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Continuous Pontine Cholinergic Microinfusion via Mini-Pump Induces Sustained Alterations in Rapid Eye Movement (REM) Sleep[1]

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SHIROMANI, P. J. AND W. FISHBEIN. *Continuous pontine cholinergic microinfusion via mini-pump induces sustained alterations in rapid eye movement (REM) sleep.* PHARMACOL BIOCHEM BEHAV **25**(6) 1253–1261, 1986.—Although there is much evidence that single microinjections of cholinomimetics into the pontine reticular formation (PRF) evokes rapid eye movement sleep (REMS), no study has yet demonstrated whether protracted manipulations of PRF cholinergic levels can produce sustained alteration of this sleep state. In this study, in rats, an indwelling, chronically implanted osmotic mini-pump was used to infuse carbachol, scopolamine, or saline solutions into various brainstem regions or the fourth ventricle for a period of five consecutive days. Throughout the period of pump operation, carbachol infusions chiefly in the PRF produced sustained REMS augmentation primarily during the night cycle, whereas scopolamine produced a sustained decrease in REMS primarily during the day cycle. The findings provide considerable support for a PRF cholinergic hypothesis of REMS generation and regulation and suggest that the alterations in REMS result from a muscarinic receptor mediated change in PRF neuronal activity.

Rapid eye movement sleep (REMS) Carbachol Scopolamine Pontine reticular formation (PRF) Chronic microinfusion

THERE is much evidence to suggest that cholinergic mechanisms within the pontine reticular formation (PRF) play an important role in triggering rapid eye movement sleep (REMS). In cats, for instance, acute microinjection of cholinergic agonists such as carbachol, bethanechol, or neostigmine directly into the PRF readily evokes some or all of the tonic and phasic components of REMS for the first few hours immediately following infusion [1, 3, 4, 18, 22, 32, 34–36, 38], while scopolamine blocks the cholinomimetic induced REMS [35]. Midbrain or medullary infusions, on the other hand, fail to evoke REMS [3,34]. Intraventricular infusion of hemicholinum, which inhibits the synthesis of acetylcholine (ACh) by blocking the transport of the essential precursor choline across the membrane of the terminal bouton, decreases REMS [12,21]. In addition, increased ACh is found during REMS in cortex [7,24] and striatum [16] of normal cats, and in ventricular perfusates of conscious dogs [20]. In normal humans [39], intravenous infusion of physostigmine or arecoline during non-REM sleep decreases the latency to REMS although infusion during or immediately after REMS prolongs arousal. In narcoleptic dogs [5,11], cholinomimetics increase the incidence of cataleptic episodes while muscarinic receptor blockers delay these episodes; nicotinic agents have no effect. Moreover, in narcoleptic dogs, increased muscarinic receptor binding is found in several pontine sites [5].

REMS is a sustained biological state that persists throughout the life-span of mammals. Much of the strongest evidence in support of a cholinergic-REMS trigger link is based on acute studies in which administration of cholinomimetics produces only short-term changes in REMS. While these studies document a cholinergic REMS triggering role for the PRF, they do not provide clues regarding the role of cholinergic mechanisms in REMS regulation over the long-term. Therefore, the purpose of the present study was to determine whether continuous microinfusion of cholinergic agents produce sustained alterations in REMS. The study employed an Alzet osmotic mini-pump to deliver solutions of

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either carbachol, scopolamine, or saline to various brainstem sites or the fourth ventricle, continuously for five days.

METHOD

Fifty-one male Holtzman rats weighing 400 g were used. The rats were housed individually in clear Plexiglas cages with wood shavings. Food and water were freely available, with lighting (7 a.m.–7 p.m., light on) and temperature (21°C±2) patterns constantly maintained.

The rats were implanted under Nembutal anesthesia (35 mg/kg, IP) with four extradural screws for recording electroencephalogram (EEG) activity, two wires sewn into dorsal nuchal muscles to record electromyogram (EMG) activity, and an L-shaped cannula. The cannula was a 1.5 cm length 21 gauge stainless-steel tube attached to a 1.5 cm piece of flexible PE tubing. Before implanting in the brain, the cannula and PE tubing were filled with 0.9% normal saline and the PE tubing was heat sealed. The cannula was lowered into the brain under stereotaxic control (David Kopf Instruments), and the part lying on the surface of the skull secured with dental cement.

One week after recovery from surgery the experiment was started. The animals were allowed two days to adapt to the recording chamber and cables. Beginning at 0900 hr on the third day a 24 hr baseline recording was obtained. At 0900 hr the following morning the Alzet osmotic mini-pump was surgically connected to the previously implanted cannula. The salient feature of this method of drug delivery is that small quantities of drug can be continuously infused at a constant rate (1.0 μl/hr) over a period of 7 days without any constraint in the animal's ability to move about. The pump infused solutions of either carbachol (0.5 μg/μl/hour), scopolamine (9.0 μg/μl/ hour) or saline (1 μl/hr) into discrete regions of the brainstem or fourth ventricle. Carbachol and scopolamine were dissolved in normal saline solution. The dose specific concentrations were derived from our own pilot studies which demonstrated that higher doses of carbachol (2 μg–10 μg per hour) produced progressively intense arousal and motor abnormalities while lower concentrations of scopolamine produced minimal REMS deficits.

At the time of pump implant, the animals were lightly anesthetized with ether. A one cm incision was then made in the skin to expose the underlying PE tubing. The PE tubing was cut to one cm length and the pump connected to it. The skin was then sutured and topical antibiotic applied to prevent infection.

Immediately after pump implant nine consecutive days of polygraph data were obtained. The animals were then removed from the recording chamber and housed in the laboratory vivarium for two weeks. They were then returned to the recording chamber, and after two days of adaptation a second 24 hour baseline sleep recording was obtained. The two baseline recordings were combined since there was no significant difference between the two sessions. Unless otherwise indicated all comparisons are within group comparisons to the pooled baseline. Following the second baseline sleep recording session the animals were administered a lethal dose of Nembutal and sacrificed. The brains were removed and placed in 10% Formaldehyde for later histological examination of cannula placement. Histological localization was made by consulting the rat brainstem atlas of Palkovits and Jacobowitz [33], and examining 40 μm thick frozen coronal sections stained with cresyl violet and luxol fast blue.

The EEG data were hand scored for waking, slow wave sleep (SWS) and REMS according to the standardized procedures employed in our laboratory over the past decade [14]. Briefly, waking was characterized by the presence of low-voltage fast EEG activity (6–12 Hz) and high EMG tone. SWS was noted when the EEG displayed high-voltage slow activity (1–3 Hz), and the EMG was decreased relative to waking. REMS was characterized by EEG low-voltage theta activity (4–8 Hz) and diminution of EMG tone relative to SWS. Throughout the recording session the animals were not disturbed except to replenish food and water. In addition, the behavior of the animals was monitored via a closed-circuit television system. Data analysis compared changes in percent total sleep time (TST), percent SWS of total recording time, and percent REMS of TST. Moreover, in order to discern the effect of the chronic infusions on the sleep rhythm, the 24 hr recordings were divided into 12 hr day (lights on), 12 hr night (lights off) periods (0700 hr–1900 hr day).

TABLE 1

THE TABLE SUMMARIZES THE TREATMENT CONDITIONS, INFUSION SITES AND THE NUMBER OF SUBJECTS IN EACH CONDITION

Treatment	Group	Infusion site	N
Saline	A	Pontine	5
	B	Medullary	6
	C	Fourth Ventricle	4
Carbachol	A	Pontine	5
	B	Medullary	8
	C	Left-side Medullary	5
	D	Fourth Ventricle	4
Scopolamine	A	Pontine	5
	B	Medullary	5
	C	Fourth Ventricle	4
			N=51

The first 24 hr immediately following pump implant were analyzed separately since a significant sleep disturbance, presumably associated with the pump implant surgery procedure, was noted during this time period (see Table 4). Behaviorally, however, the animals were alert and mobile within one-half hour after the pump was implanted.

The original experimental design was condensed by pooling within subjects, days 2 through 5, and days 6 through 9. These days were pooled because an in-vitro pilot study, employing three pumps and cannula + PE tubing filled with India ink immersed in saline solution, revealed that the pumps failed to discharge their contents beyond day 5. Normally the pumps are designed to operate continuously for 7 days but with the additional length of the cannula and PE tubing the operating period of the pump was reduced considerably. We, of course, recognize the possibility that the drugs might discharge for periods longer (or shorter) than we saw in the India ink experiment. However, only by performing the actual experiment and analyzing the data would we be aware of behavioral effects. Therefore, by pooling within subjects, a priori, we initially compared REMS changes during pooled baseline (baseline one and two), with drug infusion (days 2 through 5) and post-drug infusion (days 6 through 9) periods. A two-way repeated measures ANOVA

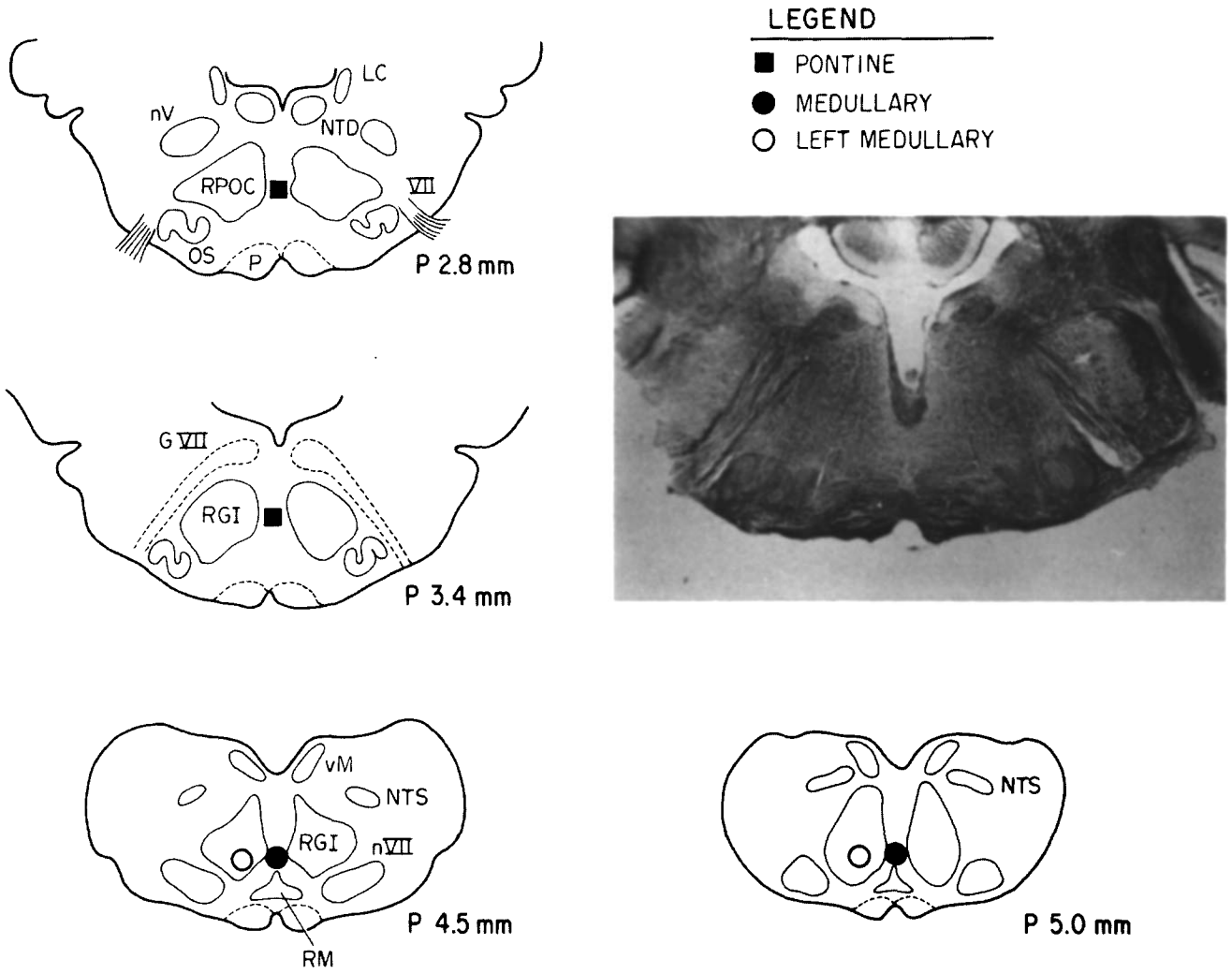

FIG. 1. The figure summarizes the location of the cannula tip for the pontine, medullary and left-medullary groups. Fourth ventricle cannula placements were at the pontine level. The symbols summarize the anterior-posterior range of the cannulae for all animals in each group. The photomicrograph depicts the location of the cannula at the level of the genu of the VII nerve (pontine group). The figures are adapted from Palkovits and Jacobowitz [33]. Abbreviations: G VII=genu of the seventh nerve; LC=locus coeruleus; NTD=dorsal tegmental nucleus; NTS=solitary tract nucleus; OS=superior olive; P=pyramids; RGI=nucleus gigantocellularis reticularis; RM=raphe magnus; RPOC=nucleus reticularis pontis caudalis and oralis; VM=medial vestibular nucleus; n V=nucleus of the fifth nerve; n VII=nucleus of the seventh nerve.

(row: infusion site; columns: baseline, drug, post-drug) was used to compare baseline with the drug infusion days. Post hoc comparisons were made using Neuman-Keul's (pairwise) or the Scheffe test (multiple pairs) [29]. In the event that post-hoc comparisons revealed significant differences between pooled baseline, drug and post-drug days, a one-way ANOVA was used to test for differences between each day of the experiment in the uncondensed original design. Dunnett's test ([29] p. 94) was then used to compare the first baseline period with each day of the experiment, including the second baseline period. In this test, in order for a pair of means to be significant, the difference between the means must exceed a critical value termed Dunnett's d'.

RESULTS

The animals were divided into four groups depending upon the histological location of the cannula: (A) midline at the level of the genu of seventh and nucleus of the sixth cranial nerves (pontine), (B) midline caudal to the genu of the seventh nerve with the caudal most cannula placement at the level of the solitary tract nucleus (medullary), (C) left-side (lateral 0.5–1.0 mm) at the level of group B (left-side medullary), and (D) the fourth ventricle at the level noted in group A. The histological classification and group assignments were made after the EEG records were scored, thus providing for blind control of the results. Independent groups of rats received either carbachol, scopolamine or saline in Groups A, B, and D. Group C consisted of rats receiving only carbachol. Table 1 indicates the number of subjects in each group and Fig. 1 summarizes the location of the cannula.

Saline: Day and Night Cycles

No statistically significant differences were found between pontine, medullary and ventricle saline groups in

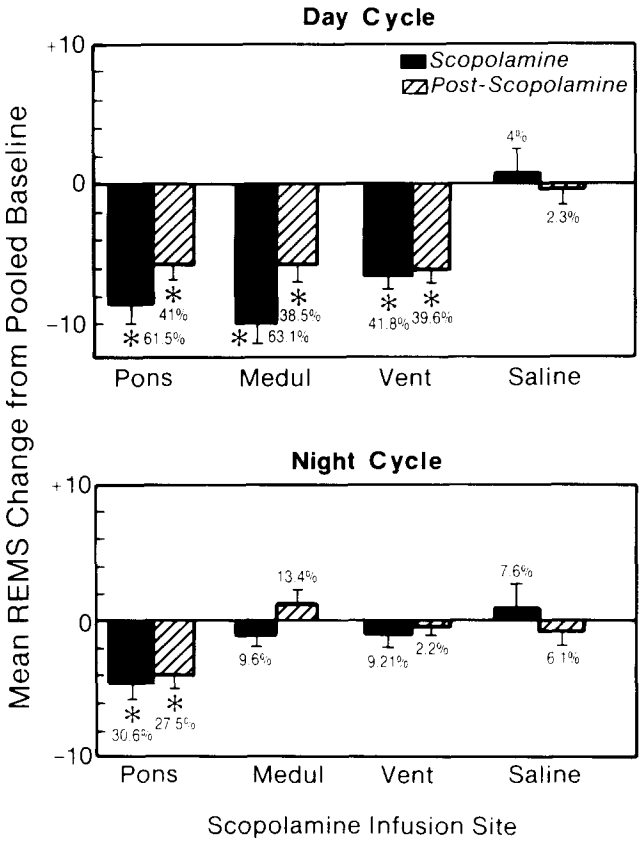

FIG. 2. Day-night changes in REMS as a result of continuous microinfusions of scopolamine into various brainstem sites. After a 24 hr baseline EEG recording the Alzet osmotic mini-pump was implanted and nine consecutive days of EEG data obtained. Two weeks later a second 24 hr baseline recording was obtained. The mean REMS change (ordinate) was derived by subracting the pooled baseline (baseline one and two) from scopolamine infusion (days 2 through 5) or post-scopolamine infusion (days 6 through 9) periods. The numbers express this difference as a percentage. During the day cycle (7 a.m.–7 p.m.) microinfusion of scopolamine produced a significant REMS decrease in all groups while during the night cycle the REMS decrease was noted only in the pontine group. The REMS decrease occurred during the period of scopolamine infusion and during the subsequent four days when the Alzet osmotic pumps had stopped working and no drug was infusing into the brainstem sites. An asterisk indicates significant difference ($p<0.05$) compared to within group pooled baseline and saline control.

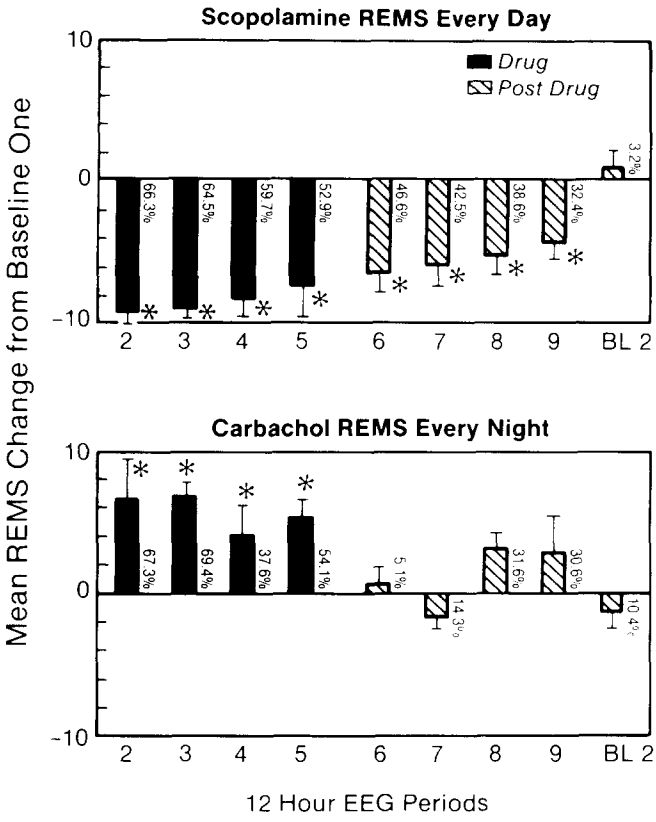

FIG. 3. Scopolamine and carbachol induced alterations in REMS. The figures summarize the day and night cycle mean (±SEM) difference in REMS from baseline one during every day of the experiment except the first day and night following pump implant. This time period was not included because REMS during the first 24 hours might have been influenced by the trauma associated with the pump implant procedure (see Table 4). Scopolamine produced a significant reduction in REMS during the period of drug infusion and during the subsequent four days. Note that the biggest REMS decrease occurred during day 2 while during the subsequent days REMS levels were not as severe even though the drug was continuously infused. In the lower figure carbachol produced a significant REMS augmentation during nights 2, 3, 4, and 5. During night 6 when the pumps stopped operating REMS returned to baseline levels. Asterisk indicates a significant difference ($p<0.05$) compared to baseline one.

REMS (Day cycle: $F(2,12)=0.54$, ns; Night cycle: $F(2,12)=0.57$, ns), TST (Day cycle: $F(2,12)=2.0$, ns; Night cycle: $F(2,12)=0.81$, ns), or SWS (Day cycle: $F(2,12)=2.49$, ns; Night cycle: $F(2,12)=1.14$, ns). Therefore, these groups were pooled (within group) into a single saline group ($n=15$), one group for the Day cycle, the other for the Night cycle and used as the control comparison for the carbachol and scopolamine groups.

Scopolamine: Day Cycle

Figure 2A summarizes the scopolamine induced change in REMS. During the period of drug infusion (days 2 through 5) scopolamine produced a significant decrease in REMS in the pontine (−61.5%; $F(2,50)=108.8$, $p<0.001$), midline medullary (−63.5%; $F(2,50)=144.7$, $p<0.001$), and the ventricle (−41.8%; $F(2,50)=65.8$, $p<0.001$) groups compared to pooled baseline. The significant reduction in REMS persisted into the subsequent four days (days 6 through 9). Dunnett's test was used to compare REMS during the first baseline period (unpooled baseline) with REMS during each day of the experiment, including the second baseline day. Figure 3A summarizes the change in REMS from baseline one during each day of the experiment in the pontine group. Scopolamine produced a significant reduction in REMS during the period of drug infusion and during the subsequent four days when the drug may not have been infusing into the brain (Dunnett's $d'=2.89$, $p<0.05$). REMS levels during baseline two were not significantly different from baseline one. A similar stepwise change in REMS was found in the

TABLE 2

MEAN PERCENT TOTAL SLEEP TIME (TST) ± SEM DURING THE DAY-NIGHT CYCLES FOR ALL GROUPS

		Baseline	Drug	Post-drug
Saline	Day	67.9	68.6	70.0
		(1.7)	(1.4)	(1.1)
	Night	38.3	43.6	41.4
		(1.8)	(2.0)	(2.6)
Scopolamine	Day			
Pontine		66.3	66.9	68.3
		(4.5)	(3.0)	(3.2)
Medullary		69.2	63.3*	61.2*
		(1.7)	(1.3)	(3.0)
Ventricle		65.7	68.3	69.0
		(2.0)	(1.1)	(1.8)
	Night			
Pontine		43.7	42.3	35.7
		(4.1)	(4.3)	(4.6)
Medullary		39.7	45.8	31.1
		(5.1)	(2.6)	(3.6)
Ventricle		41.7	41.6	37.1
		(2.3)	(1.8)	(2.8)
Carbachol	Day			
Pontine		65.9	63.7	63.2
		(5.0)	(1.7)	(1.8)
Medullary		68.6	60.5*	67.4
		(1.8)	(4.7)	(2.7)
Left-Medullary		62.4	61.7	63.3
		(2.9)	(4.4)	(2.9)
Ventricle		69.1	64.6	67.7
		(2.9)	(4.1)	(2.9)
	Night			
Pontine		34.8	40.3	30.9
		(1.6)	(3.7)	(2.7)
Medullary		37.7	42.4	37.2
		(1.7)	(2.3)	(2.0)
Left-Medullary		42.7	42.8	36.3
		(2.6)	(2.7)	(2.7)
Ventricle		45.2	45.9	35.5*
		(0.8)	(2.3)	(3.6)

*$p<0.05$ from baseline.

TABLE 3

MEAN PERCENT SLOW WAVE SLEEP TIME (SWS) ± SEM DURING THE DAY-NIGHT CYCLES FOR ALL GROUPS

		Baseline	Drug	Post-drug
Saline	Day	57.7	57.9	59.8
		(1.3)	(1.4)	(1.0)
	Night	34.0	38.4	37.0
		(1.4)	(1.6)	(2.4)
Scopolamine	Day			
Pontine		57.0	63.3*	63.1*
		(3.5)	(2.7)	(3.5)
Medullary		58.4	59.7	55.4
		(1.3)	(1.9)	(3.6)
Ventricle		55.1	61.9*	62.3*
		(1.6)	(1.4)	(1.4)
	Night			
Pontine		37.2	38.1	32.0
		(2.8)	(4.2)	(4.1)
Medullary		32.4	42.4*	28.3
		(1.7)	(1.8)	(3.4)
Ventricle		37.3	37.7	33.3
		(2.1)	(2.0)	(2.5)
Carbachol	Day			
Pontine		57.4	53.9	53.6
		(4.0)	(0.6)	(3.6)
Medullary		57.8	49.7*	55.3
		(1.5)	(3.7)	(1.8)
Left-Medullary		53.9	52.3	54.2
		(2.5)	(4.7)	(2.8)
Ventricle		57.1	52.3	55.4
		(2.8)	(3.3)	(2.7)
	Night			
Pontine		31.6	34.3	27.2
		(1.4)	(3.5)	(2.7)
Medullary		33.6	36.2	32.2
		(1.3)	(1.8)	(1.7)
Left-Medullary		35.9	35.9	30.6
		(1.7)	(2.6)	(2.2)
Ventricle		39.3	41.5	33.3
		(0.8)	(1.6)	(2.4)

*$p<0.05$ from baseline.

midline medullary group wherein each day, with the exception of day nine and baseline two, was significantly different from baseline one (Dunnett's $d'=5.62$, $p<0.05$). In the ventricular group, a stepwise REMS profile was also seen, with the second baseline day not significantly different from baseline one (Dunnett's $d'=3.79$, $p>0.05$).

Tables 2 and 3 summarize the mean percent TST and SWS. In the pontine group there was no change in TST during drug or post-drug days. However, there was a significant increase in SWS from pooled baseline during drug $F(2,50)=8.23$, $p<0.01$, and post-drug, $F(2,50)=7.63$, $p<0.05$, days. In the medullary group, TST was significantly decreased during drug, $F(2,50)=9.37$, $p<0.05$, and post-drug days, $F(2,50)=17.25$, $p<0.01$; SWS was unaffected. In the ventricle group, there was no significant difference in TST, but SWS increased during drug, $F(2,50)=7.53$, $p<0.05$, and post-drug conditions, $F(2,50)=8.43$, $p<0.05$.

In order to assess the nature of the scopolamine induced REMS disruption, we analyzed the data for changes in duration and number of REMS bouts. In the pontine group scopolamine produced a significant 44% decrease in number of REMS bouts during the drug condition compared to pooled baseline (mean pooled baseline=35.8±4.0; mean drug=20.2±1.9; matched t-test=4.8, $df=4$, $p<0.05$). The duration of REMS episodes decreased significantly by 32% on drug days (mean pooled baseline=1.9±0.09; mean drug=1.3±0.1 min; $F(2,50)=37.12$, $p<0.01$). Number of SWS bouts did not change significantly (−7%) but length of SWS episodes increased by 35% (mean pooled baseline=4.8±0.22; mean drug=6.5±0.46; $F(2,50)=16.58$, $p<0.01$).

In the medulla, scopolamine reduced REMS bouts by 53% (mean pooled baseline=41.9±3.5; mean drug days =19.7±2.2; matched t-test=3.0, $df=4$, $p<0.05$), and REMS duration by 28% (mean pooled baseline=1.8±0.08; mean drug=1.3±0.15; $F(2,50)=36.6$, $p<0.01$); length of SWS

TABLE 4

THE TABLE SUMMARIZES THE CHANGES IN TST, SWS AND REMS DURING THE FIRST 24 HOURS AFTER PUMP IMPLANT

	First Day			First Night		
	TST	SWS	REMS	TST	SWS	REMS
Saline	−33.8*	−25.3*	−77.2*	+11.9	+13.4*	+6.4
Scopolamine						
Pons	−39.8*	−26.7*	−91.3*	+14.9	+25.3*	−47.9*
Medulla	−30.9*	−19.7	−89.7*	+21.4	+40.7*	−45.7
Fourth Vent	−30.3*	−19.2	−47.2*	+15.4	+19.8	−38.3
Carbachol						
Pons	−30.5*	−22.3*	−79.5*	+51.7*	+46.5*	+33.5
Medulla	−48.2*	−39.3*	−90.5*	−3.4	+2.1	−50.5*
Left Medulla	−15.7	−6.8	−65.9*	+35.0*	+33.1*	+10.4
Fourth Vent	−38.7*	−28.7*	−83.8*	−19.0	−16.5	−35.4

*$p<0.05$ from pooled baseline.

The numbers represent the percent change from within group pooled baseline (baseline one and two).

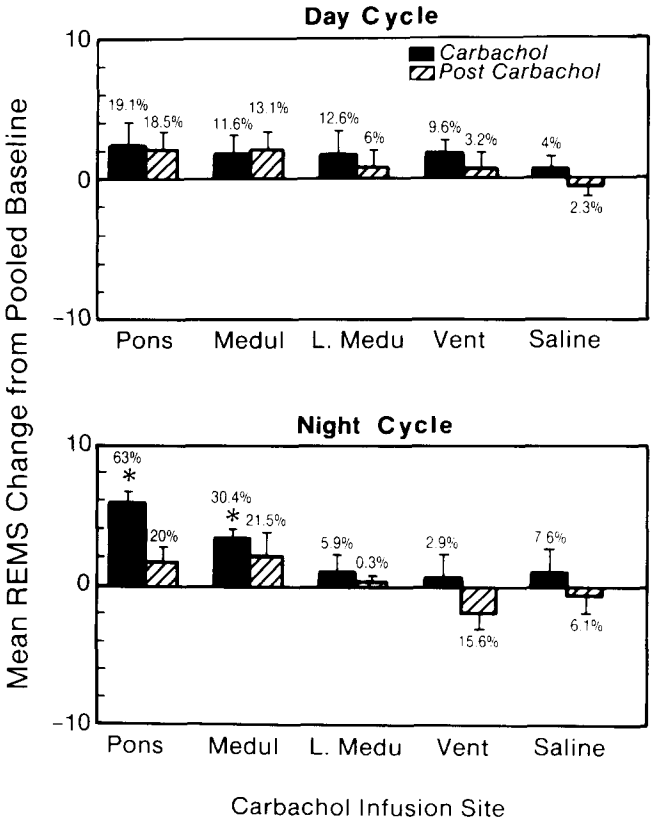

FIG. 4. Carbachol induced alterations in REMS during day and night cycles. During the day cycle carbachol did not produce a significant REM alteration. However, compared to the saline group, there was a trend towards a REMS increase. During the night cycle carbachol produced a significant REMS augmentation in the pontine and medullary groups during the period of drug infusion (nights 2 through 5). Asterisk indicates significant difference ($p<0.05$) compared to within group pooled baseline (baseline one and two) and saline control.

episodes increased significantly by 22% (mean pooled baseline=4.9±0.25; mean drug=6.0±0.44; $F(2,50)=7.95$, $p<0.05$). In the fourth ventricle group, duration (−23%) and number (−14%) of REMS bouts decreased but neither of these measures were significantly different from baseline. However, duration of SWS episodes increased by 30% (mean pooled baseline=4.4±0.20; mean drug=5.7±0.5; $F(2,50)=11.74$, $p<0.01$).

Scopolamine: Night Cycle

Figure 2B summarizes the change in REMS produced by scopolamine during the night cycle. In the pontine group there was a significant 31% reduction in REMS during the drug infusion period, $F(2,50)=13.6$, $p<0.01$. During the subsequent four nights there was a 28% REMS loss, $F(2,50)=10.9$, $p<0.01$. In the medullary and ventricle groups REMS was unchanged. In the pontine group TST and SWS were unchanged (Tables 2 and 3) during the period when REMS was significantly decreased.

Carbachol: Day Cycle

Figure 4A summarizes the carbachol induced alterations in REMS during the day cycle. In the carbachol groups there was a trend toward a REMS increase but the augmentation was not statistically significant compared to pooled baseline or saline control. The percentages of TST and SWS are summarized in Tables 1 and 2, respectively.

Carbachol: Night Cycle

Figure 4B summarizes the carbachol induced REMS alteration during the night cycle. Cabachol infusions into the pontine brainstem produced a significant 63% increase in REMS during the drug period, $F(2,64)=24.24$, $p<0.01$; during the subsequent four nights there was a non-significant 20% REMS increase, $F(2,64)=2.34$. Figure 3B profiles the REMS alterations from baseline one in the pontine group during each night of the experiment, including baseline two. Dunnett's test indicated that there was a significant increase in REMS during nights 2, 3, 4 and 5 (Dunnett's $d'=3.11$,

$p<0.05$). On subsequent nights REMS returned to baseline one levels.

The night cycle REMS augmentation in the pontine group was due to a significant 103% increase in the number of REMS bouts (mean pooled baseline=17.3±1.5; mean drug=35.2±3.0; matched t-test=13.48, $df=4$, $p<0.05$) and not the result of lengthening of individual REMS episodes (mean pooled baseline=1.4±0.2; mean drug=1.2±0.09). TST and SWS increased slightly during the period of REMS augmentation, but neither of these measures were significantly different from pooled baseline or saline control values.

Midline medullary carbachol infusion produced a significant 30% REMS augmentation during the drug infusion period, $F(2,64)=12.23$, $p<0.01$; during the post-drug period there was a non-significant 21% increase. In the midline medullary group, profile of the REMS change during each night of the experiment was similar to that seen in the pontine group but a one-way ANOVA showed that the difference was not statistically significant, $F(9,63)=1.84$). In the medullary group there was a 70% increase in the number of REMS bouts during the period of drug administration compared to baseline (mean pooled baseline=20.1±1.7; mean medullary drug=34.2±2.7; matched t-test=6.79, $df=7$, $p<0.05$). Left-side medullary or ventricular carbachol infusions produced no alteration in REMS (See Fig. 4B).

First 24 Hours of Pump Operation

Table 4 summarizes the percent change from pooled baseline of TST, SWS and REMS during the first 24 hours immediately following pump implant. In the saline group there was a significant reduction during the day cycle (following surgery) in all three sleep measures. However, by the night cycle TST and REMS levels had normalized, although SWS showed a slight increase (13%) above pooled baseline levels, $F(1,32)=5.5$, $p<0.05$.

In the scopolamine group TST and REMS were significantly reduced in all groups, while SWS was reduced significantly only in the pontine group, $F(1,25)=6.6$, $p<0.05$. During the subsequent night cycle, TST normalized but REMS levels remained lower than pooled baseline values. In the pontine and medullary groups significant reciprocal increases in SWS were noted in response to the REMS loss.

In the carbachol treated animals all groups demonstrated significant decreases in REMS during the first day cycle period immediately following surgery. TST and SWS were also significantly depressed in all groups except the left medullary group which displayed minimal TST (−15.7%) and SWS (−6.8%) loss. During the subsequent night cycle, the pontine and left medullary groups displayed an increase in TST and SWS. In the pontine group a trend toward REMS augmentation could be seen but was not statistically significant. In the midline medullary group a significant REMS suppression persisted, $F(1,32)=8.3$, $p<0.01$.

DISCUSSION

This study demonstrated that continuous infusion of cholinergic agents into the brainstem produce sustained alterations in REMS. Specifically, infusion of carbachol, a cholinergic receptor agonist, into the pontine area increased REMS, while scopolamine, a muscarinic blocker, impaired it. The results showed that REMS augmentation (63%) was obtained chiefly when carbachol was infused into the pontine reticular formation. Medullary carbachol infusion produced a lower but significant 30% REMS increase. The carbachol induced REMS augmentation was seen primarily during the night cycle and it was due to an increase in number of REMS bouts and not the result of an increase in length of REMS episodes. Scopolamine, on the other hand, decreased REMS at all sites during the day cycle and this was due to reductions in both number and duration of REMS bouts. The site-specific effect of scopolamine during the night cycle is particularly interesting because it lends support to the view that muscarinic receptor activation in the pontine region is necessary for the occurrence of physiological REMS, even though a non-muscarinic site of action cannot be ruled out [9].

In order to control for the diffusion of drugs to neighbouring brainstem areas, the cholinergics were infused into midline medullary, left-side medullary or the fourth ventricle. The results indicate that the optimum location of the cannula is in the midline pontine area where a few cholinoceptive reticular neurons have been found [27, 28, 33, 40]. Recently it was reported that iontophoretic application of acetylcholine excites medial pontine neurons [19] while, in freely behaving cats, local carbachol microinfusion in the medial PRF tonically increases firing of some pontine reticular neurons in conjunction with the carbachol induced REMS [36]. This area has long been suspected to produce REMS [25] and recent transection studies [37] and acute carbachol microinfusion studies in the cat [3, 4, 34–36, 38] continue to support this position. In short, the changes seen in the carbachol and scopolamine groups are consistent with previous studies.

One explanation for the carbachol induced REMS augmentation is that it occurred in response to increased REMS pressure produced by the REMS deprivation following the surgical procedure. A rebound might be expected considering that during the first nine hours following pump implant the animals demonstrated an 80% REMS loss. However, apart from drug treatment all animals underwent significant REMS loss following surgical implant of the minipump, but only the pontine carbachol infused animals displayed a REMS increase after the pumps became operational. Therefore, it is unlikely that the long-term persistence (days) of the augmentation could be easily explained by a brief 9–12 hour REMS deprivation.

At this juncture we can only speculate about the persistent effects of scopolamine beyond day 5. Whether the pumps continue to infuse the drug beyond day 5, or uptake and activation continues even after the pumps are exhausted is a matter of speculation. Only an independent technique for establishing the levels of the agents can clarify the matter.

Nevertheless, a number of studies have shown that acute administration of cholinomimetics augment REMS [1, 3, 4, 6, 18, 22, 32, 34–36, 38]. Our study extends these findings by demonstrating that continuous infusion of cholinergics produce sustained alterations in REMS. Furthermore, our findings provide support for at least one part of the reciprocal-interaction theory [23,30], namely that the generator mechanism of the REMS phase resides within a cholinoceptive REMS trigger zone in the PRF.

The reciprocal-interaction theory postulates that the two states comprising mammalian sleep cycle are the function of two brainstem neuronal cell groups displaying patterns that are reciprocally related to one another. So called "REM-on" cells in the gigantocellular tegmental field of the PRF are postulated to play a cholinergic role in generating the REMS phase. "REM-off" cells of the locus coeruleus (LC) and dorsal raphe nucleus (DRN) exert an inhibitory (or modula-

tory) restraint on the cholinergic "REM-on" cells of the PRF. Therefore, REMS is generated by the reciprocal-interaction of "REM-on" cells being disinhibited when the activity of "REM-off" cells is blocked.

The evidence supporting the idea that "REM-off" cells exert a restraint upon "REM-on" cells in the PRF gigantocellular tegmental field is based solely on correlative evidence and not on data providing a critical test of the hypothesis. The evidence, which includes studies of cell membership in distinct neurochemical groups [10], description of spike train characteristics [31], the neuroanatomical proximity of "REM-off" cells to the LC and DRN [8,31], the arrest of firing of cells following administration of pharmacological agents [17], and studies of "REM-off" cell firing patterns [2,15], can upon close scrutiny be interpreted as reflections of neuroanatomical configurations and behaviors that may or may not be related to REMS generation.

Our findings would lead us to think that cholinoceptive PRF activity might be sufficient to trigger the REMS phase, and we further suggest that muscarinic activation is necessary for occurrence of REMS. The carbachol induced REMS augmentation that we observed could be a result of a muscarinic receptor mediated depolarization of PRF neurons. Conversely, the scopolamine-induced decrease in duration and number of REMS episodes suggests that REMS episodes were aborted because scopolamine prevented the development of sustained muscarinic PRF neuronal activity necessary for the continuation of REMS. Consistent with this is the finding that chloramphenicol, a synaptic plasma membrane protein synthesis inhibitor, reduces REMS by aborting triggered REMS episodes [13]. In the same study, chloramphenicol also attenuated PRF activity, and it was suggested by the authors that the REMS reduction might be due to a chloramphenicol mediated receptor inactivation which prevented PRF neurons from achieving a minimum discharge rate necessary for REMS. Needless to say, only further research will bear out our speculation that REMS results from a muscarinic based change in PRF neuronal discharge.

Our results, therefore, suggest that the alteration in excitability of pontine cholinoceptive neurons is the necessary and perhaps, sufficient trigger mechanism for REMS. We further suggest that REMS rhythmicity is under the control of a cyclic mechanism, possibly the suprachiasmatic nucleus of the hypothalamus, which periodically modulates the sensitivity of muscarinic receptors.

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