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MECHANISMS AND MODELS OF REM SLEEP CONTROL

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1. INTRODUCTION AND OVERVIEW

The first sections of this paper review both the history and recent developments relevant to the major neurotransmitters and neuromodulators involved in REM sleep control. The last portion of this paper proposes a structural model of cellular interaction that produces the REM sleep cycle, and constitutes a further revision of the reciprocal interaction model (67, 70, 71, 72, 73).

No paper on REM sleep control should begin without mentioning the most influential of the many lesion experiments, the early transection studies in the cat by Jouvet (47). When the neuraxis was cut at the midbrain level (Fig. 1), rostral to the pons, signs of the state of REM sleep were not present rostral to the cut (with the cut between brainstem and forebrain, the pathway for all brainstem-mediated REM cortical desynchronization was absent). However, the essential signs of REM sleep were preserved in the brainstem caudal to the cut, as shown by the major REM indicator variables available for analysis in this preparation. The "pontine cat", as this preparation was called, showed periodically occurring states characterized by 1) rapid eye movements, although they were reduced in number and complexity, 2) antigravity muscle atonia, especially remarkable since they abolished the decerebrate rigidity otherwise present, and 3) spiky waves in the pontine tegmentum, the pontine component of PGO waves. The important implication of this study was that the structures caudal to the cut were necessary and sufficient for basic REM phenomena, including rhythmicity. (This is not to say that more rostral structures do not enter into elaboration of REM phenomena; the phenomena in the pontine cat are simpler than those in the intact animal). However, the presence of the major indicators of REM sleep below this transection has led to a fairly general consensus that the mechanisms for REM production are localized in the lower brainstem, although, as will be discussed, more precise specification of localization and the neurotransmitter influences is still controversial.

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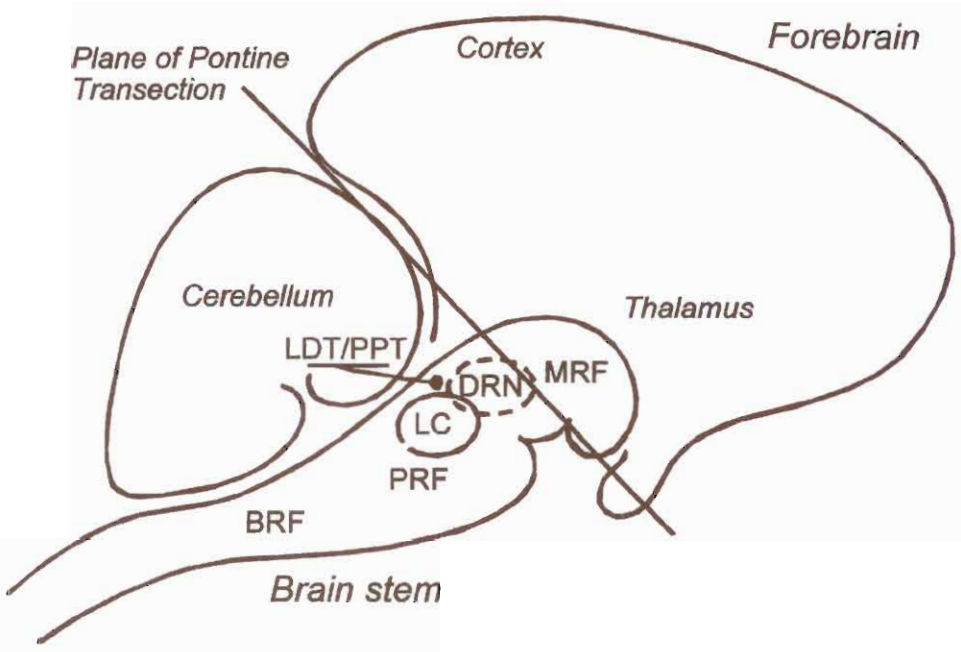


Fig. 1. - *Schematic of a sagittal section of a mammalian brain (cat) showing the location of nuclei especially important for REM sleep.*

Abbreviations: BRF, PRF, and MRF = bulbar, pontine, and mesencephalic reticular formation; LDT/PPT = laterodorsal and pedunculopontine tegmental nuclei, the principal site of cholinergic (acetylcholine-containing) neurons important for REM sleep and EEG desynchronization. LC = locus coeruleus, where most norepinephrine-containing neurons are located; DRN, Dorsal Raphe Nucleus, the site of many serotonin-containing neurons. The oblique line is the plane of transection that Jouvet (1962) found preserves REM sleep signs caudal to the transection, but abolishes them rostral to the transection (Figure adapted from McCarley *et al.*, 74).

2. CRITERIA FOR NEUROMODULATION IN REM SLEEP

The next several sections will address neurotransmitters and neuromodulators of various cell groups thought to be important in REM sleep. What are the criteria for demonstration of this putative causal role? We suggest the following, with the direction of change depending on whether the postulated influence is promoting/suppressive. In each case the prediction with promotion is listed first and suppression second.

1. *Discharge profile criterion.* Neurons utilizing the putative modulating neurotransmitter should show a discharge profile consistent with causality, there should be an appropriate increase/decrease in discharge rate prior to the putative controlled REM state or event.
2. *Anatomical connectivity criterion.* The putative modulating neurons should show connectivity with the controlled group.

3. *Neurotransmitter release criterion.* Microdialysis or other measurements of release in the controlled population should reveal the predicted increase/decrease in the controlled state.
4. *Controller activity criterion.*
   a. Effect of abolishing activity. Pharmacological or lesion silencing of the putative controlling group will decrease/increase the controlled state.
   b. Stimulation Effects. Increasing activity in the putative controlling neural population through pharmacological or electrical stimulation will increase/decrease the controlled state or event.
5. *Pharmacological agonist criterion.* Application of the agonists of putative neurotransmitter to the controlled neurons will increase/decrease the controlled event or neuronal population. Because of possible spill-over to other receptors or non-physiological effects, this demonstration should not be regarded as definitive.
6. *Pharmacological antagonist criterion.* Application of the antagonists of the putative neurotransmitter to the controlled neurons will decrease/increase the controlled event or neuronal population. Note this is a stronger criterion than number 5.
7. *Molecular biological analogs of criteria 5 and 6.* Application of antisense/RNAI directed at the putative receptor or ligand will block the effect of the putative neurotransmitter. Because of biological adaptation we do not think constitutive knockouts of receptors or a ligand constitute firm proof of a relationship or absence of a relationship, but inducible, reversible genetic knockdowns are a very useful technology.

Perhaps the most salient comment on these criteria is that *purely pharmacological criteria are never sufficient*, since one does not know whether the observed agonist/antagonist effects are, or are not, seen in natural REM sleep, a fact that should be kept in mind as the reader assays the strength of the evidence presented for REM neuromodulators in the next sections.

3 CHOLINERGIC INFLUENCES ON REM SLEEP

As a background to discussing neurotransmitter influences on REM sleep, the brainstem reticular formation should be considered as an "effector zone" with various cells and components influencing the components of REM sleep. Figure 2 sketches the dramatic alterations in membrane potential and excitability of a pontine reticular formation neuron recorded intracellularly across the sleep cycle by Ito and co-workers (43). The recording began in W; there was substantial eye movement activity in the EOG trace, low-amplitude, high-frequency EEG and also transient bursts in the EMG, indicating somatic movement. The MP in W was about -57mV with some depolarizing PSP inputs. The MP remained at about the same level with the onset of S, which began approximately at the point indicated by a single vertical arrow in the MP trace. Note also the EEG slow wave activity. At this stage, there was a low level of depolarizing PSPs, and EOG activity was also very low. However,

during the course of S, even before the onset of the first PGO wave, the MP gradually began to decrease (membrane depolarization) in association with an increased level of depolarizing PSPs. By the time of the first PGO wave, near the point labeled S~T in the figure, the membrane depolarization had reached a level sufficient for some action potential generation, as seen in the MP segment sample S~T in the bot-

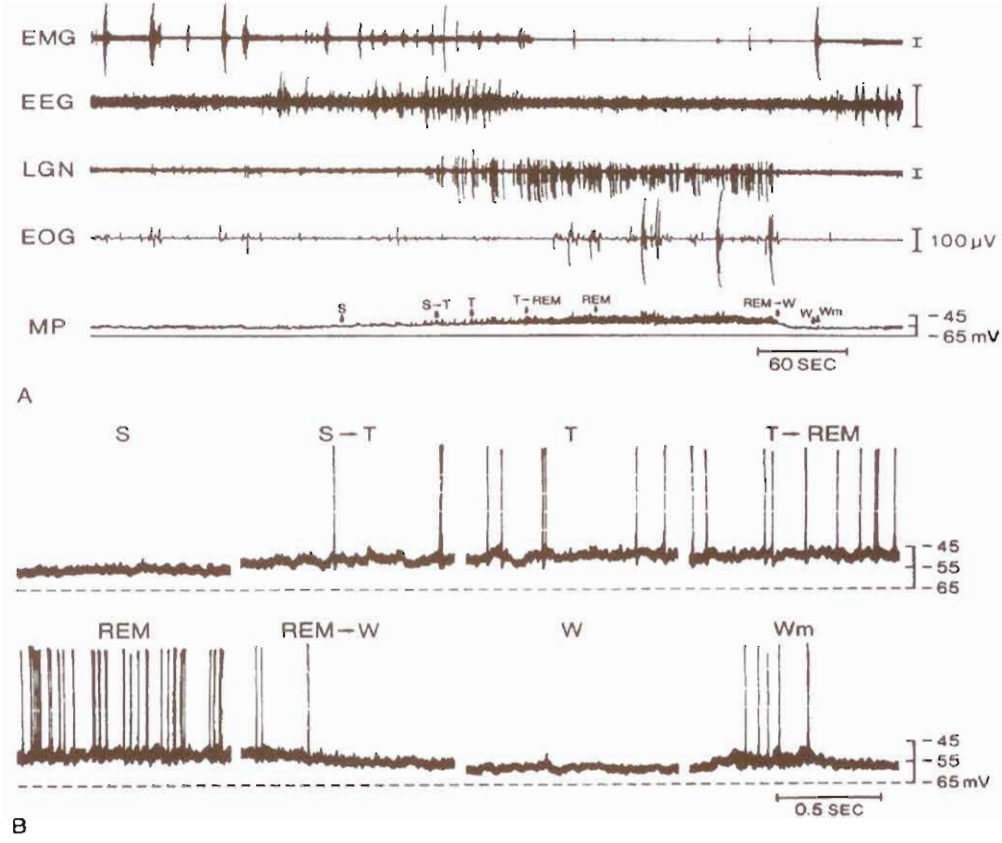


Fig. 2. - *Changes in the membrane potential (MP) of a medial pontine reticular formation neuron over a sleep-wake cycle.*

The five traces (from EMG through MP) in the top panel are a set of inkwriter recordings defining behavioral states in relationship to the MP level. Note that the inkwriter sensitivity is not high enough to trace individual action potentials (MP trace). In the bottom panel, oscilloscope photographs detail changes in the frequency of action potentials together with the MP level. The first trace of the top panel is EMG from the deep nuchal muscles. The second trace is EEG from the frontal cortex. The third trace of LGN activity shows PGO waves, which consist of high-amplitude pre-REM waves in T, irregular, high-frequency waves during REM sleep and rather high amplitude waves near the end of REM sleep. The fourth trace is EOG from the lateral rectus extra-ocular muscles. The fifth trace is the ink-writer MP record in which the many single spike-like deflections on the trace are prominent EPSPs or compounds of EPSPs and actual action potentials. The MP records in the bottom panel are eight photographs of the oscilloscope display of the tape-recorded MP. The labels indicate the corresponding segment on the inkwriter MP trace (double arrows). See also detailed text description. (Adapted from Ito *et al.*, 43).

tom panel. With the advent of more PGO waves in T and as muscle atonia (EMG trace) developed at the onset of REM sleep (49), the MP decreased to a level less negative than -50mV. With further phasic MP fluctuations, there were storms of depolarizing PSPs and accompanying high frequency action potentials (30-45 Hz at peak). At the end of REM sleep and the onset of W (segment REM-W), the membrane was re polarized to about a tonic level of -57 mV (segment W), approximately the same level as the initial segment in S. However, on this relatively stable tonic level of MP, there were sporadic depolarizing PSP inputs, as seen in the segment W. Some of these PSPs led to transient membrane depolarization and a burst of action potentials (Wm); some were associated with increased EMG activity (arrow on the EMG trace), indicating somatic movement. To be emphasized is the tonic depolarization in REM sleep that contrasts with the phasic depolarization in Wake.

One of the key questions in REM sleep control is what could cause this dramatic change in excitability, and we next consider cholinergic influences.

Cholinergic induction of REM sleep-like phenomena. – There is strong anatomical evidence of cholinergic projections to mPRF from the LDT and PPT nuclei (Fig. 3) and *in vitro* data indicate the strong excitatory response of over 60% of mPRF neurons to carbachol, administered in micromolar concentrations (33). Thus, there

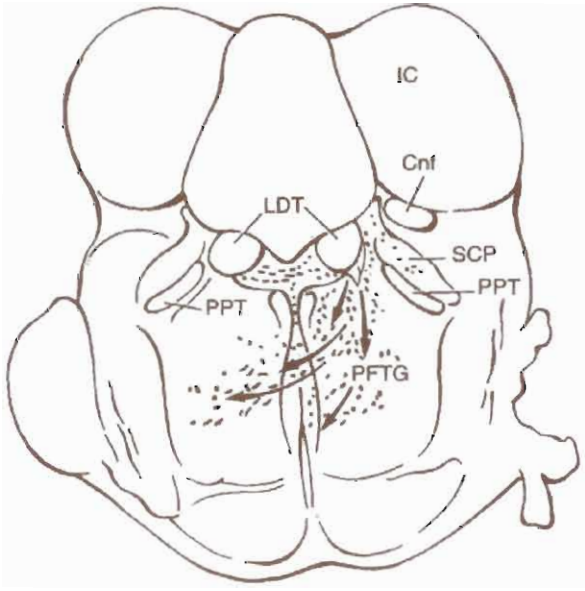


Fig. 3. - *Coronal section of the brainstem at the pons-midbrain junction showing the location of the acetylcholine-containing neurons most important for REM sleep in LDT/PPT (laterodorsal tegmental nucleus/pedunculopontine tegmental nucleus), and a schematic of projections of LDT to pontine reticular formation.*

(PFTG is an abbreviation of one component of PRF). Abbreviations: IC, inferior colliculus; Cnf, cuneiform nucleus; SCP, superior cerebellar peduncle. (Figure adapted from Mitani *et al.*, 76).

is strong supporting evidence that acetylcholine is a physiological neurotransmitter acting on mPRF neurons. (A final, but critical, piece of evidence is to show that stimulation of LDT/PPT produces PSPs with the same effects and that the PSPs are blocked by antagonists). Given this information one might, on an ad hoc basis, choose cholinergic agonists as reasonable agents to alter the excitability of reticular neuronal pools involved in production of REM phenomena.

In fact, the history of cholinergic injections into brainstem began in the 1960's on a much more empirical basis. Cordeau *et al.* (21) and George *et al.* (28) reported the induction of a REM sleep-like state by these injections. In the two decades that followed, numerous published studies have reported the elicitation of some or all components of REM sleep by brainstem injections of cholinergic agonists, with most of these studies using the cat (1, 5, 6, 7, 8, 39, 50, 54, 77, 99, 101, 102, 117, 118, 120).

In certain application sites in the pontine reticular formation, cholinergic agonists produce a "full" REM sleep-like syndrome, with the simultaneous presence of all major indicator variables; these sites tend to be in the medial pontine reticular formation rostral to VI and in the dorsal one-half. Carbachol application at other sites, such as near the peribrachial zone and hence near PPT may produce isolated PGO waves without other signs of REM sleep (120). Datta *et al.* (22) found that cholinergic stimulation of the PPT produced immediate and prolonged increases in PGO waves. Moreover, the muscle atonia of REM sleep may be produced by application of cholinergic agonists to the dorsolateral pontine reticular formation. Applications of cholinergic agonists to mesencephalic or bulbar reticular formation have not, at least in the sites so far tested, produced the full REM sleep-like syndrome (6).

Early studies in the rat also showed that carbachol was capable of producing a REM sleep syndrome (32, 100), as did later ones using microinjections (12, 23, 80, 119); compared with cats, the magnitude of the REM sleep enhancement was less, about 2-fold instead of 4-fold, and the rat episodes were shorter, close to normal duration, and had a longer latency to onset. Interestingly, Taguchi *et al.* (111) found that carbachol-induced REM-sleep like episodes of atonia and respiratory depression in the decerebrate rat were of short latency and long duration, suggesting that, in part, descending forebrain influences might account for the differential response in the chronic rat. It may also be that, in the smaller volume brainstem of the rat, diffusion of microinjected carbachol is more prone to activate ventral wakefulness-promoting sites than in the cat (55). In urethane-anesthetized rats and cats, carbachol produces partial REM signs, and Fenik *et al.* (25) have found that careful titration of the depth of anaesthesia made it possible to produce a 2-3 min state with cortical desynchronization, hippocampal theta rhythm and motoric suppression with small injections of carbachol.

Cholinergic LDT stimulation produces scopolamine-sensitive EPSPs in mPRF neurons. – Imon *et al.* (42) used single pulse electrical stimulation of the LDT in urethane anaesthetized acute cats to determine the synaptic effects on pontine reticular formation neurons, identified by antidromic activation from bulbar reticular formation and neurobiotin intracellular labeling. This stimulation produced Excitatory Post-Synaptic Potentials (EPSPs) in > 95% of recorded neurons with a latency con-

sistent with the conduction velocity of unmyelinated cholinergic fibers, 2 m/S. Also consistent with cholinergic EPSPs was their abolition by intravenous administration of the muscarinic receptor antagonist scopolamine (N = 40 neurons), by acute transverse cuts separating the LDT and the recorded neurons (N = 40), and by their reduction under barbiturate anaesthesia. These *in vivo* data thus support the anatomical and *in vitro* data indicating an excitatory, cholinergic LDT projection to pontine reticular formation.

Cholinergic unit activity during sleep and wakefulness. – We here focus on data relevant to brainstem projections and REM sleep. Some LDT/PPT neurons have markedly increased discharge activity during both states of EEG activation: wakefulness and REM sleep. We refer to these neurons as Wake/REM-on. Other LDT/PPT neurons show markedly increased discharge only in REM sleep. We refer to these neurons as REM-on. Previous presented data indicate a strong cholinergic influence in REM sleep: cholinergic agonists in pontine tegmentum produce a REM sleep-like state; cholinergic projections of LDT/PPT to pontine reticular formation that produce EPSPs, and *in vitro* data indicating cholinergic agonists produce depolarization and increased excitability in pontine reticular formation. It seems obvious that the REM-on neurons must be the ones mediating all the cholinergic REM-promoting effects save for EEG desynchronization.

Data on the discharge activity of REM-on neurons over the sleep cycle are not as extensive as extracellular recordings of mPRF neurons. Electrophysiological studies reveal that a subpopulation of LDT/PPT neurons preferentially discharges just before and during REM sleep (24, 51, 108, 112). The data from Thakkar *et al.* (112) are particularly useful since they were obtained from both LDT and PPT in freely moving cats and hence were able to use active wakefulness as a state measure while ruling out any potential confounds from the absence of head and neck movements (Fig. 4). The El Mansari *et al.* study (24) did not restrict recordings to the cholinergic PPT (although PPT was included), and the recordings were biased toward those units antidromically identified as projecting to thalamus. The Thakkar *et al.* data included both LDT (n = 11) and PPT (n = 23) neurons. These data, although limited in number, suggest that LDT neurons in general have somewhat lower discharge rates than PPT neurons and tend to show more phasic modulation during REM than PPT neurons. Figure 4 illustrates the discharge profile of this group of REM-on and Wake/REM-on neurons, and also illustrates the differential susceptibility of these two groups of neurons to a serotonin 1A agonist, a topic discussed in more detail below. Thakkar and colleagues (In Submission) also have recorded and analyzed the discharge data from 137 PPT neurons, of which 35% were REM-on, 62% were Wake/REM-on while 3% were REM-off. The REM-on neurons discharged preferentially during REM sleep and showed a statistically significant increase in discharge rate in the 50 sec prior to REM sleep compared with all nonREM states, including active wakefulness (movement). There was a much higher discharge rate in REM+ (REM with eye movements) compared with REM– (no eye movements). This within-REM selectivity suggests an important role in control of phasic events.

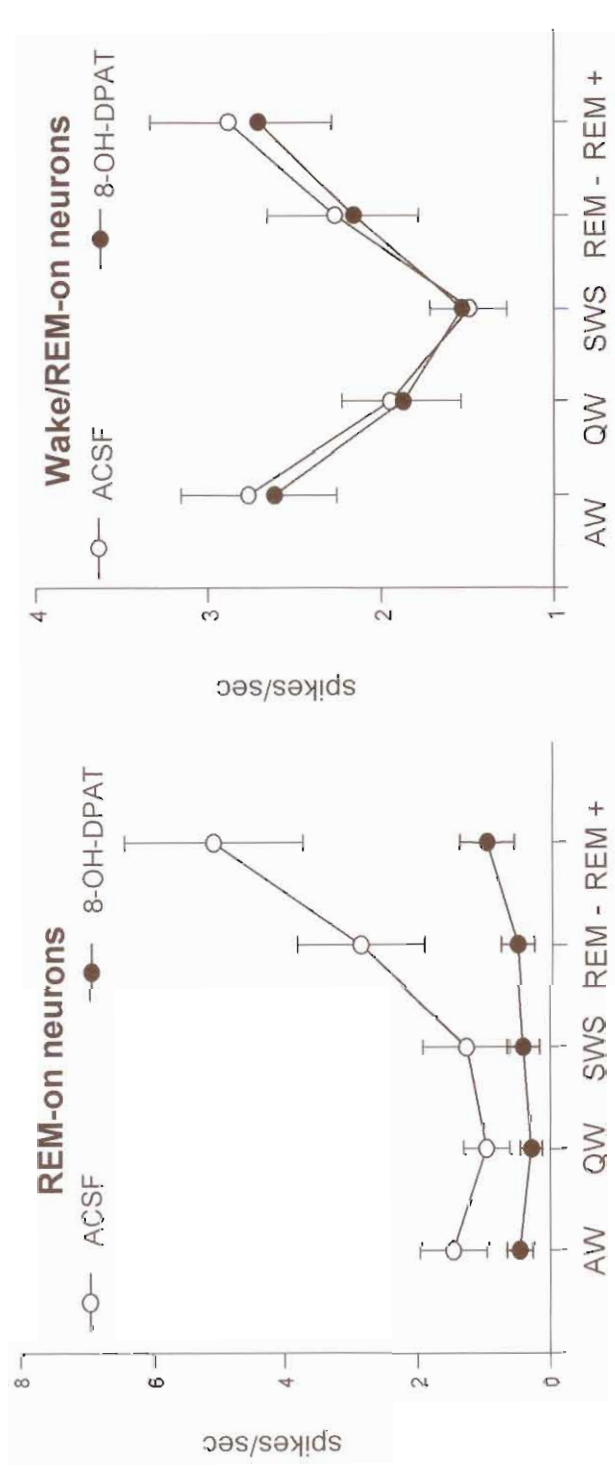


Fig. 4. - *State-related activity of units in the cholinergic LDT and PPT and the effects of a sevotonin 1A agonist applied by microdialysis.*

Left panel. REM-on units (N = 9): Grand mean (+ SEM) of discharge rate in each behavioral state before (open circle, ACSF) and after (closed circle) 10 μM 8-OH-DPAT was added to the perfusate. Note suppresssion of activity (highly statistically significant). Abbreviations are defined in text. Right Panel. Wake/REM-on units (N = 25): Grand mean (+ SEM) of discharge rate of before (open circle, ACSF) and after (closed circle) 10 μM 8-OH-DPAT was added to the perfusate. Note minimal effect of 8-OH-DPAT, not statistically significant. (Adapted from Thakkar *et al.*, 112).

Identification of the recorded neurons in the Thakkar *et al.*1998 paper (112) as cholinergic is consistent with several criteria, although tentative. First, histological reconstruction indicated that all cells were recorded in the anatomically defined cholinergic zones of LDT or PPT. Anatomical studies indicate that, in these regions about 80% of the large neurons (> 20 um cell body diameter) are ChAT positive, indicative of their being cholinergic (44, 108). Second, the recording method used fine wires of 32 and 64 μm diameter, a method that preferentially records larger cells (> 20 μm). Finally, studies have argued that cells in the cholinergic LDT/PPT with long duration action potentials (108), or slow conduction velocity (24) are likely to be cholinergic. The majority of the neurons recorded had long duration action potentials. These data suggest, but do not prove, that the large majority of REM-on and Wake/REM-on cells described are cholinergic.

4. MONOAMINERGIC INFLUENCES. REM-OFF NEURONS

The neurons described in the previous section that increase discharge rate with the advent of REM have been termed "REM-on" neurons. In contrast, groups of other neurons radically decrease and may nearly arrest discharge activity with the approach and onset of REM; these are often termed "REM-off" neurons. The typical discharge activity profile is for discharge rates to be highest in waking, then decrease in synchronized sleep and with near cessation of discharge in REM sleep. REM-off neurons are distinctive both because they are in the minority in the brain and also because they are recorded in zones with neurons that use biogenic amines as neurotransmitters. The loci include a midline zone of the brainstem raphe nuclei, and a more lateral band-like zone in the rostral pons/midbrain junction that includes the nucleus locus coeruleus, a reticular zone and the peribrachial zone. Figure 5 provides a schematic illustration of the time course of REM-on and REM-off neurons over the sleep cycle.

4.1 Raphe Nuclei

Neurons with a REM-off discharge profile were first described by McGinty and Harper (75) in the dorsal raphe nucleus, a finding confirmed by other workers (38, 62, 63, 116). Neurons with the same REM-off discharge pattern have been found in the other raphe nuclei, including nucleus linearis centralis (38, 69), centralis superior (86), raphe magnus (16, 27), and in raphe pallidus (88). Identification of these extracellularly recorded neurons with serotonin-containing neurons was made on the basis of recording site location in the vicinity of histochemically identified serotonin neurons and the similarity of the extracellularly recorded slow, regular discharge pattern to that of histochemically identified serotonergic neurons *in vitro*. Non-serotonergic neurons in the raphe system have been found to have different discharge pattern characteristics. While this extracellular identification methodology does not approach the "gold standard" of intracellular recording and labeling, the circumstantial evidence that the raphe REM-off neurons are serotonergic appears strong.

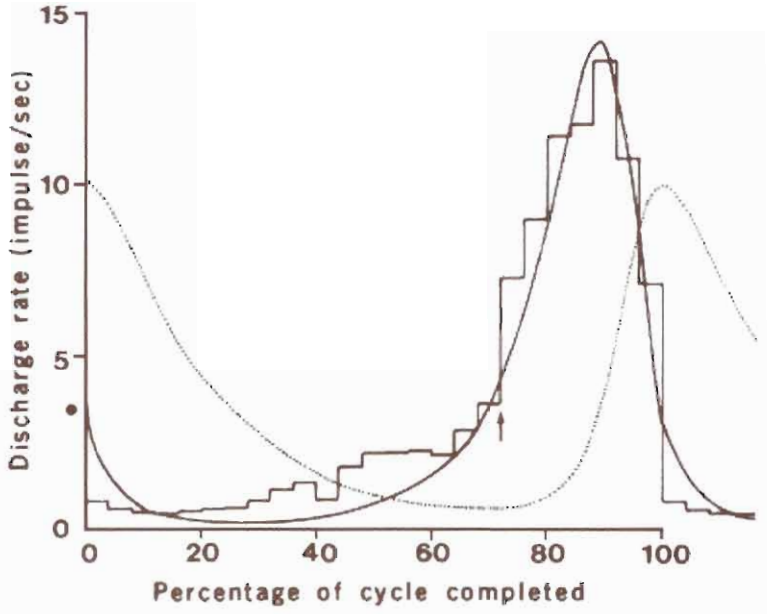


Fig. 5. - *Time course of REM-on neurons (solid lines) and REM-off neurons (dotted lines) over the sleep cycle.*

The cycle begins with the end of one REM period (0%) and ends with the end of the next REM period (100% complete). The data in bins are from averaging of the time course of a REM-on reticular neuron over many cycles and the solid smooth line is the reciprocal interaction mathematical model fit. The arrow marks the bin at which an electrographically defined REM sleep episode is most likely to begin. The REM-off data were similarly derived from Locus Coeruleus recordings (empirical data not shown here, and discharge rate is not to the same scale as REM-on neurons). (Adapted from McCarley and Hobson, 70).

4.2. Locus Coeruleus

The second major locus of REM-off neurons is the Locus Coeruleus, as described in cat (36, 37), rat (2, 3) and monkey (26). The argument that these extracellularly recorded discharges are from norepinephrine-containing neurons parallels that for the putative serotonergic REM-off neurons. Extracellularly recorded neurons that are putatively noradrenergic have the same slow, regular discharge pattern as norepinephrine-containing neurons identified *in vitro* and have the proper anatomical localization of recording sites, including recording sites in the compact locus coeruleus in the rat, where the norepinephrine-containing neurons are rather discretely localized. Thus, while the evidence that these REM-off neurons are norepinephrine-containing is indirect and circumstantial, it nonetheless appears quite strong.

Finally, the remaining groups of REM-off neurons are principally localized to the anterior pontine tegmentum/midbrain junction either in the peribrachial zone, or in a more medial extension of it, recording sites that correspond to the presence of

aminergic neurons scattered through this zone. The "stray" REM-off neurons in other reticular locations also correspond to dispersed adrenergic neuronal groups, although adrenergic identification in this case is much less secure. At this point we note that putatively dopaminergic neurons in substantia nigra and midbrain do not alter their discharge rate or pattern over the sleep-wake cycle (106), and thus are unlikely to play important roles in sleep-wake cycle control.

4.3. Do REM-off neurons play a permissive, disinhibitory role in REM sleep genesis?

The intriguing reciprocity of the discharge time course of REM-off and REM-on neurons led to the initial hypothesis of interaction of these two groups, as originally proposed for the REM-off adrenergic neurons (36, 37, 68, 70). The phenomenological, behavioral and cellular data have been sufficiently strong so that diverse groups of investigators have proposed that the REM-off neurons, as a complete or partial set, act in a permissive, disinhibitory way on some or all of the components of REM sleep, and we will here summarize these postulates, as well as presenting the phenomenology on which they are based. Many of these theories arose in the mid 1970's, as increased technical capability led to extracellular recordings of REM-off neurons.

Raphe System REM-off Neurons and PGO waves. The possibility that the dorsal raphe serotonergic neurons act to suppress PGO waves was explicitly proposed by Simon *et al.* (103), on the basis of lesion data, and the *in vivo* pharmacological experiments using reserpine (13), which depleted brainstem serotonin and simultaneously produced nearly continuous PGO-like waves. McGinty and Harper (75), in their study of extracellularly recorded dorsal raphe REM-off neurons, also noted the inverse relationship between PGO waves and dorsal raphe unit activity. With respect to REM sleep onset, the decrease in discharge activity of presumptively serotonergic raphe neurons is remarkably consistent. This time course of dorsal raphe unit activity (and other components of the sleep wake-cycle) can be averaged over multiple cycles so as to form a picture of the average time course. Using this technique, the time course of presumptively serotonergic dorsal raphe neuronal activity over the sleep-wake cycle and its relationship to PGO waves has been described by Lydic *et al.* (61). There is a clear inverse relationship between PGO waves and dorsal raphe discharge, with a premonitory increase in dorsal raphe activity prior to the end of the REM sleep episode, a phenomenon also observed and commented upon by Trulson and Jacobs (116).

On the basis of *in vivo* pharmacological experiments, Ruch-Monachon and co-workers (87) hypothesized that serotonergic neurons inhibited PGO waves, and also included adrenergic neurons as playing a suppressive role; they further suggested that cholinergic/cholinoceptive systems were actively responsible for their generation. Experiments by Cespuglio and co-workers (15) utilized local cooling of the dorsal raphe in the unanesthetized "semi-chronic cat" [spinal cord transected at T2 and deafferented above T2 (this preparation showed spontaneous sleep cycles)].

Local cooling consistently produced PGO waves and full REM sleep in 35% of the trials. Cryocoagulations at the end of the experiment produced a state with cortical desynchronization and continuous PGO waves, much as in previous pure lesion experiments (48).

Hobson *et al.* (37) and McCarley and Hobson (70) originally proposed that monoaminergic neurons might inhibit reticular REM-on neurons, thought to be cholinergic on the basis of the limited staining methods present at that time. However, stains for acetycholinesterase pointed to LDT/PPT neurons as cholinergic (76) and thus a likely major source of inhibition by monoaminergic neurons. This postulate of monoaminergic inhibition of cholinergic neurons was originally regarded as extremely controversial. However, interest was quickened in the 1990's by 1) documentation of serotonergic projections from the dorsal raphe to the mesopontine cholinergic neurons in the laterodorsal (LDT) and pedunculopontine (PPT) tegmental nuclei that are implicated in the production of REM sleep (40, 98, 107); 2) *in vitro* demonstration of serotonergic inhibition of mesopontine cholinergic neurons (56, 60); and 3) the report that microinjection of a serotonergic 5-HT1A agonist into the PPT inhibits REM sleep (94). It was also demonstrated that the level of serotonin release in the cat dorsal raphe nucleus (DRN), parallels the time course of presumptively serotonergic neuronal activity: waking (W) > slow wave sleep (SWS) > REM sleep (84), suggesting that this would also be true at axonal release sites in the LDT/PPT, since serotonin levels at distant DRN projection sites had the same behavioral state ordering of levels as those in the DRN: W > SWS > REM sleep (4, 41 in rats, 121 in cats).

Since axon collaterals of DRN serotonergic neurons inhibit this same DRN population via somatodendritic 5-HT1A receptors (105), it followed that the introduction of a selective 5-HT1A receptor agonist in the DRN via microdialysis perfusion should produce strong inhibition of serotonergic neural activity, which would be indicated by a reduction of 5-HT release in the DRN. Moreover, if the hypothesis of serotonergic inhibition of REM-promoting neurons were correct, the inhibition of DRN serotonergic activity should disinhibit REM-promoting neurons, producing an increase in REM sleep concomitant with the changes in DRN extracellular serotonin. Portas and collaborators (85) tested the effects of microdialysis perfusion of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a selective 5-HT1A receptor agonist, in freely moving cats. In perfusions during W, DRN perfusion of 8-OH-DPAT decreased 5-HT levels by 50% compared with ACSF, presumptively through 5-HT1A autoreceptor-mediated inhibition of serotonergic neural activity. Concomitantly, as illustrated in Figure 6, this 8-OH-DPAT perfusion produced a short latency, 3-fold increase in REM sleep, from 10 to 30% of the total recorded time ($p < 0.05$), while waking was not significantly affected. In contrast, and suggesting DRN specificity, 8-OH-DPAT delivery through a probe in the aqueduct did not increase REM sleep but rather tended to increase waking and decrease SWS.

These data in the cat were confirmed in the rat. Bjorvatn *et al.* (10) used microdialysis to perfuse 8-OH-DPAT (10 mM) into the DRN of rats and found a 4-fold increase in REM sleep compared to control perfusion with ACSF, while the other vigilance states were not significantly altered.

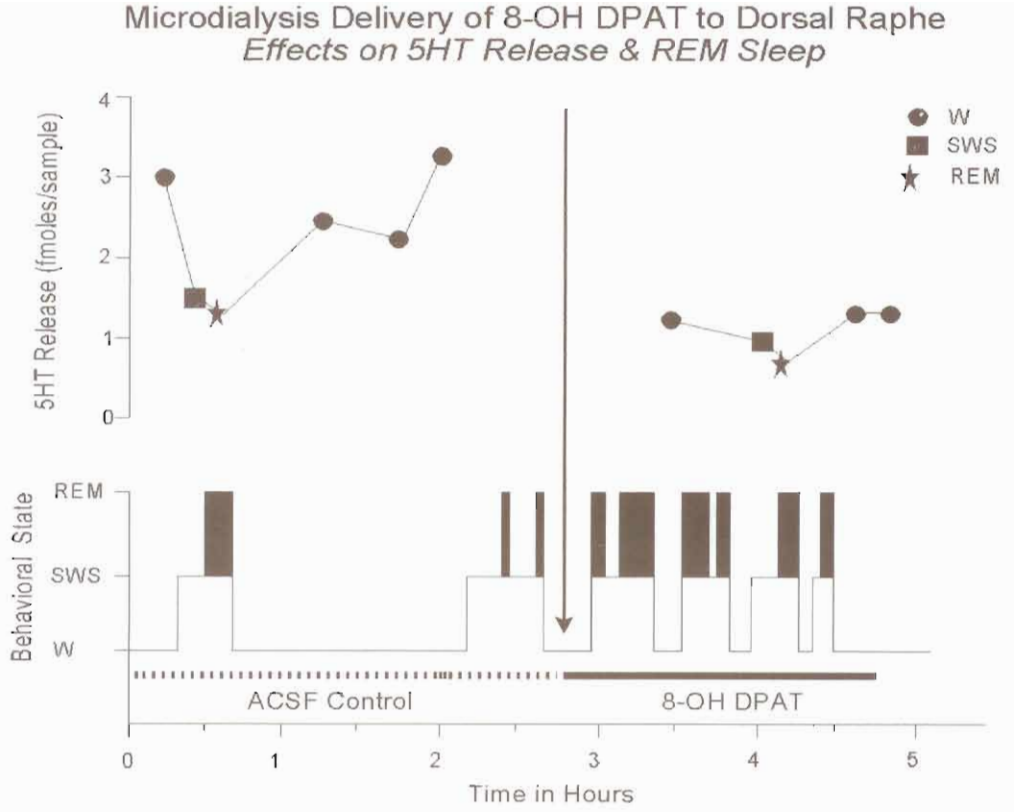


Fig. 6. - *Time course of 5-HT levels (top portion of figure) and behavioral state (bottom portion of figure) during control DRN ACSF perfusion (interrupted horizontal line) and during DRN 8-OH-DPAT perfusion (solid horizontal line) in a typical experiment.*

Note that, prior to perfusion, waking DRN 5-HT levels (circles) are higher than those in slow wave sleep (squares) and REM sleep (stars). Each 5-HT value is expressed as fmoles per 7.5 µl sample, and was obtained during an uninterrupted 5 minute sequence of the behavioral state. Upon the onset of 10 µM 8-OH-DPAT perfusion (arrow) the 5-HT level dropped quickly to levels as low as those normally present in SWS or REM. Behaviorally, 8-OH-DPAT administration markedly increased REM sleep (black bars in the hypnogram). Adapted from 85.

Sakai and Crochet (90) failed to replicate the findings of Portas *et al.* (85) in the cat and Bjorvatn *et al.* in the rat (10). Sakai and Crochet used microdialysis to perfuse 8-OH-DPAT (10, 50, 100, and 500 mM) into the DRN of cats and found a dose-dependent increase in wakefulness and decrease in slow-wave sleep (SWS), but there was no significant effect on the generation of REM sleep. They suggested that the Portas *et al.* data were flawed because the control period had "relatively high amounts of wakefulness and low amounts of REM sleep", and this allowed the comparison with REM sleep after 8-OH-DPAT to become statistically significant. However, as described above, Portas *et al.* reported the mean% time in REM sleep

after 8-OH-DPAT was 30% (18 min/hour), a value considerably higher than Sakai and Crochet's control values for any of their 7 control hours ($p < 0.007$, binomial test), and, overall, more than 2-fold higher than their average control value of about 14%. It is difficult to be certain why Sakai and Crochet did not find this effect. However, Sakai and Crochet did not, unlike Portas *et al.*, monitor 5-HT release before and during 8-OH-DPAT to verify the that the expected pharmacological effect of 8-OH-DPAT was occurring (reduction in 5-HT release), and hence indicating adequate delivery to DRN. It is thus difficult to be certain that the expected delivery of 8-OH-DPAT to a sufficiently large number of neurons in DRN occurred (although effects on single neurons were monitored); possible causes of delivery problems include gliosis on the microdialysis membrane and/or localization problems. Portas *et al.* (85) reported findings similar to Sakai and Crochet (90) of increased wakefulness and decreased SWS with 8-OH-DPAT delivery from probes not in the DRN.

The data of Portas *et al.* (85), however, did not directly demonstrate serotonergic inhibition of neurons in the cholinergic LDT/PPT. Moreover the presence of some neurons with REM-on and other neurons with Wake/REM-on activity in LDT/PPT was a puzzle in terms of the global changes in monoaminergic inhibition. McCarley *et al.* (74) postulated that while monoamines might inhibit REM-on cholinergic neurons, Wake/REM-on neurons might not be inhibited, thus explaining their continued activity in waking – since serotonergic activity is highest during wakefulness, the observed high discharge rate of Wake/REM-on neurons during wakefulness would not be consistent with a high level of serotonergic inhibition from a high level of DRN activity. *In vitro* data were also consistent with a subset, not the entire population, of LDT/PPT cholinergic neurons inhibited by serotonin acting at 5-HT1A receptors (56, 60). Thakkar and collaborators (112) developed a novel methodology allowing both extracellular single cell recording and local perfusion of neuropharmacological agents via an adjacent microdialysis probe in freely behaving cats to test this hypothesis of differential serotonergic inhibition as an explanation of the different state-related discharge activity. Discharge activity of REM-on neurons was almost completely suppressed by local microdialysis perfusion of the selective 5-HT1A agonist 8-OH-DPAT, while this agonist had minimal or no effect on the Wake/REM-on neurons, as illustrated in Figure 4, presented above. Of note, the ordering of 5-HT concentrations in the cholinergic PPT is Wake > nonREM > REM, consistent with the unit discharge data and, moreover, application of the 5HT1A agonist 8-OH DPAT to the PPT suppressed REM sleep and increased wakefulness (Strecker *et al.*, unpublished data and reference 109a, see Figure 7).

The finding that only a subpopulation of the recorded LDT/PPT cells were inhibited by 8-OH-DPAT is consistent with rat pontine slice data, where, in combined intracellular recording and labeling to confirm the recorded cell's cholinergic identity, 64% of the cholinergic neurons in the LDT/PPT were inhibited by serotonin (60). However, *in vitro*, it was obviously not possible to determine if the cells recorded *in vitro* are REM-on, or Wake/REM-on, since there were no purely electrophysiological criteria sufficient to identify the cell's state-related characteristics. The different percentages of LDT/PPT neurons that are inhibited by serotonin or serotonin agonists *in vitro* (64%) compared with the Thakkar *et al.* (112) *in vivo* findings (36.4%)

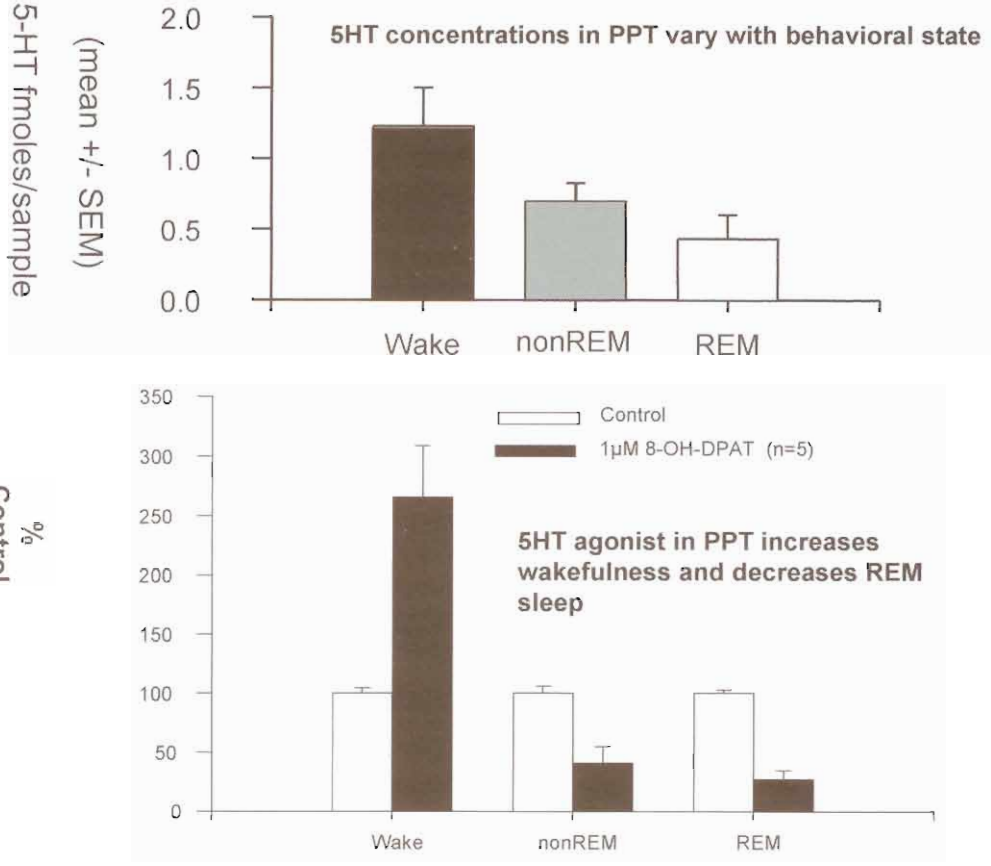


Fig. 7. - *Top Panel. Microdialysis measurements of 5HT concentrations in the cholinergic PPT parallel the behavioral state discharge rate ordering of dorsal raphe neurons.*

Adapted from 109a. Bottom panel. A microdialysis applied 5HT agonist suppresses REM sleep and increases wakefulness.
Strecker *et al.*, unpublished data.

may be due to anatomical differences between species (rat vs. cat) and/or different concentrations of agents at the receptors. Luebke *et al.* (60) did not do a concentration-response study and their bath-applied serotonin agonists may have had a higher concentration at receptor sites than in the present study. Further research will be needed to determine whether differential serotonergic inhibition in LDT/PPT results from differing serotonergic innervation, and/or different receptor distribution or sensitivity on different neurons. Anatomical studies in the cat of the percentage of mesopontine cholinergic neurons with 5-HT1A receptors are needed, as has been done in rat forebrain (52). It should be noted that, in addition to serotonin inhibition of rat mesopontine neurons *in vitro,* an additional serotonergic suppression of dendritic calcium influx has been reported (57).

Locus Coeruleus and REM sleep phenomena. – Lesion Studies. Lesion studies furnish an unclear picture of the role of the LC in REM sleep. Bilateral electrolytic lesions of LC in cat by Jones *et al.* (46) led these workers to conclude the LC was not necessary for REM sleep. In the REM-like sleep state following the lesion there was a two-fold reduction of PGO spikes while the number in deep synchronized sleep increased approximately three-fold, so that the total number of spikes remained approximately the same – a picture much like that following acute raphe lesions. Over time the total number of PGO spikes declined and the percentage of a REM sleep-like state increased from about 5% to 10%, vs. a control value of 15%. We use the term "REM sleep-like" because muscle atonia was abolished and there was in fact motor activity like that described in the previous chapter for the "REM sleep without atonia" state following tegmental lesions; this syndrome likely resulted from spread of the lesion to the reticular area subserving atonia. Other lesion effects included loss of spontaneous micturition and defecation, a rise in mean temperature from 37.1 to 38.3 degree C, loss of grooming, and a loss of coordination and balance.

The picture following *unilateral* locus coeruleus lesions was quite strikingly different. Caballero and De Andres (14) found a 50% *increase* in the percentage of REM sleep ($p < 0.001$) following unilateral electrolytic lesions of locus coeruleus in cats; cats with lesions in neighboring tegmentum and sham-operated controls showed no change. The post-operative condition of animal with unilateral lesions was much better than after bilateral lesions; in only one unilaterally LC-lesioned animal was there urinary retention, and this was transient and no "alteration in any other vegetative function was observed." Accordingly, Caballero and DeAndres (14) attributed the differences between their study and that of Jones *et al.* (46) to non-specific effects of the larger lesions that, as with almost any CNS insult, may have led to a REM sleep reduction.

Locus Coeruleus Cooling induces REM Sleep. Cespuglio and co-workers (17) performed unilateral and bilateral cooling of the locus coeruleus using the same methodology as described above for the dorsal raphe cooling. The effects of cooling were quite clear-cut. Three cats each had 5 trials of bilateral cooling, and in all 15 trials there was progression to synchronized sleep (30-60 sec) and then to the transition phase with PGO waves (2-3 min), and then, in 40-50% of the trials, to full-blown REM sleep (3-4 min after cooling onset). Unilateral cooling produced exactly the same picture in 92% of the trials. In repeated cooling trials REM sleep was repetitively induced, and the percentage of REM sleep increased by 120% over control periods. Cooling experiments produced repeatable effects, implied temporary inactivation and not destruction of neuronal elements, and also because they clearly enhanced REM. This raises the general point that non-specific effects of destructive lesions always decrease REM, as do other CNS insults. Satinoff's comment (97) about non-specific effects is that, "One might also say that rendering an animal unconscious by a blow to the head eliminates REM sleep. In a sense it does, but that sense is completely trivial." It is consequently hard to draw definitive and inter-

pretable conclusions about destructive lesions, especially those that do not enhance REM sleep. Jones, for example, concluded that her lesions showed the LC was not necessary for REM sleep, in the sense of being a region actively promoting REM (as had been proposed in the early Jouvet theory). While this interpretation appears reasonable, an important alterative interpretation was not ruled out: namely that the LC plays a permissive, disinhibitory role, but that non-specific effects of the large lesions prevented the appearance of increased REM sleep, as we have seen did take place with both unilateral LC lesions and cooling that inactivated monoamine neurons. Later studies in the Jones laboratory were consistent with this disinhibition hypothesis (65). In summary, many non-specific factors decrease REM and few, if any, increase it; consequently lesions or manipulations that increase REM are always more directly interpretable.

Site(s) of REM-off and REM-on interaction. The model for REM sleep control to be proposed discusses REM-off suppression of REM-on neurons. It must be emphasized that there are several, non-mutually exclusive possible sites of interaction. These include direct ACh-NE interactions in the LDT and PPT. For example, there is now evidence that ChAT-labeled fibers are present in locus coeruleus and it has long been known that the NE-containing LC neurons also stain intensely for the presence of acetylcholinesterase (see review of NE-ACh anatomical interrelationship in 45). NE varicosities are present throughout the reticular formation and the LDT and the peribrachial area that is the site of ChAT-positive neurons. Thus, adrenergic-cholinergic interactions may take place directly between these two species of neurons and/or may take place at reticular neurons.

5. GABAERGIC INFLUENCES AND REM SLEEP

In addition to the monoamines and acetylcholine as modulators and controllers of the sleep cycle, there is accumulating evidence that GABAergic influences may play an important role. Defining the role of GABA with certainty is difficult, however. Since GABA is a ubiquitous inhibitory neurotransmitter, purely pharmacological experiments using agents that increase or decrease GABA do not answer a key question, namely whether the results so obtained were representative of the increases or decreases in GABA that occur naturally in the course of the sleep cycle, or were simply and trivially the result of a pharmacological manipulation of GABA systems not naturally playing a role in sleep cycle control. Microdialysis is potentially a very useful way of sampling naturally occurring changes in GABA levels over the sleep cycle, but is often limited in sensitivity and hence in time resolution of when the changes occur in the sleep cycle.

This section surveys GABA data from dorsal raphe, locus coeruleus, and pontine reticular formation that are relevant to sleep-wakefulness control. From the standpoint of sleep cycle control, one of the most puzzling aspects has been defining what causes the "REM-off" neurons in the LC and DRN to slow and cease discharge as

REM sleep is approached and entered. The reciprocal interaction model (see below) which hypothesized a recurrent inhibition of LC/DRN might account for this. While recurrent inhibition is present, there is no clear evidence that it might be the causal agent in REM-off neurons turning off. Thus, the prospect that a GABAergic mechanism might be involved is of great intrinsic interest. We go into some detail about the GABA levels reported, since GABA sampling is evolving technically.

5.1 Dorsal Raphe Nucleus

Microdialysis. – Nitz and Siegel (78) obtained *in vivo* microdialysis samples from the DRN in naturally sleeping cats, noting that "cessation of firing of serotonergic dorsal raphe neurons is a key controlling event of rapid eye movement (REM) sleep." This study is the single extant microdialysis study of GABA release in DRN, and reported a significant increase in GABA levels in REM sleep (0.072 pmol/uL or 72 fmol/uL) compared with wakefulness (0.042 pmol/uL), while SWS (0.049 pmol/L) did not significantly differ from wakefulness. Glutamate and glycine release did not change over the sleep cycle. Further supporting a GABA role in REM control via inhibition of serotonergic neurons was the 67% increase in REM sleep observed with microinjections of the GABA agonist muscimol into the DRN and the observation that reverse microdialysis of the GABA antagonist picrotoxin completely abolished REM sleep. For comparative purposes we note that the increase in REM sleep observed with microdialysis application of the 5HT1A agonist 8-OH-DPAT to DRN by Portas *et al.* (85) was greater (190%) suggesting that factors other than GABA might influence serotonergic neurons.

Although the data did not directly support GABAergic inhibition as a mechanism of the slowing of serotonergic unit discharge in the passage from wakefulness to SWS, Nitz and Siegel noted that the possibility a small increase in the release of GABA, possibly beyond the resolution of the microdialysis technique, might be sufficient to reduce DRN unit discharge in SWS.

Microiontophoresis. – Levine and Jacobs (58) showed in cats that iontophoretic application of bicuculline reversed the typical suppression of DRN serotonergic neuronal activity seen during SWS but had no effect on maintained activity during W and the more complete suppression of activity occurring during PS. An interpretation of this finding offered by Nitz and Siegel was that bicuculline was able to antagonize the lesser GABAergic inhibition during SWS, but not the greater inhibition during REM sleep, since bicuculline is a competitive GABA-A blocker, unlike the picrotoxin used by Nitz and Siegel (78).

Gervasoni *et al.* (31) reported that, in the unanesthetized but head-restrained rat, the iontophoretic application of bicuculline on DRN serotonergic neurons, identified by their discharge characteristics, induced a tonic discharge during SWS and REM and an increase of discharge rate during quiet waking. They postulated that an increase of a GABAergic inhibitory tone present during wakefulness was responsible for the decrease of activity of the DRN serotonergic cells during slow wave and REM sleep. In addition, by combining retrograde tracing with cholera toxin B sub-

unit and glutamic acid decarboxylase immunohistochemistry, they provided evidence that the GABAergic innervation of the dorsal raphe nucleus arose from multiple distant sources and not only from interneurons as classically accepted. Among these afferents, they suggested GABAergic neurons located in the lateral preoptic area and the pontine ventral periaqueductal gray, including the DRN itself, could be responsible for the reduction of activity of the DRN serotonergic neurons during SWS and REM sleep, respectively.

However a report from the same laboratory in the same year described results at variance with those of Gervasoni *et al.* (31). Sakai and Crochet (89) used in vivo extracellular unit recordings combined with microdialysis infusion in the cat, but were unable to block the cessation of discharge of presumed serotonergic DRN neurons during REM sleep by either bicuculline or picrotoxin application. Rather, in subpopulations of DRN neurons, cessation of REM sleep discharge was completely blocked by either histamine (2 or 5 mM, in 19/27 neurons), or phenylephrine, an alpha1 adrenoceptor agonist (2 mM, in 10/21 neurons). (No neuron responded both to histamine and to phenylephrine). Suppression of spontaneous discharge of DRN neurons during quiet wake and SWS occurred with microdialysis application of mepyramine, a specific H1 histamine receptor antagonist (N = 9 histamine-responsive neurons) or prazosin, a specific alpha1 adrenoceptor antagonist (N = 2 phenylephrine-responsive neurons). Sakai and Crochet (89) concluded that the suppression of REM-off neuronal discharge was the result of SWS and REM sleep disfacilitation of excitatory histaminergic and noradrenergic projections to DRN. No data were presented on whether DRN application of mepyramine and/or prazosin was able to increase REM sleep percentages, as were GABA antagonists and serotonin agonists, as described above. Sakai and Crochet (91) found an increase in DRN neuronal antidromic excitability during REM sleep, mainly due to a decreased spontaneous discharge rate, a cause that could be due to either disfacilitation or inhibition. While it is entirely possible that GABA phrmacological actions could differ radically in the cat and rat, the most parsimonious interpretation is that the Gervasoni *et al.* studies (31) in the rat and Sakai and Crochet (89) in the cat differed in technical aspects. The argument for a different pharmacology in the cat and rat is weakened also by the results of Nitz and Siegel (78) which agreed with the Gervasoni *et al.* (31) rat data.

5.2 Locus Coeruleus

Microdialysis. – The single published study on sleep-wake analysis of GABA release in the Locus Coeruleus region placed microdialysis probes on the border of LC or in the peri-LC region in the cat (79). GABA release was found to increase during REM sleep (1.9 fmol/μL) as compared to both waking values (1.2 fmol/μL) and SWS (1.6 fmol/μL). GABA release during SWS showed a trend-level significance ($p < 0.06$) when compared with waking. The concentration of glutamate and glycine in microdialysis samples was unchanged across sleep and wake states. These data, because of the SWS differences, appear to offer more direct support for LC than for DRN neurons for the hypothesis of GABA-induced inhibition causing the reduction in LC/DRN discharge in SWS and virtual cessation of firing in REM sleep.

Incidentally, the authors did not explicitly comment on the reason for their finding a 35-fold greater GABA concentration in the DRN than in the LC during waking; this may have been due to various methodological differences, and thus calls to attention the difficulty in measuring GABA.

Microiontophoresis. – Gervasoni and colleagues (30) applied their methodology of microiontophoresis and single-unit extracellular recordings in the LC of unanaesthetized, head-restrained rats. Bicuculline, a GABA-A receptor antagonist, was able to restore tonic firing in the LC noradrenergic neurons during both REM sleep (in contrast to its effects in the DRN) and SWS. Application of bicuculline during wakefulness increased discharge rate. These data, combined with Nitz and Siegel (79), are thus consistent with GABAergic inhibition in the LC during REM and SWS.

5.3. Source of state-related GABAergic input to DRN and LC.

Overall, the DRN and LC studies just surveyed are consistent with, but don't prove the hypothesis that increased GABAergic inhibition leads to REM-off cells turning off. The increased GABAergic tone could simply be a *consequence* of other state-related changes without causing these changes. Here, as with other neurotransmitters, it would be helpful to have unit recordings of GABAergic neurons with inputs to LC/DRN. One could see if these neurons had the requisite lead times and state-related discharge time course to cause the changes. Where might these neurons be located?

Periaqueductal Gray? The Gervasoni *et al.* (31) study on DRN pointed to the periaqueductal gray (PAG) as a possible source of the GABAergic input proposed to inhibit DRN neurons. The PAG is involved in the control of a number of behavioral and physiological functions, many related to autonomic and visceral function, as well as to pain (reviewed in 9). With respect to behavioral state control, there are reports that suggest the ventrolateral division of the PAG (vlPAG) may be involved in the regulation of REM sleep, since both vlPAG lesions (83) and muscimol injections (96) produced a large increase in REM sleep. Thakkar and colleagues (113) thus decided to record vlPAG unit activity in freely behaving cats to determine if neurons selectively increased their tonic discharge activity before and during REM sleep, and hence might furnish GABAergic inhibition of monoaminergic neurons. Several types of state-specific neuronal populations were found in the periaqueductal gray, but none of the 33 neurons showed a tonic discharge increase before and during REM, and rather were phasic in pattern and increased discharge rate too late in the cycle to be a cause of the DRN SWS suppression. These data thus suggest that, although vlPAG neurons may regulate phasic components of REM sleep, they do not have the requisite tonic pre-REM and REM activity to be a source of GABAergic tone to monoaminergic neurons responsible for their REM-off discharge pattern. This (and any) study with negative findings can be critiqued on failure to study more potential exemplars. However, compared with other unit studies, this study did record more neurons than most; its negative findings would suggest that, at a minimum, neurons with the requisite activity are not abundant in the vlPAG.

Ventro-lateral preoptic area (VLPO)?. This forebrain site was retrogradely labeled by Gervasoni *et al.* (31) as projecting to the DRN. Forebrain influences on REM sleep are discussed in the next chapter, but the Jouvet transection experiments suggest, however, these are not essential for the basic REM cyclicity found in the pontine cat.

5.4. GABA and the pontine reticular formation: disinhibition and REM sleep

Pharmacological studies in cats on the behavioral state effects of GABA agents. In 3 chronic, unanesthetized cats, Xi *et al.* (123) microinjected GABA, muscimol (GABA-A receptor agonist) and bicuculline (GABA-A receptor antagonist), separately, into the nucleus pontis oralis (NPO) in a region about 2 mm lateral to the midline and more than 1 mm ventral to LC, a region where carbachol induced a short latency (< 4 min) onset of REM sleep. The injection of either GABA or muscimol induced wakefulness; SWS and REM sleep were suppressed. In contrast, the injection of bicuculline induced a prolonged state that was similar to naturally-occurring REM sleep with muscle atonia, EEG desynchronization and rapid eye movements. This REM-like state was 3-fold more prominent that natural REM sleep (36% and 12% respectively of recording time).

Xi *et al.* (124) elaborated on their 1999 (123) data and microinjected GABA-B agents baclofen (GABAB agonist) and phaclofen (GABAB antagonist) as well as muscimol and bicuculline into the NPO. Microinjection of 10.0 mM muscimol into RPO in cats significantly and immediately increased wakefulness at the expense of REM and NREM, whereas 10.0 mM bicuculline enhanced REM, producing increases in the percentage of time in this state, its frequency and reducing the latency to onset. In contrast, injections of bicuculline or phaclofen produced active sleep. The percentage of time spent in active sleep and the frequency of active sleep increased while the percentage of time spent in wakefulness and the latency to active sleep was significantly reduced. The effects of baclofen and phaclofen were similar to the GABA-A agents, but less strong. These data suggested that pontine GABAergic processes acting on both GABA-A and GABA-B receptors might play a critical role in generating and maintaining wakefulness and in controlling the occurrence of the state of REM sleep.

Pharmacological studies in rats on the behavioral state effects of GABA agents. In the head-restrained rat, Boissard *et al.* (11) used microiontophoresis of the GABA-A antagonists bicuculline and gabazine in the pontine reticular formation just ventral to the locus coeruleus and LDT, termed the dorsal and alpha subcoeruleus nuclei by the Paxinos and Watson atlas (82) and the sublaterodorsal nucleus (SLD) by Swanson (110). These agents produced a REM-like state with some, but not all, of the electrographic characteristics of REM sleep. Muscle atonia was a prominent and consistent feature, and an anatomical study showed anterogradely labelled fibres originating from the SLD were apposed on glycine- and C Fos-positive neurons (labelled after 90 min of pharmacologically induced REM-like state) in the medullary gigantocellular and parvicellular reticular nuclei, likely sources of muscle inhibition during REM sleep. The EEG spectrum of natural REM sleep was

not consistently present in this pharmacological state; the EEG spectrum was either intermediate between W and REM (39% of trials) or similar to W (61% of trials), including little theta activity. Rapid eye movements and penile erections were notably absent in this pharmacologically induced state. In contrast to the cat, carbachol applied to the SLD in these head-restrained rats produced wakefulness and not REM sleep. The authors interpreted these data as supporting the production of the REM-like phenomena through GABA disinhibition, especially the muscle atonia.

Sanford *et al.* (95) assessed REM after bilateral microinjections into RPO and RPC of muscimol and bicuculline in rats during the light (inactive) period. In RPO, muscimol (1,000 uM) suppressed REM while bicuculline (1,000 uM) enhanced REM. In RPC, muscimol (200, 1,000 uM) suppressed REM, but bicuculline (1,000 uM and less) did not significantly affect REM. Higher concentrations of BIC (10,000 uM) injected into RPO and RPC produced wakefulness, circling, and escape behavior. Of note, bicuculline induced an overall increase in REM across the 6-h recording period that was greatest in the third and fourth hours after the injection, but the pronounced short-latency, long-duration increase in REM seen in cats (123) was not observed.

This observation is similar to the absence of short latency, long duration enhancement of REM sleep with carbachol in the rat and its presence with these very different agents suggests a species difference in REM organization, perhaps related to the more circadian rats. Repeating the experiments of Sanford *et al.* (95) and Boissard *et al.* (11) in the dark active phase would be of interest in determining whether circadian phase is an important variable in response to these agents in the rat.

Microdialysis measurements of GABA in the pontine reticular formation. Recently Thakkar *et al.* (unpublished data and 114) have studied GABA release in pontine reticular formation of freely moving cats (Figure 8). They validated GABA measurements by increasing/decreasing GABA release by local microdialysis perfusion of 1) high (100 mM) K+; 2) GABA uptake blockers (SKF 89976A and nipecotic acid); and 3) 10% procaine. The sensitivity in 5 uL samples was 0.04 pmol/sample. In the four PRF sites thus far tested, multiple episodes of REM sleep had consistently lower levels of GABA than Wakefulness (Figure 8). Although wake was not statistically different from SWS, there was a trend was toward lower GABA levels in SWS. These data provide very preliminary but direct evidence compatible with GABA disinhibition in the pontine reticular formation during REM sleep.

5.5. The pedunculo-pontine tegmental nucleus (PPT)

Torterolo *et al.* (115) microinjected muscimol and bicuculline into the PPT of 4 chronic cats. Muscimol increased the time spent in REM sleep by increasing the frequency and duration of REM episodes; this increase was at the expense of the time spent in wakefulness. A decrease in PGO density during REM sleep was also observed following the microinjection of muscimol. On the other hand, bicuculline decreased both REM sleep and SWS and increased the time spent in wakefulness. These data were somewhat paradoxical in that an inhibitory agent increased REM

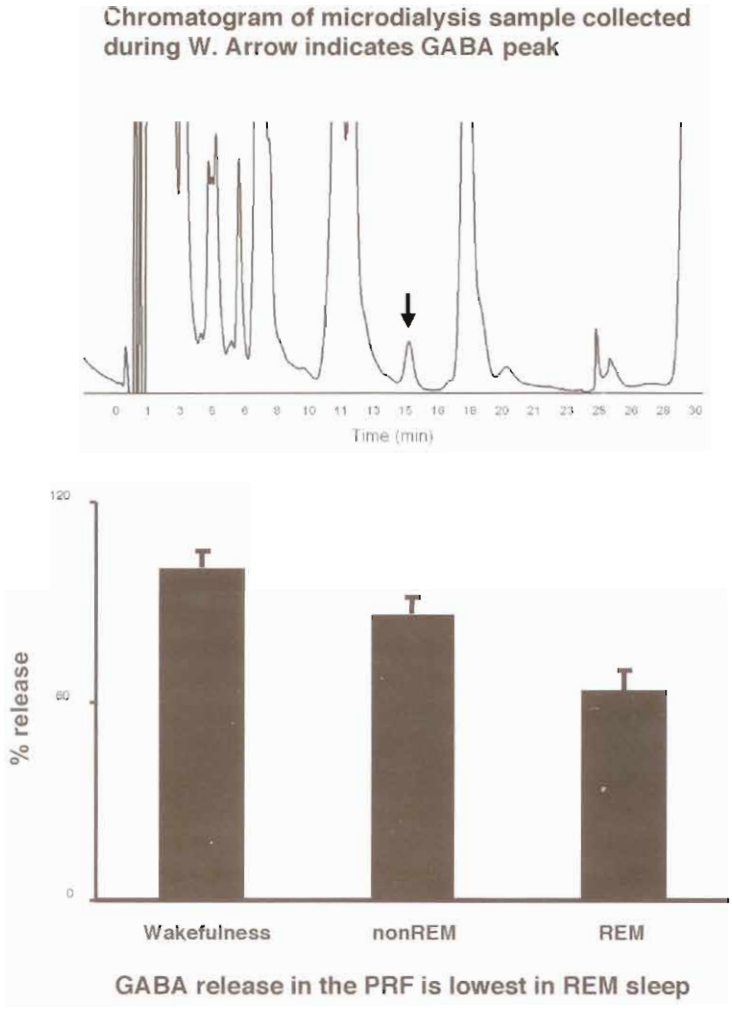


Fig. 8. - *Thakkar, Tao and McCarley, unpublished data and adapted from reference 114*

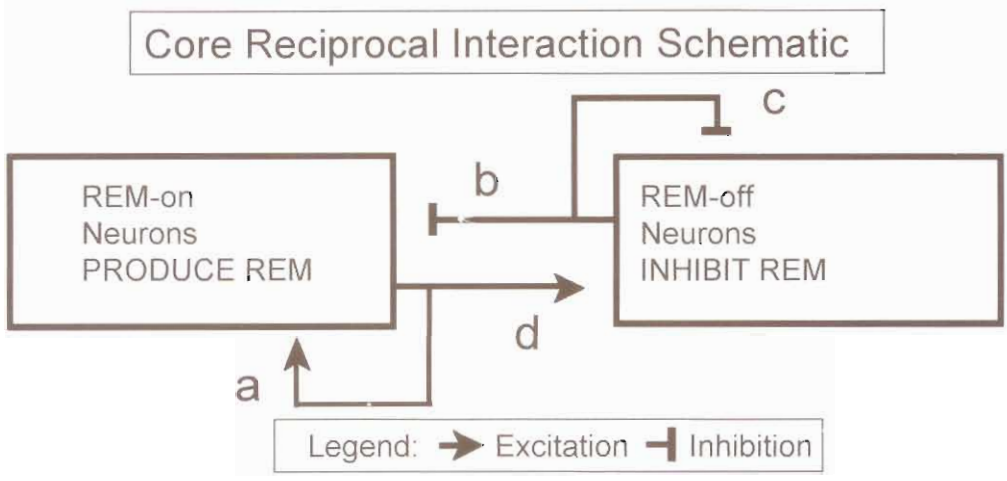
sleep; the authors suggest that the GABA-A agonist acted primarily to suppress the activity of wakefulness-promoting PPT neurons, which are in a majority. We think there is an alternate explanation: GABA agonists may primarily act to inhibit the GABAergic PPT neurons inhibiting cholinergic REM-on neurons. Since the PPT Wake/REM-on neurons are active in natural REM sleep as well as in Wake, it is highly unlikely they would act to suppress REM sleep. Similarly, bicuculline might act to disinhibit PPT GABA neurons; however their actions on PPT cholinergic neurons would also be blocked, as would other incoming GABAergic influences from outside the ipsilateral PPT, thus producing a net disinhibition of PPT cholinergic neurons.

6. A STRUCTURAL MODEL OF REM SLEEP GENERATION

This section presents a structural model of REM sleep cyclicity, based on the data discussed above, with a very brief sketch of a mathematical model incorporating a limit cycle. The history of the development of structural models encompasses the history of discovery of neurons and neurotransmitters important in REM sleep, and is one of growing complexity. The first formal structural and mathematical model was presented in 1975 by McCarley and Hobson (70). This model, termed the Reciprocal Interaction model, was based on the interaction of populations of REM-on and REM-off neurons and mathematically described by the Lotka-Volterra equations, derived from population models of prey-predator interaction. This paper will suggest that the basic notion of interaction of REM-on and REM-off neuronal populations is a very useful one for modeling and conceptualization, even though the description of the populations of neurons characterized as REM-on and REM-off has been altered and made much more detailed. Before getting into the details of the anatomy and the interaction, we first point the reader to Figure 9 which describes the "core" features of the structural and mathematical model, and provides a non-mathematical description of the dynamics. The subsequent parts of this section elaborate on this core model in terms of current knowledge of the physiology and anatomy.

6.1 REM-on neurons and the postulate of self excitation (positive feedback) and exponential growth-term "a" in Figure 9. –

The 1975 model (70) was constructed before ChAT identification of cholinergic neurons and the REM-on population was simply characterized as pontine reticular formation neurons. Today we view a subset of cholinergic neurons in the LDT and PPT as being the principal REM-on neuronal population; they are conceptualized as interacting with pontine reticular neurons in a mutually excitatory positive feedback relationship (initially described by 73). We have described strong in vitro and in vivo data indicating the excitatory effects of acetylcholine (ACh) on pontine reticular formation (PRF) neurons and clear anatomical evidence of cholinergic projections from the mesopontine laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) to both PRF and bulbar reticular formation (BRF). These projections depolarize and excite neurons in the effector systems important in REM sleep, including those for rapid eye movements, PGO waves, muscle atonia, and EEG desynchronization. The conjoint activation of these neuronal groups produces the state of REM sleep. In terms of details of modeling the positive feedback system, a key consideration is the fact that acetylcholine hyperpolarizes (inhibits) the cholinergic neurons (56), and thus the exponential growth of REM-on neuronal activity can not be due to a positive feedback within the LDT/PPT neuronal population. Instead the positive feedback for exponential growth in REM-on activity likely stems from the inclusion of reticular formation neurons in the loop: LDT/PPT-->PRF-->LDT/PPT. The reader is referred to Figure 10 which describes this structure of the REM-on population, and should be referred to for details of the current anatomical/structural model. (Additional positive feedback occurs via reticulo-reticular connections; for simplicity this feature is not graphed in Figure 10).



Core Reciprocal Interaction equations (Lotka-Volterra equations)

$X'(t) = aX - bXY$

$Y'(t) = -cY + dXY$

where

X = Time course of activity of REM-on neurons,

Y = Time course of activity of REM-off neurons

Fig. 9. - *Summary of the "core" features of the reciprocal interaction model.*

The REM-on neuronal population has a positive feedback so that activity grows (see connection labeled "a"). This activity gradually excites the REM-off population (connection "d"). The REM-off population then inhibits the REM-on population (connection "b"), terminating the REM episode. The REM-off population is also self-inhibiting (connection "c"), and as REM-off activity wanes, the REM-on population is released from inhibition and is free to augment its activity. This begins a new cycle of events. This interaction is formally described by the Lotka-Volterra equations, where X = REM-on activity and Y = REM-off activity.

Supporting data for the PRF–>LDT/PPT excitation include the presence of reticulo--> LDT/PPT projections (34, 35), evidence that excitatory amino acids (EAA) are the principal excitatory transmitters of PRF (109), and *in vitro* evidence for excitation of LDT/PPT neurons by EAA (92, 93). Further supporting the concept of PRF-LDT/PPT interaction during REM sleep are data showing that a medial PRF microinjection of carbachol that induced REM sleep also increased the release of acetylcholine in the medial PRF contralateral to the injection site, presumably as a result of PRF excitation of LDT/PPT neurons (64). Finally, as described above, unit recording data indicate that a subset of LDT/PPT neurons becomes selectively active just before and during REM sleep, as would be expected of neurons promoting this state.

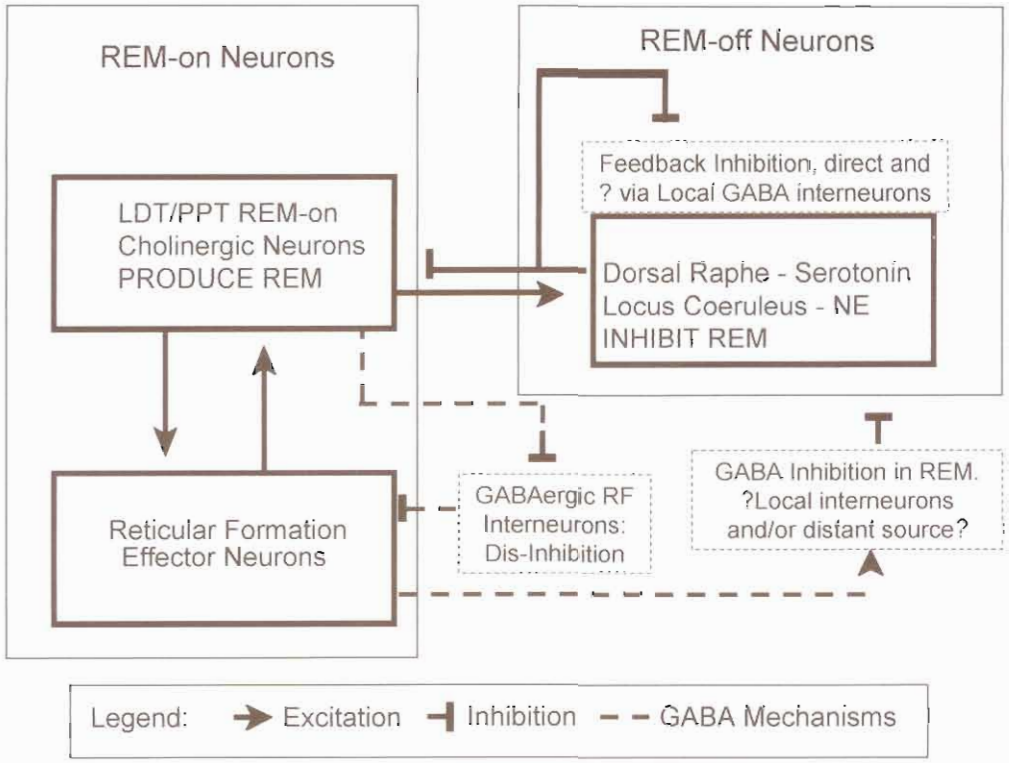


Fig. 10. - *A structural model of REM sleep control.*

See text for description.

6.2 Reticular formation and GABAergic influences. –

Not only may LDT/PPT cholinergic input excite PRF neurons but there is the intriguing possibility that inhibitory LDT/PPT projections from REM-on neurons impinge onto GABAergic PRF interneurons with projections onto PRF neurons. This would have the effect of disinhibiting glutamatergic PRF neurons as REM sleep was approached and entered. Gerber *et al.* (29) found that about one-fourth of PRF neurons *in vitro* were inhibited by muscarinic cholinergic agents. Whether these neurons that were inhibited were GABAergic or not, however, is still not known. Preliminary data in the cat support cholinergic inhibition of GABAergic neurons, since microdialysis application of carbachol to the PRF not only induced REM, but also decreased GABA concentrations in samples from the same microdialysis probe (114). Moreover, as outlined above, there is considerable evidence that reduction of GABA inhibition in the PRF might play a role in production of REM sleep. First, there are preliminary microdialysis data in both the cat (114) and the rat (66) that GABA levels in the PRF are decreased during REM sleep compared to wakefulness, and the Thakkar *et al.* (114) data indicate that levels in nonREM sleep are interme-

diate between wakefulness and REM sleep. Second, pharmacological experiments support this concept since GABA antagonists applied to the rostral PRF produced REM sleep in both cat (123, 124) and rat (95). This postulated pathway of LDT/PPT muscarinic inhibition of GABA PRF neurons during REM sleep is illustrated in Figure 10. The dotted lines for this and other GABAergic pathways indicate the more tentative nature of identification of both the projections and their source. This figure graphically emphasizes that the inhibition of PRF GABAergic neurons that inhibit PRF neurons would "Dis-Inhibit" the PRF neurons and so constitute an additional source of positive feedback. Of note, the GABA levels in wake and in REM in the PRF (114) are almost the exact inverse of Nitz & Siegel's (79) measurements of GABA in LC (see Chapter 11) suggesting a possible common source in the REM-on neuronal activity of disinhibition in PRF and inhibition in LC REM-on neurons (PRF Wake/REM ratio = 1.7 and LC REM/Wake ratio = 1.7).

6.3 Excitation of REM-off neurons by REM-on neurons (Figure 10 term "d"). – There is anatomical evidence for cholinergic projections to both locus coeruleus (LC) and dorsal raphe nucleus (DRN) (44). *In vitro* data indicate excitatory effects of acetylcholine (ACh) on LC neurons, but data do not support such direct effects on dorsal raphe (59). The REM-on neuronal excitation of dorsal raphe neurons may be mediated through the reticular formation; there is *in vitro* evidence for EAA excitatory effects on both LC and DR neurons.

6.4 Inhibition of REM-on neurons by REM-off neurons (Figure 10 term "b"). – As noted in Chapter 11, for many years, this aspect of the model was most controversial, since the indirect evidence from *in vivo* data, although generally supportive, was subject to alternative explanations. Now *in vitro* data indicate a subpopulation of cholinergic neurons in the LDT are inhibited by serotonin (60). Inhibition is especially consistent for the population of LDT neurons that fire in bursts; such burst firing has been shown by *in vivo* extracellular recordings to be tightly correlated with lateral geniculate nucleus PGO waves, which other data indicate are cholinergically mediated. The action potential burst itself is caused by a particular calcium current, the *low threshold spike (LTS)*, which causes calcium influx and depolarization to a level that produces a burst of sodium-dependent action potentials. Some non-burst cholinergic neurons are also hyperpolarized by serotonin. Other data indicate effects of norepinephrine (NE) on LDT/PPT cholinergic neurons are also inhibitory (122). Moreover, non-cholinergic, presumptively GABAergic interneurons, are excited by NE (53); GABAergic interneurons acting to inhibit cholinergic neurons would furnish yet another possible mechanism of inhibition of cholinergic mesopontine neurons by NE, thus further strengthening the model's postulates.

6.5 Inhibitory feedback of REM-off neurons (Figure 10 term "c"). – There is strong *in vitro* physiological evidence for NE inhibition of LC neurons and of serotonergic inhibition of DR neurons, and anatomical studies indicate the presence of recurrent collaterals. These recurrent collaterals, which have been demonstrated to be inhibitory, could be the source of suppression of raphe activity,

with the prolonged silence during REM resulting from long duration neurotransmitter effects, perhaps from coupling with second or third messengers. It has been suggested that there may also be an unconventional mode of serotonin release during REM sleep that leads to increased extracellular serotonin, and hence to inhibition. *In vivo* voltammetry data from Cespuglio and co-workers (19) in the Jouvet laboratory suggest that, even though action potential activity in dorsal raphe neurons during REM sleep is low, the levels of serotonin metabolites increase. This suggests a release of serotonin not coupled with soma depolarization. While this at first might be viewed as a highly improbable mechanism, Pan and Williams (81) have obtained *in vitro* data compatible with such an unconventional release of serotonin in dorsal raphe neurons. However, direct measurements by *in vivo* microdialysis of serotonin release in the cat DRN parallels the time course of presumptively serotonergic neuronal activity: waking (W) > slow wave sleep (SWS) > REM sleep (84) (Fig. 7), and so it seems unlikely that there is a large serotonin release in DRN during REM sleep.

6.6 GABAergic influences in the DRN during REM sleep. –

From the standpoint of sleep cycle control, one of the most puzzling as aspects has been defining what causes the "REM-off" neurons in the LC and DRN to slow and cease discharge as REM sleep is approached and entered. While LC-LC and DRN-DRN recurrent inhibition is present, there is no clear evidence that it might be the causal agent in REM-off neurons' turning off. Thus, the prospect that a GABAergic mechanism might be involved is of great intrinsic interest. As reviewed above, supporting a GABAergic mechanism in the DRN is the *in vivo* microdialysis finding of Nitz and Siegel (78) in naturally sleeping cats that there is a significant increase in DRN GABA levels in REM sleep (0.072 pmol/μL or 72 fmol/μL) compared with wakefulness (0.042 pmol/μL), while SWS (0.049 pmol/μL) did not significantly differ from wakefulness. Moreover, as discussed above, the balance of pharmacological studies support a GABA-induced suppression of DRN activity. We think it important to emphasize that the issue of GABAergic and serotonergic inhibition as important in suppression of DRN discharge is not an either/or but likely one of joint influences. For example, we note that the 190% increase in REM sleep observed with microdialysis application of the 5HT1A agonist 8-OH-DPAT to DRN by Portas *et al.* (85) was greater than that observed with the GABA agonist muscimol by Nitz and Siegel (78), suggesting that factors other than GABA might influence serotonergic neurons. Determination of whether the GABA time course of release parallels the decrease in activity of DRN serotonergic neurons during SWS as REM is approached awaits better technology for measurement of GABA.

6.7 GABAergic influences in the LC during REM sleep. –

Nitz and Siegel (79) placed microdialysis probes on the border of LC or in the peri-LC region in the cat. GABA release increased during REM sleep (1.9 fmol/μL) as compared to both waking values (1.2 fmol/μL) and SWS (1.6 fmol/μL) (79). GABA release during SWS showed a trend-level significance ($p < 0.06$) when compared with waking. These data, because of the SWS differences, appear to offer more direct support for LC than for DRN neurons for the hypothesis of GABA

induced inhibition causing the reduction in LC/DRN discharge in SWS and virtual cessation of firing in REM sleep. In pharmacological experiments, Gervasoni and colleagues (30) found that microiontophoresis application of bicuculline, a GABA-A receptor antagonist, during extracellular recordings in the LC was able to restore tonic firing in the LC noradrenergic neurons during both REM sleep (in contrast to its effects in the DRN) and SWS in unanaesthetized, head-restrained rats.

6.8 Source of GABAergic inputs to LC and DRN. –

The major missing piece of evidence on GABAergic inhibition of LC/DRN and REM-off neurons is the recording of GABAergic neurons whose activity has the proper inverse time course to that of LC and DRN neurons (See Chapter 11 review). In our diagram of the brainstem anatomy of REM sleep cycle control we have suggested that GABAergic neurons in the PRF might provide the input to DRN/LC. Certainly neurons in the PRF have the requisite time course of activity, but there is, to date, no evidence that these are GABAergic neurons. Within the LC and DRN, Maloney *et al.* (65) found the extent of C-Fos labeling of GAD-positive neurons in DRN and LC to be inversely correlated with REM sleep %, and to decrease in recovery from REM sleep deprivation. This is of course compatible with a local source of GABA increase during REM. However, unit recordings in DRN and LC have not found evidence for neurons with an inverse time course to that of the presumptively monoaminergic LC and DRN neurons.

6.9 Postulated dynamics of REM-off and REM-on neurons during the REM cycle. –

Figure 5 (time course of REM-off and REM-on neurons) and Figure 10 (structural model) should be referred to in this discussion.

1. The slowing and near-cessation of firing of REM-off neurons disinhibit the population of REM-on neurons (0-25% of cycle duration).

2. As a result of this disinhibition, the population of REM-on neurons becomes increasingly active and this activity augments because of: a) the excitatory interconnections in this REM-on population (LDT/PPT- reticular interaction) and b) disinhibition of reticular effector neurons from reticular GABAergic neurons which are inhibited by increasing LDT/PPT activity. Note also that Figure 10 shows that REM-off neurons may also be inhibited by GABAergic inputs from REM-on neurons. This augmenting of REM-on population activity continues until the REM episode is produced (25-75% of cycle duration).

3. The REM-off population becomes active as a result of excitatory input from the REM-on population (75-100% of cycle duration). When the REM-off population becomes sufficiently active, the REM episode is terminated because of the REM-off neurons' inhibition of the REM-on population.

4. The population of REM-off neurons is postulated to become less active because of inhibitory feedback, and this leads to step 1 and a resumption of the cycle. The exact circuitry responsible for REM-off neurons turning off over the sleep cycle remains undefined, and is one of the principal current questions in REM sleep control. The sketch in Figure 10 indicates that REM-off neuronal autoinhibition might *not only occur through monoaminergic autoreceptors (the original postulate of the*

reciprocal interaction model), but also through GABAergic mechanisms, with REM-off neuronal activity producing GABA-ergic inhibition in the initial stage of the sleep cycle (0-25% in Figure 5) either through local interneurons and/or distant sources.

6.10 Modeling considerations and orexin effects. –

These can only be summarized briefly in this review. Mathematically, a limit cycle model best describes the dynamics of the REM cycle, which retains its basic cyclicity no matter how it is set into motion (for discussion, see 67, 71, 72, 73). The other important feature not addressed in the simple model is circadian variation. Figure 11 sketches the modeling of the normal course of a night of REM activity in entrained humans. This smaller amplitude and shorter initial first cycle, as well as the absence of REM activity during the day is modeled by having the REM oscillator shut off and modulated by excitatory input to the REM-off neurons. When this excitatory input to the REM-off neurons was not present, it allowed the REM oscillator to become active (67, 71, 72, 73). One of the exciting possibilities is that orexin could be this factor (or one of the factors) exciting the REM-off neurons, consis-

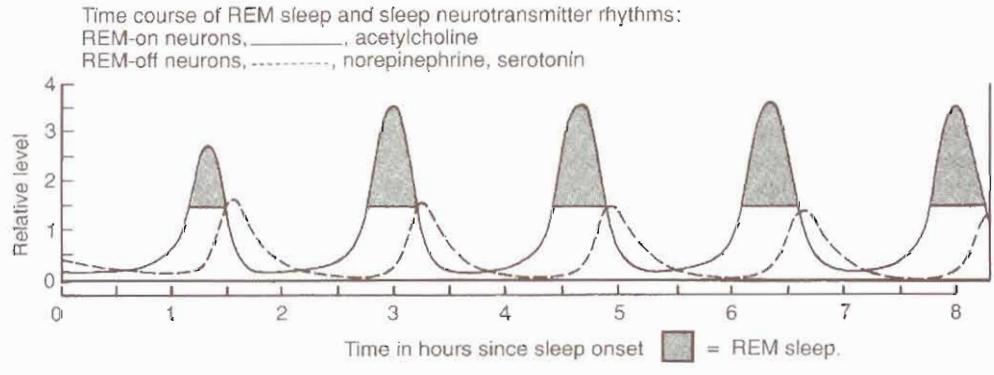


Fig. 11. - *Schematic of a night's course of REM sleep in humans showing the occurrence and intensity of REM sleep as dependent upon the interaction of populations of "REM-on" (= REM promoting neurons), indicated by the solid line and REM-off neurons, indicated by the broken line).*

Circadian influences are also modeled. As the REM-promoting neuronal activity reaches a certain threshold, the full set of REM signs occurs (black areas under curve indicate REM sleep). Note, however that, unlike the steplike EEG diagnosis of stage, the underlying neuronal activity is a continuous function. The neurotransmitter acetylcholine is thought to be important in REM sleep production, acting to excite populations of brainstem reticular formation neurons to produce the set of REM signs. Other neuronal populations utilizing the monoamine neurotransmitters serotonin and norepinephrine are likely REM-suppressive; the time course of their activity is sketched by the dotted line. These curves mimic actual time courses of neuronal activity, as recorded in animals, and were generated by a mathematical model of REM sleep (the limit cycle reciprocal interaction model of McCarley and Massaquoi (71), and this figure is adapted from this paper). Note that GABAergic neurons postulated to inhibit REM-off neurons should have a time course of activity inverse to the REM-off neurons. Note further that the effect of circadian modulation is to produce a lesser intensity, shorter duration first REM period and a much smaller increase in REM duration over subsequent REM periods.

tent with its effects on LC and DRN neurons. Experiments in which the orexin ligand is either knocked down or orexin neurons are destroyed will be useful in determining if these manipulations destroy the circadian modulation of REM sleep, as would be predicted by this hypothesis. The breakthrough of REM-like phenomena during the day in narcolepsy, a disorder characterized by a loss of orexinergic neurons, would be consistent with this hypothesis (see review of orexin and narcolepsy in 104).

SUMMARY

The first sections of this paper survey the history and recent developments relevant to the major neurotransmitters and neuromodulators involved in REM sleep control. The last portion of this paper proposes a structural model of cellular interaction that produces the REM sleep cycle, and constitutes a further revision of the reciprocal interaction model

This paper proposes seven criteria to define a causal role in REM sleep control for putative neuro-transmitters/modulators. The principal criteria are measurements during behavioral state changes of the extracellular concentrations of the putative substances, and electrophysiological recording of their neuronal source. A cautionary note is that, while pharmacological manipulations are suggestive, they alone do not provide definitive causal evidence.

The extensive body of *in vivo* and *in vitro* evidence supporting cholinergic promotion of REM sleep via LDT/PPT neuronal activity is surveyed. An interesting question raised by some studies is whether cholinergic influences in rat are less puissant than in cat. At least some of the apparent lesser REM-inducing effect of carbachol in the rat may be due to incomplete control of circadian influences; almost all experiments have been run only in the daytime, inactive period, when REM sleep is more prominent, rather than in the REM-sparse nighttime inactive period.

Monoaminergic inhibition of cholinergic neurons, once thought to be the most shaky proposal of the reciprocal interaction model, now enjoys considerable support from both *in vivo* and *in vitro* data. However, the observed time course of monoaminergic neurons, their "turning off" discharge activity as REM sleep is approached and entered would seem to be difficult to produce from feedback inhibition, as originally postulated by the reciprocal interaction model. New data suggest the possibility that GABAergic inhibition of Locus Coeruleus and Dorsal Raphe monoaminergic neurons may account for the "REM-off" neurons turning off. However, the source(s) of GABAergic influences suggested by anatomical studies has yet to be definitively identified by electrophysiological recordings of GABAergic neurons that show the requisite inverse time course of activity relative to monoaminergic neurons.

New and still preliminary microdialysis data suggest that reticular formation neurons, the effector neurons for REM sleep phenomena, might be disinhibited during REM sleep by decreased GABAergic influence, perhaps stemming from REM-on

cholinergic neuronal inhibition of reticular formation GABAergic neurons. Whether the postulated cholinergic inhibition of GABAergic neurons is present is testable with *in vitro* recordings and double labeling.

Taking into account the observed data on neuro-modulators/transmitters, a structural model incorporating interaction of REM-on and REM-off neurons and GABAergic influences is proposed. Finally, with respect to orexin and REM sleep, it is hypothesized that orexinergic activity may be a principal factor controlling REM sleep's absence from the active period in strongly circadian animals such as rat and man.

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