal and iteration of REMS-like state annotated at the tips to visually assess clustering. The R package *ape* (Paradis et al., 2004) was used to format the hierarchical dendrogram.

The R package *Ecdist* (Goslee and Urban, 2007) was used to perform Mantel tests between the pixel intensity distance matrix and binary matrices for animal identity and REMS-like iteration. Each binary matrix was compared against the pixel distance matrix.
naturally behaving animals. Data were analyzed via model comparison using size-corrected Akaike information criterion (AICc), which penalizes the addition of excess parameters. We compared two models: one null model represented by the intercept-only model and one model where state (awake, QS, REMS-like) was the only fixed predictor. Models contained animal identity as a random effect. We used the R package lme4 (Bates et al., 2015) to create and compare models using AICc scores. The R package AICcmodavg (http://CRAN.R-project.org/package=AICcmodavg) was used to calculate parameter estimates, standard errors and confidence intervals.

RESULTS

Animals primarily maintained some variation of the mottle pattern (Chiao et al., 2009) unless feeding or disturbed by human activity. The number of hours of video recorded for each animal was as follows: Hippo, 282 h; zZ, 187 h; MrChips, 159 h; Medusa, 167 h; Dumbo, 70 h; and Clyde, 54 h. During sleep-like bouts, when the animals were not in the REMS-like state, body patterns consisted mostly of mottle variants. In total, 55 REMS-like iterations were observed (Table S1). All six animals were recorded demonstrating at least one sleep-like bout with the REMS-like state alternating with the QS state (Fig. 3; Fig. S2). The number of sleep-like bouts and REMS-like iterations for each animal was as follows: Hippo: 2, 15; zZ: 1, 10; MrChips: 2, 6; Medusa: 3, 11; Dumbo: 1, 9; and Clyde: 1, 4. The duration of the REMS-like iteration was on average 2.42±0.22 min (mean±s.e.m., n=55) and the time between REMS-like iterations (i.e. QS state intervals, n=45) was 34.01±1.49 min (Table S1). We defined the periodicity as the time between the onset of one REMS-like iteration and the onset of the next REMS-like iteration (Fig. 3B; marked by eye ‘squinting’, discussed below); this resulted in a 36.34±1.46 min periodicity. On average, animals spent a total of 13.36 min (range 1.93–23.07 min) in the REMS-like state during a 176.89±36.71 min sleep-like bout (Table S1; n=10). Therefore, cuttlefish spent approximately 7% of the sleep-like state in the REMS-like state.

We observed arm movements by every individual in 55 iterations and eye movements in 54 out of 55 (side view was obscured once by condensation on the tank glass). Eye movements and concurrent chromatophore patterning around the eyes and brain (collectively known as the ‘head’ in cephalopods) included peculiar features that best characterize the REMS-like state of S. officinalis. The eyes shifted fore and aft (or left and right when viewed from the side) in all animals, and the W-shaped slit pupil sometimes dilated to a round shape. But the more common eye behavior was a gross ‘squinting’ of the whole eye socket in which various eye muscles contracted and squeezed the surrounding tissue to occlude the pupil. A close-up example of these strong eye movements can be seen in Movie 1. Often, this behavior was accompanied by sudden darkening of all the chromatophores around each eye and between the eyes, forming a ‘dark head bar’ that has not been seen in normally behaving cuttlefish during the daytime (Hanlon et al., 2009; Hanlon and Messenger, 1988). The darkening of the skin in cuttlefish is a result of direct neural stimulation from lower motor centers in the brain to muscles that contract to stretch the chromatophore pigment sac (Messenger, 2001), while muscle relaxation is concomitant with pale or white skin coloration.
Sometimes the whole head was flattened as if the cuttlefish was ducking. It is worth mentioning with regard to the expectation of loss of muscle tone at the onset of REMS that Movie 2 shows an animal with arms raised, which relax just as the REMS-like iteration begins. Also, a white head bar was always observed to precede the REMS-like state but was not useful in determining whether and when a REMS-like state would occur. These various muscular contractions of the eyes, head, chromatophores and arms seem akin to the myoclonic twitching and phasic activity in birds and mammals in the REM state.

Arm movements included slight twitching, raising of the first pair, and movement of all arms (see Movie 2 for arm raising). The only arm movement observed in all 55 iterations was slight arm twitching. Collectively, these represent conspicuous dramatic behaviors that are rarely or never seen in cuttlefish outside of the QS and REMS-like states; that is, they are REMS-like state specific and their order of appearance was not fixed but variable. The white head bar was present in all animals just prior to the REMS-like state and all animals performed this gross squinting, which coincided with the rapid chromatophore darkening at the start of what we considered the REMS-like state. The eye squint was used to mark the start of the REMS-like state for duration measurements.

The videos revealed episodic expression, during the REMS-like state, of several of the 34 chromatic components of body patterns that *S. officinalis* is known to use for signaling and camouflage (Hanlon and Messenger, 1988) (Movie 6). For example, cuttlefish showed each of the following: (i) mottle patterns (Chiao et al., 2009), a common camouflage pattern for primary defense; (ii) various expressions of deimatic (threat, startle) patterns, such as the unilateral or bilateral paired mantle spots, which are secondary defenses; and (iii) dark flashing of the mantle or whole body, or the ‘passing cloud’ pattern, which are examples of protean defense. These expressions were transitory and were not usually full or normal expressions of the patterns as seen in field or laboratory behavioral studies (Adamo et al., 2006; Hanlon et al., 2009; Hanlon and Messenger, 1988, 2018; Langridge, 2006; Langridge et al., 2007; Staudinger et al., 2013). Most of the body patterns exhibited during the REMS-like state were not observed in these animals outside of the REMS-like state.

The rate and nature of change in body patterning differed greatly between the REMS-like state and the QS and awake states (summarized in Table 1; raw data are provided in Table S2). In ~28 min of the REMS-like state, cuttlefish changed body patterns...
null model with supported state (awake, QS, REMS-like) as a predictor over the (Fig. 4). In further analysis, AICc model selection strongly from those of naturally behaving, awake (eyes open) animals chromatic components shown during REMS-like sleep differed noteworthy that in over 90% of the cases, body pattern and chromatic components shown during REMS-like sleep differed from those of naturally behaving, awake (eyes open) animals (Fig. 4). In further analysis, AICc model selection strongly supported state (awake, QS, REMS-like) as a predictor over the null model with $\Delta$AICc92 and an AICc model weight of 1. Standard practice requires a minimum of $\Delta$2 to consider a model better at describing the data than others in the set (Burnham and Anderson, 2013). Model-averaged parameter estimates (relative to the awake state) calculated the rate of pattern change in the REMS-like state as $\Delta$0.08±3.33 (+unconditional s.e.) with [43.56, 56.61] unconditional 95% confidence interval. The parameter estimate for the QS state is $\Delta$0.06±3.54 relative to the awake state, with [−7.61, 6.28] as the 95% confidence interval.

To quantify the variability of skin chromatophore patterning activity (to corroborate the species-specific skin displays), the distance matrix containing all pair-wise comparisons of REMS-like iterations was visualized in a hierarchical clustering dendrogram with animal ID and iteration tip labels for the two body regions (head: Fig. 5; mantle: Fig. S3). No robust clustering by animal or by iteration (1st, 2nd, 3rd, etc., REMS-like iteration within a cycle) was found for either body region. A Mantel statistical test comparing the pixel series distance matrix to a binary matrix for the head region showed a weak effect of animal identity: Mantel $r=0.09$, two-tailed $P<0.05$, and 95% confidence interval for Mantel $r$ of [0.06, 0.14]. Results for pixel intensity distance data from the mantle of the cuttlefish were similar but not significant with Mantel $r=0.07$, $P=0.19$, and 95% confidence interval of [0.04, 0.12]. Tests showed no effect of iteration for either head or mantle regions: Mantel $r=0.002$, two-tailed $P=0.97$, and 95% confidence interval of [−0.03, 0.04] for the head region and Mantel $r=-0.006$, $P=0.92$, and 95% confidence interval of [−0.11, 0.04] for the mantle region. A Mantel correlogram was computed to examine the underlying structure of the data where pixel series distance correlated significantly with animal identity. We found that REMS-like iterations that were more similar could be attributed to the same animal with Mantel $r \approx 0.14$ and that iterations that were more dissimilar could be attributed to different animals with Mantel $r \tilde{=} -0.07$ (Fig. S4).

**DISCUSSION**

Slow-wave sleep and REMS states have been demonstrated in birds, mammals (Siegel, 2008) and reptiles (Libour et al., 2018; Shein-Ideles et al., 2016). Here, we show that cuttlefish in a quiescent, sleep-like state periodically undergo a REMS-like state, characterized by (i) general immobility with occasional muscle twitching (arms and neuromuscular chromatophore organs), (ii) rapid horizontal movements of the eyes, (iii) alternating QS and REMS-like states in a predictable ultradian rhythm and (iv) a significant increase in the number and unusual combinations of neurally controlled skin pattern changes compared with those in the awake state. In octopuses, similar observations of quiescent behavior have been reported, including (i) circadian rhythms in activity, (ii) quiet periods characterized by closed eyes and a lack of activity, (iii) rebound when quiescence was prevented and (iv) heightened sensory thresholds during quiet periods (Brown et al., 2006; Meisel et al., 2011). There are also anecdotal reports in octopuses of body patterning inconsistent with camouflage during this quiescent state (Meisel et al., 2011). If borne out by more

<table>
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<th>Total no. of changes</th>
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At least three different iterations of the REMS-like state were analyzed for each of three animals and approximately 40 min were analyzed per animal for the QS and awake states.
Frank et al. (2012) also tested juvenile cuttlefish and observed homeostatic regulation of the QS state; however, they did not observe the REMS-like state in juveniles. One possible explanation presented in Frank et al. (2012) is potential neural immaturity of sleep-specific brain areas. Alternatively, the REMS-like state may be decreased or absent in situations that are stressful and demand increased vigilance. For example, most species show a circadian periodicity of REM; however, marine mammals, such as pinnipeds, show significantly decreased durations of REM sleep while in the water as opposed to when on land (Lyamin et al., 2012, 2018) and the frigatebird (Fregata minor) decreases REM sleep while in flight (Rattenborg et al., 2016). Frank et al. (2012) also noted that the juvenile cuttlefish in their study may not have been provided with a sufficiently long acclimation period. Technical issues prevented uninterrupted recording of cuttlefish in our study; however, the longest continuous recordings allowed us to observe two animals (Hippo and zZ) for 53 and 40 h, respectively. Although the animals appear to experience the QS state, they did not show REMS-like states during these continuous recordings. The REMS-like state had been observed in Hippo prior to this time block, suggesting the animal was acclimated. If we allow that stress effects are not responsible for altering the expression of the REMS-like state in these animals, an alternative explanation may be that it is not physiologically necessary for S. officinalis to experience this state every 24 h. Elephants (Loxodonta africana) and walruses (Odobenus rosmarus) have been reported to forego REM sleep for as long as 4 days (Gravett et al., 2017; Pryslova et al., 2009). It is important to consider that these rapid chromatophore pattern changes likely interfere with successful camouflage and may be costly in terms of predation risk. This may reflect a trade-off between meeting the presumed physiological need for the REMS-like state and limiting periods of risky conspicuousness. Future behavioral studies in juvenile and adult cuttlefish will be necessary to explore these possibilities.

Pixel intensity changes in small patches of chromatophores recorded in REMS-like iterations were not identical within individuals, nor were they identical between animals (Fig. 5; Fig. S3). Moreover, the expression of individual chromatic components, as well as whole body patterns, was always highly transitory in the REMS-like state and usually different from the way they are expressed in awake cuttlefish (Fig. 4). They were expressed in a seemingly disorganized manner, i.e. a typical camouflage pattern such as ‘mottle’ was shown often but very briefly (seconds), whereas in awake animals this chronic pattern is maintained for many minutes or hours. The mottle pattern was only shown acutely in cuttlefish in the REMS-like state, however, and was preceded or followed by patterns that function as conspicuous displays such as ‘deimatic’ and ‘passing cloud’ (Adamo et al., 2006). There were some similarities among REMS-like iterations (Movie 6), however. For example, all animals performed the passing cloud display and most performed symmetrical/asymmetrical activation of mantle deimatic spots. These displays are associated with antipredator behavior and are therefore normally expressed in response to a perceived threat (Hanlon and Messenger, 1988; Langridge, 2006). None of the animals were in visual contact with other animals or with humans; it is possible – but highly unlikely – that the body pattern changes likely interfere with successful camouflage and may be costly in terms of predation risk. This may reflect a trade-off between meeting the presumed physiological need for the REMS-like state and limiting periods of risky conspicuousness. Future behavioral studies in juvenile and adult cuttlefish will be necessary to explore these possibilities.

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quantitative measures (brain as well as behavioral), collectively this would suggest that some cephalopods including the common cuttlefish, S. officinalis, and possibly the common octopus, O. vulgaris, experience a state akin to REM sleep.

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and the REMS-like state was not observed in multiple cuttlefish simultaneously. Thus, it is highly likely that the cuttlefish were indeed exhibiting a typical behavior associated with the sleep-like state.

We recognize our methods may underestimate the similarity in gross pattern performance within and between animals because we compared pixel intensity changes for relatively small areas of the animal over temporally short periods of time. The animals actually performed many recognizable components of their patterning repertoire as mentioned above, but did not express these components in the same order or at the same time across REMS-like iterations. For example, all individuals displayed, either bilaterally or unilaterally, the third pair of deimatic spots on the mantle; however, the appearance of the spots did not occur at similar time points within the REMS-like iteration or even in all REMS-like iterations by the same individual (Movie 6). The only recognizable

Fig. 6. Two REMS-like iterations demonstrating common patterns of rapid brightness changes of skin patterning. (A,B) Top panels show the full-length sequence for the head (green) and the mantle (blue) and bottom panels show an expanded time series of the rapid onset of the REMS-like state (left panels) and later in the REMS-like state (right panels) as indicated by the x-axis time scale. Insets in the top panels show the cross-correlation function between head and mantle time series. See Results for a detailed explanation.
common chromatophore pattern that was displayed in a temporally predictable manner was the presence of the white head bar, which turned dark (never before seen) and was accompanied by the eye squint, marking the start of the REMS-like state. All other components were recognizable but the order in which they were performed and the duration of appearance was variable. Many of these components are normally expressed in response to predators or perceived threats or are used in camouflage, yet the testing environment was static with regard to substrate, structures and visual cues.

Our findings suggest that during the REMS-like state, fragments of normal waking activity in these areas are spontaneously re-activated, but in combinations not observed in wakefulness. This is reminiscent of mammalian REM sleep, which also contains fragments of waking patterns of activity in motor, limbic and cortical circuits (Matarazzo et al., 2011). This is revealed most dramatically in mammals that lack the normal paralysis of REM sleep. Under such conditions, during REM sleep animals will display components of complete waking behaviors, including aggressive displays, orientation and stalking behavior (Dumonoul Bridi et al., 2015; Louie and Wilson, 2001; Morrison, 1979). Moreover, in REM sleep, remote and recent memories are accessed and combined in complex ways not typically experienced in the waking state (Hobson et al., 2000). While speculative, it is possible that the unusual combinations of chromatophore activity during REMS-like states reflect a similar process in S. officinalis.

Although not the prime target of this study, the dynamics of brightness changes would be worth investigating more quantitatively. Of particular note: (1) all REMS-like iterations begin with a rapid darkening that is synchronized between head and mantle regions of interest (bottom left panel in Fig. 6A), but can also involve delays of several seconds (bottom left panel in Fig. 6B); (2) overall, brightness changes in the head and the mantle region are correlated, often with a delay of 30–100 ms (insets in top panels in Fig. 6A,B); (3) such correlations are not present, however, in the fine-scale brightness change on short time scales (Fig. 6, bottom panels); (4) rapid brightness changes occur in discrete steps, even before the REMS-like state starts (Fig. 6, bottom panels) but (5) can also involve bursts of smooth oscillatory changes (Fig. 6, bottom right panels); (6) both head and mantle regions tend to brighten slowly throughout the course of a REMS-like iteration (top panels in Fig. 6A,B). Given that these brightness changes reflect chromatophore dynamics and the activity of neural networks, it would be interesting to compare the temporal organization of brightness changes in awake and ‘sleeping’ cuttlefish and to eventually relate them to changes in chromatophores and to the neural activity orchestrating them.

If future work reveals the cephalopod QS and REMS-like states to be analogous to vertebrate slow-wave and REM sleep, then it is worth remarking here that the periodicity of the REMS-like state in the cuttlefish appears more similar to the periodicity of REM sleep in small mammals than to that of birds or reptiles (Shein-Idelson et al., 2013). The average time an animal spent in the REMS-like state across a single QS–REMS-like cycle (~7%) is similar to that for small mammals and birds. Regardless of whether sleep in animals resulted from single or multiple evolutionary origins, this phylogenetic distribution of patterns of periodicity, total duration of specific sleep states and flexibility to delay REM sleep (or REMS-like states) promises a fruitful avenue towards identifying ecological pressures that shape sleep behavior (Aulsebrook et al., 2016).

The possible occurrence of sleep and REMS-like sleep in a clade of organisms that is distantly related to any clade where REM sleep has been observed previously poses a challenging and important opportunity to broaden our understanding of sleep and REM sleep (Corner, 2013; Libourel et al., 2018; Siegel, 1995; Tobler, 1988). Cephalopods have the largest and most complex brains – and the most diverse repertoire of behaviors – of any invertebrate animal (Hanlon and Messenger, 2018; Nixon and Young, 2003). Whether future work demonstrates that cuttlefish adhere to all the criteria currently deemed necessary for a legitimate sleep state or prove to be an exception to the general pattern described in the literature for vertebrates, examining these questions in an organism that is ecologically distinct and phylogenetically distant from current model organisms should serve to inform the broader questions of how to accurately define sleep, discover its functions and understand its evolutionary origins.

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