

## **SLEEP IN MONOTREMES; IMPLICATIONS FOR THE EVOLUTION OF REM SLEEP**

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Sleep onset in mammals is associated with slowing of the dominant EEG frequencies and increases in EEG amplitude. EEG slow waves are intermixed with sleep spindles in the lighter stages of sleep, while slow waves increase in the deeper nonREM stages. Levels of muscle tone are reduced. Arousal thresholds are increased. NonREM sleep alternates with periods of REM sleep, characterized by rapid eye movements, EEG desynchrony, hippocampal theta, ponto-geniculo-occipital (PGO) spikes, twitching, autonomic irregularity and muscle atonia.

Not all animals show all of these signs of REM sleep. For example, complete REM sleep muscle atonia is not present in the rabbit or dog (Pivik et al., 1981; Siegel et al., 1991) and most birds (Amlaner and Ball, 1989). Rapid eye movements are absent or minimal in REM sleep in animals with few waking eye movements such as the mole (Allison and Van Twyver, 1970), opossum and owl (Susie and Kovacevic, 1973; Berger et al., 1972). While increased heart rate variability is a consistent feature of REM sleep in humans and most

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other species, the mole actually has reduced heart rate variability in REM sleep (Allison and Van Twyver, 1970). Thus a cluster of variables, rather than a single one, must be used to identify the state of REM sleep.

Using these variables, REM sleep had been observed in all orders of mammals, with the exception of the monotremes. Marsupials have large amounts of REM sleep, with the opossum *Lutreolina crassicaudata* having 6.6 hours per day, one of the largest amounts of any species. Insectivores (in contrast to the ant eating echidna) have between 1 and 4 hours of REM sleep per day. Other orders that have been found to have substantial amounts of REM sleep include primates (humans have 2 hours of REM sleep/day), lagomorpha (rabbits), rodents, carnivores, pinapeds (seals), proboscidea (elephants) and grazing and farm animals such as the horse, pig, giraffe, goat and sheep (Zepelin, 1989; Allison and Cicchetti, 1976).

The only mammal other than the echidna that has been reported to lack REM sleep is the dolphin (*Tursiops truncatus* and *Phocena phocena*) (Mukhametov, 1987; Mukhametov et al., 1977). The evidence for the absence of REM sleep in the dolphin is not comparable to that which existed for the echidna. No recordings of hippocampal theta or other subcortical indicators were carried out in this animal. No tests of arousal threshold or brain temperature were conducted. The animals were confined when studied, raising the possibility that normal sleep patterns were not observed. The related cetacean, the pilot whale *Globicephalus scammoni*, does have REM sleep in small amounts (Shurley et al., 1969). REM sleep may be present but difficult to observe in confined dolphins. Further study of unrestrained dolphins would be desirable.

### **Monotremes**

"Plesiomorphic" or ancestral characteristics can be inferred by their presence in several orders, since it is more parsimonious to hypothesize the presence of such characteristics in the common ancestor than to assume its evolution on multiple occasions. The question posed in the present paper in its simplest form is "is REM sleep a plesiomorphic state in mammals?"

All mammals living today are descended from ancient egg laying reptiles called therapsids. Mammals are classified into three taxa, the eutheria (placentals), the metatheria (marsupials) and the prototheria. The monotremes are the only living representatives of the

prototheria. DNA hybridization analyses indicate that monotremes diverged from the other mammalian lines approximately 130 million years ago (Kemp, 1982; Westerman and Edwards, 1992). Thus their divergence was during the early cretaceous period, when theropods such as *allosaurus*, sauropods such as the giant *diplodocus* and ornithischians such as *stegosaurus* flourished. At this time the continents were fused in Gondwanaland. The monotreme divergence occurred at or before the development of the placentals and marsupials. The echidna and platypus lines diverged from each other 60-80 million years ago (Westerman and Edwards, 1991), at the end of the period when *tyrannosaurus*, *triceratops* and related dinosaurs were dominant. The separation of the platypus and echidna lines coincides with the mass extinctions of the dinosaurs and the major radiations of the eutherian and metatherian lines. It is likely that ancestors of the amphibious platypus were able to occupy vacated niches on land, evolving into echidnas. DNA hybridization studies date the divergence of the short and long beaked echidna to within the last 200,000 years (Westerman and Edwards, 1992).

The platypus has shown a remarkably conservative evolutionary course since its divergence from the rest of the mammalian line. For example fossil teeth from *Steropodon galmani* dated at 110 million years ago, are quite similar to the vestigial teeth of the current day platypus *Ornithorhynchus anatinus*. Analyses of fossilized skull remains indicate remarkably little change in platypus morphology over at least 60 million years (Archer et al., 1992; Pascual et al., 1992).

The low level of speciation throughout the fossil record is another indicator of the uniquely conservative lineage of monotremes. The 130 million years of platypus evolution, has produced no species radiation, although the fragmentary skull evidence available for the identification of *Monotrematum sudamericanum*, has led to its tentative classification as separate taxon. The echidna has had a similar history. The classification of the giant echidna *Zaglossus hacketti*, is uncertain because of the lack of cranial material (Griffiths, 1978). However, apart from this specimen, there has only been the relatively recent divergence of the short and long beaked echidna over the echidnas 60 million year long evolutionary course. Closely related, structurally similar, mammalian species have similar sleep structure (Zepelin, 1989). Thus sleep structure in the present day platypus and echidna should be similar to that of their lineal progenitors.

The echidna and platypus occupy distinct ecological niches and are behaviorally and morphologically quite different. For example the platypus has webbed feet, is amphibious, eats water dwelling crustaceans, has fine fur covering most of its body and does not hibernate. In contrast, the short beaked echidna avoids water, eats only certain kinds of ants, is covered with sharp spines, has large amounts of fur only on its ventral surface and hibernates seasonally. However the echidna and platypus also have many striking similarities. They both regulate body temperature at approximately 32°C. They both lay eggs. The platypus has only vestiges of teeth, while teeth are completely absent in the echidna. They both have a single cloacal opening. They are the only two mammals known to have electroreception (Scheich et al., 1986), with both echidna and platypus having arrays of mucous gland electroreceptors in their bills. Females of both species secrete milk from glands in their ventral skin, rather than having teats for nursing. Males platypuses have venomous spurs on their hindlimbs, while male echidnas have vestigial spurs on their hindlimbs. Monotremes are the only venomous mammals. While the brain of the echidna is highly convoluted, the platypus's brain is lissencephalic. However, cortical mapping studies have revealed clear homologies between the platypus and echidna in the location and size of sensory cortices (Krubitzer et al., 1991). Thus there is morphological and physiological justification for hypothesizing either similar or different forms of sleep in the echidna and platypus.

### **Sleep in monotremes**

The first study of sleep in monotremes was carried by Allison et al. (1972). A major motivation of this study, was to address the issue of whether REM sleep is a phylogenetically old state or whether it evolved relatively recently. The finding that pontine neurons were critically involved in REM sleep generation and the large amounts of REM sleep early in life, are arguments for the early evolution of this state. The reduction in thermoregulatory responses in REM sleep has some similarity to reptilian thermoregulation and is consistent with an early evolution of this state. On the other hand, the absence of REM sleep in most studies of reptiles (Hermann et al. 1964; Tauber, et al., 1966) and the "rudimentary" REM sleep in birds (Amlaner and Ball, 1989) argue for a more recent evolution of REM sleep. The link between REM sleep and dreams in humans, with their rich symbolic

content, has been taken to indicate a higher function of REM sleep, consistent with a more recent evolution of this state.

Allison et al. (1972) found that the echidna has periods of EEG synchrony, which are accompanied by elevated arousal thresholds (Allison et al., 1972). While EEG synchrony was seen, long term recordings from the echidna found that no periods of EEG desynchrony with rapid eye movements occurred while the echidna was quiescent. Since Allison et al. (1972) found that they could observe few eye movements in waking in these animals, they sought other possible signs of REM sleep during periods of EEG desynchrony without movement. They dubbed these periods "PS?," to reflect the possibility that they were periods of REM (or paradoxical) sleep. They tested arousal thresholds in PS? and found that unlike REM sleep, which in most species is accompanied by increased arousal thresholds, PS? was accompanied by lower thresholds relative to synchronized sleep. Since theta activity is a consistent feature of mammalian REM sleep, they recorded hippocampal activity in the echidna. Theta was present in waking with movement but not in PS?. They looked for cardiac irregularity, a sign of REM sleep in most mammals. This was not present during PS?. They monitored cortical brain temperature and found no abrupt increases characteristic of REM sleep during PS?. Finally they attempted PS? deprivation to see if a PS? rebound akin to REM sleep rebound occurred. They found no evidence of a rebound. Rather the PS? state decreased after the deprivation procedure. Allison et al. interpreted all these findings as consistent with PS? being waking, rather than REM sleep. Hence they concluded that REM sleep did not exist in the echidna.

Allison et al. (1972) carefully considered the implications of their finding. If the echidna is representative of the monotremes and monotremes lack REM sleep, what aspect of the biology of monotremes is responsible for this major difference in state organization and what does this tell us about the evolution of REM sleep? They consider several unusual aspects of monotreme physiology. Monotremes have a low body temperature, though they are homeotherms. So Allison et al. studied the armadillo. They found that it exhibited copious amounts of REM sleep at body temperatures comparable to those seen in the echidna (Van Twyver and Allison, 1974). *Myotis*, a bat that exhibits diurnal torpidity has REM sleep at temperatures as low as 25°C (Brebbia and Paul, 1969). Allison et al., note that the echidna is a hibernator. However hibernators as a group

have relatively large amounts of REM sleep (Van Twyver, 1969). Surveying the phylogenetic data, Allison et al. found a strong correlation between REM sleep amounts and depth of sleep, noting that "deeply sleeping" animals, usually those that are safe while sleeping, have large amounts of REM sleep (Allison and Cicchetti, 1976). However, Allison concluded, the echidna, which sleeps burrowed into the dirt with only its razor sharp quills protruding, is a deep sleeper. Thus the positive correlation between sleep depth and amount of REM sleep would predict a large amount of REM sleep in the echidna. Another feature of the echidna is their immaturity at birth. However, this feature also seems to powerfully predict increased rather than decreased amounts of REM sleep. Precocial animals such as the guinea pig have low levels of REM sleep, whereas altricial (born immature) animals such as humans and opossums have large amounts of REM sleep (Zepelin, 1989).

Having rejected body temperature, sleep safety, hibernation and immaturity at birth as explanations for the echidna's lack of REM sleep, Allison et al. (1972) conclude that REM sleep is related to the live birth (viviparity) seen in all other mammalian orders. However, there is no obvious functional connection between viviparity and REM sleep. Furthermore the other class of egg laying animals, the birds, does have REM sleep.

### **Theories of REM sleep function**

Many theories of REM sleep function have postulated that REM sleep is related in some way to intellectual function. In one variation on this theme, Crick and Mitchison (1983) hypothesized that REM sleep was related to the forgetting of random associations. According to this hypothesis, the echidna's lack of REM sleep has led to a compensatory overdevelopment of prefrontal cortex (Divac et al., 1987). This prefrontal cortex compensated for the reduced efficiency in cortical processing caused by the absence of REM sleep. However, Krubitzer et al. (1991) have physiologically mapped echidna cortex and found that prefrontal cortex while large, is not so disproportionately large as to set echidnas apart from all other mammals. Rather, the folding pattern differs in echidnas, with prefrontal sulci being relatively shallow. This causes the echidna to appear to have a larger prefrontal cortex when viewed *in situ*. An analysis of the encephalization quotient of the echidna (the ratio of the actual brain weight to that expected for a given body weight) concluded that the echidna's brain

size in relation to its body weight is comparable to or less than that of the cat (Zepelin, 1989).

### **REM sleep at the neuronal level**

Some investigations have suggested that REM sleep may exist in reptiles (Ayala-Guerrero et al., 1991; Huntley 1987), while other studies find no evidence for amphibian or reptilian REM (Hobson, 1967; Flanigan et al., 1973, 1974; Meglasson and Huggins, 1979; Warner and Huggins, 1973). [see chapter By Rial] The presence of REM sleep in birds (Amlaner and Ball, 1989) along with its absence in monotremes and in most studies of reptiles led to the idea that REM sleep evolved more than once (Allison et al., 1972; Zepelin, 1989). This hypothesis in its simplest form strains the concept of convergent evolution. A functional appendage such as a wing can evolve many times in the animal kingdom. However, the structure of the wing differs radically in the bird, fly and bat. In contrast, REM sleep "structure" as expressed in polygraphically recorded slow wave variables, does not appear to differ substantially in the bat, human and bird. The phylogenetic evidence pointing to the repeated evolution of sleep in birds and mammals, suggests that there must be a very near precursor to REM sleep in the common ancestor. Some features of the state from which REM sleep evolved might be present in monotremes because of their ancient divergence from the mammalian line and their conservative evolutionary course. For this reason we felt that the study of neuronal activity during monotreme sleep might indicate the nature of this precursor state.

There is widespread agreement that REM sleep is generated by neurons in the dorsolateral pons, while medullary and hypothalamic regions are critical to its normal expression and timing. Some of the critical pontine generator neurons are cholinergic while others contain excitatory amino acids including glutamate and peptides including corticotropin releasing factor (Lai and Siegel, 1988, 1991, 1992). Descending projections from the pons to the medulla produce tonic motor inhibition during REM sleep. Ascending projections emanating from cholinergic neurons in the pedunculopontine (PPN) and laterodorsal tegmental nucleus (LDT) produce tonic EEG desynchrony and hippocampal theta (Steriade et al., 1990; Vertes 1981). Noradrenergic neurons in and adjacent to the locus coeruleus and serotonergic neurons in the raphe nuclei are tonically active during waking and fire at a reduced rate during nonREM sleep. During REM

sleep, discharge in both of these cell groups cease, presumably due to active inhibition (Nitz and Siegel, 1993). Within the pontine reticular formation, the nonREM sleep pattern of minimal, regular activity, is replaced by a burst-pause pattern of discharge in REM sleep, with a marked elevation of mean rate. The burst-pause pattern is visible in polygraph or computer displays of action potentials and is sufficient to identify REM sleep. The characteristic burst discharge pattern of reticular cells can be quantified using spectral analysis of the spike train (Yamamoto and Nakahama, 1983; Yamamoto et al., 1986). Such analyses reveal that the spectral density of discharge differs in reticular neurons in REM and nonREM sleep. REM sleep is characterized by a spectral density inversely proportional to frequency, while nonREM sleep discharge patterns have a flat "white noise" spectrum. This reflects the much greater low frequency fluctuation of firing in reticular cells in REM sleep compared with nonREM sleep (Yamamoto et al., 1986). The "simplified dependency" measure expressing this fluctuation is greatest in REM sleep, intermediate in waking and minimal in nonREM sleep. Therefore, if the reticular unit discharge during sleep is "REM sleep-like" in the echidna, the dependency measure will be higher in sleep than in waking. If echidna sleep is like nonREM sleep, the simplified dependency measure will be lower in sleep than in waking.

Brainstem neurons firing in bursts during sleep, include cells with monosynaptic connections to motoneurons (Siegel and Tomaszewski, 1983; Siegel et al., 1983; Takakusaki et al., 1989; Peterson et al., 1979). The bursting discharge of these neurons is responsible for the rapid eye movements, the twitches that in the cat and human break through atonia and for the respiratory irregularities seen in REM sleep. Lateral pontine neurons synapsing on lateral medullary autonomic nuclei are likely to be responsible for the phasic autonomic changes of REM sleep.

Viewed at the neuronal level, nonREM sleep in placental mammals consists of a reduction of activity in most brainstem reticular neurons. REM sleep consists of a widespread periodic phasic activation of mesopontine reticular cell groups (REM-waking-on cells).

If REM sleep evolved several times, there must have been a precursor state present in the ancestors of the early mammals. We hypothesized that certain elements of REM sleep such as the bursting activity of pontine reticular units or the complete cessation of activity

in locus coeruleus and raphe cells, were present in the pre-mammalian forms giving rise to both birds and therian mammals. The monotremes, located near the junction of the mammalian, avian and reptilian lines and having an extremely conservative evolutionary course, might display this precursor state.

### **Neuronal activity during sleep in the echidna**

In a search for this precursor state, we have re-examined sleep in the echidna, looking at the neuronal activity of the brainstem to more completely characterize state (Siegel et al., 1996). Unit recording electrodes were placed in the pontine tegmentum, an area that has been shown to be critically involved in REM sleep generation. Electrodes were also placed in the midbrain reticular formation, the brain region whose sleep cycle discharge has been most thoroughly studied in placental mammals (Siegel, 1994).

Neck muscle tone was absent whenever the echidna was not actively moving. Thus, there was no further reduction in tone with the EEG synchrony of sleep. Periods during which the echidnas had little movement or EMG activity, but showed a desynchronized EEG tended to follow active periods during which the echidnas ate, locomoted and explored the environment. The echidna, which has small, laterally placed eyes, has few eye movements during waking. We saw no periods of EOG (electrooculogram) activity during sleep with high voltage EEG waves. We also saw no periods of EOG activity with desynchronized EEG when the animal was not actively locomoting. These observations are in accord with Allison et al.'s (1972) conclusion that the echidna does not exhibit the polygraphic signs of REM sleep.

Forty three units were recorded from the midbrain reticular formation (n=26) and the subcoeruleus/reticularis pontis oralis region of the pons (n=17) of the echidna. Units were recorded for a minimum of three hours and a maximum of 30 hours. Active waking was characterized by high and irregular discharge rates. During quiet waking, the EEG remained desynchronized, and reticular formation unit activity slowed and became more regular. Thus during waking, echidna unit discharge was like that of placental mammals.

Mean rates were 4.7 Hz in quiet waking (QW) and 3.3 Hz in sleep. Sleep discharge rates were significantly lower than the rates in quiet waking ( $F=3.8$ ,  $p<.05$ ). Therefore the rate decrease during sleep in the echidna resembled that in placental mammals during nonREM sleep (Siegel et al., 1994).

Despite a reduction in discharge rate, analogous to the change in unit activity in placental nonREM sleep, most echidna reticular units had substantially increased discharge variability (burst firing) throughout sleep. In placental mammals, virtually all midbrain and pontine units show unchanged or decreased discharge variability in nonREM sleep relative to quiet waking and increased variability only in REM sleep.

The increased variability of echidna reticular formation unit discharge in sleep relative to quiet waking was quantified by calculating the variability in discharge frequency during consecutive ten sec. epochs. The same calculation was made for units recorded in the same reticular nuclei in the dog and cat during waking, nonREM sleep and REM sleep. Ten sequentially encountered reticular cells in the dog, 13 in the cat and the 22 echidna cells whose records we were able to digitize across the sleep cycle were compared. In the dog and cat, all units decreased variability as the animal went from waking to nonREM sleep, while in the echidna, 17 of 22 increased variability. The increased variability of reticular unit discharge in sleep in the echidna differed significantly from the decreased variability seen in nonREM sleep in the cat and dog ( $p < .001$ , Mann Whitney U test). Whereas the increase of variability resembled that of REM sleep, the magnitude of the variability increase seen in sleep in the echidna was lower than that in REM sleep in the dog and cat.

We did not see any periods of phasic motor activity in the EMG or in our infra-red video observations during these periods of unit discharge irregularity. The complete atonia during sleep may have blocked the motor expression of phasic brainstem unit activity. Another explanation for the lack of motor activity, is that whereas the bursting activity of brainstem neurons during REM sleep is synchronized across the entire neuronal population in placental mammals (Siegel et al., 1981), bursting is not synchronized in the echidna. Cross correlation analyses at 1, 25 and 200 msec binwidth of 20 units recorded in 10 pairs, revealed that echidna units, even when recorded from the same microwire, fired their bursts asynchronously in sleep.

Thus whereas echidna sleep does not appear to have the pattern of behavioral or EEG variables that define REM sleep in eutherians, its pattern of brainstem neuronal activity is not like that of nonREM sleep. Rather the "state" of neurons in the echidna brain during sleep appears

to be intermediate between the state of eutherian neurons in REM and that in nonREM sleep. In other words it is not simply the case that the echidna does not have REM sleep. Rather it has a sleep state that combines aspects of REM and nonREM sleep.

### Sleep in the platypus

The platypus, the most primitive monotreme, can provide the most direct insight into the nature of sleep in the earliest mammals. Obstacles in recording sleep from this animal are many. The Australian government strictly regulates access to the platypus. Part of the reason for this is that few platypuses survive for long in captivity, even in Australian zoos. Only with the development of new techniques for managing the platypus, including the construction of an enclosure that reduced stress on the platypus by minimizing electrical noise (Manger and Pettigrew, 1995), did it become practical to record from them.

A further impediment to work in the platypus is their aquatic nature. Since they cannot be confined without lethally stressing them, it is necessary to use telemetry to record from them. We have utilized a system that allowed us to continuously monitor the platypus as it swam in the tank, as well as when it stayed in the dry "burrow" area. We found to our surprise that the predominant sleep state in the platypus was REM sleep.

When the platypus was observed to be awake, EEG voltage was at its lowest level. **Wake** periods occupied 9.7 h/day. No periods with comparable low voltage EEG occurred during sleep. At sleep onset, EEG amplitude increased, in Quiet Sleep with Moderate voltage EEG (**QS-M**), which occupied 4.4 h/day. Periods with high voltage EEG, (**QS-H**) occupied 1.4 h/day.

REM sleep, as defined by muscle atonia, > 3 phasic EMG potentials exceeding 10  $\mu$ V/min and rapid eye movements, was always accompanied by an EEG which was of moderate (**REM-M**, 7.6 h/day) or high amplitude (> 100  $\mu$ V, **REM-H**, 0.9 h/day). Thus the platypus has 8.5 h of REM sleep/day, much more than in any other animal. The phasic EOG and EMG potentials of REM were correlated with visually observed rapid movements of the eyes, neck and bill.

Our work in the platypus and the echidna suggests a new view of how sleep states in mammals evolved. The predominant sleep state in the common mammalian ancestor, as represented by the platypus, was a state that meets the behavioral definition of REM sleep. Eyes

move, the bill twitches and the animal remains asleep. However, the EEG is high in voltage. Neither the echidna nor the platypus have the low voltage EEG of eutherian sleep. Between the platypus and the more derived echidna, the amount of motor activity during the "brainstem activated, cortex synchronized" sleep state has decreased. This could be due to the reduced synchrony between the bursting brainstem units compared to that in eutherian REM sleep. It may be that the monotreme genome or environmental conditions allowed the echidna to evolve a "brainstem activated-cortex synchronized" sleep state. By preventing phasic movement, it decreased its potential signalling to predators. Twitching of the spines of the echidna would reveal its position and be quite maladaptive.

I would predict that more complete study may reveal other animals with high voltage EEG during "REM" sleep as in the monotremes. However, REM sleep with synchronized EEG is in fact common in neonates. Neonatologists have often used the term "active sleep" to describe the state of phasic movement during sleep with EEG synchrony seen in neonates. We know that this state is developmentally continuous with adult REM sleep. So in development it appears that we are seeing ontogeny recapitulating phylogeny. This extends to the pattern of unit activity during development. Work in neonates has found a less bursty pattern during early REM, or active sleep, compared to the adult (Corner and Bour, 1984). This rather exactly parallels the differences between the monotremes, with their EEG synchrony and twitching and the eutherian mammals.

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