

Dolphin Continuous Auditory Vigilance for Five Days.

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Summary

The present report describes the first study of continuous vigilance in dolphins. Two adult bottlenose dolphins (*Tursiops truncatus*), WEN a male and SAY a female, maintained very high level of paddle press responses to randomly presented, infrequent, 1.5 s target tones in a background of frequent 0.5 s equal-amplitude tones over five continuous 120-hour sessions. The animals were able to maintain high levels (WEN 97, 87, 99 percent; SAY 93, 96 percent) of target detection without signs of sleep deprivation as indicated by behavior, blood indices or marked sleep rebound during 24 hours of continuous post-experiment observation. Target response time overall ($F=0.384$; $p=0.816$) did not change between day one and day five. However, response time was significantly slower ($F=21.566$, $p=0.019$) during nighttime (2100 to 0400) when the dolphins would have ordinarily been resting or asleep.

Introduction

It has long been known that dolphins can swim continuously for very long periods (Townsend, 1914). Rest is indicated by the animal floating at the surface without significant progressive swimming or by slow circular swimming in a stereotyped manner. After a dolphin calf is born the mother and offspring have been observed swimming continuously for several weeks (Lyamin et al., 2005). Nursing occurs while the mother is swimming slowly presenting first one side then the other enabling the calf to nurse underway (McBride and Kritzler, 1951; Cockcroft and Ross, 1990).

Dolphins have shown slow wave sleep (SWS) electroencephalograms (EEG) in one brain hemisphere while producing waking EEG in the other (Mukhametov, et al., 1977; Mukhametov, 1984, 1987; Ridgway, 2002). Left and right hemispheres alternate SWS and waking by some yet unknown mechanism. Independent eye movement and closure (McCormick, 1969; Dawson et al.,

1981; Lyamin et al., 2001), observations of behavior in nocturnal rest periods (Flanigan, 1974; Goley, 1999), a small corpus callosum (Tarpley and Ridgway, 1994), complete crossing of the nerves at the optic chiasm (Tarpley et al., 1994), absence of an arterial Circle of Willis (McFarland et al. 1979) all suggest a degree of hemispheric independence. No studies, however, have demonstrated a physiological or behavioral advantage of "uni-hemispheric sleep" or documented long-term vigilance in dolphins.

It has been suggested that uni-hemispheric sleep functions to "assure that the animal is always scanning his environment with at least half of it's afferent inputs" (Lilly, 1964). In other words, in order to maintain vigilance (Mukhametov, 1984). We have investigated the ability of the dolphin to maintain a high level of vigilance continuously, ostensibly by the use of uni-hemispheric sleep. The hypothesis was that, when one hemisphere is tired, the dolphin can switch to the other (Lilly, 1964; Mukhametov, 1984, 1987; Ridgway, 2002) awake and vigilant hemisphere. Here we report our findings on auditory vigilance over five separate 120-hour sessions.

Materials and Methods

Two adult bottlenose dolphins (*Tursiops truncatus*), WEN a male (age 21, 196 kg in weight, 250 cm in length) and SAY a female (age 26, 246 kg, 274 cm) were trained to discriminate an infrequent, random, underwater 1.5 s, 70 kHz target tone from a background of frequent 0.5 s, 70 kHz tones of equal source level projected every 30 s as they swam freely in their 9 X 9 m open water enclosures (Fig. 1A). The dolphin's approach to the underwater target paddle was illuminated at about 850 nm wavelength by infrared (IR) light sources and recorded by IR camera (Fig. 1A and B). Our studies indicate that the dolphin's pupil does not respond to this IR illumination nor is there any behavioral indication that the dolphin can see this wavelength (data not shown). Much of the time, the dolphin's eye state (open or closed) could be observed on the recorded video.

Under computer control, the 1.5 s target stimulus randomly replaced the 0.5 sec tone at intervals between 4 and 24 minutes. The dolphin was required to press a paddle on one side of its enclosure within 20.5 s of a target tone. When the paddle was pressed within 20.5 s of the target tone, the computer sounded a buzzer as a bridging stimulus to the dolphin and to signal the trainer to come out of the equipment hut and give the dolphin a fish reward (Fig. 1A). The rewards were dispensed on a side of the enclosure away from the target paddle. If the dolphin pressed the paddle in response to a 0.5 s tone – a false alarm – the wait period for the next tone was extended for 30 seconds. The dependent variables were the time to respond to the signal and the number missed or ignored targets.

Prior to the vigilance trials, the dolphins were fed a standard amount of fish each day during the daylight hours – 10 kg for WEN, 12 kg for SAY. During the vigilance sessions the total daily food consumption was maintained and spread out over a 24-hour period. The animals were fed around-the-clock with small amounts being given for each correct target response. The trainers randomized the amount of reward, giving one to four small fish for each correct target so that the day's standard ration (in kilocalories) was delivered by the end of each 24-hour period of the vigilance session.

After conducting some shorter training sessions, five continuous 120-hour vigilance sessions were conducted, three for WEN (A,B,C) and two for SAY (A,B). Infrared light sources and cameras were used for observation and for the video recording (Fig. 1B). Other dolphins were present in nearby netted enclosures and their vocalizations could be heard by the subject dolphin. In addition to the 120-hour continuous vigilance session a video record was made for 24 hours before and 24 hours after the experiment for a total of 192 hours of observation per vigilance session.

Respiratory rate (number of breathes per five minutes) was recorded every hour throughout the sessions. The video record was scored for behavioral indices of sleep (McCormick, 1969; Flanigan, 1974; Goley, 1999; Lima et al., 2005) at 30 s intervals. Two types of behavior were scored as sleep; slow stereotyped circular swimming and floating at the surface with only an occasional beat of the tail to bring the blowhole above the surface to breathe. Comparison of behavior during the 24-hour period before and after the session was used as an indicator of sleep rebound following the 120-hour vigilance session.

The dolphins were trained to present their flukes for blood sample collection (Fig. 2). On two trials with WEN (A,B), non-fasting blood samples were collected for complete blood cell count (CBC), serum chemistry, catecholamines, and selected hormones analysis (Table 1). Blood was collected, at the same time of the day, both immediately prior to the start and at the end of the 120-hour vigilance sessions. Samples were analyzed by two reference laboratories (Quest Diagnostics, San Diego, CA; and ARUP Laboratories, Salt Lake City, Utah) with experience performing these specific analyses on dolphin blood. Handling of blood was in accordance with previously established protocols by Romano et al., 2004.

Data were analyzed with Systat 10 (Systat Software, Inc, Point Richmond, CA, USA). Repeated measures analysis of variance (ANOVA) was used to evaluate the respiratory rate and response time to the target tone between 24-hour periods within the 120-hour vigilance session and between daytime (0900-1600) and nighttime (2100- 0400). Target response time or respiratory rate were used as the dependent variable with 24hour periods of the vigilance session (1-24 hrs; 25-48 hrs; 49-72 hrs; 73-96 hrs; 97-120 hrs), time of day (daytime vs. nighttime), and animal as the independent variables. Logistic regressions were used to evaluate changes in the accuracy (correct

vs. missed trials) by 24 hour periods and by time of day (daytime vs. nighttime) within each trial. Probabilities less than 0.05 were considered significant.

Results

The dolphins detected 94.2 ± 2.26 percent (SAY) and 94.36 ± 6.33 percent (WEN) of the target stimuli throughout all sessions with little vigilance decrement as indicated by response time and accuracy (Fig. 3). When compared with the 24 hours before the start of the vigilance session, there was not a substantial increase in sleep behaviors (McCormick, 1969; Flanigan, 1974; Goley, 1999) or signs of sleep deprivation (Oleksenko et al., 1992; Dinges et al., 1994; Dukas and Clark, 1995) compared to the 24 hours following the vigilance sessions (Fig. 4).

The dolphins showed no indications of a vigilance decrement during the five days. For three of the trials (WEN A, WEN C, SAY B) the number of missed targets was very low, and the dolphins did not miss more targets on day 5 compared to day 1. However, for two of the trials, WEN B (logit 4.418, $p < 0.001$) and SAY A (logit 2.483, $p = 0.003$) the number of missed trials did significantly increase over the session. Specifically, more targets were missed on day 5 (97-120 hr period) than during previous days of the session.

The change in target response time was less than 1 second per 24 hour period of the all vigilance sessions (Table 2). We suspect that, such a small difference in the response time suggests an adaptation to the routine nature of the task rather than the dolphin becoming tired due to sleep deprivation. Additionally, Fig. 4 indicates there was not a substantial increase in sleep behavior for the 24 hours after the 120-hour vigilance sessions for either dolphin.

It is apparent from Fig. 3 that a diurnal pattern was present in the target response time and respiratory rate of both dolphins. The target response time ($F = 0.384$, $p = 0.816$) and respiratory rate ($F = 1.224$, $p = 0.425$) did not significantly change over the course of the vigilance sessions. However,

there was a significant decrease in target response time ($F=21.556$, $p=0.019$) during daytime (0900-1600) compared to nighttime (2100-0400). While a diurnal pattern appears to be present in the respiratory rate, there was not a significant difference in respiratory rate ($F=8.426$; $p=0.211$) between daytime (SAY 9.97 ± 1.40 ; WEN 12.22 ± 2.17) and nighttime (SAY 8.20 ± 1.23 ; WEN 11.11 ± 1.49). During the night most of the trainers were absent from the facility, other dolphins were not being fed, and the dolphins displayed most of their quiescent hanging behavior (QHB) or slow circular swimming (SCS) suggesting that they were sleeping.

Despite the increase in response time during the night (Fig. 3 and Table 3.), three of the sessions (WEN A, WEN C, SAY B) did not have significant change in accuracy (number of missed targets) over the course of the sessions or between daytime and nighttime hours. One session, WEN B, which had an overall accuracy of 87 percent did have a significant increase of missed targets over the vigilance session (logit 4.418, $p<0.001$) and during the nighttime (logit 595.65, $p<0.001$). While SAY A did show a significant increase of missed targets over the course of the session (logit 2.483, $p=0.003$) she maintained an overall accuracy of 93 percent and she was not more likely to miss targets at night (logit 7.123, $p=0.252$).

Target response times significantly differed between the two dolphins ($F=24.814$, $p=0.016$), ranging from 4.18 to 19.06 s for SAY (9.45 ± 3.01) and from 0.11 to 18.40 s for WEN (6.84 ± 1.7). During the day, especially between the hours of 0900 to 1600 (when trainers were working with dolphins in nearby enclosures) both dolphins were actively swimming around their enclosures, obviously alert to their surroundings. While respiratory rates did follow a diurnal pattern, increasing during the day while target response time decreased (Fig. 3), there was not a significant difference between day and night ($F=13.127$, $p=8.426$). Nor did the respiratory rate change over the course of the vigilance sessions ($F=1.224$, $p=0.425$). During quieter times, after training activity with other

dolphins in nearby enclosures slowed or ceased, WEN would usually float near the center of his enclosure for long periods, broken occasionally by a slow circular swim around the enclosure. In response to the target tone, WEN would pump his tail flukes two or three times to propel himself to the response paddle; then, after taking a fish reward, returned to the floating position near the center of the enclosure. Although there was some slowing of his response time during the night WEN's overall response times were not nearly as variable as those of SAY (Fig. 3 and Table 3).

In contrast to the WEN's behavior "after hours", SAY floated less and usually continued to circle the enclosure. Especially at night this circling became very slow and stereotyped as has been previously described by McCormick, (1969), Flanigan (1974), and Goley (1999).

Eye closure could not always be determined from our infrared illuminators and underwater camera system (Fig. 1). Video records never clearly indicated simultaneous closure of both eyes. The dolphin might approach the target at any angle thus recognition of eye state (open or closed) was especially difficult at night. However, it is interesting and possibly significant that closure of either eye was only observed once during the nighttime hours (Fig. 6).

Sleep deprivation is known to change results of many clinical blood measurements (Dinges et al., 1994; Suchecki, et.al, 1998; Heiser, et. al, 2000). Overall, we quantified 57 parameters from whole blood, serum, and plasma samples taken at the start and at the end of two of the vigilance sessions (WEN A and B). Results from 16 parameters are presented in Table 1. For example, leukocyte measures, cortisol, epinephrine, norepinephrine, and dopamine showed no consistent changes before and after the vigilance sessions and all parameters of interest remained within normal ranges (St. Aubin et. al, 1996; Ridgway et. al, 1970).

Discussion

The dolphins' lack of a significant vigilance decrement over the 120 hours sharply contrasts both field observations and laboratory studies of other species (Dukas and Clark, 1995). The range of response times from target tone to paddle press probably depended to some extent on where an individual dolphin happened to be in the enclosure when the target tone sounded. During the vigilance sessions, SAY did not remain near the response paddle or the stimulus hydrophone on another side of the enclosure. Rather the animal continued to swim around the enclosure much in her usual manner. The subject dolphins could hear the vocalizations of other dolphins in the group generally located more than 20 m away from the outside border of the subject's enclosure.

Vocalizations from the non-participants may have at times masked or delayed the subject dolphin's response to the target tone, however such times were surely infrequent since the slowest response times in the middle of the night were coincident with the periods when vocalizations from other dolphins were very infrequent compared to the daylight hours (data not shown). The 70 kHz frequent tones and the 70 kHz target tone were near threshold and not likely heard by the non-participants who were away in other enclosures. The high frequency of 70 kHz was selected because both dolphins could hear this frequency and because 70 kHz would be absorbed rapidly in seawater (Urick, 1982) and not be an annoyance to other animals further away in other enclosures in the area.

SAY's slowed response time, especially during the hours between 2100 to 0400 hours, probably depended somewhat on where she was in her slow circle when the target tone sounded. If she was swimming away from the response paddle, she tended to complete the circle and press the paddle as she passed it. In contrast to SAY, WEN tended to spend more time simply floating near the center of the enclosure. His response times were shorter (6.83 ± 0.56 vs. 9.65 ± 0.61) on average

and did not show as much slowing during the night (Fig. 3 and Table 3). However, his response times during the night were significantly slower than during the day.

Both dolphins had lower respiratory rates in the middle of the dark period at times when they would ordinarily have been resting or sleeping (Fig. 4). The slowed respiratory rates corresponded with periods of the slower response time for paddle press to the target tones. The slowed respiratory rates and response times also coincided with periods of floating posture or quiescent hanging behavior (WEN) and slow circular swimming (SAY). Since the amount food reward delivered after correct target tone response was randomized by the observers and controlled to deliver the same amount of food over the 24-hour period, food consumption cannot account for differences in response time or respiratory rate. For these experiments, dolphins appeared to shift seamlessly from consuming their daily ration in an 8-hour period to having the same ration spread over the 24-hour period.

Sleep rebound is a universal finding in sleep deprivation in humans and other mammals (Tobler, 1985; Benington and Heller., 1999; Franken P., 2002). Neither dolphin showed marked sleep rebound after any of the 120-hour vigilance sessions as indicated by our observations of behavior and posture (McCormick, 1969; Flanigan 1974) comparing the 24 hours before and after the 120-hour vigilance session (Fig. 3). The ability to sleep with one hemisphere and to have each brain hemisphere sleeping alternately (Mukhametov, 1984, 1987; Ridgway, 2002) seems the most likely explanation for the dolphin's ability to detect and respond to the acoustic targets randomly presented over the continuous five days of these sessions.

During EEG recordings of dolphin uni-hemispheric sleep, as indicated by slow waves, the eye contra lateral to the sleeping hemisphere was closed about 75% of the time in the studies of Lyamin et al. (2004). In the current vigilance studies it seems remarkable that closure of either eye

was observed only once during nighttime target paddle approaches (Fig. 6). Video records never clearly indicated closure of both eyes at night or during daylight. Eye closure could usually, but not always, be determined from our infrared illuminators and underwater camera system. The dolphin might approach the target at any angle thus recognition of eye state (open or closed) was often difficult at night. Occasional eye closure was observed during daylight hours (Fig. 6), however, it is interesting and possibly significant that closure of either eye was only observed once during the nighttime hours. An animal with both eyes below the surface at night may have little need for eye closure to reduce illumination that might interfere with sleep. On the other hand, bright sun light penetrating surface waters during daylight hours might influence the need for eye closure.

The absence of eye closure during nighttime target approaches (Fig. 6) suggests that if the dolphin were asleep in either hemisphere, the target tone caused an arousal during the target approach and paddle press followed by collection of the fish reward near the hydrophone station on the adjacent side of the enclosure (Fig. 1). During the nighttime periods of slowed respiration and slowed response time, the dolphin's posture and behavior was consistent with sleep (McCormick, 1969; Flanigan, 1974; Goley, 1999; Lima et al., 2005) except during the brief periods of 25 seconds or so required for target approach and taking of the food reward. The slowed response times during the nighttime period could be viewed as an increased arousal threshold. If the slowed response time is an indication of increased arousal threshold, it suggests that the target tone response involves an arousal even from uni-hemispheric sleep. It seems remarkable that the dolphin can discriminate the target tone from the non-salient background tones, swim to the target response paddle, then swim to the adjacent side of the enclosure and eat the fish reward and repeat this 40 or 50 times during the nighttime period and show no signs of sleep deprivation.

