

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-01-0188	
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PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 2006		2. REPORT TYPE Journal Article		3. DATES COVERED (From - To)	
4. TITLE AND SUBTITLE Dolphin continuous auditory vigilance for five days				5a. CONTRACT NUMBER N66001-05-C-0040	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
				5d. PROJECT NUMBER	
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				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) ¹ SSC San Diego ² UCSD ³ BIOMIMETICA Marine Mammal Program School of Medicine 7951 Shantung Drive 53560 Hull Street 9500 Gilman Drive Santee, CA 92071 San Diego, CA 92152-5001 La Jolla, CA 92093				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited. <i>US Govt Joint Work.</i>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT This report documents the first use of magnetic resonance images (MRIs) of living dolphins to register functional brain scans, allowing for the exploration of potential mechanisms of unihemispheric sleep. Diazepam has been shown to induce unihemispheric slow waves (USW), therefore we used functional imaging of dolphins with and without diazepam to observe hemispheric differences in brain metabolism and blood flow. MRIs were used to register functional brain scans with single photon emission computed tomography (SPECT) and positron emission tomography (PET) in trained dolphins. Scans using SPECT revealed unihemispheric blood flow reduction following diazepam doses greater than 0.55mg kg⁻¹ for these 180-200 kg animals. Scans using PET revealed hemispheric differences in brain glucose consumption when scans with and without diazepam were compared. The findings suggest that unihemispheric reduction in blood flow and glucose metabolism in the hemisphere showing USW are important features of unihemispheric sleep. Functional scans may also help to elucidate the degree of hemispheric laterality of sensory and motor systems as well as in neurotransmitter or molecular mechanisms of unihemispheric sleep in delphinoid cetaceans. The findings also demonstrate the potential value of functional scans to explore other aspects of dolphin brain physiology as well as pathology. Published in <i>The Journal of Experimental Biology</i>, Vol. 209, 2006, pp. 2902-2910.					
15. SUBJECT TERMS dolphin <i>Tursiops</i> functional imaging diazepam SPECT scan MRI scan PET scan brain unihemispheric sleep slow wave hemisphere autonomy					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON James J. Finneran
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (Include area code) (619) 767-4098
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Dolphin continuous auditory vigilance for five days

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Accepted 26 June 2006

Summary

The present report describes the first study of continuous vigilance in dolphins. Two adult bottlenose dolphins (*Tursiops truncatus*), WEN (male) and SAY (female), maintained a very high detection rate of randomly presented, infrequent, 1.5-s target tones in a background of frequent 0.5-s equal-amplitude tones over five continuous 120-h sessions. The animals were able to maintain high levels (WEN 97, 87, 99%; SAY 93, 96%) of target detection without signs of sleep deprivation as indicated by behavior, blood indices or marked sleep

rebound during 24 h of continuous post-experiment observation. Target response time overall ($F=0.384$; $P=0.816$) did not change between day 1 and day 5. However, response time was significantly slower ($F=21.566$, $P=0.019$) during the night (21.00–04.00 h) when the dolphins would have ordinarily been resting or asleep.

Key words: dolphin, *Tursiops*, vigilance, diurnal rhythm, brain, uni-hemispheric sleep, hemisphere autonomy.

Introduction

It has long been known that dolphins can swim continuously for very long periods without resting (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 (McCormick, 1969; Flanigan, 1974; Goley, 1999; Lima et al., 2005). 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; Mukhametov, 1987; Ridgway, 2002). Left and right hemispheres alternate between SWS and waking by some still 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) and 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 to '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 its afferent inputs' (Lilly, 1964), in order to maintain vigilance (Mukhametov, 1984). We have investigated the ability of dolphins to maintain continuous high levels of vigilance, ostensibly by the use of uni-hemispheric sleep. The hypothesis was that when one hemisphere is tired, the dolphin can switch to the other awake and vigilant hemisphere (Lilly, 1964; Supin et al., 1978; Mukhametov, 1984, 1987; Ridgway, 2002). Here we report our findings on auditory vigilance over five separate 120-h sessions on two dolphins.

Materials and methods

All experiments were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committees of the Navy Marine Mammal Program, Space and Naval Warfare Systems Center, San Diego, CA, USA.

Two adult bottlenose dolphins (*Tursiops truncatus* Montagu 1821), WEN (male, age 21, mass 196 kg, length 250 cm) and SAY (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 9×9 m open air and open water enclosures (Fig. 1A) under ambient light conditions in San Diego Bay. Behavioral hearing tests with both dolphins showed that they could hear the 70-kHz tones well and reliably respond at a level about 20 dB above their audiometric threshold in the bay based on behavioral thresholds measured at 1 m from the source (Finneran and Houser, 2006). The high frequency of 70 kHz was chosen because of the low ambient San Diego Bay noise at that frequency and because 70 kHz would be absorbed rapidly in seawater (Urlick, 1982) and not be an annoyance to other animals further away in other enclosures in the area.

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 cameras (PC503IR, Supercuits, Liberty Hill, TX, USA; Fig. 1A,B). Our observations 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.

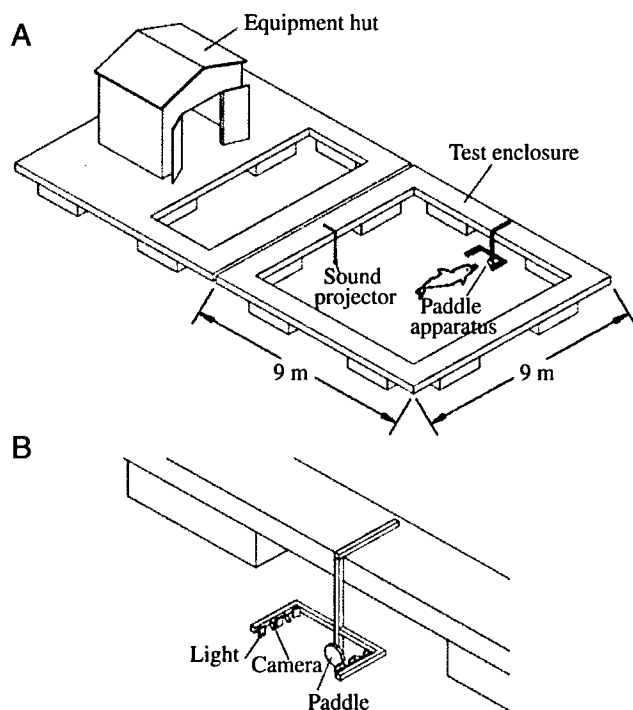


Fig. 1. Experimental setup for the dolphin vigilance sessions. (A) Equipment hut and test enclosure showing location of response paddle and the sound projector on the adjacent side of the pen where the dolphin was rewarded. (B) A close-up drawing showing the response paddle apparatus, the underwater infrared cameras, and infrared light sources that allowed for visualization of the dolphin's eyes on target approaches.

Under computer control, the 1.5-s target stimulus randomly replaced the 0.5-s tone at intervals between 4 and 24 min. 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 both 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 s. (The false alarm wait period was installed because during the initial training period, dolphin WEN would sometimes rest against the response paddle for long periods during the first few nights of training.) The dependent variables were the time to respond to the signal and the number of missed or ignored targets.

Prior to the vigilance sessions, 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-h 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-h period of the vigilance session.

After conducting some shorter training sessions, during which false alarms and WEN's night time paddle resting behavior were eliminated, five 120-h vigilance sessions were conducted between July and February as dolphins and human observers were available. The sessions required observation for 7 days continuously, including a 1-day observation with a video record before the 5 days of continuous vigilance and 1 day after the 5 days with video record to observe possible sleep rebound. Three vigilance trials were conducted for WEN (A, B, C) and two for SAY (A, B). Infrared lights, monitors 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.

Respiratory rate (number of breaths per 5 min) 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-h period before and after the session was used as an indicator of sleep rebound following the 120-h vigilance session.

The dolphins were trained to present their flukes for blood sample collection. On two trials with WEN (A and B), non-fasting blood samples were collected for complete blood cell count (CBC), serum chemistry, catecholamines and selected hormone analyses (Table 1). Blood was collected at the same

Table 1. Sixteen blood parameters from blood samples collected before and after two 120-h vigilance sessions

Blood variables	WEN before A	WEN after A	WEN before B	WEN after B
White blood cells ($\times 10^{-3} \mu\text{l}^{-1}$)	8.8	8.5	6.5	6.0
Seg. neutrophils (%)	61	55	64	66
Lymphocytes (%)	12	13	19	13
Monocytes (%)	2	8	4	5
Eosinophils (%)	25	20	13	16
Hemoglobin (g l^{-1})	146	148	141	145
Hematocrit (%)	42.4	42.8	41.3	41.9
Sedimentation rate (60 min)	6	7	15	12
Glucose (mg l^{-1})	1190	1050	1190	1130
Globulins (g l^{-1})	24	27	26	27
Albumen/globulin ratio	1.8	1.6	1.6	1.7
Calcium (mg l^{-1})	8.8	9.4	9.0	9.6
Cortisol ($\mu\text{g l}^{-1}$)	6.6	3.6	4.4	6.1
Dopamine (pg ml^{-1})	26	24	23	>20
Norepinephrine (pg ml^{-1})	564	568	806	868
Epinephrine (pg ml^{-1})	44	50	79	59

time of the day both immediately prior to the start and at the end of the 120-h vigilance sessions. Samples were analyzed by two reference laboratories with experience in performing these specific analyses on dolphin blood (Quest Diagnostics, San Diego, CA; and ARUP Laboratories, Salt Lake City, UT, USA). Handling of blood was in accordance with previously established protocols by Romano et al. (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-h periods within the 120-h vigilance session and between daytime (09.00–16.00 h) and night time (21.00–04.00 h). Target response time or respiratory rate were used as the dependent variables, and 24-h periods of the vigilance session (1–24 h, 25–48 h, 49–72 h, 73–96 h, 97–120 h), time of day (daytime *versus* night time), and animal as the independent variables. Logistic regressions were used to evaluate changes in the accuracy (correct *vs* missed targets) by 24-h periods and by time of day (daytime *vs* night time) within each session. Probabilities less than 0.05 were considered significant.

Results

It is apparent from all five vigilance sessions that a diurnal pattern was present in the target response time and respiratory rate of both dolphins. This is illustrated in Fig. 2 for one of the sessions with each dolphin. 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, target response time was significantly slower ($F=21.556$, $P=0.019$) during the night time (21.00–04.00 h) compared to daytime (09.00–16.00 h). Whereas a diurnal pattern

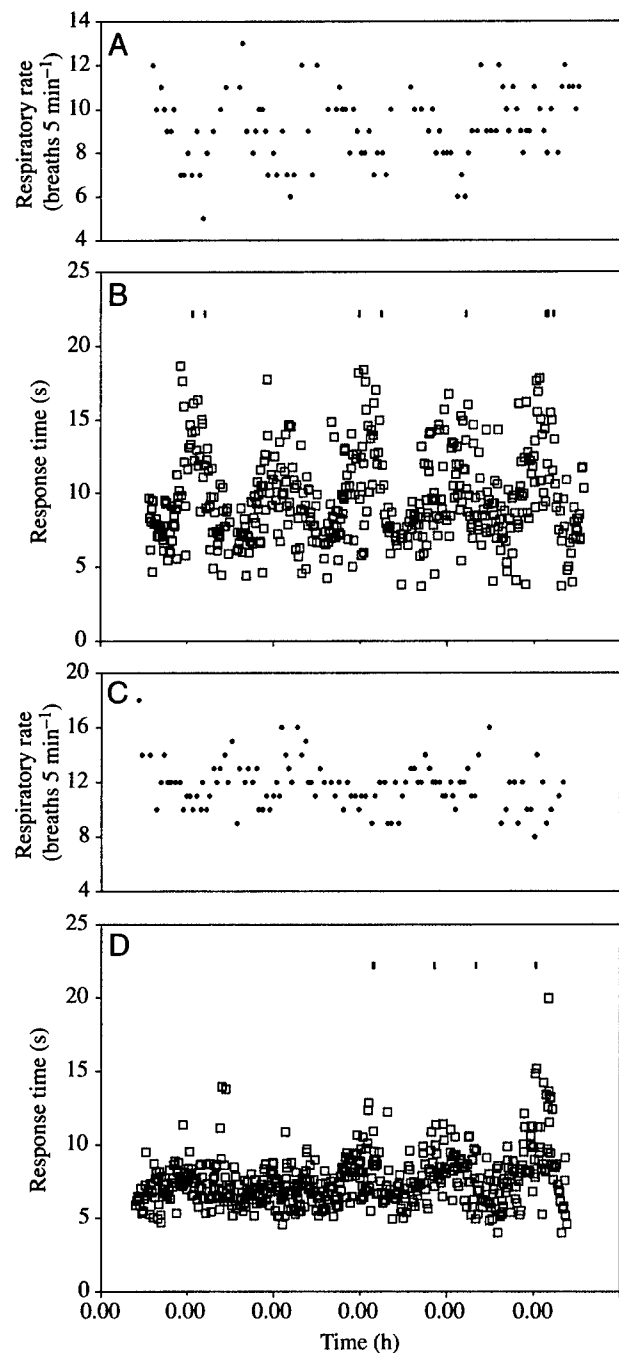


Fig. 2. (A,C) Respiratory rates (plus signs) and (B,D) responses to target tones (squares, correct trial; dashes, missed trial) during 120 h of continuous vigilance for trials SAY B (A,B) and WEN C (C,D). 0.00 indicates midnight.

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 night time (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

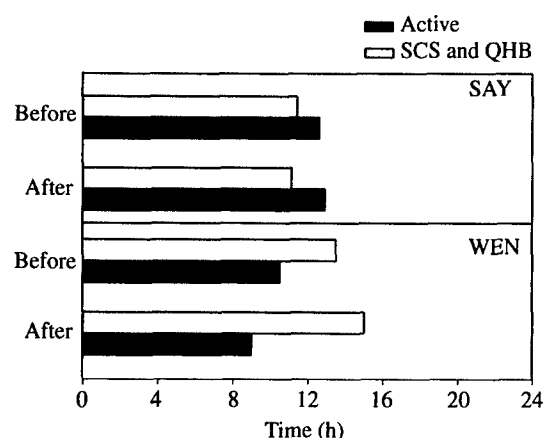


Fig. 3. Behavior scored (30-s intervals) from video recordings during 24 h before and 24 h after 120-h vigilance sessions for SAY and WEN. QHB (quiescent hanging behavior) and SCS (slow circular swimming) indicate sleep behaviors.

hanging behavior (QHB) or slow circular swimming (SCS) suggesting that they were resting.

The dolphins detected $94.2 \pm 2.26\%$ (SAY) and $94.36 \pm 6.33\%$ (WEN) of the target stimuli throughout all sessions with little

vigilance decrement as indicated by target response time and accuracy (Fig. 2). When the 24 h before the start of the vigilance session was compared with the 24 h following the vigilance sessions (Fig. 3) 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).

The dolphins showed no indications of a vigilance decrement during the 5 days. For three of the sessions (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 sessions, WEN B (logit 4.418, $P < 0.001$) and SAY A (logit 2.483, $P = 0.003$), the number of missed targets did significantly increase over the session. Specifically, more targets were missed on day 5 (97–120-h period) than during previous days of the session.

The change in target response time was less than 1 s per 24-h period for 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. 3 indicates that there was not a substantial increase in sleep behavior for the 24 h after the 120-h vigilance sessions for either dolphin.

Table 2. Descriptive statistic for target responses of both dolphins for each 24-h period of the vigilance sessions

	Time period (h)	Correct targets	Range	Response time (s)				Missed targets
				Min.	Max.	Mean	s.d.	
SAY A	1–24	89	13.55	4.18	17.73	8.39	2.51	0
	25–48	112	14.67	4.39	19.06	9.54	3.27	3
	49–72	85	13.90	4.56	18.46	10.48	2.87	3
	73–96	106	12.14	5.00	17.14	10.08	2.66	4
	97–120	84	14.12	4.94	19.06	10.35	3.25	10
SAY B	1–24	89	13.95	4.45	18.40	9.50	3.08	4
	25–48	92	5.16	4.39	9.55	9.04	2.49	1
	49–72	91	5.05	3.79	8.84	9.67	2.49	1
	73–96	100	5.22	3.68	8.90	9.85	3.32	0
	97–120	92	6.52	3.68	10.20	9.59	2.80	3
WEN A	1–24	104	7.86	3.13	10.99	6.25	1.84	2
	25–48	93	10.17	3.07	13.24	6.17	1.32	0
	49–72	94	5.93	3.46	9.39	6.06	1.08	3
	73–96	102	6.59	3.40	9.99	5.92	1.20	2
	97–120	88	10.00	0.11	10.11	5.67	1.40	2
WEN B	1–24	96	7.36	3.63	10.99	7.39	1.76	7
	25–48	90	9.29	3.95	13.24	6.76	1.55	7
	49–72	93	7.30	2.09	9.39	6.46	1.34	2
	73–96	95	6.09	3.90	9.99	6.80	1.18	8
	97–120*	74*	5.77*	4.34*	10.11*	6.95*	1.21*	22*
WEN C	1–24	101	9.23	4.72	13.95	7.28	1.38	0
	25–48	108	9.23	4.56	13.79	6.97	1.29	0
	49–72	106	7.91	4.94	12.85	7.56	1.54	2
	73–96	101	7.90	4.95	12.85	7.85	1.57	0
	97–120	104	15.93	4.01	19.94	8.33	2.67	1

*During the last night time of the WEN B test the dolphin ignored all target stimuli for 4 h and appeared to be asleep.

Table 3. Descriptive statistic for daytime (09.00–16.00 h) and night time (21.00–04.00 h) within each 24-h period of the vigilance sessions

	Time period (h)	Daytime						Night time					
		Correct targets	Response time				Missed targets	Correct targets	Response time				Missed targets
			Min.	Max.	Mean	s.d.			Min.	Max.	Mean	s.d.	
SAY A	1–24	25	4.18	12.52	8.19	2.14	0	29	5.27	17.74	9.72	2.83	0
	25–48	28	4.50	19.01	9.08	3.21	3	33	4.78	18.79	10.49	2.98	0
	49–72	17	4.56	13.34	8.88	2.50	0	27	7.08	14.77	11.32	2.45	3
	73–96	34	5.16	16.87	10.03	2.55	1	30	5.00	17.08	10.81	2.96	3
	97–120	25	4.94	12.25	8.23	2.03	3	25	5.66	19.06	11.40	3.66	7
SAY B	1–24	23	4.45	10.60	7.99	1.60	1	25	5.55	18.67	12.29	3.34	3
	25–48	26	4.84	11.10	7.80	1.48	0	27	4.61	17.74	10.49	2.68	1
	49–72	24	3.79	14.88	7.18	1.99	0	30	5.00	18.40	11.81	3.59	1
	73–96	26	4.01	14.34	8.57	1.78	0	30	5.38	16.75	11.09	2.92	0
	97–120	30	3.95	14.34	7.91	2.19	0	26	3.79	17.80	12.14	3.29	3
WEN A	1–24	29	3.13	18.40	5.85	2.83	1	33	3.63	8.85	6.32	1.16	1
	25–48	25	3.51	9.55	6.12	1.46	0	26	4.22	8.01	6.29	1.06	0
	49–72	26	3.96	8.19	5.72	1.04	3	31	5.10	8.84	6.51	0.95	0
	73–96	30	3.40	8.13	5.63	1.14	1	31	3.90	8.95	6.11	1.40	1
	97–120	26	3.84	7.19	5.72	0.91	1	25	0.11	10.22	6.02	1.99	1
WEN B	1–24	25	3.63	10.11	6.78	1.64	7	31	4.84	10.99	8.19	1.96	0
	25–48	24	4.01	8.84	6.44	1.28	4	29	5.05	10.49	7.35	1.31	3
	49–72	24	4.18	7.85	6.15	1.06	2	30	2.09	9.39	6.65	1.63	0
	73–96	28	4.73	9.34	6.31	1.07	3	27	3.90	8.95	6.95	1.12	5
	97–120*	30*	4.34*	8.35*	6.33*	1.00*	0*	11*	5.66*	8.35*	6.86*	0.90*	22*
WEN C	1–24	28	4.95	13.95	7.13	1.96	0	31	5.77	11.37	7.77	1.03	0
	25–48	32	5.16	13.79	7.16	1.66	0	35	4.56	10.88	7.15	1.29	0
	49–72	34	4.94	9.00	6.69	0.94	0	32	5.49	12.85	8.54	1.71	2
	73–96	27	5.00	9.62	6.77	1.08	0	32	6.21	11.42	8.64	1.43	0
	97–120	28	4.01	9.12	6.44	1.31	0	33	5.27	15.16	9.90	2.28	1

*During the last night time of the WEN B test the dolphin ignored all target stimuli for 4 h and appeared to be asleep.

Despite the slower response time during the night (Fig. 2 and Table 3), three of the sessions (WEN A, WEN C, SAY B) did not have a significant change in accuracy (number of missed targets) over the course of the sessions or between daytime and night time hours. One session, WEN B, which had an overall accuracy of 87%, did have a significant increase of missed targets over the vigilance session (logit 4.418, $P < 0.001$) and during the night time (logit 595.65, $P < 0.001$). The significant increase in missed targets during this session and the night time can be attributed to a 4-h non-responsive period during the night time of the 97–120-h period (Table 3). Although in session A, SAY showed 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% and was not more likely to miss targets at night (logit 7.123, $P = 0.252$).

Target response times (Fig. 4) significantly differed between the two dolphins ($F = 24.814$, $P = 0.016$), ranging from 4.18 to 19.06 s for SAY (9.45 $\pm$ 3.01) and from 0.11 to 18.40 s for WEN (6.84 $\pm$ 1.70). During the day, especially between the hours of 09.00 to 16.00 (when trainers were working with dolphins in nearby enclosures) both dolphins were actively swimming

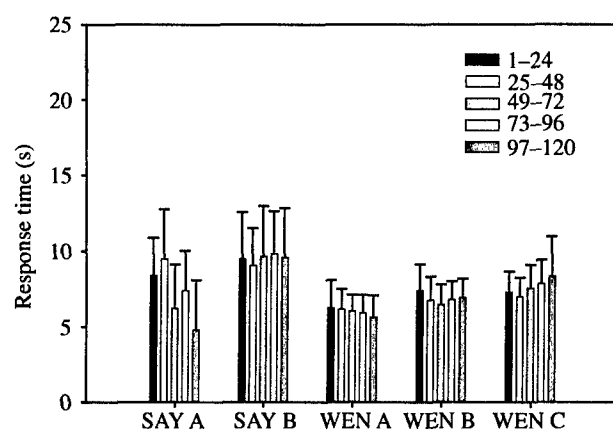


Fig. 4. Mean target response time ($\pm$ s.d.) for each 24-h period (see key) within the 120-h vigilance sessions.

around their enclosures, obviously alert to their surroundings. 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. 2 and Table 3).

In contrast to 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 Flanigan (Flanigan, 1974) and Goley (Goley, 1999).

Eye closure could not always be determined from our IR 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, closure of either eye was only observed once during the night time hours (Fig. 5).

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 h sharply contrasts both field observations and

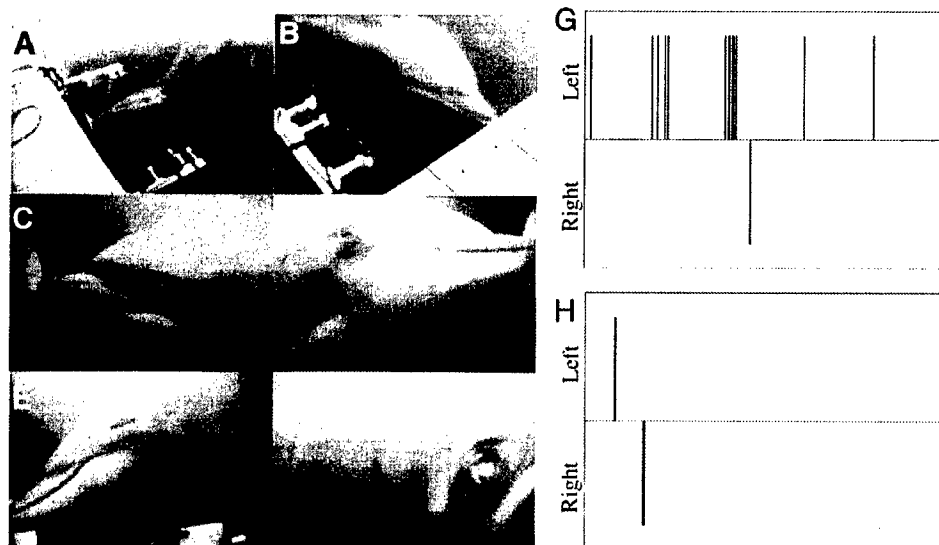
laboratory studies of other species (Dukas and Clark, 1995; Siegel, 2005). It is an almost universal finding that prolonged periods of sleep deprivation cause a reduction in vigilance performance (Davies and Parasuraman, 1982; Beaumont et al., 2001; Horne and Pettitt, 1985; Gillberg and Akerstedt, 1998). 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 either the response paddle or the stimulus hydrophone on the other 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 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 to be heard by the non-participants who were away in other enclosures.

SAY's slowed response time, especially during the hours between 21.00 to 04.00, probably depended somewhat on where she was in her slow circle swim 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 on average shorter (6.83 ± 0.56 vs 9.65 ± 0.61) and did not show as much slowing during the night (Fig. 2 and Table 3).

The review by Klinowska (Klinowska, 1986) or any other studies of which we are aware did not consider diurnal

Fig. 5. A dolphin approaches the response paddle, as seen from above (A,B). Underwater camera showing left eye closed (C,E) and right eye open (D,F) as the dolphin hits the response paddle during vigilance sessions. Bar graph showing observed eye closures during 120-h vigilance sessions for SAY A (G) and WEN C (H). Shaded areas indicate night time observations.



variability of respiratory rates in dolphins. In the current study, both dolphins had lower respiratory rates in the middle of the night time period, at times when they would ordinarily have been resting or sleeping (Fig. 3). The slowed respiratory rates corresponded with periods of slower target response times. 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 of 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-h period, food consumption cannot account for differences in response time or respiratory rate. During these experiments, dolphins appeared to shift seamlessly from consuming their daily ration in an 8-h period to having the same ration spread over the 24-h period.

Sleep rebound is a universal finding in sleep deprivation in humans and other mammals (Tobler, 1985; Benington and Heller, 1999; Franken, 2002). Neither dolphin showed marked sleep rebound after any of the 120-h vigilance sessions as indicated by our observations of behavior and posture (McCormick, 1969; Flanigan, 1974) comparing the 24 h before and after the 120-h vigilance session (Fig. 2).

It is known that some birds can utilize uni-hemispheric sleep to maintain visual vigilance (Rattenborg et al., 1999; Lima et al., 2005). The dolphin's ability to sleep with one hemisphere and to have each brain hemisphere sleeping alternately (Supin et al., 1978; Mukhametov, 1984; Mukhametov, 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 5 days of these sessions without signs of sleep deprivation.

During EEG recordings of dolphin uni-hemispheric sleep, as indicated by slow waves, the eye contralateral to the sleeping hemisphere was closed about 75% of the time in the studies of Lyamin et al. (Lyamin et al., 2004). In the current vigilance studies it seems remarkable that closure of either eye was observed only once during night time target paddle approaches (Fig. 5). 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 IR 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. 5). Bright sun light penetrating surface waters during daylight hours or visible light used for observation at night might influence the need for eye closure. We used IR illumination at 850 nm to illuminate the dolphin approaching the target paddles both at the surface and underwater because these events were not visible to our trainers without the infrared system.

It is interesting and possibly significant that closure of either eye was only observed once during the night time hours. Goley observed night time eye closures by visible light (Goley, 1999); however, an animal with both eyes below the surface at night where visible light is minimal may have little need for eye closure to reduce illumination that might interfere with sleep.

Supin et al. have observed slow waves in the hemisphere opposite open eyes, and mentioned that eye closure was not a reliable indicator of sleep in the opposite hemisphere (Supin et al., 1978). Mukhametov noted that the open eye could perform a sentinel function 'regardless of whether the contralateral hemisphere was asleep or awake' (Mukhametov, 1984).

The absence of eye closure during night time target approaches (Fig. 5) suggests that if the dolphin was asleep in either hemisphere with one or both eyes closed, 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 night time periods of slowed respiration and slowed response time, the dolphin's posture and behavior was consistent with rest (McCormick, 1969; Flanigan, 1974; Goley, 1999; Lima et al., 2005) except during the brief periods of 25 s or so required for target approach and taking of the food reward. The slowed response times during the night time 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. The dolphin might be able to process the auditory cue, awaken, and initiate the behavioral response. Almost certainly the dolphin auditory system is well connected to brainstem alerting systems, however, 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 25 or 35 times during the night time period and show no signs of sleep deprivation.

Evoked potential studies have shown that the sleeping human brain can detect and discriminate auditory stimuli in an odd-ball paradigm that bears some similarity to the task put to our dolphins (Cote, 2002). However, the time between the dolphin's targets (the odd stimulus) is much longer than in the human electrophysiological experiments. In addition, our dolphins made a distinct behavioral response by swimming to the side of the enclosure to press a paddle and then go to the adjacent side to receive and eat their fish reward.

Only short-term periods of bi-hemispheric sleep have been documented in un-anesthetized dolphins (cf. Supin et al., 1978; Ridgway, 2002). McCormick has observed dolphins in a behavioral state that appeared to be sleep with both eyes closed (McCormick, 1969). It is possible that the 4-h non-responsive period in one of the sessions (WEN B) represented periods of bi-hemispheric sleep but this is not possible to determine from our observations.

The findings in this study document for the first time that dolphins can maintain continuous auditory vigilance for 5 days (120 h). There was a prominent diurnal pattern present in the target response time and an apparent diurnal pattern in the respiratory rates. The dolphins were able to maintain a high level of vigilance even during night time periods of slowed target response time when they appeared to be resting or sleeping.

The animal care and training staff of the Space and Naval Warfare Systems Center were critical for the training and care of the dolphin subjects. We thank Dr Douglas Deutschman for his help with statistical analysis, and Drs Mark Xitco, Eric Jensen, Cynthia Smith, John Carney, Brett Giroir, Amy Kruse and Lisa Ely for support and encouragement. This study was supported by the Defense Advance Research Projects Agency (DARPA) under SSC San Diego Contract N66001-05-C-0040.

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