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Lesions producing REM sleep without atonia disinhibit the acoustic startle reflex without affecting prepulse inhibition

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This study determined whether the brainstcm motor inhibition system that mediates muscle atonia during rapid eye movement (REM) sleep is involved in the elicitation and prepulse inhibition of the acoustic startle reflex. Electrolytic or neurotoxic (glutamate) lesions were made in the dorsolateral pontine tegmentum or the medial medulla, respectively, to produce the syndrome of REM sleep without atonia. Startle responses were released during REM sleep following the lesions. However, the amount of startle suppression produced by auditory prepulse after the lesion did not differ from that seen in intact controls. We conclude that REM sleep suppression of the acoustic startle responses is mediated by the system responsible for tonic motor inhibition, but auditory prepulse inhibition of the acoustic startle is not.

'Prepulse inhibition' refers to the suppression of the startle reflex by an immediately preceding stimulus[11]. Prepulse inhibition is a robust phenomenon that does not require conditioning and has been demonstrated in many species such as pigeons, rats, cats and humans[11,41]. The diminution of startle responses in the presence of the preliminary stimulus has been used as an indicator of signal detection and information processing, and has been used successfully in assessing normal and abnormal sensory processing in both humans and animals[2,12,27,39].

The neural mechanism responsible for prepulse inhibition is unclear. The available evidence suggests that the site of modulation may occur at or before the pontine reticular formation in the startle pathway[38,41], although further modulation in the spinal cord is possible[4,37]. Previous lesion studies suggested that structures rostral to the superior colliculus are not essential for auditory prepulse inhibition[5,7,8,15]. On the other hand, lesions of the lateral tegmental area at the mesopontine level were found to reduce or eliminate prepulse inhibition (both auditory and visual) in the rat[19]. However, those lesions were extensive and included most of the midbrain reticular formation, parabrachial region, nucleus cunei-formis (NC), locus coeruleus (LC), and rostral dorsal pontine tegmentum[19]. It is thus not clear what specific structure(s) may be responsible for the diminished prepulse inhibition produced by the lesion.

The rostral dorsolateral pontine tegmentum is important in mediating muscle inhibition during rapid eye movement (REM) sleep[9,13]. The critical area within the pontine tegmentum has been identified as the peri-locus coeruleus *a* (peri-LC *a)* region just ventral to the LC and medial to the brachium conjunctivum[29]. In both cats and rats, lesions of this area induce a phenomenon called 'REM-without-atonia' in which the animal retains skeletal muscle tonus during REM sleep and can, depending on the extent of the lesion, either raise its head and upper body or display quadrupedal locomotion while in REM sleep[9,10,13,23]. A similar syndrome has also been described in humans with REM sleep behavior disorder[21]. Conversely, stimulation of this area either chemically[3,6,17.24,34,36] or electrically[16] produces muscle atonia.

Since the pontine region responsible for the suppression of muscle tone in REM sleep is within the region implicated in prepulse inhibition[19], we hypothesized that animals with pontine lesions producing REM sleep without atonia would have a deficit in prepulse inhibition. Furthermore, the startle response is greatly suppressed during REM sleep[40], suggesting that the REM sleep suppression of startle may be mediated by the tonic motor inhibition system of this state.

The peri-LC a region projects caudally to the medial medulla[29,30,35]. Electrical or chemical stimulation of the medial medulla produces muscle atonia in the decere-brate animal[16,20]. It has recently been shown that lesions of this medullary region also produce REM sleep without atonia[32]. Thus the medial medulla is part of the descending pathway mediating muscle tone suppression in

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REM sleep. In the present study we have evaluated the effect of REM-without-atonia caused by either pontine or medullary lesions on the elicitation and prepulse inhibition of the acoustic startle reflex.

The subjects were 15 adult cats. These cats all had conventional chronic electroencephalogram (EEG), electrooculogram (EOG), lateral geniculate (for the recording of ponto-geniculo-occipital (PGO) waves), and neck electromyogram (EMG) electrodes implanted, for monitoring their sleep/waking states as has been described previously[41]. Three cats were further implanted with chronic 29-gauge cannulae aimed at the medial medulla to be used for later neurotoxic lesions. One week of recovery was allowed for these animals before baseline sleep/waking patterns and startle response levels were measured. Pre-lesion baseline startle response and prepulse inhibition were determined in 3 cats subsequently used for pontine lesions. The remaining 7 lesioned cats received only post-lesion tests.

Seven cats received electrolytic lesions (cathodal current, 3 mA for 30 s) aimed at the pontine peri-LC *a* area. Three cats received medial medullary lesions by microinjection of glutamate (0.5 µl, 0.2 M, dissolved in Ringer's solution and adjusted to pH 7.4) through the implanted cannula. All lesions were made bilaterally. Drug infusion was done using a 33-gauge needle connected to a 1-µl Hamilton microsyringe through a plastic tubing. The remaining 5 cats received no lesion and served as controls. Animals' sleep/waking patterns were monitored for at least 4 weeks following the lesion. The magnitude of startle and prepulse inhibition was established 1 week after the lesion. Three pontine-lesioned cats received a 2nd post-lesion test 1 month later.

The startle response was elicited with a 20-ms, 115-dB (re 20 µN/cm^2, SPL†) noise burst. The prestimulus was a 20-ms, 70-dB noise burst delivered 100 ms before the eliciting stimulus. Both the startle-eliciting and the preliminary stimuli had a 5 ms rise-decay time. A total of 30 trials was presented with 1-2 min separation between trials. Half of the trials were presented with the startle-eliciting stimulus alone (control trials), and the other half with the prepulse preceding the eliciting stimulus (prepulse trials). The control and the prepulse trials were presented in a counter-balanced manner. The number of trials in quiet waking (QW) and non-REM sleep states in the control and the prepulse trials was kept the same for all the animals. Since prepulse inhibition in non-REM sleep is comparable to that in QW[40], data from these two states were combined in the analysis.

The startle response was measured with either a head-mounted accelerometer or directly from the neck EMG. These signals were filtered, full-wave rectified, and then integrated for 200 ms following the onset of the eliciting stimulus, using an IBM PC/XT equipped with a CED signal averaging package and interface (Cambridge Electronic Design, Ltd, Cambridge, U.K.). The amount of startle reduction produced by the prepulse was calculated as the difference in the average startle amplitude between the control and the prepulse trials and expressed as the percentage of the control startle level.

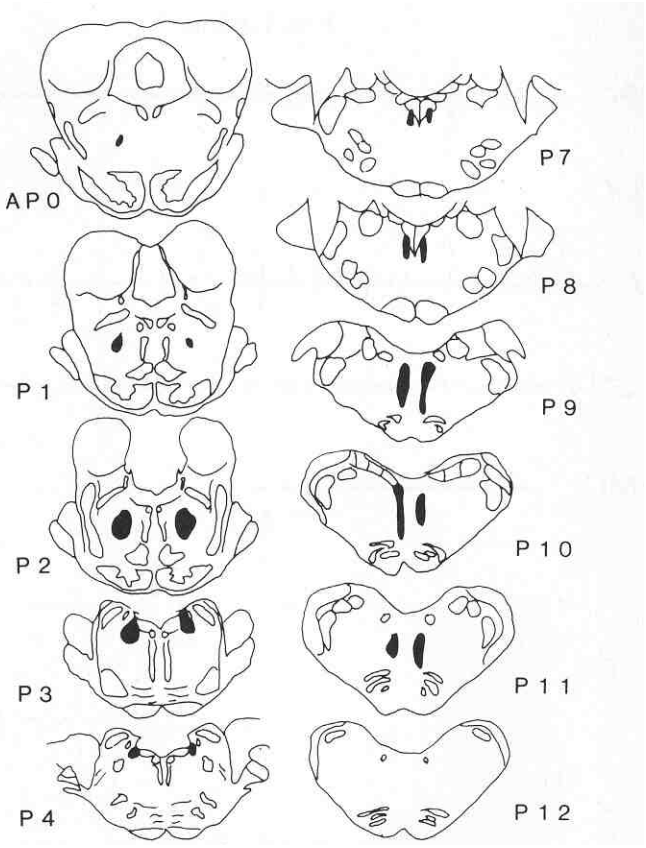

Fig. 1. Representative histological reconstructions of the dorsolat-eral pontine (left) and medial medullary (right) lesions from 2 cats.

Lesioned animals were sacrificed with a Nembutal overdose at the end of the experiments. The site and the extent of the lesion were determined for each animal with 5O-µm brain slices stained with Cresyl violet following conventional histological procedures.

Fig. 1 shows histological reconstructions of the pontine and medullary lesions in two cats. The pontine lesions generally involved the dorsolateral pontine tegmentum between APO and P4 and included peri-LC a, LC, laterodorsal tegmental nucleus, and part of the nuclei reticularis pontis oralis and caudalis. The medial medullary lesions, which were characterized by cell loss and fiber damage as well as extensive gliosis, were found in the caudal nucleus gigantocellularis, nucleus magnocellularis, and rostral nucleus paramedianus. All the cats that received lesions in the pons or medulla showed the REM-without-atonia syndrome shortly after the lesion, as shown in Fig. 2, similar to the Group 1 or Group 2

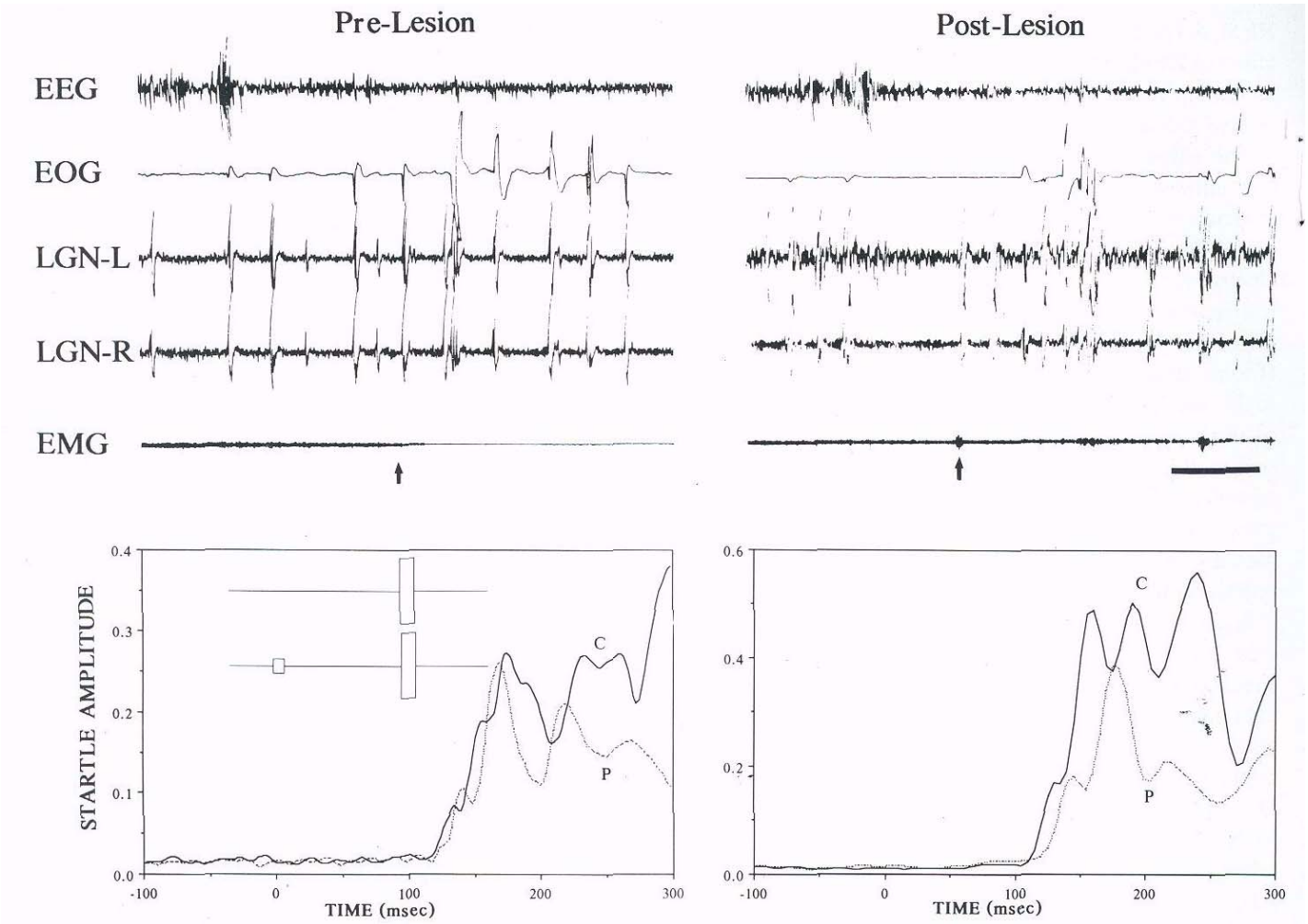

Fig. 2. Upper panels demonstrate the normal REM sleep atonia before the pontinc lesion and REM-without-atonia after the lesion. Note the frequent association of muscle twitches with PGO waves and rapid eye movements in REM sleep after the lesion. Arrows indicate the transition from non-REM sleep to REM sleep. Paper speed calibration is 5 s. Lower panels show the startle response, with (P, 70 dB + 115 dB) and without (C, 115 dB alone) the prepulse, measured through the head-mounted accelerometer before and after the lesion. The inset demonstrates the stimulus configurations for the control and the prepulse trials. The startle amplitude is expressed in arbitrary units. Note the scale of the ordinate is different for the pre- and post-lesion figures. The startle response following the lesion increased 68% over the pre-lesion level. The prepulse induced 32% reduction in the startle response before the lesion and 47% reduction after the lesion.

syndrome, according to Morrison's criteria[26]. These animals were able to raise the upper body or the head during REM sleep with the presence of sustained neck muscle tonus (pontine-lesioned cats) or with sustained muscle tone intermixed with muscle atonia (meduliary-iesioned cats). These animals also displayed frequent orofacial and pinna twitches, as well as head and limb movements during REM sleep. Very often these phasic events were associated with PGO waves and/or rapid eye movements (Fig. 2). The REM-without-atonia syndrome lasted at least 2 weeks for all the lesioned cats. Partial recovery was seen in some pontine-lesioned cats after the second week, in that intermittent muscle atonia was seen during REM sleep, similar to that seen in the medullary-lesioned cats. However, none of the lesioned animals (pontine or medullary) showed a full recovery of muscle atonia during the course of the experiments.

In addition to the increased spontaneous phasic head-body jerks which were often associated with PGO waves and/or eye movements, all lesioned animals would also startle during REM sleep. This is rarely seen in normal animals, presumably as a result of tonic muscle inhibition of this state. Two pontine-lesioned cats showed an increase of startle response during QW and non-REM sleep, 50% over their pre-lesion levels, one pontine-lesioned cat had a large decrease in startle response (< 5% of the pre-lesion level), and one medullary cat had much larger than normal startle response (205% of average intact animal). But, overall, there was no significant difference in the startle level between the intact and the lesioned animals, 100.0 ± 30.2 vs 96.7 ± 23.3 (mean ± S.E.M., normalized score), $t_{13} = 0.09$, *P*

> 0.5. The post-lesion data of the one lesioned cat that showed greatly diminished startle were not included in the analysis of prepulse effects on startle.

In intact animals, the 70-dB prepulse produced an average of 30.9% (± 4.1) reduction in the startle response. Lesioned animals (6 with pontine lesion and 3 with medullary lesion) had an average prepulse-induced startle reduction of 36.7% (± 6.4), not significantly different from that of the intact animal (t_{13} = 0.67, F > 0.05). Compared to their pre-lesion scores, one of the three pontine-lesioned animals that were tested before lesioning showed increased prepulse inhibition while the other 2 showed decreased inhibition, and the overall results were also not statistically significant (46.5 ± 18.7 vs 39.0 ± 15.3, t_2 = 0.57, P > 0.05). The second post-lesion test, which was conducted 1 month after the first one, also did not show any deficit in prepulse inhibition (53.1 ± 14.1, t_2 = 1.71, P > 0.05). Fig. 2 shows an example of prepulse inhibition before and after the pontine lesion.

The present experiments determined if the peri-LC *a* area and the medial medulla, which have been shown to be required for the mediation of REM sleep muscle atonia, are also required for prepulse inhibition of the acoustic startle reflex. Animals with lesions in these two areas lost muscle inhibition during REM sleep. Startle responses could be elicited during REM sleep in the lesioned animals, but startle amplitude in QW and non-REM sleep was not changed. Prepulse inhibition of the acoustic startle in the lesioned animals did not significantly differ from that seen in the intact controls. These results indicate that REM sleep suppression of the acoustic startle is mediated by the tonic REM-associated motor inhibition mechanism, while auditory prepulse inhibition of the acoustic startle is not.

Stimulation of a more lateral area that surrounds the nucleus cuneiforrnis and the Pedunculopontine nucleus (PPN) has been shown to inhibit the startle response[28]. This area is involved in locomotor control[25,33] and in the generation of the PGO wave[18,22,31]. In the decerebrate and anesthetized animal, muscle suppression or excitation, depending on the stimulation parameters, can be elicited from the same sites around NC and PPN[14,17]. PGO waves can be evoked with a variety of sensory stimuli[1,40]. Intense auditory stimuli elicit PGO waves simultaneously with the startle response[1,40]. At lower intensities, e.g. 70-80 dB, a period of phasic muscle suppression is elicited along with the PGO wave[40]. This auditory-evoked phasic muscle suppression continues to be present during REM sleep in animals with the REM-without-atonia syndrome following dorsolateral pontine lesions (Wu and Siegel, unpublished observations). Thus this phasic inhibitory mechanism is intact, and could mediate prepulse inhibition in animals with the REM-without-atonia syndrome as well as in normal animals.

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