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Research Report

Effects of tetrodotoxin (TTX) inactivation of the central nucleus of the amygdala (CNA) on dark period sleep and activity

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ABSTRACT

The amygdala has been implicated in emotional arousal and in the regulation of sleep. Previously, we demonstrated that tetrodotoxin (TTX), a sodium channel blocker that temporarily inactivates neurons and tracts, microinjected into the central nucleus of the amygdala (CNA) during the light period significantly reduced REM, shortened sleep latency, and increased EEG delta power in rats. TTX inactivation of CNA also reduced activity in the open field. These findings suggest that the amygdala modulates arousal in a variety of situations. To test the hypothesis that the amygdala may influence spontaneous arousal, we examined the effects of TTX inactivation of CNA on sleep and activity during the dark period when rats show higher arousal and less sleep. EEG and activity were recorded via telemetry in Wistar rats (n = 8). Bilateral microinjections of TTX (*L*: 2.5 ng/0.1; *H*: 5.0 ng/0.2 μl) or SAL (saline, 0.2 μl) were administered before lights off followed by recording throughout the 12-h dark period and following 12-h light period. Microinjections were given at 5-day intervals and were counterbalanced across condition. TTX significantly shortened sleep latency, increased NREM time, decreased REM time, and decreased activity. TTX increased NREM episode duration, whereas the number and duration of REM episodes were decreased. The present results indicate that TTX inactivation of CNA can increase NREM time when spontaneous arousal is high, suggesting a broad role for the amygdala in regulating arousal. The results suggest that understanding the ways in which the amygdala modulates arousal may provide insight into the mechanisms underlying altered sleep in mood and anxiety disorders.

1. Introduction

The amygdala appears to play a critical role in mediating the effects of the environment on arousal state. In wakefulness, it has been linked to evaluating and coding the emotional significance of stimuli (Geschwind, 1965; Jones and Mishkin, 1972; Weiskrantz, 1956) and in generating the appropriate behavioral responses (Geschwind, 1965; Amaral et al., 1992). Electrical stimulation of the central nucleus of the amygdala (CNA) produces "alerting" behaviors along with EEG desynchronization (Stock et al., 1981; Kreindler and Steriade, 1964; Kapp et al., 1994), and cells in the amygdala, in particular in CNA, respond to novel and significant stimuli (Bordi et al., 1993). Electrical stimulation of CNA also increases the amplitude of elicited ponto-geniculo-occipital (PGO) waves, a neural marker of alerting (Sanford et al., 1992, 1993), in response to habituated auditory stimuli (Deboer et al., 1999). Thus, the

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amygdala may be vital for the interface between emotion, significant environmental events, and their impact on arousal and alerting. It is neuroanatomically positioned to modulate behaviors in wakefulness based on emotional experiences and significance and to mediate the influence of these factors on sleep–wake regulation.

Chemical inactivation of the CNA with the $GABA_A$ agonist, muscimol, decreased rapid eye movement sleep (REM) without significantly altering non-REM sleep (NREM) (Sanford et al., 2002). By comparison, inactivation of CNA with the sodium channel blocker, tetrodotoxin (TTX) (Martin and Ghez, 1999), produced similar or greater decreases in REM but also significantly shortened latency to NREM and increased NREM during the first hour of post-injection recording (Tang et al., 2005b). TTX-induced changes in arousal were accompanied by significant increases in EEG delta power during wakefulness and sleep (Tang et al., 2005b). Differences in the effects of muscimol and TTX could be due to differences in the effects they have locally in the amygdala. Muscimol inactivates only cell bodies in CNA, whereas TTX blocks both cell bodies and fibers of passage and has been used as a tool for producing temporary lesions (Martin and Ghez, 1999). Some fibers of passage coursing through CNA originate in the basal amygdala (Amaral et al., 1992; Davis and Whalen, 2001) which has been implicated in the control of NREM. For instance, bilateral electrolytic and chemical lesions of the basolateral amygdala (BLA) have been reported to increase NREM and total sleep time, whereas activating BLA with microinjections of L-glutamate decreased NREM and total sleep time (Zhu et al., 1998). Thus, the influence on NREM after TTX microinjection into CNA may have involved output of BLA.

TTX inactivation of CNA also reduced locomotion and rearing in an open field, an environment that normally produces arousal in rodents (Tang et al., 2005b). Reduced locomotion in the open field may indicate fear (Tang and Sanford, 2005; Tang et al., 2002, 2005a). However, lesions of the amygdala also significantly reduce emotional autonomic or somatic reactions in response to fearful stimulation (e.g., reviewed in Cardinal et al., 2002), suggesting that reduced locomotion may have reflected a reduction in fear-induced arousal.

Our previous study involved microinjections of TTX during the light period, where inactivation of CNA and amygdalar output may have facilitated an existing higher sleep drive. A number of studies have indicated that the effects of experimental manipulations on sleep may differ depending on whether the procedure was conducted in the light or dark portion of the cycle and on the natural arousal level of animals (e.g., Roky et al., 1993, 1994; Yi et al., 2004). This raised the question of whether TTX inactivation of CNA (cell bodies and fibers of passage) impacts sleep differently when administered at a time of high spontaneous arousal. We tested this possibility by microinjecting TTX prior to the onset of the dark period and examining the effects on sleep and activity.

2. Results

2.1. *Microinjection sites*

Fig. 1 shows the location of the injections sites in the amygdala. Though there were variations in the placements among animals, the histology indicated that TTX or SAL would have been infused into CNA and immediately adjacent areas in all rats that were used in the study. Therefore, all eight rats were used in the analysis of sleep and activity. The data from seven rats were used in the EEG power analysis (one animal was excluded from this analysis due to questionable calibration).

2.2. *Sleep*

The initial analyses of the dark period revealed that the effects on sleep of TTX microinjected into CNA were limited to the first 8 h of recording, and we concentrated our efforts on this time. Analyses of the following light period did not find any significant differences between the SAL or TTX conditions for total sleep ($P = 0.742$), NREM ($P = 0.724$), REM ($P = 0.921$), or activity ($P = 0.837$), and the light period recordings were not further analyzed.

Fig. 2 presents total sleep time, NREM and REM plotted in 2-h blocks across treatment conditions. The ANOVA revealed no significant differences in total sleep time (NREM + REM) within blocks (Fig. 2A). However, compared to SAL, both dosages of TTX increased NREM (Fig. 2B), but reduced REM

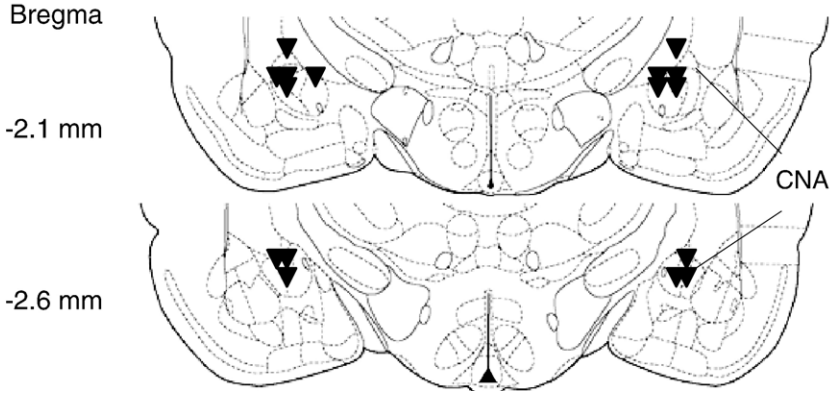

Fig. 1 – Line drawings illustrating microinjection sites in animals ($n = 8$). Injections sites are indicated by inverted triangles (▾). CNA: central nucleus of the amygdala.