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Research report

A functional role for REM sleep in brain maturation

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Abstract

The biological function of REM sleep is defined in terms of the functions of neural processes that selectively operate during the REM sleep state. The high amounts of REM sleep expressed by the young during a period of central nervous system plasticity suggest that one function of REM sleep is in development. The phenomenon of activity-dependent development has been clearly shown to be one mechanism by which early sensory experience can affect the course of neural development. Activity-dependent development may be a ubiquitous process in brain maturation by which activity in one brain region can influence the developmental course of other regions. We hypothesize an ontogenetic function of REM sleep; namely, the widespread control of neuronal activity exerted by specific REM sleep processes help to direct brain maturation through activity-dependent developmental mechanisms. Preliminary tests of the hypothesis have been conducted in the developing feline visual system, which has long been known to incorporate information derived from visual experience in establishing neuronal connectivity. We find that suppression of REM sleep processes by an instrumental REM deprivation procedure results in a significant enhancement of the effects of altered visual experience by monocular occlusion. Bilateral brainstem lesions that selectively block the occurrence of ponto-geniculo-occipital (PGO) waves are sufficient to produce similar results. These data indicate that the propagation of phasic influences during REM sleep interacts with other processes subserving neural development. This source of influence appears not to derive from the environment but rather stems from an intrinsic source of genetic origin. Examination of the neural activity associated with PGO waves in the lateral geniculate nucleus reveals a distribution of facilitatory influence markedly different from that induced by visual experience. We conclude that REM sleep directs the course of brain maturation in early life through the control of neural activity.

Key words: Development; Visual system; Plasticity; Ponto-geniculo-occipital wave; Activity dependency; REM deprivation; Monocular occlusion; Lateral geniculate nucleus

1. Introduction

Biological function is defined as the specific contribution of a tissue, organ, system or biological process to the inclusive fitness of a living organism. Inclusive fitness is the ability of the organism to pass its genes to future generations [19]. Thus, biological functions keep individuals alive to reproduce and provide enough viable offspring to continue the process in future generations. Behaviors are processes that exhibit biological function. The value to inclusive fitness of behaviors such as feeding, mating and parental care are obvious. In contrast, the function of other behaviors such as certain courtship rituals may require a detailed examination to ascertain their purpose. We will present evidence that the behavior of REM sleep has biological function by serving to direct the course of brain maturation.

Sleep is in the behavioral repertoire of all mammals and birds studied [1,7,71]. Sleep is typically not a singular process; rather, it is composed of two distinct and alternating stages, each possessing unique neural mechanisms and physiological indicators [26,38]. Beneath sleep's mask of behavioral quiescence, these two vastly different modes of central nervous system activity cyclically succeed each other. Slow-wave (SW) or non-rapid eye movement (NREM) sleep, is characterized by a general reduction in neural activity [22,60,61,62]. During the other stage, REM sleep, active inhibition of motor activity conceals a global, central activation.

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Despite abundant data regarding the manifold neural activities that characterize the two states of sleep, the biological functions of NREM and REM sleep remain enigmatic. One obstacle to full understanding of their functions lies in the difficulty of studying behaviors typified by gross motor inactivity and lack of an obvious object to which the behavior is directed. Increasing theoretical attention has been paid to the likelihood that sleep behavior is directed internally at the central nervous system. This is most compelling in regard to REM sleep because of its seemingly paradoxical combination of motor inhibition and elevated neural activity. A major challenge to the understanding of the function of the sleep states is to identify the effects of state on the central nervous system.

2. Defining 'function of a state of arousal'

What is the function of a particular state of arousal? Many definitions are possible. The definition we use is: state of arousal creates a central nervous system condition that permits the operation of specific and selective biological processes to occur; the functions of these processes constitute the functions of a state. To attribute the functional significance of a biological process to a particular state, a degree of specificity must exist with respect to the expression of the process to that state. For example, the heart pumps blood in every state of arousal and, though permitted to do so in waking, this action is not considered solely a function of waking. On the other hand, modulation of heart rate is a state-related phenomenon and, accordingly, may have state-related functional consequences. The term 'processes' that we use in the definition of state is deliberately broad so that it may apply to many levels of organization, from behavior and gross organ function to neural systems, subsystems, small neural assemblies and, ultimately, to the processes of individual cells and the expression of their genetic material.

To illustrate the various levels of organization involved, let us examine a function of waking in the development of the mammalian visual system. This example is important to the later discussion. Many animals have considerable overlap in the visual field perceived by the two eyes. Due to the horizontal displacement of the eyes, the image falling on the retina in the region of overlap is displaced in each eye relative to the other. This disparity increases with decreasing distance of objects to the eyes and thus provides information about the distance of objects. Binocular disparity is a very powerful perceptual cue to depth and the three dimensional nature of the space around us.

For neural mechanisms that process visual stimuli to extract accurately this information, knowledge of the relative location of the eyes is required. The developing visual system utilizes information from the view supplied by each eye to determine eye position. This fact is supported by the work of Hubel and Wiesel [24,25], showing that in cats, during a critical period from approximately the time of eye-opening to 3 months after birth, thalamocortical synaptic connectivity of neurons specific to the binocular visual field is particularly sensitive to manipulations creating asymmetries in input to the two eyes. It would appear that the final 'hard wired' connections in the visual system remain plastic for a period of time after the initial synaptic contacts are made so as to utilize information inherent in visual experience. Inasmuch as visual experience is a process normally restricted to waking, we can conclude that one early function of the waking state is development of the visual system.

The mechanisms underlying this process are not yet completely understood. Visual experience determines the discharge activity expressed by neurons in the visual system. Variations in neuronal activity, therefore, must interact with inherent developmental processes in making and breaking synaptic connections. Full understanding of these mechanisms ultimately will require knowledge of the molecular events involved in synapse formation and its modulation by events accompanying generation of action potentials. Accepting incomplete understanding of the underlying mechanisms, the phenomenon of activity-dependent development has nevertheless been identified in several sensory systems in addition to the visual system, such as the auditory and somatosensory systems [36,45,59,64]. Activity-dependent development may be a widespread mechanism directing neural connectivity throughout the brain. To the extent that neural activity is under the control of waking sensory experience, a function of waking in early life is directing central nervous system maturation.

Activity-dependent development has been primarily regarded as a mechanism playing a role in the formation of neural circuitry by means of which early experience provides information about the environment. It is reasonable to assume, however, that such a mechanism could be utilized by other sources of information to influence development. Shatz has shown in cats that even before birth, spontaneous, correlated, neural activity within each retina is necessary for the anatomical segregation of eye-specific synaptic connections in the lateral geniculate nucleus (LGN) [40,70]. This spontaneous intra-retinal activity is clearly not dependent upon visual experience. It becomes clear then that development of neural connections in particular structures can be influenced by activity under the control of other structures. The reliance on activity-dependent processes could be a general principle of brain development. The source of most of this type of information is probably encoded in genes. Genetic information is

the ultimate source of the circuitry and intrinsic properties of neurons in the immature retina that result in their spontaneous, correlated activities. Though this endogenous retinal activity probably is not affected by state of arousal, state-related neuronal activity is a widespread phenomenon; in adults, neurons in every region of brain are observed to alter their rate and/or pattern of discharge systematically with shifts in state of arousal [22]. It is our thesis that in early development neuronal activity specific to a particular state of arousal has functional consequences through activity-dependent developmental processes.

3. The ontogenetic hypothesis of REM sleep function

During the late fetal and early neonatal stages of central nervous system development, a relatively high percentage of time is spent in REM sleep [27,39,48,50,57]. As maturation proceeds, both the absolute amount of REM sleep and its proportion of total sleep time decrease [27,48,57]. The relatively high amounts of REM sleep in early life when nervous system maturation is proceeding at a high rate led Roffwarg et al. [48] to first postulate that the neural activity controlled by REM state mechanisms may be developmentally functional, contributing significantly to widespread physiological and structural maturation within the central nervous system during the intrauterine and early postnatal periods. They further suggested that the REM sleep experienced by the last-trimester fetus and young infant is incremental and complementary to whatever exogenous, sensorial stimulation is available to the organism. On this basis, REM sleep was viewed as executing a critical role in satisfying the early-stimulation requirements of the growing brain [48].

Identification of the phenomenon of activity-dependent, neural development now provides a more specific mechanism by which sensory experience and endogenously controlled, spontaneous neural activity, interactively and independently, participate in directing the course of nervous system development. Our current, ontogenetic hypothesis of REM sleep function is that neural processes selectively operating in REM sleep result in specific patterns of activity in neuronal populations whose development is dependent upon activity.

The challenge to testing this hypothesis is in first specifying its various components. What REM sleep processes might perform this function? Several possible candidate processes are known that result in altered neuronal discharge. These include, the high mean rates of spontaneous firing observed in widespread areas of brain during REM sleep [22], as well as the phasic, discontinuous discharges associated with ponto-geniculo-occipital (PGO) waves commencing before and continuing through REM sleep [5,23,52]. REM sleep-specific neuronal activity is also represented by decreased spontaneous discharge, as observed in several aminergic cell populations [8,37,47]. The cessation of cell firing by noradrenergic locus ceruleus neurons [8], for example, may not only affect the development of the noradrenergic system but may influence the course of maturation at its numerous postsynaptic targets [34]. Though much is known about the different neural phenomena expressed selectively during REM sleep, this knowledge is not complete. Processes that remain to be identified might also operate in REM sleep and be important to the brain's development. This is particularly so with respect to the neonate and to the fetus in which a paucity of information exists [41]. In addition, it must also be remembered that many processes observed to operate selectively in REM sleep in the adult may not be present in the very young. The need for more data from very young animals is great.

What neural systems might REM sleep processes influence during development? Given the hypothesis that activity-dependent development is the underlying mechanism, several sensory systems have been identified in which this phenomenon is expressed [36,45,59,66]. These data have been interpreted mainly in support of the role of sensory experience in early development but, as argued above, endogenous sources of neural control can be a sufficient stimulus [70]. Again, we believe that REM sleep is capable of providing such a source of control. The best documented system expressing activity-dependent development is the visual system. As such, it is a likely candidate for demonstrating a REM sleep developmental influence. Activity-dependent development could be the basis of a ubiquitous process by which the innervation of a brain region undergoing development is influenced by the activity of neurons in another region. More work is needed to identify these systems and to delineate the underlying molecular mechanisms.

Our theoretical view posits that specific processes selectively operating during the REM sleep state participate in directing the course of the brain's maturation through the control of neuronal activity. We believe that this endogenous influence derives from a phylogenetic source of information promoting adaptive, species-specific brain development, thus contributing to the inclusive fitness of the organism. We present new data below indicating support for this developmental function of REM sleep.

4. REM deprivation alters the course of visual system development

Guided by the ontogenetic REM sleep hypothesis, our experimental strategy is to identify a REM sleep-specific

process, demonstrate that manipulation of this process alters the developmental course of a particular neural system, and delineate the mechanisms responsible for these results. In an initial attempt, however, we believed it efficacious to take a more modest approach. Inasmuch as the relevant REM sleep processes have not been identified, total REM sleep deprivation (RD) was used to block the operation of all REM sleep-specific processes.

We were greatly encouraged by the work of Corner, Mirmiran and co-workers, who in a series of studies [10,42,43] showed that pharmacological suppression of REM sleep early in postnatal development in rats results in behavioral, anatomical and biochemical deficiencies lasting into adulthood. These data suggest that REM sleep is involved with maturational processes. From the perspective of activity-dependent development, however, the continuous action of drugs and their effects on neuronal activity *independent* of their action on REM sleep can be expected to influence the course of neural development, especially in systems undergoing critical aspects of their development at the time of drug administration. We chose to utilize behavioral methods of RD so as to achieve a higher level of specificity than can be achieved by pharmacological means [9,32,68].

Despite problems associated with using RD, an initial test of the developmental function hypothesis under the proper conditions was able to yield interpretable results valuable to determining future directions of the work. We chose to investigate the effects of RD on the developing visual system of the young cat. As a model of neural development, the visual system presents many advantages for demonstrating an influence of REM sleep. First, it is a system that has been extensively studied so that much is already known. Second, the visual system clearly exhibits activity-dependent development [54,64], making it a logical candidate for investigation of REM sleep influences. Third, REM sleep-specific neuronal activity is expressed at both thalamic and cortical levels in the visual system [3,6,15,44]. Finally, the maturational process within the visual system of cats extends several months after birth, beyond the time in which REM sleep has taken on adult-like characteristics [27]. The absence of sleep-onset REM episodes at this age permits the use of an instrumental method of RD, thus avoiding the problems associated with systemic drug administration.

4.1. The binocular segment

It has been clearly established that the developing visual system of certain mammals during a delimited critical period is dependent upon activity driven by visual experience [17,25,56]. The classical demonstration of this process is the striking physiological and structural reorganization of the system that results from depriving patterned vision to one eye during the critical period [56]. The effect is an alteration of the innervation of visual cortex particularly by those neurons residing in the binocular segment of the LGN that mediate binocular vision [18,33,55]. The cortical terminals of LGN neurons receiving input from the deprived eye branch less frequently and form fewer, as well as morphologically abnormal and smaller terminals in cortex than LGN neurons receiving input from the seeing eye [16,65]. Another correlate of this process is the resulting reduction in soma cross-sectional area of LGN neurons receiving input from the deprived eye [69]. Whereas visual deprivation of both eyes produces minimal effects in binocular segment neurons [20], cell-size reduction is greatly enhanced by the activity in the seeing eye [18]. The mechanisms underlying this phenomenon have been described in terms of a competition among geniculocortical afferent terminals for synaptic space in the cortex [18,20]. The neurons deprived of visual experience fare poorly in this competition, while the non-deprived neurons expand their synaptic space [16,65] and correspondingly increase in size [20,56]. With monocular deprivation (MD), the increase in soma size is less pronounced than the cell-size reduction [56] and is thought to occur as a secondary consequence of axonal arbor proliferation into the space previously vacated by the 'deprived' neurons [16].

One way by which REM sleep deprivation may alter visual system development is by influencing the outcome of the competitive process. Pompeiano and Corvaja, in a preliminary report [46], performed total sleep deprivation in young kittens experiencing MD and found significant cell-size reduction in those cells of the LGN receiving input from the occluded eye. The size reduction was larger than that in normally sleeping, MD animals. This amplification of the effects of MD on the developing visual system could have resulted from removing an influence of REM sleep. Alternatively, it could have resulted simply from increased monocular visual experience of the sleep deprived animals accompanying increased wake time in the light.

We have performed an experiment more specifically directed to the role of REM sleep (Oksenberg et al., manuscript in preparation). On post-natal day 34 or 35, utilizing aseptic technique under deep pentobarbital anesthesia, eight kittens were surgically implanted with an array of electrodes for sleep recording. An opaque occluder was sutured beneath the lid of one eye to accomplish MD. On post-natal day 42, animals were removed from the litter and placed individually in separate recording chambers. To control for waking-light experience, all animals were placed on a 1.5/4.5 h light/dark schedule. During the 1.5 h light (2000 lux) periods, kittens were allowed to run free and interact with each other, but were maintained awake.

During the 4.5 h periods of absolute darkness, the kittens remained in recording chambers and were electrophysiologically monitored. This procedure equalized the waking light exposure of all the animals (6 h/day) whether or not they were REM deprived. Non-experimental animals remaining in the home cage on a 12/12 h light/dark cycle receive approximately 4.8 h of waking light exposure per day.

Day 42 constituted a baseline (BL) recording day in which all animals resided on large platforms (32 × 43 cm) while in the chambers. For the next 7 days following BL, four kittens remained on large platforms (MD-only) while the remaining four were deprived of REM sleep by the multiple, small platform technique (MDRD) [67]. The MDRD group's recording chambers contained three circular platforms (17 cm) surrounded by water to a level just below their surface. The platforms were spaced such that a sleep posture could only be maintained on a single platform. Its small size was such that loss of muscle tone would cause the head to droop, the face to touch water and the animal to arouse preventing entrance into REM sleep. This method was very effective at reducing REM sleep (see Table 1). Compared to BL levels, the mean reduction in REM sleep in the MDRD group was 82.5%; the MD-only group showed a 3.5% decrease. Wake time was significantly increased in the MDRD group. After the 7-day sleep manipulation, these experimental animals and five additional age-matched, unmanipulated animals (12/12 h, L/D) (NORM) were killed. Coronal sections through the thalamus were prepared for cell morphometry.

The natural segregation of eye-specific input to LGN into alternating laminae allows the simple identification of cell populations innervated by the occluded or unoccluded eye. The most dorsal lamina (A) receives input exclusively from the contralateral retina, the next ventral lamina (A1) receives input from the ipsilateral retina. The procedures of Kalil [29] and Sanderson [53] were employed to collect data on soma cross-sectional areas of 100 cells in each of the A and A1 lamina of the binocular segment of LGN ipsilateral to the occluded eye. The differential-laminae effect of MD was assessed by computing the mean cell-size ratio (A1:A) for each animal [2]. Table 2 summarizes the results. A comparison of the MD-only and NORM groups reveals the well established MD effect [2,20]: MD resulted in decreased size of cells innervated by the occluded eye and increased size of the cells innervated by the unoccluded eye. The natural tendency for lamina A1 cells to be larger than those in lamina A produces a ratio slightly greater than unity in the NORM group. Following MD, this ratio was significantly reduced. The direction of change in cell-size between NORMs and MD-only's was further accentuated when RD was added to the MD manipulation, resulting in a significant decrease in lamina ratio between the MDRD and MD-only groups.

These data indicate that the removal of some REM sleep-process has an effect upon the developmental competitive mechanisms by which the visual system adapts to binocularly disparate visual inputs. We suggest that these findings demonstrate the involvement of REM sleep in neural development. Elucidation of the specific role played by REM sleep in development will require a method other than REM deprivation.

4.2. The monocular segment

The visual field is topographically distributed across the individual lamina of the LGN such that a microelectrode descent perpendicular to the surface reveals neurons having receptive fields in similar locations of visual space [53]. As the electrode within the binocular segment traverses one lamina and enters the next, the same field is represented though mediated by the other eye. This is not the case in the monocular segment. The monocular segment of lamina A receives input from that portion of the peripheral visual field available to the contralateral eye that does not overlap with the field that can be viewed by the ipsilateral eye. There is, therefore, no corresponding region in lamina A1. With respect to alterations in cell-size induced by altered visual experience during development, the monocular segment does not express the competitive mechanism defined in the binocular segment. Asymmetric visual experience produced by MD in the critical period results in very little effect upon cell-size in monocular segment LGN neurons [18,56]. Binocular visual deprivation, which has little effect in the binocular segment, results in

Table 1
Mean percent change from baseline (± S.E.M.) in stage-scored amounts of rapid eye movement sleep (REM), slow wave sleep (SW) and waking (AW) in animals that were only monocularly deprived (MD-only) and in those that also received a week of REM deprivation (MDRD)

MD-only			MDRD		
REM	SW	AW	REM	SW	AW
98.2 (7.9)	104.8 (4.6)	102.5 (1.5)	16.8* (7.4)	104.6 (7.6)	158.2* (12.2)

* Significantly different from 100% ($P<0.05$).

Table 2
Mean binocular segment, A1/A lamina cell-size ratios (± S.E.M.) in the LGN ipsilateral to the patched eye

Norms	MD-only	MDRD
1.04 (0.017)	0.89 (0.055)	0.78 (0.021)

All significantly different from each other ($P<0.05$).

significant reductions in cell-size or rate of growth in the monocular segment [21,28,56]. It is evident that the underlying developmental processes operating within these two regions of the visual system are not the same. This offers the opportunity to determine whether REM sleep has a more generalized developmental influence than that restricted to the specialized competitive mechanism of the LGN binocular segment.

One hundred LGN cells were sized in the monocular segments ipsi- and contralateral to the occluded eye in the eight experimental animals described above (manuscript in preparation). A summary of the results is shown in Table 3. In the MD-only group, monocular segment cells on the side receiving input from the occluded eye are slightly smaller than the cells on the contralateral side that receive input from the seeing eye. This reduction is not statistically significant. The difference in cell-size in the monocular segments of MDRD group members between the ipsi- and contralateral sides is statistically different. The biggest differences found, however, are the significant reductions in cell-size in both ipsi- and contralateral monocular segments of the MDRD group compared to the MD-only. It appears that the RD procedure not only results in smaller cells that are also deprived of patterned vision but also in size reductions in those monocular segment-cells receiving input from the seeing eye.

The results of our experiment in the monocular segment are a little more difficult to interpret than those in the binocular segment. The design of the studies controlled for the amount of asymmetric visual input experienced by each animal, which was 6 h per day. Due to the fact that RD animals have more waking, the MDRD and MD-only groups differed in their waking dark visual experience (mean 7.5 and 3.25 h/day, respectively). The cell-size reductions in the monocular segment could have been due to this increase in waking-dark. This is unlikely to have occurred in the binocular segment inasmuch as no size reduction is observed in cells receiving input from the unoccluded eye, indicating no general tendency for all cells to be smaller. In addition, the effect measured in the binocular segment (A1/A ratio) is the result of the asymmetric visual input of MD, which is not active in total darkness. We are currently conducting an experiment to determine whether increased waking dark experience alone is capable of producing the magnitude of cell-size changes we have observed in the monocular segment. Despite these remaining issues, the data in hand are extremely supportive of the view that REM sleep plays a role in directing the course of central nervous system development and does so by interacting with a variety of underlying developmental processes.

Table 3
Mean cell size (± S.E.M.) in the LGN monocular segment contralateral to the unoccluded (UNOC) and patched (OC) eye.

MD-only (μm^2)		MDRD (μm^2)	
UNOC	OC	UNOC	OC
274.6 (8.5)	250.6 (6.0)	233.9† (4.7)	206.3*† (5.0)

* Significantly different from contralateral side ($P < 0.02$).
† Significantly different from MD-only ($P < 0.02$).

5. Suppression of phasic activity alters the course of visual system development

The limitations of the RD paradigm preclude elucidation of the specific REM sleep processes and sites of action by which REM sleep influences development. With respect to visual system development, the cell-size changes we observe are an indirect indicator of an altered thalamocortical innervation that is susceptible to influence in the LGN, visual cortex or both loci. Many identified REM sleep processes are theoretically capable of exerting an effect at both sites. The next step in our investigation is to determine which REM sleep process or processes may be mediating developmental influences.

One of our current projects (in which we have preliminary data) was inspired by the reports of Davenne and Adrien [11,12]. These workers showed that bilateral lesions of the ponto-mesencephalic isthmus in neonatal cats, which block the occurrence of PGO waves in the LGN, result in cell-size reductions and alterations in LGN activity later in life. PGO waves are field potentials recorded in a variety of brain loci, including LGN and visual cortex, which appear in REM sleep and in NREM sleep just before REM onset [5,14]. These phasic events represent one of the fundamental processes that selectively operate during REM sleep and appear to be one mechanism capable of controlling neuronal activity in widespread regions of the brain [3,35,52]. The results of PGO wave blockade to the developing LGN strongly implicate this process of REM sleep as at least one mechanism involved in the maturation of the brain. These data, however, can be alternatively interpreted in terms of a postsynaptic degenerative effect rather than manipulation of a REM sleep mechanism. Bilateral brainstem lesions, which sever ascending pathways, will result in degeneration of the presynaptic innervation of its targets. This non-physiologic process could result in a failure of the postsynaptic cells to grow as rapidly in a developing brain region. We reasoned that our visual-deprivation paradigm combined with PGO wave suppressing lesions would provide an additional test of the proposed Davenne and Adrien PGO

wave mechanism. If the effects of the bilateral lesions are due to postsynaptic degeneration, then, irrespective of retinal innervation, cells in the binocular segment should be reduced in size. Alternatively, if the effect is due to the removal of the influence of a REM sleep process, then we should observe exacerbation of the effects of MD, a size reduction restricted to LGN cells innervated by the occluded eye and a tendency towards increased size in the cells innervated by the unoccluded eye.

Several adaptations were made to the original experimental design. At initial surgery on day 35, multipolar electrodes were chronically implanted bilaterally in the brainstem in addition to the other electrodes for sleep recording and occlusion of one eye. The lighting schedule was maintained on 12/12 h light/dark. On BL day 42, the quality of the PGO wave recording was assessed in each animal. Animals with poor quality recordings were assigned to the sham-lesion group. On day 43, when in our other study RD commenced, all animals were anesthetized (ketamine/xylazine) and DC current was passed through the lesioning electrodes of members in the lesion group only. Following recovery from anesthesia, animals were placed back in the recording chambers and monitored for 7 days. Quantification of LGN cell-size were carried out as previously described.

Thus far, we have analyzed the cell-size data in the binocular segment of six sham-lesioned controls and in four lesioned animals in whom complete suppression of discriminable PGO wave activity in the LGN was obtained. Although we have not completed analysis of the sleep data from these animals, previous reports indicate that PGO wave suppressing lesions do not reduce the temporal amount of REM sleep [11]. Our initial impression is that these successfully lesioned animals continue to express high amounts of REM sleep, differing in electrographic characteristics from normal REM sleep only by virtue of absence of PGO waves. A summary of the cell-size data is shown in Table 4. The MD-effect in the sham control group is evident in the reduction of the A1/A lamina ratio to a number less than unity. The further, significant reduction of this ratio in the lesioned group clearly indicates that PGO suppression amplifies the effects of MD as does suppression of the REM sleep state with RD. Indeed, these preliminary data indicate that the effect is even greater with PGO wave suppression than RD (0.72854 vs. 0.88 compared to 0.78005 vs. 0.89). The reduction in the A1/A ratio is mainly due to an increase in mean cell-size in lamina A, innervated by the unoccluded eye. This is an unusual response to MD and requires further investigation. The lack of evidence of a generalized cell decrease, however, substantiates the conclusion that the main result of the brainstem lesions on LGN cell-size is not due to postsynaptic degenerative effects but rather to suppression of PGO wave activity. Furthermore, these data support the conclusion that propagation of phasic influences throughout the brain represents at least one REM sleep process capable of interacting with the course of development.

Table 4
Mean LGN binocular segment laminae cell sizes and ratios (± S.E.M.)

SHAM lesion			Lesion		
UNOC	OC	A1/A	UNOC	OC	A1/A
254.0 (22.4)	223.5 (15.7)	0.88 (0.03)	295.5 (12.3)	215.2 (16.7)	0.73* (0.05)

* Significantly different from sham lesion ($P < 0.02$).

6. Mechanism of REM sleep phasic activity in development

By what mechanism might the REM sleep phasic influence effect visual system development? Based on our hypothesis, we sought evidence in the control of neuronal activity in LGN that accompanies the occurrence of PGO waves. Classic reports demonstrated that the majority of LGN neurons in the cat express facilitation of discharge rate with the appearance of PGO waves [3,52]. Even in rat LGN, from which field potential, PGO waves can not be recorded, 70% of neurons increase their firing rate in conjunction with hindbrain PGO waves [35]. REM sleep phasic input to LGN is presumed to be transmitted via a cholinergic pathway from the brainstem [23,58]. Cholin-

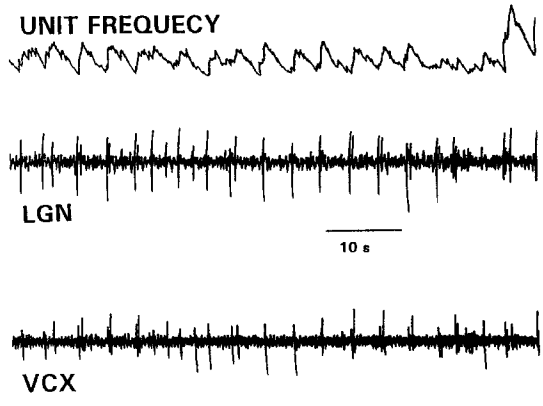

Fig. 1. Ponto-geniculo-occipital (PGO)-like waves appear spontaneously in the lateral geniculate nucleus (LGN) and visual cortex (VCX) of the adult, urethanized (0.8 g/kg) cat pretreated 2 h earlier with RO4-1284 (20 mg/kg). The top trace represents the simultaneous discharge activity of a single LGN neuron (mean discharge rate of all LGN neurons recorded was 6.7 Hz). Upward deflections indicate increases in discharge rate. Note the gross correlation of Unit discharge and occurrence of PGO-like waves.

ergic synapses are anatomically distributed upon neural elements within the LGN similar to the synapses of retinal origin [13]. Thus, facilitatory control of thalamocortical relay cells of the geniculate may be a common pathway by which visual experience and also endogenous REM sleep activation instruct the course of visual system development. The retinal and cholinergic innervation of the LGN, however, appear to differ in other aspects of their organization [56,63,66]; retinal input segregates into eye-specific channels whereas the brainstem input probably does not. This difference in organization may result in REM sleep phasic activation occurring simultaneously across eye-specific visual channels. When visual experience is altered via MD, an imbalance in the stimulation derived from each eye is created that operates through the competitive developmental processes to produce inter-lamina cell-size disparity in LGN. If REM sleep, phasic activation delivers balanced, simultaneous input to all LGN neurons independent of their eye-specific innervation, then during the time spent in REM sleep (greater than

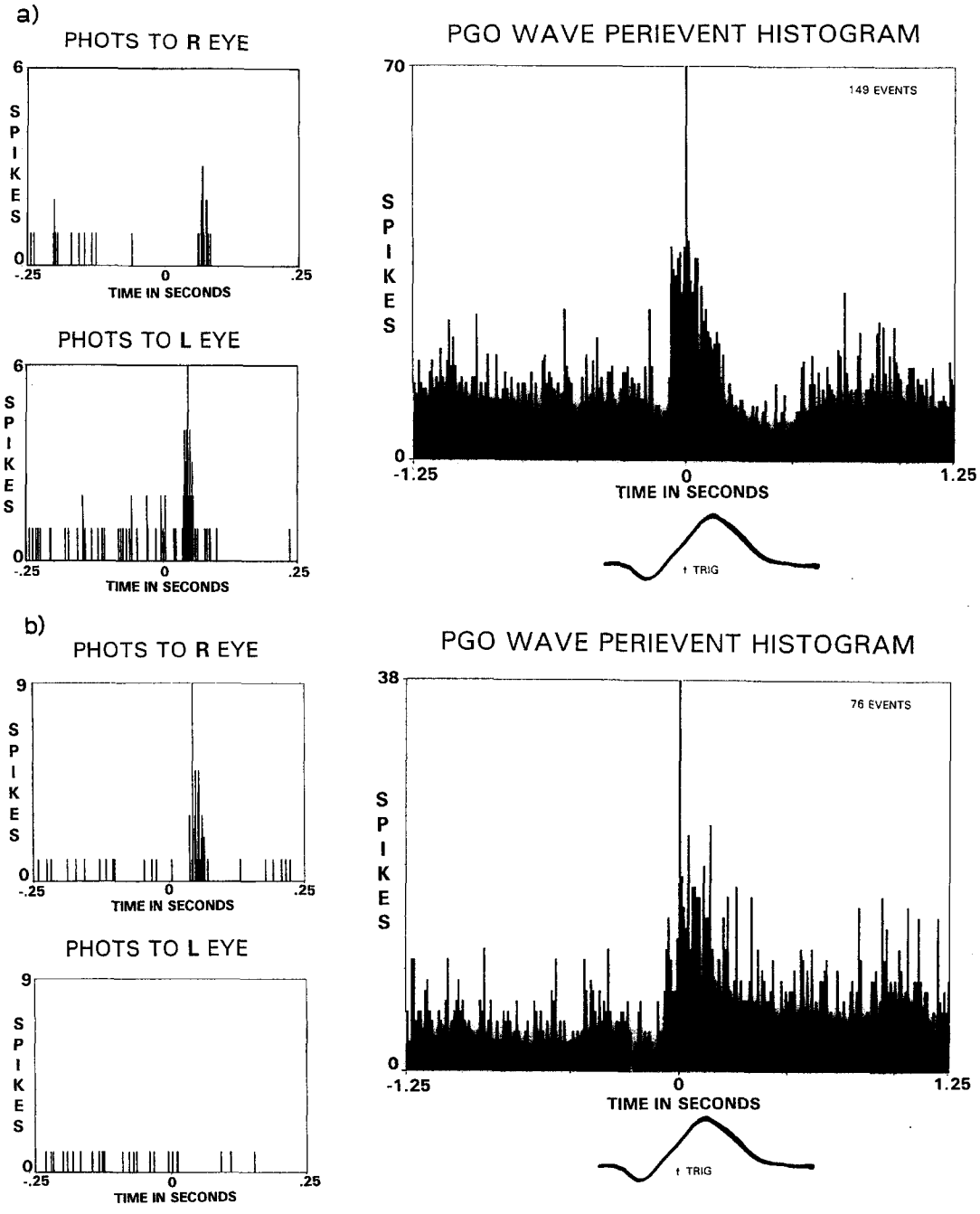

Fig. 2. Perievent histograms of the evoked and PGO-related spontaneous activity of two (panels a and b) neurons located in different LGN laminae in the same cat. Histograms to the left show responses to 10 photic flashes (phots) delivered to each eye (2 ms bin size, ± 250 ms window). The histogram to the right shows activity summated over many PGO wave-events (10 ms bin size, ± 1.25 s window). a: neuron in left LGN lamina A1 responding to stimulation of the left eye; b: neuron in lamina A responding to stimulation of right eye. See text for discussion.

20% of the day at the age of the animals in our experiments), this balanced stimulation would tend to oppose the effect of the asymmetrical input produced by MD (experienced for 25% of the experimental day). Suppressing REM sleep-control of activity by RD or suppressing PGO wave activity by brainstem lesions would remove this opposing influence and result in exacerbation of the effects of MD, as we observe.

To test the lack of lamina specificity of the REM-phasic input to LGN, we studied the spontaneous and evoked activity of single LGN neurons in four urethanized adult cats pretreated with RO4-1284, a reserpine-like compound that depletes monoamines [49]. Under these acute conditions, regular and continual appearance of LGN PGO-like waves is observed (see Fig. 1). These PGO-like waves have been shown to share a common mechanism of generation with spontaneous, REM sleep PGO waves [23]. Neurons were identified as residing in lamina A or A1 by short-latency (mean latency, 59.1 ms), selective response to photic flash presented to the contra- or ipsilateral eye, respectively. Perievent histograms, summed over many PGO waves recorded from a fixed electrode in each animal, were generated for each identified neuron ($N = 35$). The activity of two representative neurons is shown in Fig. 2. Significant facilitation of discharge was expressed in 94% of neurons at the rising slope of the PGO-like wave that was followed by a significant inhibition in 80% of neurons (inhibition not present in Fig. 2b). No proportional difference was observed for the presence or absence of either facilitation or inhibition between laminae within each animal or combined across animals. In addition, the mean magnitudes and mode times for facilitation or inhibition did not differ between laminae.

These data suggest that though some variability exists in the association of spontaneous LGN neuronal activity with occurrence of PGO waves, this variability is not associated with lamina location; that is, neurons in LGN, irrespective of lateralized retinal innervation, appear to be synchronously facilitated at the time of PGO wave occurrence. We propose that the cross-lamina, synchronous input underlying REM sleep phasic activity opposes the developmental response to asymmetric visual input produced by MD in the binocular segment. Suppression of PGO waves removes this opposing influence and results in amplification of the effects of MD. Inasmuch as the competitive process is not operating in the monocular segment, a different mechanism is needed to account for the possible effects of RD in this cell population. Perhaps, withdrawal of the bilateral, phasic facilitatory influence produces effects of RD analogous to those of dark-rearing, which has been shown to result in significant cell-size reductions in the monocular segment [56]. We are in the process of analyzing the cell-size data in the monocular segment in kittens with PGO wave-suppressing lesions.

In addition to phasic activity, probably other REM sleep processes mediate developmental effects. PGO waves do not appear in the cat until after eye opening [4], yet the majority of brain development occurs before this time. Accordingly, much work remains to be done to uncover the mechanisms that mediate REM sleep function in brain development.

7. Conclusion

It is our view that the relatively abundant amounts of REM sleep expressed by young mammals and birds functions in directing the course of brain maturation. We believe that the mechanism by which this is accomplished is through the expression of one or more REM sleep-specific processes controlling neuronal activity in widespread regions of the central nervous system. The phenomenon of activity-dependent development has been clearly demonstrated in a variety of sensory systems and may be a ubiquitous feature in the development of complex nervous systems.

Activity-dependent developmental processes have been well documented in the visual system of the cat. This mechanism has been proposed to underlie the means by which environmental information through visual experience is incorporated into the system in preparation for a lifetime of interacting with the environment. The endogenously generated influences of REM sleep appear to derive from a phylogenetic source of information and, as such, may reflect a history of adaptation to a variety of environments. This dual source of information, possibly acting through a common, activity-dependent mechanism, may serve to promote adaptive, species-specific, brain development that contributes to the inclusive fitness of the organism. Our data are not yet sufficient to determine the specific nature of REM sleep's influence on the developing visual system but it is clear that it is present and differs from that of visual experience, suggesting a contribution mutually exclusive of waking experience.

It was the correlation of high REM sleep amounts with maturational indices that first prompted the proposal of a functional association. The expression of REM sleep, however, is not restricted to the developmental period but continues throughout life [48]. The increasing awareness that changes do occur in the adult brain leaves open the possibility that certain mechanisms of neural plasticity operate throughout the lifetime of the organism [30]. Processes that operate in REM sleep play a role in the development of the visual system and, we believe, in other systems that have a dependence upon activity for development. In a similar way, these same REM sleep pro-

cesses may influence such mechanisms as those underlying learning in adulthood [31]. Though much remains to be determined about the nature and mechanisms of REM sleep function, we suggest that the REM sleep state could prove important to the general process of modeling the nervous system.

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