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INFLUENCE OF FEAR CONDITIONING ON ELICITED PONTO-GENICULO-OCCIPITAL WAVES AND RAPID EYE MOVEMENT SLEEP

L.D. SANFORD[1], A.J. SILVESTRI[1], R.J. ROSS[2,3] AND A.R. MORRISON[1,2]

[1] Laboratory for Study of the Brain in Sleep, Department of Animal Biology, School of Veterinary Medicine, [2]Department of Psychiatry, School of Medicine, University of Pennsylvania and [3] Veterans Affairs Medical Center, Philadelphia, PA, USA

INTRODUCTION

Fear conditioning involves creating an association between a neutral stimulus (generally a light or auditory stimulus) or situational context and an aversive stimulus (usually a footshock) (12, 13). The physiological consequences of fear conditioning closely mimic those seen in human anxiety disorders (12,13). Indeed, the concepts of anxiety and fear are closely related although fear is considered to be stimulus specific whereas anxiety is more generalized (12,13). Both anxiety and fear appear to involve the amygdala. Fear potentiated startle, the output measure usually studied after fear conditioning, has been used as a model of anticipatory anxiety (12, 13). Fear potentiated startle appears to be regulated by the amygdala because lesions of the central nucleus of the amygdala (CNA) block the effects of conditioned fear on the acoustic startle response (12, 13, 20). In addition, several indices of fear, such as freezing, changes in heart rate and blood pressure, and increased startle can be induced by electrical stimulation of CNA (5, 22, 25, 46, 47, 48, 62).

Evidence suggests that the amygdala has a role in modulating both alerting and state of arousal. Electrical stimulation of CNA can produce 'alerting' behaviors with EEG desynchronization (26, 30, 62). The CNA projects massively into brainstem regions involved in alerting during wakefulness and in the generation of rapid eye movement sleep (REM) and one of its characteristic signs, the ponto-geniculo-occipital (PGO) wave (2, 21, 37, 41). An elicited analog of the PGO wave (PGO_E) may be obtained in sleep and wakefulness by auditory stimuli in rats (28) and cats (7, 50, 51, 52, 66), suggesting that the PGO wave reflects the activation of an internal alerting system (50, 51, 52). The amplitudes of PGO_E are significantly greater in response to novel or newly re-introduced stimuli in wakefulness (15, 52), leading to the suggestion that the amplitude of PGO waves correlates with the orienting response (52). Le Doux (31, 32) has hypothesized that the amygdala plays a role in coding the emotional significance of stimuli in producing appropriate neurobehavioral responses to relevant stimuli. In turn, this suggests a functional link between PGO waves as an index of alerting and the amygdala. That REM can be seen as a state with features akin to alert wakefulness (39) is in keeping with Kleitman's (29) concept of a basic rest-activity cycle, REM substituting for alert wakefulness during sleep.

Electrical stimulation of the amygdala can increase subsequent REM (57,58), and electrically stimulating CNA during sleep influences the occurrence and amplitude of PGO waves in cats (8) and rats (14). Electrical stimulation of CNA prior to the presentation of an auditory stimulus enhances the amplitude of PGO_E (15) and the acoustic startle response (15, 47, 48). There is also a growing body of evidence that pharmacological manipulations of CNA can influence arousal state, especially REM (9, 54).

Thus, direct manipulations of the amygdala, particularly CNA, indicate a strong modulatory role in the control of alerting and arousal. The purpose of the present study was to examine the effect of fear conditioning on the PGO_E response to auditory stimuli, and on sleep and wakefulness, to assess the usefulness of it as a potential model to study the effects of fear and anxiety on alerting and arousal.

METHODS

Eight male Sprague-Dawley rats (ages 90-120 days at the time of surgery) were maintained on a 12 h light : 12 h dark cycle (lights on at 7.00) and given *ad lib* food and water for the duration of the experiment. The rats were anesthetized with IM injections of ketamine (85 mg/kg) and xylazine (12 mg/kg). Under aseptic conditions the rats were implanted with screw electrodes for recording the electroencephalogram (EEG). Stainless-steel wire electrodes were implanted in the neck musculature for recording the electromyogram (EMG). Bipolar electrodes (diameter 0.2 mm) were stereotaxically implanted bilaterally in the vicinity of the locus coeruleus (LC) [coordinates AP -0.3 (intra-aural zero), ML 1.0, DV 7.0 (40) for recording spontaneous PGO waves and PGO_E (17, 28, 34). Leads from the recording electrodes were routed to a nine pin miniature plug. The rats received the antibiotic gentamicin (6 mg, IM), and buprenorphine (0.5 mg/kg, SC) to control potential postoperative pain. A minimum of 7 days elapsed between surgery and using the animals in the experiment.

The rats were adapted to the sleep recording chamber and cable for 6 hours during the light period (11:00 A.M. to 5:00 P.M. EST). On a subsequent day, a 4-hour recording of EEG, EMG and PGO wave activity was obtained to determine baseline sleep amounts and to determine whether each rat exhibited spontaneous PGO waves during REM.

Following baseline sleep monitoring, the rats were trained in a fear conditioning procedure. The rats were presented with 15 light – shock pairings (light: 5 s duration; shock (0.5 mA) during the last 0.5 s) on four consecutive days. Shock was produced by a Coulbourn Instruments Precision Shock Generator (Model E13-14) and presented to the rats via the grid floor of a Coulbourn Instruments Habitest operant cage (Model E10-18RF). Immediately after each training session, the rats were taken to a separate room for polygraphic monitoring of wakefulness and sleep.

On each fear conditioning day, four-hour polygraphic studies were conducted between 12 pm and 4 pm. Wakefulness, non-REM (NREM) and REM were determined by trained observers. The parameters examined were: total recording period (TRP), time spent asleep (TSA), sleep efficiency (TSA/TRP), wakefulness, NREM and REM percentage (REM time /TSA), number and mean duration of wakefulness, NREM, transition and REM episodes, and REM latency (to the first REM episode post-training). Post-conditioning sleep was compared to sleep during the same hours on days in which fear conditioning was not performed.

Twenty-four to 48 hours after the last fear-conditioning trial, amplitudes of PGO_E in response to white-noise bursts and white-noise bursts coupled with the light-conditioning stimulus (CS) were measured. The rats were first presented with 200 white noise bursts (100 dB, 20 ms duration, 3 s ISI) to habituate the PGO_E response. During the subsequent testing phase, the rats were presented with 25 additional white noise bursts alone and 25 in the presence of light. All

test trials were presented in a randomized order with a 5 to 10 s ISI. A General Radio Company random noise generator (Model # 1382) generated white noise. Output was gated to a Harmon/ Kardon amplifier (Model # PM655 Vxi) and routed to a JBL loudspeaker (Model # TL600). Both training and test trials occurred in a darkened, sound-dampened chamber. For training the animals were placed in a cage with a grid floor. Testing was conducted with the rats in their home cage, but in the same chamber as that used for training.

The experiments were controlled, and the PGO_E data were collected, using a DataWave Experimenter's Workbench software module running on an IBM compatible Pentium computer. For test trials, data were collected at a sampling rate of 1 kHz and stored to disk in 1 s bins for later off-line analysis. Data were collected beginning immediately with the onset of the CS, with the associated white-noise stimulus being presented 3.5 s after light onset so that it would not coincide with the exact time shock had been presented. For control trials, data collection was begun immediately prior to the onset of the white noise stimulus.

PGO_E occurring between 20 and 40 ms after the onset of white noise were accepted for analysis. Events were detected when the amplitude of PGO_E increased above a threshold set individually for each animal. Each event was later evaluated off-line by the experimenter, and movement artifacts were removed from the data set. Non-detected responses were considered to be of 0 amplitude. The mean amplitude (mean peak amplitude of responses, excluding trials with no detectable response), magnitude (peak amplitude/no. of stimulus presentations) and proportion (no. trials with PGO_E/total no. of trials) of PGO_E were determined for each experimental block. For analysis purposes responses were averaged across blocks of 5 stimuli. The first and last five blocks (25 stimuli) of the habituation trials (pre- and post-habituation blocks, respectively) and the five blocks with light (25 stimuli) and five blocks without light (25 stimuli) in the test phase were compared for potential differences.

After the experiment was completed, the rats were overdosed with sodium pentobarbital (50-100 mg/ 100 g IP) and perfused intracardially with 9% saline and 10% formalin. The brains were processed to determine electrode placements. For this purpose, 40 µm slices were made through the areas of interest, and the sections were stained with cresyl violet. Figure 1 demonstrates the positioning of the PGO_E electrodes in a representative animal.

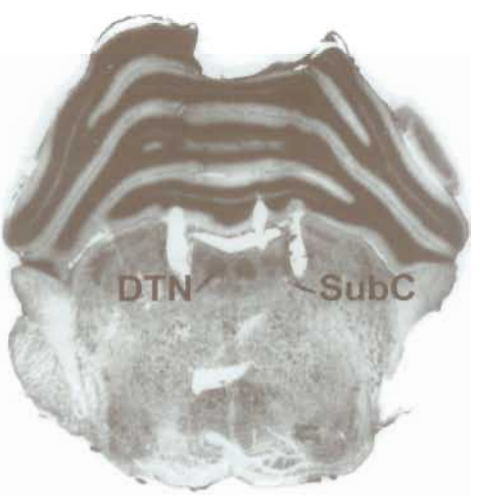

Fig. 1. - *Photomicrograph demonstrating electrode localization for recording pontine PGO waves in rats.*

DTN: Dorsal tegmental nucleus; SubC: Subcoeruleus nucleus.

RESULTS

Influence of fear conditioning on elicited PGO waves.

Six rats had unilateral or bilateral evoked responses that met our criteria for acceptance as PGO_E. Elicited responses in the remaining two rats were of longer latency and were not included in the analyses. Significance across conditions was tested with within subjects analysis of variance (ANOVA) tests.

The PGO_E data were analyzed with 4 (stimulus condition) X 5 (block) within subjects ANOVAs. The overall ANOVA resulted in a significant main effect for stimulus condition, $F(3,15) = 5.68$, $p = .008$, a significant main effect for block, $F(4, 20) = 6.68$, $p = .001$, and a significant interaction, $F(12, 60) = 2.01$, $p = .039$. The data were then examined for overall differences between stimulus conditions and for block by block differences. Examples of averaged waveforms are presented in Figure 2. Overall, the amplitudes of PGO_E were significantly greater in trials in which the light was present compared to those during post-habituation trials, $F(1,5) = 19.40$, $p = .007$. Pre-habituation PGO_E amplitudes were also higher

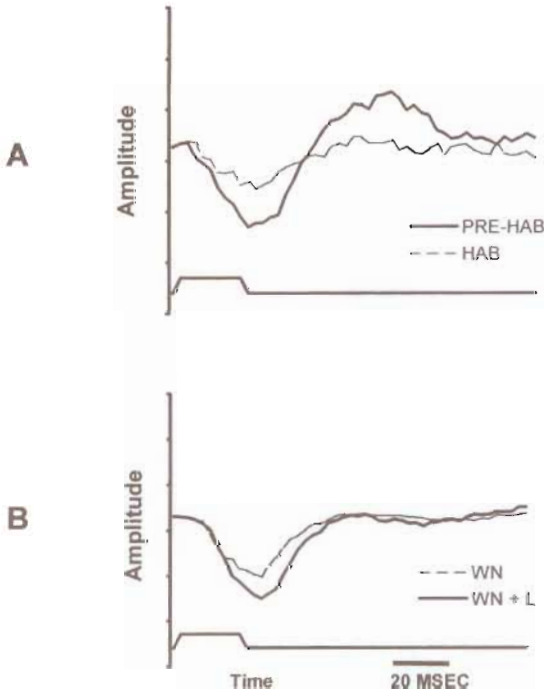

Fig. 2. - *PGO_E waves in the fear-conditioning paradigm.*

Illustration of a comparison of a representative wave before (PRE-HAB) and after (HAB) habituation (A) and of enhancement of PGO_E wave amplitude following a fear conditioned stimulus, noise plus light (WN + L), compared to noise alone (B). The elevated portion of the time marker represents duration of the stimulus.

compared to those after habituation (p = .025), but not significantly so after the Bonferroni correction. No other comparisons were significant. The amplitudes of PGO_E elicited in response to auditory stimuli during testing without the presence of the light had intermediate values between those of pre-habituation and those with the light, and those after habituation.

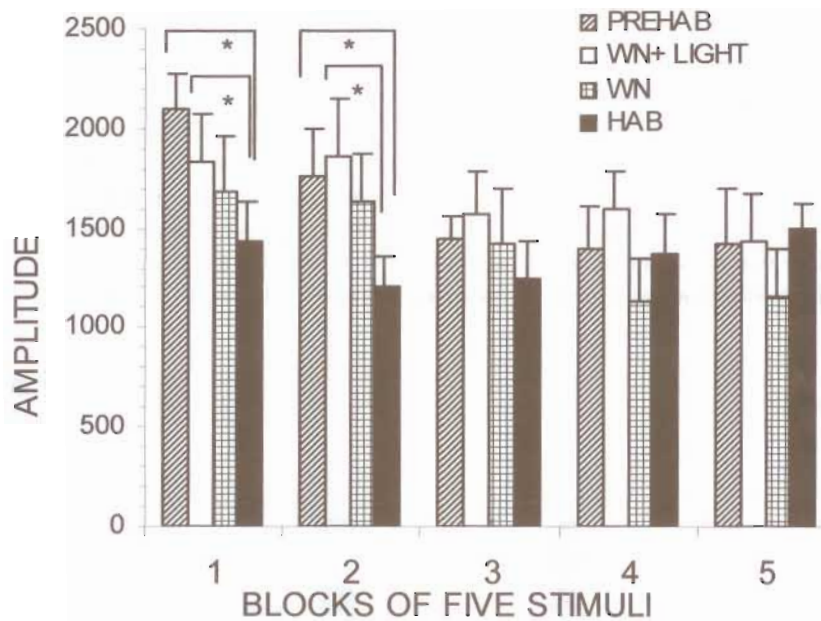

Fig. 3. - *Influence of fear-conditioning stimulus on* PGO_E.

Mean PGO_E amplitudes across blocks. 25 test trials are grouped in blocks of 5 stimuli. PGO_E amplitudes were significantly increased when light was presented with white noise (WN) compared with post-habituation (HAB) values in the first and second blocks, p = < 0.05 with Bonferroni correction. Pre-habituation (PRE-HAB) and HAB are values from the first and last 25 of 200 presentations of WN alone. Lack of significant effects in the last three blocks indicates that habituation occurred in all conditions during the test day itself.

Responses across blocks are presented in Figure 3. The data were examined on a block by block basis using 1 X 5 (block) ANOVAs. Overall ANOVAs were significant for the first 2 blocks, but not the last two, indicating that habituation had taken place in all conditions. Responses in the presence of light were significantly greater than those after habituation during blocks 1 (p = .001) and 2 (p = .005). Pre-habituation responses were also greater than those after habituation during block 2 (p = .003), but did not retain significance during block 1 (p = .021) after a Bonferroni correction. No other comparisons were significant.

The overall ANOVA for magnitude found a significant main effect for block, $F(4,20) = 12.22$, p = .001, and a significant stimulus condition by block interaction,

F(12,60) = 2.98, p = 0.003. The analyses of simple effects for magnitude followed a similar pattern to that of amplitude, but did not reach significance after application of the Bonferroni correction.

No significant differences were found for proportion of responses.

Influence of fear conditioning on wakefulness and sleep.

The data were analyzed with 5 (condition) X 4 (hour) within subjects ANOVAs. As expected, significant main effects for hour were found in almost every analysis, reflecting the normal tendency for sleep to vary across the recording period. The relevant analyses involved main effects for condition and the condition X hour interactions. Main effects for condition were found for NREM percentage, F(4,28) = 5.46, p=.002, REM percentage, F (4, 28) = 5.99, p= .001, number of REM episodes, F (4, 28) = 3.87, p=.013, and number of minutes in REM, F (4, 28) = 6.24, p = .001. The average duration of REM epsiodes was not significantly altered. The suppression of REM was most pronounced during the first two hours of recording and had returned to baseline levels by hour 4 (Figure 4).

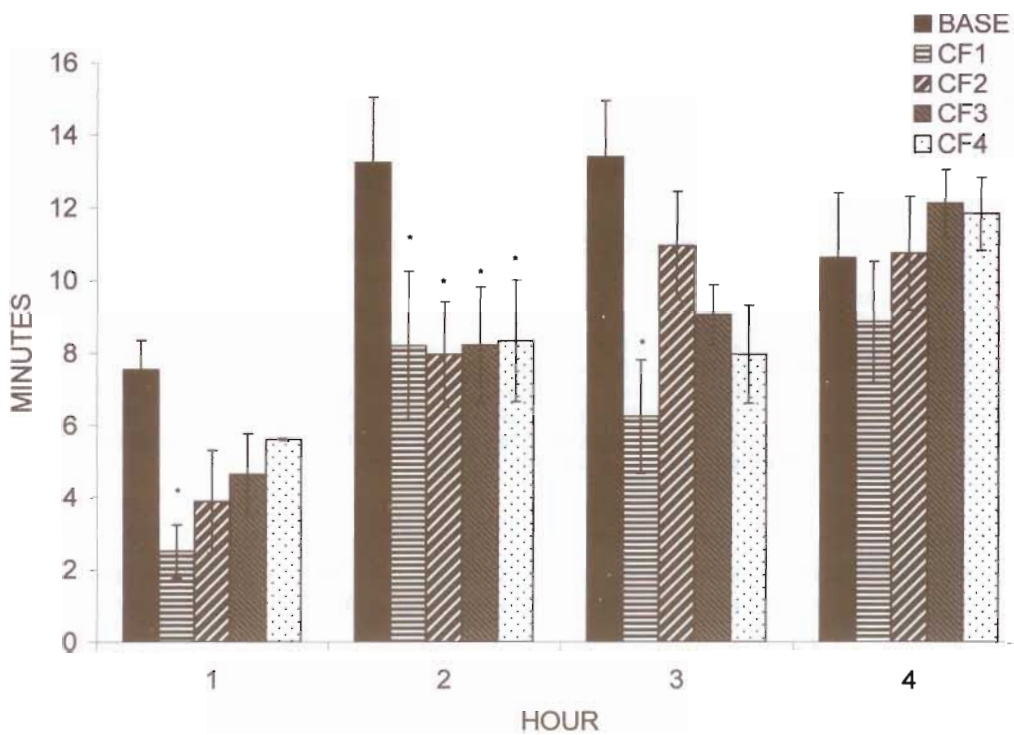

Fig. 4. - *REM over 4 hours after conditioning on each of 4 days.*

REM was significantly suppressed, whereas NREM was not reduced. (Base, pre-conditioning recording; CF1CF4, post-conditioning days 1-4). Significant differences from baseline are indicated by *, p = < 0.05 with Bonferroni correction.

Table 1. - *Effects of fear conditioning on sleep and wakefulness.*
All significant effects refer to comparisons to baseline sleep. All values are mean per hour.

	Baseline Mean ± SD	Confear1 Mean ± SD	Confear2 Mean ± SD	Confear3 Mean ± SD	Confear4 Mean ± SD
Wake percentage	22.62 ± 18.30	34.59 ± 24.15	27.37 ± 21.21	26.61 ± 18.25	33.01 ± 20.53
Wake duration (mins)	0.88 ± 0.65	1.43 ± 1.58	1.04 ± 1.04	0.92 ± 0.98	3.44 ± 10.5
Wake episodes	15.58 ± 11.19	16.75 ± 8.83	16.38 ± 8.87	19.47 ± 9.02	14.28 ± 7.51
TOTAL WAKE (mins)	13.57 ± 10.98	20.75 ± 14.49	16.41 ± 12.72	15.97 ± 10.95	19.81 ± 12.32
NREM percentage	76.20 ± 6.70	83.06 ± 9.75 [a]	83.06 ± 9.62 [a]	80.96 ± 8.50	82.82 ± 18.67
NREM duration (mins)	2.37 ± 1.14	2.13 ± 1.40	2.24 ± 1.52	1.94 ± 1.07	2.67 ± 1.51
NREM episodes	17.04 ± 6.89	18.05 ± 7.25	18.84 ± 6.68	21.19 ± 7.43	15.47 ± 7.98
TOTAL NREM (mins)	35.03 ± 7.48	31.89 ± 11.26	35.70 ± 10.28	35.56 ± 9.26	34.23 ± 11.44
REM percentage	23.80 ± 6.70	16.94 ± 9.75 [a]	16.94 ± 9.62 [a]	19.04 ± 8.50	14.05 ± 11.26
REM duration (mins)	1.55 ± 0.60	1.18 ± 0.63	1.16 ± 0.58	1.40 ± 0.56	1.20 ± 0.69
REM episodes	8.13 ± 4.54	5.6 ± 3.56 [a]	6.66 ± 4.27	6.69 ± 3.63	4.75 ± 3.73
TOTAL REM (mins)	11.40 ± 4.78	7.37 ± 5.16 [a]	7.85 ± 4.89 [a]	8.47 ± 4.15	5.96 ± 4.70 [a]
REM latency (mins)	18.57 ± 18.93	63.34 ± 41.73 [a]	46.50 ± 25.16	30.10 ± 23.08	51.79 ± 43.09
Sleep efficiency	77.38 ± 18.30	65.41 ± 24.15	72.63 ± 21.21	73.39 ± 18.25	66.99 ± 20.53

[a] Significant at $p < 0.05$ level with Bonferroni correction.

REM latency was analyzed with a 1 X 4 (hour) within subjects ANOVA which was significant, $F(4, 28) = 3.08$, $p = .032$. Comparisons among means revealed that fear conditioning significantly increased REM latency on day 1 of conditioning, $F(1, 7) = 25.44$, $p = .001$. REM latency was also increased on day 2, $F(1, 7) = 9.16$, $p = .019$ after the Bonferroni correction.

Significant interaction effects (condition X hour) were found for the number of wakefulness episodes, $F(12, 84) = 2.17$, $p = .021$, and the number of NREM episodes, $F(12, 84) = 1.88$, $p = .049$. These interactions were due to significantly fewer changes in behavioral state during the first hour of recording on day four of fear conditioning relative to the first hour on day 3 of fear conditioning. Comparisons amongst means are presented in Table 1.

DISCUSSION

Influence of fear conditioning on elicited PGO waves.

The amplitudes of PGO_E were greater in response to a white noise auditory stimulus presented in the presence of a light CS previously associated with footshock. This result complements and extends previous findings that electrical stimulation of CNA during REM increases spontaneous PGO wave frequency in cats (8) and spontaneous PGO wave amplitude in rats (14), and that electrical stimulation of CNA immediately prior to the presentation of an auditory stimulus enhances the amplitudes of PGO_E (15). The present findings demonstrate that the presence of a fear-inducing stimulus enhances the amplitudes of PGO_E responses. This suggests that emotional tone, possibly set by the amygdala, can influence brainstem alerting mechanisms. Interestingly, responses to auditory stimuli alone that were interspersed with those paired with the light CS also had somewhat elevated amplitudes, though not significantly so. This could have been due to contextual cues (the animals were tested in their home cages placed inside the same chambers they had been trained in) or to residual effects produced by repeated presentations of the CS.

A consistent finding in studies in which we present series of auditory stimuli is that the initial presentations of an auditory stimulus elicit high-amplitude PGO_E, whereas PGO_E with lesser amplitudes are elicited on further presentations of the stimulus (15, 52). Rapid habituation of amplitude is one of the defining characteristics of the orienting response (59), and the observed pattern of PGO_E responses led us to suggest that high amplitude PGO waves reflects orienting. According to this conception, the mere presence of a PGO_E signals the registration of a stimulus, whereas novel and/or important stimuli would elicit PGO_E of significantly greater amplitude, which, in turn, could reflect an augmentation in the processing of sensory information in wakefulness (52).

Activating the amygdala does not appear to induce PGO wave or PGO_E generation in the pons, but rather acts to enhance both spontaneously generated and elicited waves. We (15) previously suggested that the increase in PGO_E amplitude after electrical stimulation of CNA could be produced by amygdalar input to the

pons acting to raise the level of excitability of PGO-wave generating neurons (56, 61), thereby making them more likely to respond to external stimuli. We observed a detectable PGO wave after electrical stimulation of CNA alone on only 22% of all electrical stimulations. Occurrence of these waves could have resulted from CNA-enhanced responses to incidental sounds or to unknown external stimuli (15).

This hypothesis, that the amygdala accentuates, but does not initiate PGO activity fits with the current data as well. Presentation of the CS alone, which could act to activate CNA, did not produce a detectable response, but did enhance the amplitudes of PGO_E elicited in response to auditory stimuli. Given the posited role of the amygdala in evaluating environmental stimuli (2, 18, 23, 31, 65), it seems reasonable that this structure could influence, perhaps enhance, responsivity in brainstem alerting systems. For example, CNA modulation of pontine regions involved in alerting in wakefulness could reflect emotional expectations of stimulus importance.

There is also evidence that the amygdala can influence the activity of pontine neurons underlying PGO wave generation. The same putative cholinergic neurons thought to underlie the generation of PGO waves can be activated by auditory stimulation in cats (60) and rats (44), thereby inducing a PGO-like wave in the LGB of cats (50, 51, 66) and the pons of rats (28). A recent study (56) examined the influence of electrically stimulating CNA on putative cholinergic neurons in the rabbit pedunculopontine tegmentum. One category of neuron exhibited "burst" firing in response to acoustic stimuli similar to that observed in cells with firing that precedes PGO waves in cats. Stimulation of CNA produced activation in 43% of the "PGO-related" neurons recorded, suggesting that activation of CNA can affect the activity of putative cholinergic neurons that produce PGO waves (8, 56). Noradrenergic neurons, which appear to play an inhibitory role in PGO wave generation (60), are also influenced by CNA. Train electrical stimulation (67) and perfusion of glutamate (Sanford et al. unpublished observations) into CNA suppress firing in LC. Therefore, stimulation of CNA could remove noradrenergic inhibition from PGO generator sites and make the occurrence of spontaneous or elicited PGO responses more likely.

Influence of fear conditioning on sleep.

A second prominent finding of this study was the relatively selective suppression of REM produced by fear conditioning. This conclusion is supported by the finding of minimal effects on wakefulness and NREM. Total minutes in NREM and wakefulness were fairly consistent across conditions, suggesting that the significant increase in NREM percentage was due to actual decreases in REM and not to an increase in the amount of NREM. Thus, REM contributed less to TSA, thereby accentuating the percentage contributed by NREM.

Adrien *et al.* (1) found that shock presented during the training phase of the learned helplessness paradigm suppressed REM, and suggested that the suppression was due to stress associated with shock. However, factors other than stress (globally defined) may be involved. Immobilization stress has been reported to

significantly increase REM (6,38,43), up to 92% over the subsequent 10 hr period (43). This increase in REM has been suggested to result from the hormonal effects of intense stress (43). An increase in endogenous CRH during the prolonged (1-2 hr) stress and continuous stimulation of LC by CRH could deplete brain noradrenaline which would, in turn, be permissive to REM (38). In fact, the increase in REM is prevented by i.c.v. microinjection of the CRH antagonist, (-helical CRH (38). The hypothesis that prolonged stress depletes noradrenaline could account for the apparent discrepancy between the increase in REM produced by immobilization stress and our results. Shock given intermittently over a 15 min period presumably would produce activation of LC via CRH, but would not have the same depleting effect on noradrenaline as 1-2 hr of continuous stress and the concomitant continuous elevation of CRH. Interestingly, CRH-immunoreactive neurons in CNA are retrogradely labelled from CRH-immunoreactive areas of LC, suggesting a substrate by which CNA may influence the response of LC to stress (63).

Because footshock activates LC (10), which could, through its silence, play a permissive role in REM generation (60), it is reasonable to suggest that the REM suppression we observed after fear conditioning was due to activation of LC by shock, and did not involve the amygdala or the conditioning process. However, we have demonstrated that microinjections of CRH into CNA reduce total REM and REM percent (33). Thus, it is difficult, at this point, to suggest the most likely cause of REM suppression, or whether both amygdala and LC are involved. We are currently conducting studies to determine whether exposure to conditioned stimuli (cues and context) alone after training will produce a conditioned suppression of REM. This would more clearly link the amygdala to REM suppression.

The conditioning regimen we used involved training the rats to make an association between a specific stimulus (light) and shock. The training portion of the learned helplessness paradigm can produce associations between the context of the training and the presentation of shock. Both conditioning procedures involve differential activation of brain regions compared to that produced with immobilization stress. Explicitly cued and contextual conditioning to shock produces c-fos activation in CNA and the basolateral amygdala (BLA), among other brain regions (42). Immobilization stress produces low c-fos activation in CNA and BLA, though other regions are activated (11). Interestingly, pre-exposure to context and shock prevents increased c-fos activation in CNA and the hippocampus, among other areas (42). This would suggest that c-fos activation in those areas that normally participated in making the association between the context and shock was reduced by the pre-exposure to the stimuli.

These results may have relevance to the proposed role REM has in learning. Fear conditioning involves making an association between a specific neutral stimulus or situational context and an aversive stimulus. Explicitly cued fear conditioning produces long-term potentiation-like changes in the lateral amygdala (45) and contextual fear conditioning involves the hippocampus (16) suggesting information storage within these regions. In fact, learning is reported to be associated with increases in REM (3, 57, 58). Thus, one might have predicted increased REM

following fear conditioning. However, our data and that of Adrien et al (1) do not indicate that successfully forming an association to shock leads to increased REM, unless it occurs in the form of a post-suppression increase in REM. However, Adrien et al. (1) found no evidence of a REM rebound within 24 hr post-training.

CONCLUSIONS

Fear conditioning enhances the amplitude of PGO_E and suppresses REM in a relatively selective fashion. The biological relevance of enhanced PGO_E wave amplitude may relate to the role CNA plays in evaluating and responding to significant stimuli (2, 18, 23, 31, 32, 65) and to the evidence that the PGO wave reflects orienting and alerting (50, 51, 52). The increase in PGO_E amplitude in the presence of the CS suggests that fear-inducing (or threatening) situations can enhance activity in pontine circuitry involved in information gathering processes. Under normal circumstances, this would promote survival by ensuring that the organism attended to, and rapidly or efficiently processed potentially important stimuli. In contrast, inappropriate or prolonged activation of the circuitry could have adverse consequences. This could have relevance for disorders in which sleep is affected. For example, anxiety, in which the amygdala is implicated (12, 13), appears to play a role in insomnia (64). It also could be a factor in posttraumatic stress disorder (PTSD), which is characterized by hypervigilance and exaggerated startle during wakefulness, and by repetitive stereotypical anxiety dreams during sleep (4). The latter may be a function of increased phasic activity exemplified in animals by the PGO wave during REM (49). Inappropriate activation of this circuitry may also play a role in the cataplectic attacks characteristic of narcolepsy (35, 36); these are produced, paradoxically, by arousing stimuli (19). Recent evidence has linked neuronal degeneration in the amygdala to cataplexy in narcoleptic dogs (55).

The internal emotional and drive status of the organism, its environment and the interaction between internal and external factors are important influences on sleep and wakefulness. In general, these influences have received little attention in basic sleep research, though their impact is recognized in many human sleep disorders. It may be that the role of the amygdala in evaluating and storing the emotional significance of events is a key component of its involvement in sleep-wake regulation. The use of fear conditioning, as a validated behavioral model in wakefulness, may be useful in gaining insight into the processes by which the amygdala affects the regulation of brainstem alerting systems and of arousal state.

SUMMARY

The amygdala plays a central role in fear conditioning, a model of anticipatory anxiety. It has massive projections to brainstem regions involved in rapid eye movement sleep (REM) and ponto-geniculo-occipital (PGO) wave generation.

PGO waves occur spontaneously in REM or in response to stimuli. Electrical stimulation of the central nucleus of the amygdala enhances spontaneous PGO wave activity during REM and the amplitude of both the acoustic startle response and the elicited PGO wave (PGO_E), a neural marker of alerting. This study examined the effects of fear conditioning on REM and on PGO_E. On conditioning days, the number of REM episodes, the average REM duration and the REM percentage were decreased while REM latency was increased. The presentation of auditory stimuli in the presence of a light conditioned stimulus produced PGO_E of greater amplitudes. The results suggest that fear, most likely involving the amygdala, can influence REM and brainstem alerting mechanisms.

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