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Central Administration of Two 5-HT Receptor Agonists: Effect on REM Sleep Initiation and PGO Waves

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SANFORD, L. D., R. J. ROSS, A. E. SEGGOS, A. R. MORRISON, W. A. BALL AND G. L. MANN. *Central administration of two 5-HT receptor agonists: Effect on REM sleep initiation and PGO waves.* PHARMACOL BIOCHEM BEHAV **49**(1) 93–100, 1994. – Cholinergic neurons in the pedunculopontine tegmental (PPT) and the laterodorsal tegmental (LDT) nuclei are implicated in the generation of rapid eye movement sleep (REM) and ponto-geniculo-occipital (PGO) waves. Serotonin (5-HT) has a role in sleep–wake regulation and appears to inhibit PGO wave generation. We studied the effects of the central infusion of the relatively specific 5-HT_{1A} receptor agonist 8-hydroxy-2-(n-dipropylamino)tetralin (DPAT) and the less specific 5-HT_1 receptor agonist 1(3-chlorophenyl)piperazine (mCPP) on the regulation of REM and on PGO wave generation. DPAT (0.0, 0.002, 0.01, 0.08, and 0.8 μg/0.5 μl normal saline) and mCPP (0.0, 0.02, 0.2, 2.0, and 20.0 μg/0.5 μl normal saline) were infused unilaterally into the peribrachial region of PPT (PB) in cats. Additionally, DPAT (0.01 μg/0.5 μl) was infused bilaterally into PB in a separate experiment. Low dosages of DPAT (unilateral or bilateral) decreased successful entrances into REM (0.01 μg) and time spent asleep (0.002 μg and 0.01 μg) without affecting outward behavior. No dosage of mCPP significantly decreased the number of REM episodes, and neither drug decreased REM episode duration once REM had been entered. Neither drug affected the rate of PGO waves independently of modulating behavioral state. We propose that 5-HT_{1A} receptor mechanisms have an inhibitory role in actual REM initiation, possibly by facilitating endogenously generated excitation of brainstem startle mechanisms at the onset of REM.

DPAT mCPP PGO waves Sleep Rapid eye movement sleep Serotonin

PONTO-GENICULO-OCCIPITAL (PGO) waves recorded from the lateral geniculate body (LGB), pons, and occipital cortex are identifying neural markers of rapid eye movement sleep (REM) in cats. The generation of PGO waves in LGB has been traced to presumed cholinergic neurons in the pedunculopontine tegmental and laterodorsal tegmental nuclei (PPT/LDT) that fire in bursts 15 to 25 ms prior to a PGO wave (35,39,44). Several other classes of neurons in this area have more recently been linked in specific ways to PGO wave generation (51). REM onset also appears to depend on activity of cholinergic neurons in PPT/LDT that have input into a cholinoceptive site in the pons (2).

Serotonin (5-HT) has been implicated in the inhibition of PGO waves as well as the regulation of REM. The administration of 5-HT-depleting drugs, such as parachlorophenylalanine (PCPA) and reserpine, releases PGO waves into states other than REM (5,6,7,14,27). Reserpine also depletes norepinephrine, but its effect on PGO waves is thought to be due to 5-HT depletion because 5-HT precursors block reserpine-induced waves more effectively than do catecholamine precursors (7). Lesions of the dorsal raphe nuclei (DRN) and cuts between the DRN and the dorsolateral pons release PGO waves into wakefulness (W) (50). These findings are complemented by reports that electrical stimulation of the DRN suppresses PGO wave activity during REM (26).

Spontaneous neural activity in the DRN is related to PGO

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waves and behavioral state. Cells (putatively serotonergic) in DRN fire most often in W, less frequently during NREM and generally cease firing during the transition from NREM into REM (T) and during REM, when PGO waves are observed (8,33,34,36,54). Forced locomotor activity, which alters sleep cycle timing, does not abolish the relationship of DRN neuronal firing to the sleep cycle and PGO waves, suggesting that DRN has a role in the regulation of the sleep cycle (33,34). Luebke et al. (32) have found that cholinergic burst neurons in infant rat LDT are inhibited by 5-HT agonists in vitro in a manner consistent with the hypothesized role of DRN in the control of PGO waves. However, neurons with bursting patterns of firing were not found in in vivo recordings of LDT in mature rats (29).

To date, most pharmacological studies seeking to determine the role of 5-HT in PGO wave and REM generation have used systemic administration of drugs. Interpretation of the results of such studies is limited, either by the use of nonspecific drugs or by the inability to determine the specific site of drug action. Microinjecting drugs with known specificity for certain 5-HT receptor subtypes into PPT/LDT should circumvent many of these problems. The purpose of the present work was to examine the effect of the infusion into PB of drugs active at specific 5-HT receptor subtypes to examine the role of 5-HT receptor mechanisms in sleep/wake patterning and PGO wave generation. We chose the relatively specific 5-HT_{1A} agonist 8-hydroxy-2-(n-dipropylamino)tetralin (DPAT), which has affinity for the 5-HT_{1A} receptor subtype of approximately 1 nM and affinities for 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_2 receptor subtypes of 10 μM or greater (25). We also chose the less specific 5-HT_1 receptor agonist 1(3-chlorophenyl)piperazine (mCPP), which has similar affinities for 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptors (100 nM to 1 μM) and slightly higher affinity (10 nM) for 5-HT_{1C} receptors (25).

METHOD

Seven adult, female cats were implanted with a standard array of electrodes used for sleep recording. Stainless steel screw electrodes were placed in the frontal sinuses for recording the electrooculogram (EOG) and electroencephalogram (EEG); wire electrodes were inserted into the nuchal muscles for recording the electromyogram (EMG). Tripolar stainless steel electrodes (tip separation 1 mm) were implanted in LGB bilaterally [coordinates AP: +6.0; ML: ±10; DV: +2.7; Berman (3)] for recording PGO wave activity according to Brooks (4). Twenty-two gauge stainless steel guide cannulae were implanted unilaterally with their tips aimed 2 mm above PB (n = 2) or bilaterally with the tips aimed 4 mm above PB (n = 4). For microinjections, a 30 gauge, stainless steel cannula was lowered into PB [coordinates AP: −2.0; ML: ±3.0; DV: +0.5; Berman, (3)].

All surgical procedures were performed stereotaxically under sterile conditions using halothane or pentobarbital (42 mg/kg) anesthesia. Subcutaneous injections of nalbuphine (2.5 mg/kg) were used to control potential postoperative pain.

The cats were allowed a minimum of 14 days to recover from surgery prior to being entered in any experimental protocols. Following recovery, four cats were unilaterally infused with saline followed by successive doses of DPAT (0.002, 0.01, 0.08, μg/0.5 μl normal saline); two of these cats also received a higher dose (0.8 μg/0.5 μl). A second group of five cats was bilaterally infused with saline and the most effective dose of DPAT (0.01 μg/0.5 μl). Five cats were unilaterally infused with saline followed by successive doses of mCPP (0.02, 0.2, 2.0, 20.0 μg/0.5 μl normal saline). Some cats served in both experiments, and all trials with one drug were completed before tests of the next drug were initiated. The total volume of each infusion was 0.5 μl. Both drugs were administered in a counterbalanced order. A minimum of 7 days elapsed between drug doses. Six-hour uninterrupted sleep studies were conducted for each dose. Behavioral state was determined by experienced observers from Telefactor videotape records.

Analyses of the number of REM episodes, episode duration, and PGO wave frequency (rate/minute) were conducted in all instances where the defining electrophysiological parameters of REM (low-voltage, high-frequency waves on the EEG, rapid eye movements, muscle atonia, and PGO waves) were present for 1 min or more. Such periods of less than 1-min duration were considered aborted REM episodes. This criterion is more than three standard deviations below the mean lengths (5 to 6 min) of normal REM episodes (55). The actual observed length of aborted REM episodes in this study ranged between 5 and 45 s.

We also examined the total sleep time (TST), sleep efficiency (TST/total recording time) and REM percent (time in REM/TST). PGO waves ipsilateral to the injection site were counted using Brainwave Experimenter's Workbench software. The data for both DPAT and mCPP were analyzed with single-factor, within-subjects (subject × drug dose) analyses of variance with repeated measures on the factor dose. Differences among means were analyzed with Tukey's HSD test.

After completion of the experiment, the cats were injected with an overdose of pentobarbital (22–23 mg/kg, IV) and perfused with saline and 10% formalin. The brains were removed from the skull and postfixed in 10% formalin. For histological localization, brain tissue was cut in 40 μm sections with a freezing microtome, mounted on slides and stained with cresyl violet. All histological sections that contained a lesion made by the microinjector were localized using the atlas of Berman (3). The brain of one cat (SP8) was lost and one cat is still being used in studies.

RESULTS

Histological Analyses

The line drawing in Fig. 1 shows the localization of the injection sites in the sagittal plane. All effective injection sites were localized in the vicinity of PB, and encompassed regions previously reported to contain PGO burst neurons (39).

Unilateral Microinjections

Sleep–wake states. There was a significant difference in TST across doses of DPAT, $F(3,9) = 5.5$, $p < 0.02$. TST was reduced 18–20% by low doses of DPAT (0.002 μg and 0.01 μg). A slight (6%) increase in TST after injection of 0.08 μg DPAT compared to these decreases resulted in the significant difference among means (Tukey, $p < 0.05$). The reduction in TST was accompanied by a slight, nonsignificant reduction in sleep efficiency at the 0.01 μg dose of DPAT, and a significant difference in REM percent across doses of DPAT, $F(3, 9) = 6.5$, $p < 0.012$ (Fig. 2). REM percent was reduced at the 0.01 μg dose of DPAT (Tukey, $p < 0.05$) relative to saline. REM percent at the 0.002 μg and 0.08 μg doses of DPAT was virtually identical to that with saline alone. This was not due to a rebound in REM late in the recording period because the distribution of REM episodes with these two dosages approximated that recorded with saline alone.

Two cats given the highest dose of DPAT (0.8 μg) were

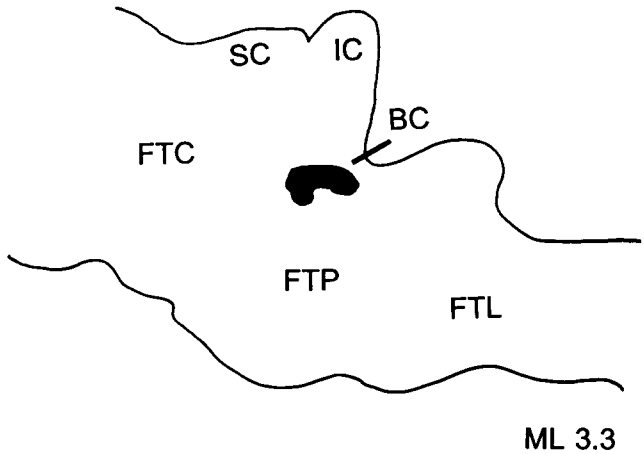

FIG. 1. Histological localization of injection sites. The injection site for each cat was localized on a sagittal section using the atlas of Berman (3). The darkened area encompasses the extent of all injection sites. All effective injection sites were histologically located in areas previously demonstrated to contain PGO burst cells (41). SC: superior colliculus: IC: inferior colliculus. BC: brachium conjunctivum. FTC: central tegmental field. FTG: gigantocellular tegmental field. FTL: lateral tegmental field.

restless and unable to maintain a quiet position. No statistical analyses were performed on the data obtained from these two cats; however, W was markedly increased compared to saline (Table 1). In both cats, there were long periods of W and NREM interrupted by frequent arousals.

There was no significant effect of any dose of mCPP on TST, sleep efficiency, or REM percent (Fig. 2).

Number of REM episodes. DPAT had a significant dose-related effect on REM initiation, $F(3, 9) = 7.4$, $p < 0.008$ (Fig. 3A). The number of REM episodes (≥ 1 min) was significantly decreased by DPAT (0.01 μg) relative to saline alone, Tukey, $p < 0.01$. A higher dose of DPAT (0.08 μg) did not significantly decrease the number of REM episodes.

The decrease in the number of REM episodes (≥ 1 min) by a low dose of mCPP (0.02 μg) was not as pronounced as that observed with DPAT and did not reach significance (Fig. 3A).

Aborted REM episodes. The occurrence of frequent PGO

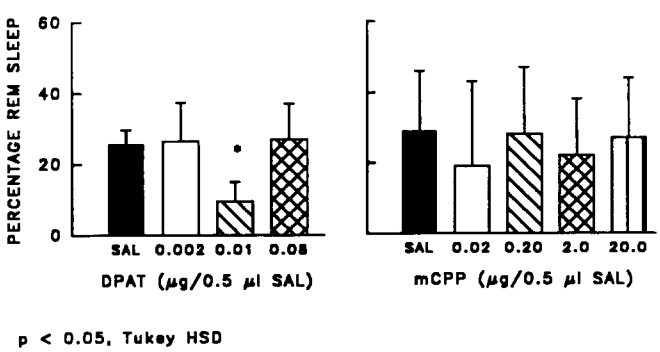

FIG. 2. Mean REM percent occurring during 6-h observation periods for each unilateral dosage of DPAT and mCPP compared with saline controls. DPAT: $N = 4$. mCPP: $N = 5$. Vertical lines indicate standard deviation in this and other graphs. DPAT (0.01 μg) significantly reduced the percentage of time a cat spent in REM.

TABLE 1

TIME IN REM AND NREM AFTER SALINE AND HIGH DOSAGES OF DPAT (0.8 μg) UNILATERALLY MICROINJECTED INTO PB

	REM		NREM	
Cat	Saline	DPAT	Saline	DPAT
SP6	36	70s	117	30
SP7	56	42s	199	98

Data is presented in minutes except as noted.

waves in NREM usually indicates the beginning of transition into REM (T). We combined observed instances of trains of high amplitude PGO waves in NREM that abruptly ended in arousal, T that ended in abrupt arousal and aborted REM episodes (<1 min) in a measure to determine whether the propensity for REM was affected by the drugs. As can be seen in Fig. 3B, the total number of such instances was high under all doses of DPAT, whereas under mCPP, there was no apparent difference from saline. All cats given DPAT exhibited an increase in aborted REM episodes (as defined here), but the increase for an individual animal could be variable across dosages, resulting in statistically nonsignificant differences with the four animals tested. Although this measure of the propensity for REM is indirect and highly variable between cats, we believe it is reasonable to conclude that DPAT did not diminish the actual drive for REM.

REM episode duration. There was no significant effect of low doses of DPAT on REM episode duration once a cat actually entered REM (Fig. 3C). REM episode duration was not significantly altered by any dose of mCPP (Fig. 3C).

PGO wave frequency in REM. DPAT and mCPP did not appear to affect PGO wave generation independently of their effects on REM. PGO wave frequency in REM episodes (>1 min) was not significantly different from the saline control (Fig. 4). Even at the highest dose of DPAT (0.8 μg), where REM was virtually abolished, PGO wave frequency in REM was apparently normal.

Wave frequency in W and NREM. Waves recorded from LGB were counted during four quiescent W periods and four NREM episodes, one from each quarter of the 6-h recording period. W periods ranged from 4 to 8 min. NREM episodes did not include aborted REM episodes. Wave counts were analyzed with a 4 (DPAT drug condition) × 4 (episode) or a 5 (mCPP drug condition) × 4 (episode) within-subjects ANOVA. There were no significant differences in wave frequency for either drug either across drug conditions or across episodes.

Bilateral Microinjections

REM after bilateral microinjections of DPAT. The effects of bilateral microinjections of 0.01 μg DPAT on REM are summarized in Table 2. Bilateral microinjections of drug were compared to bilateral injections of saline in the same cats. Statistical analyses were conducted with dependent *t*-tests. In general, the results of bilateral injections of DPAT followed those for unilateral injections with significantly reduced TST, REM percent, and number of REM episodes compared to saline, whereas REM episode duration and overall sleep efficiency were not significantly affected. The latency to REM was somewhat longer after DPAT, but the increase was not significant. As with unilaterally administered DPAT, the number of aborted attempts to enter REM (defined earlier)

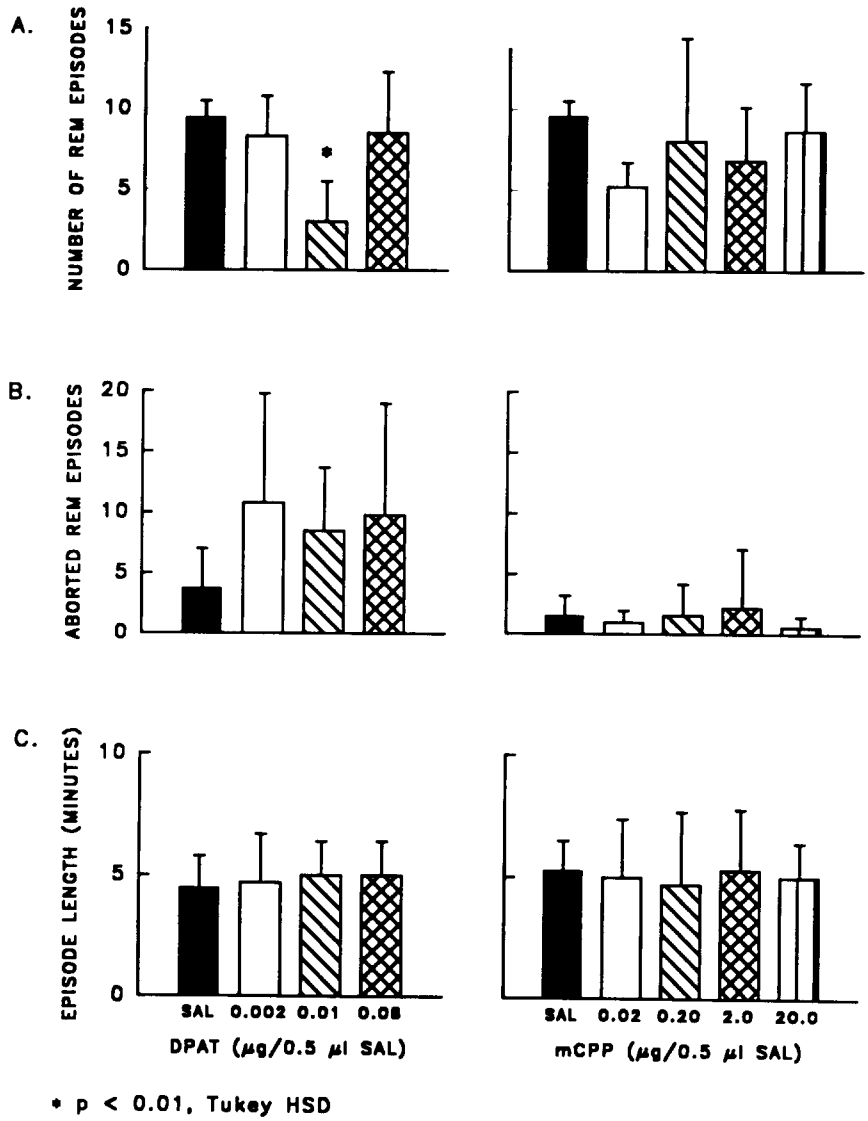

FIG. 3. (A) Mean number of REM episodes occurring during 6-h observation periods for each unilateral dosage of DPAT and mCPP. (B) Mean number of aborted REM episodes occurring during 6-h observation periods for each dosage of DPAT and mCPP. Aborted REM is defined here as periods of high PGO wave activity in NREM that terminate in W; episodes of T, including spindles and decreased EMG amplitude but without rapid eye movements that terminate before REM is actually entered; and aborted REM episodes with durations of less than 1 min. (C) Mean length of REM episodes occurring during 6-h observation periods for each dosage of DPAT and mCPP. DPAT: $N = 4$. mCPP: $N = 5$.

was highly variable across cats and the analysis did not reach significance.

Wave frequency after bilateral microinjections of DPAT. Wave frequency was examined in W, NREM, and REM after bilateral microinjections of saline and the most effective dosage of DPAT (0.01 μg/0.5 μl). Waves were counted during four quiescent W periods and four NREM periods, one from each quarter (1.5 h) of the 6-h recording period as described above. The data were analyzed with 2 (drug) × 4 (episode) within-subjects ANOVAs. PGO wave frequency was also examined during REM after bilateral injections of DPAT. Due to the variability in the number of REM episodes in the drug condition, the mean PGO wave frequency after DPAT was compared with that after saline with a dependent *t*-test. There were no significant differences for any measure with these analyses, indicating that bilaterally injected DPAT had no effect on waves recorded from LGB independently of altering behavioral state.

DISCUSSION

Studies using various investigative means have affirmed the initial suggestion by Jouvet (28) that 5-HT is involved in the regulation of sleep and wakefulness. Studies examining the effects of drugs preferentially active at different 5-HT receptor sites have indicated that 5-HT_{1A} (1,15,21,41) and 5-HT_2

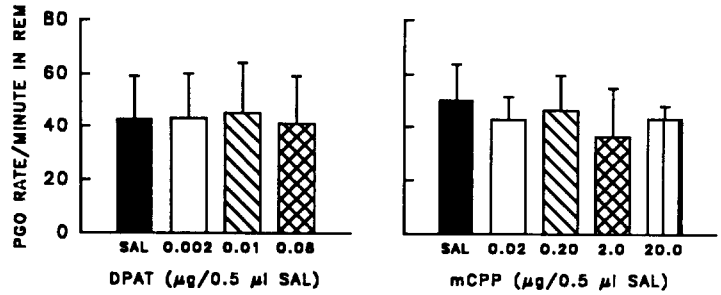

FIG. 4. PGO wave frequency ploted as mean rate/minute in REM episodes ≥ 1 min during 6-h observation periods. Neither DPAT nor mCPP significantly alter PGO wave frequency during REM. DPAT: $N = 4$. mCPP: $N = 5$.

(19,20,49,53) receptors participate in modulating sleep and W though their roles remain to be clarified. The results of this study support the idea of involvement of 5-HT_{1A} receptor mechanisms in the regulation of REM (15,16,21,41). We propose a refinement of this role by suggesting that 5-HT_{1A} receptor mechanisms suppress the initiation of REM. This suggestion is based on the observation that a low dose of DPAT (0.01 μg), an agonist that is relatively specific for 5-HT_{1A} receptor sites and has minimal efficacy at 5-HT_{1B}, 5-HT_{1C}, and 5-HT_2 receptor sites (25), significantly decreased successful entrances into REM without eliminating the drive for REM, in contrast to saline alone or mCPP. Nevertheless, recent anatomical information (discussed below) urges caution in relying on 5-HT as the sole transmitter of importance in this instance.

Adrien and Hamon (1) reported that IP injections of the 5-HT_{1A} agonists CM 57493 and ipsapirone inhibited PGO wave activity, reduced REM, and increased W in a dose-dependent manner. Systemic administration of DPAT decreased the number of PGO waves induced by reserpine (15). Similarly, Quattrochi et al. (41) reported that eltoprazine, a 5-HT_1 agonist, given IP, reduced PGO wave activity and suppressed REM. Thus, one could expect the local infusion of DPAT into PB to inhibit PGO wave generation; however, in our study, DPAT did not significantly affect PGO wave generation independently of its effect on behavioral state. Once REM had been successfully entered (defined here as an episode that lasted at least 1 min) PGO wave frequency was consistent regardless of the dosage of DPAT. We also found no difference between wave frequencies recorded in W and NREM after saline or drug.

On close examination, our results may not conflict with those of Quattrochi and his colleagues (41). They counted overall hourly PGO wave activity without examining PGO wave frequency in individual REM episodes. Thus, a reduction in REM would necessarily have resulted in reduced counts of total PGO waves. This may also be the case in the Adrien and Hamon (1) study, which did not describe how PGO waves were actually counted. We did find that PGO wave activity was more variable under mCPP, but there was no statistically significant decrease at any dose.

Because we used unilateral injections for most of the experiments in this study, the possibility existed that generator mechanisms in the contralateral PPT could have been sufficient to prevent a reduction in PGO wave activity. To address this concern, we bilaterally microinjected the most effective dose of DPAT (0.01 μg) in altering REM and obtained results that essentially paralleled those found with unilateral injections. Unilateral lesions of PPT decrease ipsilateral PGO waves in LGB by 50% (45), and neuroanatomical evidence indicates that connections between PB and LGB are greater ipsilaterally although contralateral connections are fairly extensive (52). Furthermore, unilateral injections of carbachol into a more caudal region of PB are sufficient to increase PGO waves in the ipsilateral LGB (12). Thus, there is reason to believe that unilateral injections of an effective serotonergic inhibitory agent should decrease PGO waves ipsilateral to the injection site, even if there is influence from the contralateral PB.

Although considerable evidence from lesion (50), stimulation (26), pharmacological (5,6,7,14,27), in vivo cellular recordings (8,36,54), and, recently, in vitro studies (31,32) implicates 5-HT and the DRN in the inhibitory modulation of PGO wave generation, the neuroantomical pathways by which serotonergic efferents from DRN affect presumed cholinergic PGO wave generator mechanisms in PPT/LDT have not been well delineated. One would expect a fairly prominent afferent projection; however, two studies indicate that, at best, there is low to moderate input to the PPT/LDT areas from DRN in rats (10,22). Also, while there is moderate ^{3}H-cynanoimipramine binding to 5-HT uptake sites in the rat PPT, indicating some serotonergic innervation of this region, there is little ^{3}H-DPAT binding and probably few 5-HT_{1A} receptor sites (47). A preliminary study reporting on two cats (42) found evidence of only a weak projection from DRN to LDT. We

TABLE 2

SLEEP AFTER BILATERAL MICROINJECTIONS OF SALINE OR 0.01 μg DPAT INTO PB

	Saline	DPAT
	Mean ± SD	Mean ± SD
Total sleep time	239.04 ± 119.95	205.68 ± 101.04*
Latency to REM	99.90 ± 24.91	171.04 ± 116.80
Sleep efficiency	0.53 ± 0.10	0.47 ± 0.18
REM percent	29.00 ± 7.42	15.00 ± 10.09†
Aborted REM episodes	4.40 ± 5.04	6.20 ± 8.99
Number REM episodes	10.60 ± 1.50	4.20 ± 2.89‡
REM episode duration	5.64 ± 2.05	6.38 ± 2.37

*$p < 0.02$
†$p < 0.001$
‡$p < 0.003$

are unaware of studies describing projections from DRN to PPT in cats.

This suggests that a neuroanatomical substrate outside the DRN may also be involved in 5-HT-mediated influences on behavioral state and PGO wave generation. The serotonergic neurons that have been found intermingled among the noradrenergic and cholinergic neurons in the predominantly cholinergic PPT/LDT area of cats (48) present a possibility. Due to the close association of these neurons (and to the effects we observed), it is worth noting that DPAT has moderate antagonistic effects at α_2-adrenoreceptors (11) in addition to its strong affinity for 5-HT_{1A} receptors such that a nonserotonergic mechanism may have participated in the effects we observed. Further anatomical work in cats will be required to clarify this issue.

The lack of efficacy of DPAT at the 0.08 μg dosage may possibly be explained by the activation of additional receptor populations. The lack of efficacy at this dosage underlines the inherent difficulty of assigning a single effect on REM to 5-HT neurons. Recent evidence suggests that interactions among different 5-HT receptor subtypes may modulate certain behaviors (24). Increased doses may activate more than one population of receptors; thus, the effect of 5-HT agents will depend both on the site of action and the dose.

This may explain our results with the less specific 5-HT_1 agonist mCPP, which is generally considered a 5-$HT_{1B/1C}$ receptor agonist yet has some affinity for 5-HT_{1A} and 5-HT_2 receptor sites (25). In fact, no consistent, statistically significant effect was found for mCPP on any sleep/waking parameters we examined. The literature on the effects of mCPP on sleep is limited; however, inconsistent effects of mCPP on sleep and drowsiness in humans have been reported, with self reports of drowsiness increased in some studies (9,30) and decreased in others (38).

The propensity for REM (as indicated by abrupt terminations of trains of PGO waves in NREM, T, and REM) remained high at all levels of DPAT. There could be a far greater number of aborted attempts to enter REM after DPAT, although this varied greatly among cats. However, the cats rarely were able to enter and sustain REM successfully at the 0.01 μg dosage (unilateral or bilateral). Instances of increased PGO wave frequencies in NREM that did not lead into REM were often terminated by head jerks and ultimately W. Our data do not suggest that alterations in PGO wave generators have a major role in these terminations. However, pontine generators responsible for other phasic events may be responsible.

There are great changes in the motor system during the transition from NREM to REM (23). The EMG in a normal transition from NREM to REM exhibits a gradual reduction of muscle tone with brief pauses in muscle activity (23). In REM, muscles twitches occur on a background of muscle inhibition (23). These phasic muscle twitches resemble startle and may ultimately be produced by the same medullary reticulospinal startle system. The stimulus for startle comes from external stimuli, whereas the impetus for the muscle twitches of REM putatively comes from the pontine phasic event generator (23). Spinal intrathecal injections of DPAT increase the acoustic startle reflex in rats (13). Although the neuroanatomical circuitry of phasic events and the startle reflex has not been established for cats, it is interesting to speculate that DPAT infused into PB may facilitate an endogenously generated excitation of the startle system such that the cat awakens. A possible entrance into the startle circuit could be via PB projections (37) into the pontine reticular formation where startle-related cells exist in cats (56).

Because startle is highly variable among cats (46), the relative effectiveness of DPAT in facilitating endogenous startle could also vary across cats. These individual differences could account for the high variability we observed in aborted attempts to enter REM after DPAT. Our hypothesis that aborted REM is due to activation of startle circuitry could be tested by introducing auditory stimuli normally not effective in arousing a cat (46) in the transition period in combination with DPAT infusion. One would predict an increase in abortive episodes, and that this increase would be related to the amount of startle each cat normally exhibited.

The protein synthesis inhibitor, chloramphenicol, administered systemically or perfused into pontine and midbrain reticular formation sites, also reduces the frequency of REM episodes but does not decrease episode duration (17,18,40,43). Drucker-Colín et al. (17) also found that chloramphenicol perfused into the pontine reticular formation increased abortive REM, but did not affect PGO waves. They reasoned that protein synthesis inhibitors may affect some mechanism involved in the transition into REM, possibly by attenuating the firing of cells that show increased activity as REM is entered (17). The remarkable similarities between the effects of DPAT and chloramphenicol suggest they may be operating on some common mechanism. However, we have thus far found the effective injection sites for DPAT to be circumscribed locally in PB (see Fig. 1), a site similar to the midbrain reticular formation injection site of Drucker-Colín et al. (17) which did not produce increases in abortive REM episodes in that study. Thus, while the similarities between the effects of DPAT and protein synthesis inhibitors is tantalizing, further work will be required to determine if they are affecting the initiation of REM through common neural mechanisms.

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