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Ponto-Medullary Interactions in the Generation of REM Sleep

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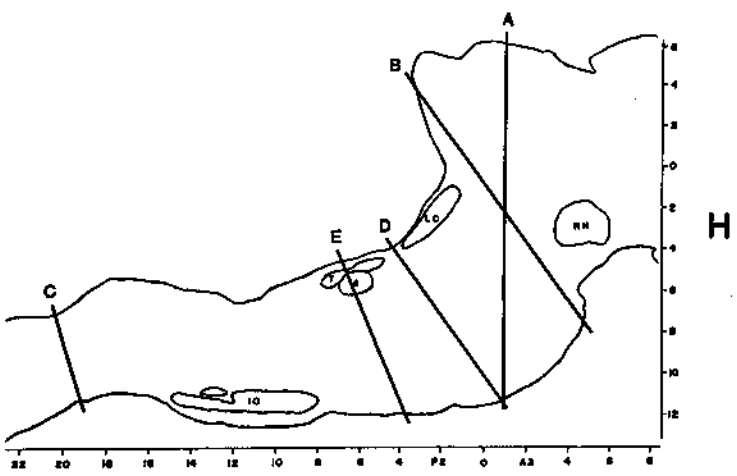
There has been considerable uncertainty about the localization and anatomical extent of the brainstem regions critical for REM sleep control. While recording experiments have shown that many brainstem cell groups change discharge rate and pattern in REM sleep, the key experimental evidence on the localization of areas critical for REM sleep control must be derived from manipulations, with stimulation and lesion techniques, of the areas suspected of controlling REM sleep. The loss of REM sleep after brainstem lesions may be due to nonspecific factors. This is particularly true in medullary regions, where stimulation and lesions interfere with the regulation of respiration and blood pressure. Therefore, the medullary role in behavioral state control has remained largely unknown. Recently identified anatomical connections between lateral pontine regions and the medulla have increased interest in this role (see Jones, Sakai, this volume).

While the loss of REM sleep may be a result of nonspecific factors, the presence of REM sleep after the removal of a brain area is definitive evidence that the removed area is not required for generating this state. Therefore, we have performed a series of brainstem transection studies in the hope that we might find positive evidence bearing on the contribution of both pontine and medullary regions to the pattern of physiological events and periodicities that constitutes REM sleep.

Figure 1 illustrates the key transection experiments in the localization of REM sleep control mechanisms. Transection at levels A and B prevent REM sleep signs in the rostral portion of the brain, but allow all of the brainstem signs of REM sleep caudal to the cut, including rapid eye movements, extreme miosis, neck muscle atonia and normal durations and periods of recurrence for this REM sleep-like state (3,5,21,). Work in cats with spinal transections (Fig. 1C) and in humans with spinal injury has shown that the spinal cord makes no essential contribution to the brainstem signs of REM sleep (1,13). This positive evidence



PONTO-MEDULLARY INTERACTIONS IN REM SLEEP

FIG. 1. Key transection levels plotted on sagittal plate. LC, locus coeruleus; IO, inferior olive; 6, abducens nucleus; 7, genu of the facial nerve; 3, occulomotor nucleus. A and B are transections at the junction of pons and midbrain, C is at junction of spinal cord and medulla, D is midpontine transection and E is a transection at the ponto-medullary junction.

clearly establishes that the ponto-medullary brainstem is sufficient for the generation of REM sleep. Further evidence on the localization of REM sleep control mechanisms has relied largely on inferences from pontine lesion studies. It has been demonstrated that lateral pontine areas are critical for certain REM sleep phenomena. Very small lesions restricted to the peri-locus coeruleus a region prevent REM sleep atonia (Morrison, Sakai, this volume). Large lesions of the dorsolateral pons can permanently prevent REM sleep (Jones, Sakai, this volume). While such studies demonstrate that pontine areas are required for REM sleep, they clearly do not indicate the extent of areas sufficient to generate REM sleep pheonomena.

Critical evidence further localizing the brainstem regions needed for REM sleep could be derived from transection through the mid pons or ponto-medullary junction (Fig. 1. D,E). Such transections would leave the regions whose destruction disrupts REM sleep rostral to the cut. If these regions are sufficient for all or some of the REM sleep phenomena, then the pons and forebrain should exhibit these phenomena. Similarly certain aspects of REM sleep might appear caudal to the cut, identifying potential medullary contributions to REM sleep control.

Several problems of technique and interpretation are encountered in attempting to analyze state control in preparations with

transections at the mid pontine or caudal medullary level. Since small pontine lesions prevent REM sleep atonia but not the other signs of REM sleep, the absence of periods of atonia cannot be considered persuasive evidence that a REM sleep state does not exist In regions caudal to the transection. Therefore, we have used chronic unit recording techniques to monitor unit activity in the medullary nucleus gigantocellularis. This region has a characteristic discharge pattern in REM sleep (14). In addition, we have monitored neck electromyogram (EMG), EKG and respiration to assess medullary state.

Unit activity in the midbrain was recorded to facilitate analysis of forebrain states. In addition, ponto-genicluo-occipital (PGO) spikes and sensorimotor EEG were observed. In the cat with complete brainstem transection, eye movements may originate either from the abducens nucleus, i.e., from the medulla; or from the trochlear or oculomotor nuclei, i.e., from the forebrain. In order to establish independent recordings of forebrain and pontine commanded eye movements, we cut the abducens nerve on the left side, the trochlear nerve bilaterally at its decussation, and the oculomotor nerve on the right side. This procedure resulted in a cat in which movements of the left eye were produced by the forebrain and movement of the right eye were produced by the brainstem.

After midbrain transections a progressive normalization of brainstem function occurs over a period of one to two weeks. It is likely that a similar progression would be seen after lower transections. Therefore we developed procedures that allowed us to maintain these preparations for as long as 30 days post-transection (17,18).

Midpontine-Inratrigeminal Preparation

Our first series of transections passed just caudal to the locus coeruleus complex dorsally and through the trapezoid body ventrally. The transection went through the trigeminal nucleus and nerve (Fig. 2); therefore, we are labelling it the "midpon-tine-intratrigeminal" preparation to distinguish it from the more rostral "midpontine-pretrigeminal" preparation .(2). As has been reported with midbrain transection, complete transections at the midpontine level produced two independent state generators in the same cat, a medullary and a forebrain generator.

Medullary States

Figure 3 shows the behavioral states observed in the caudal portion of the preparation. Most of the time was occupied by what can be described as a quiescent state. EMG amplitude was comparable to that seen during baseline conditions in nonREM sleep (Fig. 4). EKG rates were regular and also comparable to nonREM sleep values (18). This quiescent state was periodically

FIG. 2. Cat with transection at the midpontine level.

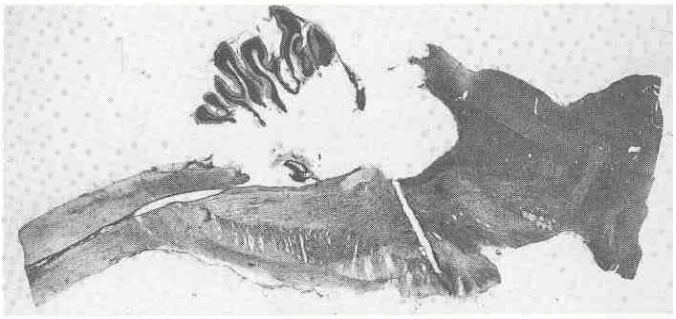
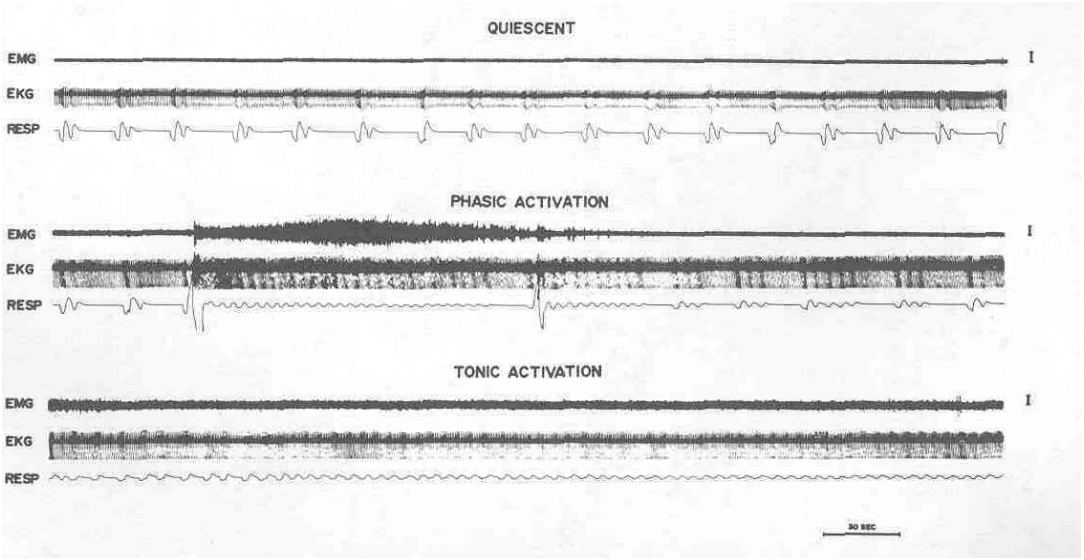


FIG. 3. States **seen in** caudal portion of cat after midpontine transection. EMG, dorsal neck electromyogram; EKG, electrocardiogram; Resp, thoracic strain gauge. EMG calibration, 50yV, {ref. 18).



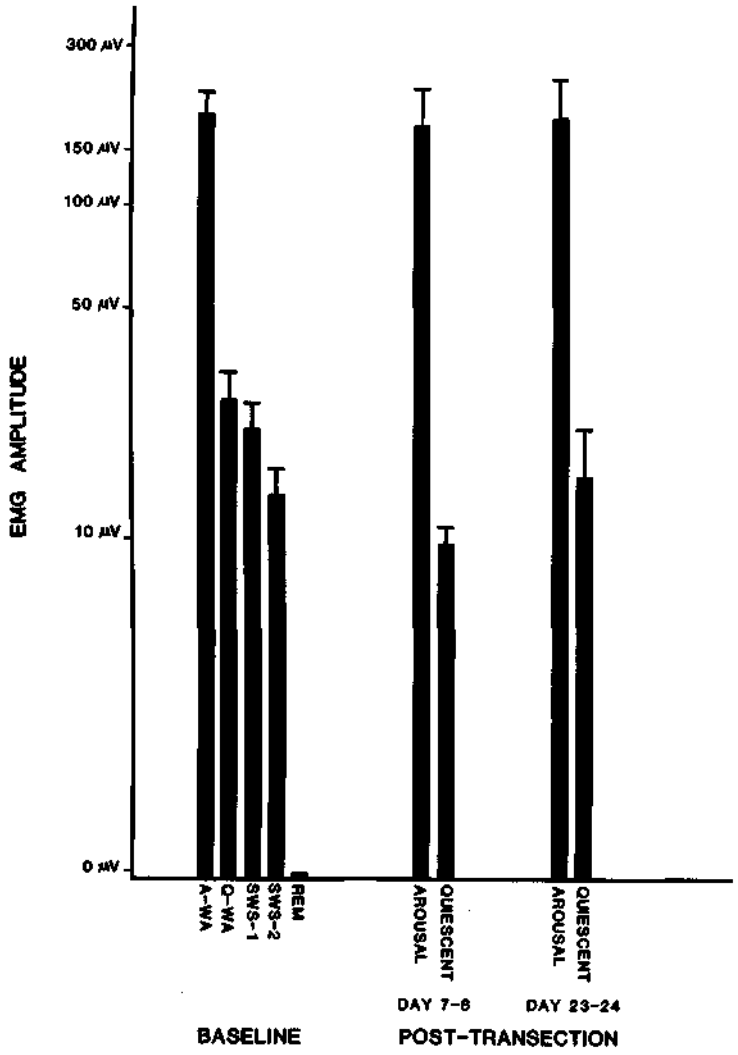


FIG. 4. Neck EMG amplitude in intact cats during active waking (A-WA), quiet waking (Q-WA), light and deep slow wave sleep (SWS-1, SWS-2) and REM sleep, compared to EMG levels seen during arousals and in quiescent state after transection in same cats (ref. 18).

punctuated by "phasic arousals." These arousals recurred spontaneously and could also be induced by noxious stimulation. During arousals, EMG activity increased to the levels observed during active waking in the intact cat (Fig. 4). Righting

attempts and extensions of the limbs and axial skeleton occurred at these times. In most cases, the quiescent condition returned within 1-2 minutes after the phasic arousal. However, during the first two post-transection weeks, a sustained increase in EMG levels and respiratory rate ("tonic arousal") could, in some cases, last for as long as 2-4 hours (Fig. 3).

The phasic arousal episodes recurred with great regularity in the medullary cat (Fig. 5). At 7 days post-transection, activations recurred on average at 9.4 minute intervals while at 23 days post-transection the mean interval was 22.6 minutes.

No periods of rapid eye movements were seen in caudal brain-stem systems after transection. Only isolated nictitating membrane blinks were observed (Fig. 6). The occurrence of these blinks did not correlate with changes in any of the other recorded variables.

A total of 50 units were recorded in the nucleus gigantocellu-laris (Fig. 7). Units discharged regularly during quiescent periods (Fig. 8). This regularity could be best seen in the repetitive peaking of the autocorrelations of medullary units (Fig. 9). In contrast, such rhythmic "pacemaker" discharge is not seen in medial medullary units recorded in the intact cat, where autocor-relograms are virtually always unimodal (14). Discharge rates increased by an average of 129% during phasic arousals (Fig. 10; Table 1). Rates during phasic arousals were comparable to the rates seen in the intact cat in active waking (14). We never saw units which increased activity substantially without a correlated phasic activation.

TABLE 1. Discharge rates of nucleus gigantocellularis units.

	Quiescence	Phasic Rousal
mean	16.2	37.1
n	19	19
S.D.	16.6	38.5
range	0.7-71.3	0.0-150.0

Despite continuous recording for periods of up to 30 days, we never saw periods of atonia in the neck musculature. The loss of atonia could be explained as resulting from a loss of the pontine projections that activate the medullary inhibitory region in REM sleep (Horrison, Sakai, this volume). Therefore, we directly stimulated the "inhibitory" region in two chronic medullary cats. We were surprised to find that this stimulation produced excitation rather than inhibition at most sites within the medial medulla (16). In contrast, the same stimulation applied throughout the medial medulla of midbrain decerebrate cats could produce

FIG. 5. Twenty-four hour plot (in arbitrary units) and activity in a medullary unit. Unit rate counter reset every 30 sec. Note positive corrilation of EMG and unit activ ity, and periodicities of Increases in these parameters.

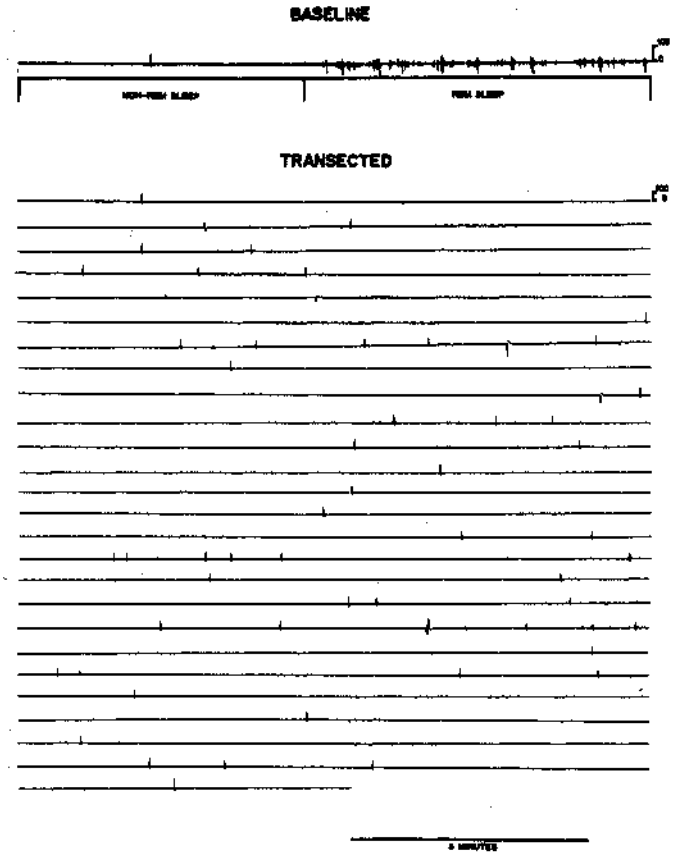


FIG. 6. Abducens controlled eye movements prior to and after brainstem transection. In baseline recording, periods of rapid eye movement can be observed during REM sleep. After transection, only isolated nictitating membrane blinks are observed. Calibration, 100 µV (ref. 18).

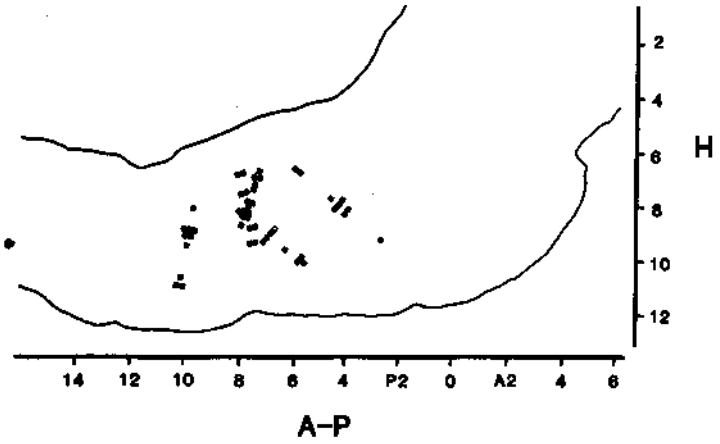


FIG. 7. Locations of medullary units recorded after transection. Squares, units with rates >4.0/sec in quiescence; circles, units with rates <4.0 sec. Empty square, unit decreasing rate during phasic arousal (ref. 18).

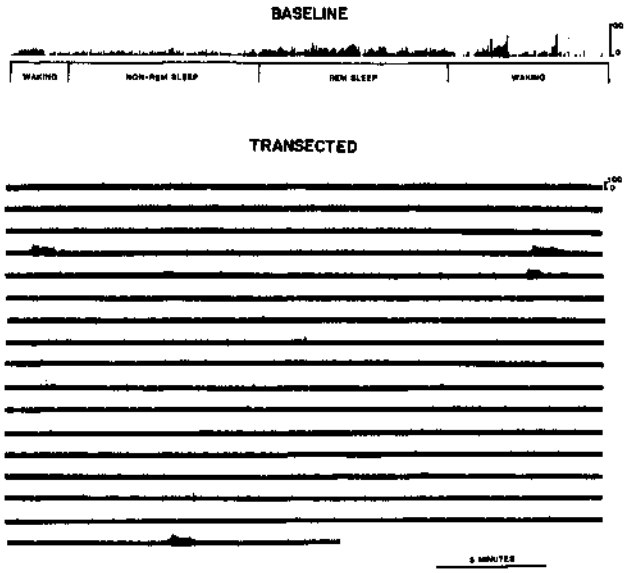


FIG. 8. Medullary reticular formation (RF) unit activity during sleep-waking cycle in intact cat, and after brainstem transection. Tracing is output of a digital counter resetting at one second intervals. Note the long periods of accelerated and irregular unit activity during waking and REM sleep in the intact cat. RF cells in the transected cats have extremely regular discharge rates, interrupted by short periods of increased unit activity occurring in conjunction with phasic arousals (ref. 18).

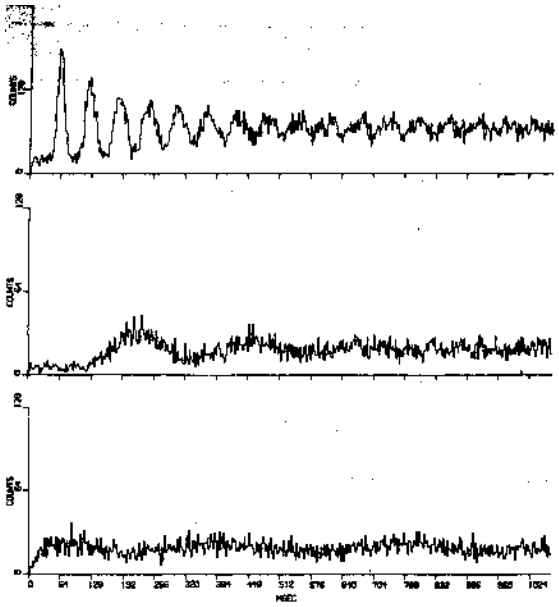


FIG. 9. Autocorrelations of three medullary RF units in transected cats. Note the repetitive peaking, with different period, in each unit. This pattern indicates regular "pacemaker" discharge.

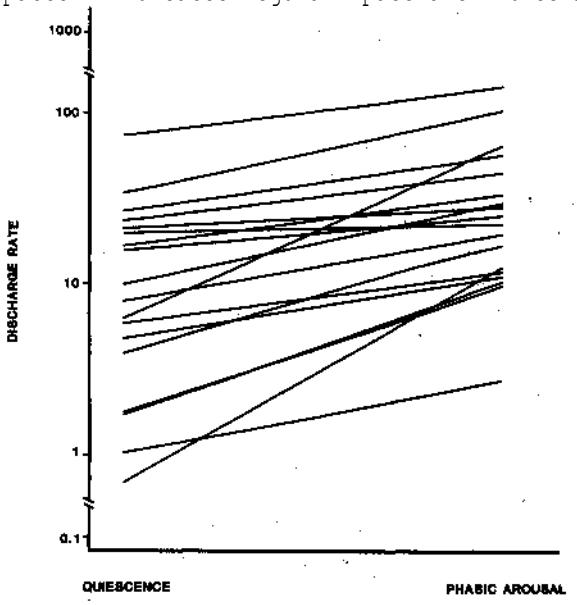


FIG. 10. Increases in discharge rates of medullary units with phasic arousal from quiescent state baseline (ref. 18).

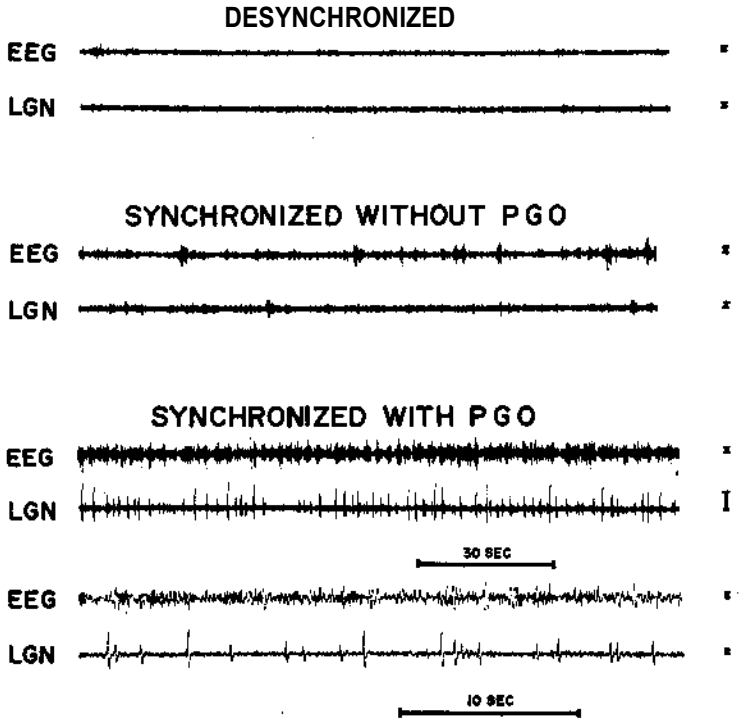


FIG. 11. Forebrain states seen after midpontine transection. EEG, sensorimotor electroencephalogram; LGN, lateral geniculate nucleus. Calibration 50μV.

complete bilateral inhibition of the antigravity muscles. Therefore pontine mechanisms contribute to medullary induction of atonia, even when the medulla is directly stimulated. Pontine influences may act as part of a recruitment loop facilitating the spread of medullary excitation at medullary or pontine levels. Pontine influences may also act by altering respiratory and cardiac control. In recent studies we have found that small reductions in blood pressure can block medullary inhibition (19). Consequently, the loss of atonia in the medullary cat may be due to changes mediated by systemic variables in addition to any loss of ponto-medullary excitation.

Conclusions: Medullary Cat

The medullary cat has an aroused state, which resembles a crude, short duration rudiment of the waking state. It also has a quiescent state, analogous to nonREM sleep. We do not see the cluster of physiological signs that define REM sleep in the medullary cat. However, the medullary cat does show a regular cyclicity in the recurrence of phasic arousals.

Forebrain States

Three states could be seen in forebrain systems after midpon-tine transections: a desynchronized state with no PGO spikes, a state with intermittent EEG synchronization and a state with continuous EEG synchronization and PGO spikes (Fig. 11). The duration of the state of EEG synchrony with PGO spikes and the time between consecutive states of EEG synchrony with PGO spikes (cycle time) were much greater than those seen in intact-cats (Table 2). No periods of EEG desynchrony with PGO spikes were seen. Unit activity in the mesencephalic reticular nucleus did not change markedly as a function of forebrain state (Table 3). In the intact cat, units in this same region show dramatic rate increases in REM sleep and active waking (15).

TABLE 2. Forebrain state durations, midpontine transections.

	Mean	Range
cycle length	232.0	52.9-737.0
desynchronized	18.2	9.4- 46.3
intermittent synchronization	6.8	2.5- 22.3
synchronized	35.99	5.8- 97.0

TABLE 3. Midbrain unit discharge rates, midpontine transections.

	State		
	Desynchronized no PGO	Synchronized no PGO	Synchronized with PGO
Mean Rate S.D. range	17.9 13.3 0.5–51.6	17.4 13.3 0.5–53.7	17.5 15.2 0.3–62 .1

Conclusions: Forebrain States After Midpontine Transection.

Both EEG and unit data lead to the conclusion that the pattern of forebrain activity seen in the intact cat during REM sleep is not observed after midpontine transections. However, the rostral pons is sufficient to generate PGO spikes and PGO spike bursts analogous to those seen in normal REM sleep.

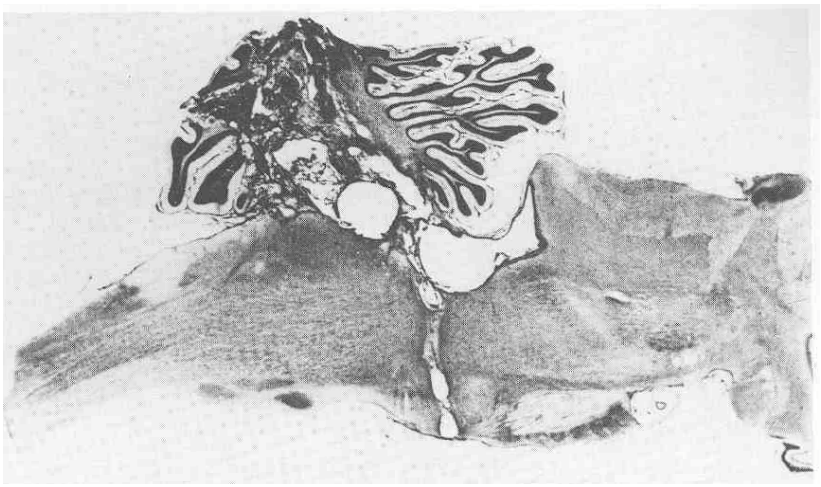


FIG. 12. Cat with transection at ponto-medullary junction. Intact locus coeruleus and subcoeruleus are visible rostral to the transection.

Forebraln States After Transection at the Pon to - Medullary Junction

Since REM sleep was not seen in either rostral or caudal brain regions after midpontine transection, we performed a second series of transections at the ponto-medullary junction (Fig. 12). This allowed us to determine if the addition of caudal pontine structures would be sufficient to produce any further signs of REM sleep in the forebrain.

We observed three forebrain states: A state of EEG desyn-chrony without PGO spikes, a state of EEG synchrony with PGO spikes and a state of EEG desynchrony with PGO spikes (Fig. 13). Hippocampal theta was prominent in this last state, as it is in REM sleep. Periods of rapid eye movement were concentrated in this state although they also occurred at other times. States of EEG desynchrony without PGO spikes could be induced during EEG synchronization by stimulation of the mesencephal ic reticular nucleus. This stimulation also terminated the state of PGO spiking with desynchrony. In the intact cat such stimulation triggers a desynchronized waking state without PGO activity (9).

Since the motor nucleus of the trigeminal nerve is intact rostral to the cut, this preparation provides a unique opportunity to observe changes in motor control with state in the absence of medullary influences. We therefore implanted the masseter muscles for EMG recording. As can be seen in Fig. 13, muscle tone

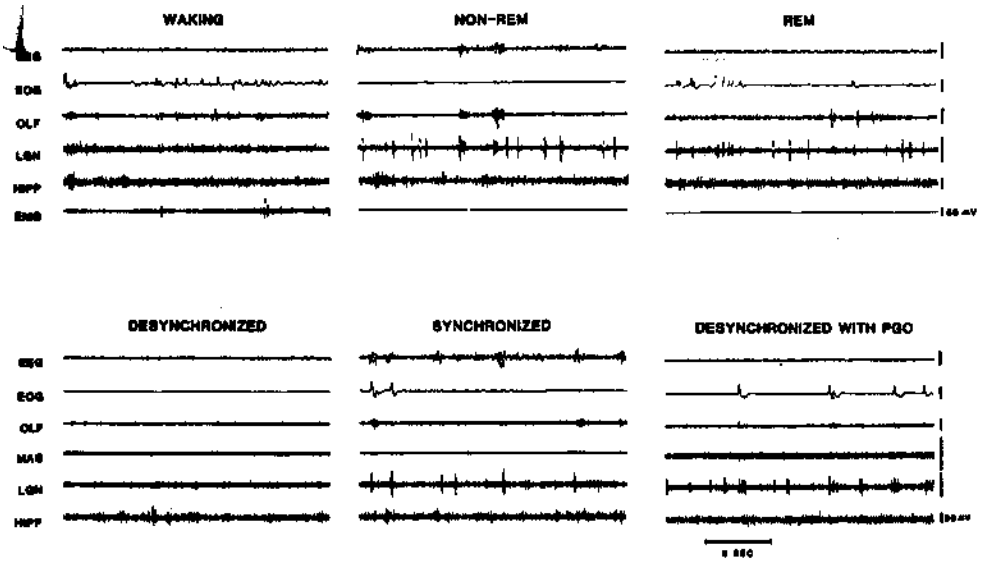


FIG. 13. States seen in forebrain after transection at ponto-medullary junction compared to baseline data from same cat. OLF, olfactory bulb; HIPP, hippocampus (ref. 17).

was present in all states. During periods of EEG synchronisation, muscle tone was greatly reduced. Therefore, this reduction of muscle tone, which is also seen during nonREM sleep in the intact cat, does not require medullary mechanisms. However, during "REM sleep-like" periods of desynchrony with PGO spikes, masseter tone increased. This increase is reminiscent of the increased muscle tone seen in the state of REM sleep without atonia, although the pontine region whose ablation produces this syndrome was intact in our animals (6). This strengthens the evidence that a descending loop to the medulla is required for the inhibition of trigeminal motoneurons in REM sleep (see Chase, this volume). However, the central motor excitation which accompanies REM sleep can be generated by the pons in the absence of medullary input.

We recorded midbrain unit activity in 17 mesencephalic reticu-lar units (Table 4). Unit rates were maximal during EEG desynchrony with PGO spikes. This increase was particularly marked in some units (Fig. 14) and was dramatic during PGO spike bursts.

The timing of forebrain states was quite different from states seen in the intact cat. In particular the interval between "REM sleep-like" episodes after transection was shorter (Table 5) and the episode durations were far more variable than those seen prior to transection (17).

TABLE 4. Midbraln unit rates after transection at ponto-medullary junction.

Mean S.D. Range	Desynchronized no PGO	Synchronized with PGO	Desynchronized with PGO
	5.0 6.0 0–24.3	4.9 6.3 0.4–28.0	11.0 16.3 1.11–59.2

TABLE 5. Forebrain cycle length and state durations after transection at ponto-medullary junction

Day 7 post-transection		Baseline REM sleep	"desynchronized with PGO"
Cycl	e length mean	35.6	11.0
	range	3.6-199.6	2.4-54.0
State	duration		
	mean	4.4	6.4
	range	1.2-10.8	1.2-49.2

Conclusions: States Generated by Pons-Forefarain After Transection at Ponto-Medullary Junction.

The pons and attached forebrain can generate states resembling those seen in the intact cat, i.e., waking, nonREM and REM sleep. However the timing of the REM sleep-like state is abnormal.

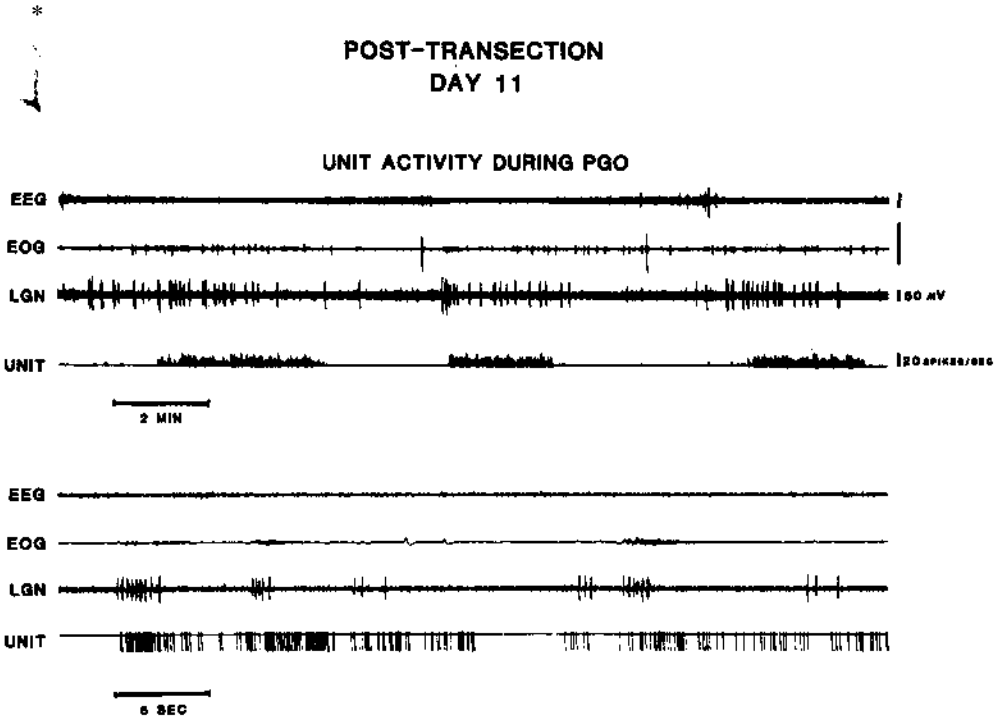


FIG. 14. Unit activated during EEG desynchrony with PGO spikes, shown at two different polygraph speeds. In slow trace, unit channel displays output of digital counter resetting at one sec intervals.

DISCUSSION AND CONCLUSIONS

These results demonstrate that medullary mechanisms are not required to generate the pattern of PGO activity, rapid eye movements, EEG desynchrony, hippocampal theta and midbrain unit activity that constitute the defining signs of REM sleep in the forebrain. It remains possible that some critical feature of REM sleep may be identified that would distinguish it from the state we have observed in the forebrain of the animals with transections at the ponto-medullary junction. However, it is clear that the conjunction of these physiological signs, seen in the intact cat only in REM sleep, can be generated by pontine-forebrain mechanisms.

It has long been known that Isolated PGO spikes could be generated under acute conditions by pontine regions. There has been some uncertainty over the localization of brainstem regions critical for the synthesis of PGO bursts (11,12). The present results indicate that rostral pontine regions located above our roidpontine projections are sufficient to generate PGO spike bursts similar to those seen in the intact cat. The most caudal regions of the pons are required for the appearance of these bursts during EEG desynchrony. Therefore we hypothesize the ex-istance of an area facilitating PGO burst generation in pontine regions between the abducens nucleus and the locus coeruleus complex. One possible substrate of this facilitation might be the "long lead" PGO burst cells recently identified in the caudo-medial pons (4).

We have seen that both pontine and medullary regions can independently modulate muscle tone as a function of behavioral state. The medulla generates the motor activation seen during phasic arousals and the low level of electromyographic activity during the quiescent state. The pons-forebrain can cause reduction of electromyographic activity during EEG synchrony and motor activation during desynchrony with PGO spikes. The atonia seen during REM sleep requires both pontine and medullary mechanisms and may in addition require a certain level of baroreceptor activity (19).

Another function that apparently requires ponto-medullary interaction is the normal temporal sequencing of the REM sleep cycle. This 15-30 minute cycle is also thought to underlie the basic rest activity rhythm in waking performance (7,8,10,20). The medullary brainstem disconnected from pontine regions is sufficient to generate such a rhythm of "phasic arousals" in spinal and respiratory motor activity. However the forebrain of the "midpontine-intratrigeminal" preparation does not generate such a rhythm. The addition of caudal pontine regions brings a regular ultradian rhythmicity to the forebrain along with the state of desynchrony with PGO spikes. While the REM like state recurs regularly in the forebrain of these cats, the duration of this state is significantly more variable than in the intact cat. These observations lead to the following conclusions. 1) The anatomical substrates for regulating the duration and period of recurrence of the REM sleep state are distinct from those generating the state itself. 2) Caudal pontine mechanisms are sufficient to generate a basic rest-activity cycle in the forebrain. 3) Medullary regions are required for the regulation of REM sleep duration. 4) Since forebrain cycles are abnormally short in the forebrain of the medullary cat, medullary regions may contribute to the regulation of the duration of the basic rest-activity cycle.

SUMMARY

1) We have studied patterns of brainstem and forebrain neur-onal activity after transections at the midpontine or ponto-medullary junction level. We found that medullary regions exhibited quiescent and active states after both levels of transection. No periods of muscle atonia were observed. No states resembling REM sleep were seen in the medulla. However, a regular ultradian rhythm in the occurrence of active states was seen.

2) After transections at the midpontine level, states of EEG desynchrony and of EEG synchrony with and without PGO spikes were observed in the forebrain. Unit activity and eye movement data indicated that REM sleep was not present. No ultradian rhythm approximating that of the basic rest activity cycle was seen.

3) After transection at the ponto-medullary junction, a REM sleep-like state was seen in forebrain regions. This state had patterns of unit activity, PGO spikes and eye movement similar to those seen in the intact cat. An ultradian rhythm with shorter and more variable period than that seen in the intact cat was present. Muscle tone was increased during periods otherwise resembling REM sleep.

4) We conclude that medullary regions are not required to generate the constellation of physiological signs used to identify REM sleep. Pontine regions caudal to the locus coeruleus and rostral to the abducens nucleus are critical in the generation and timing, of the REM sleep state. Medullary regions are required for the regulation of the duration of REM sleep and for the generation of atonia in REM sleep.

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