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BRAIN RESEARCH

Research Report

State-dependent pattern of Fos protein expression in regionally-specific sites within the preoptic area of the cat

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ABSTRACT

Clinical and experimental data have shown that the preoptic area of the hypothalamus (POA) is involved in the generation and maintenance of NREM sleep. However, the activity of specific populations of POA neurons during REM sleep, NREM sleep and different waking conditions is still not firmly established. Consequently, we performed a quantitative, regionally-specific analysis of the Fos immunoreactivity of neurons in the POA of the cat during NREM sleep and REM sleep induced by microinjections of carbachol into the nucleus pontis oralis (REMc), as well as during quiet and alert wakefulness. We observed that while the total number of Fos immunoreactive neurons in the POA did not change as a function of these behavioral states, state-specific differences in neuronal activity were detected in restricted regions of the POA. An increase in the number of Fos+ neurons was observed in the rostral tip of the suprachiasmatic nucleus (SCN) during NREM (83.4±25.6) compared to quiet wakefulness (5.1±1.3, $p<0.05$) but not with the other behavioral states. In the median preoptic nucleus (MnPN), the number of Fos immunoreactive neurons was greater during NREM sleep (39.5±6.1) compared with quiet wakefulness (13.5±1.4, $p<0.05$) and REMc (16.2±2.0, $p<0.05$). State-specific Fos immunoreactive neurons were not observed in the ventro-lateral preoptic nucleus (VLPO). Finally, there was no significant increase in the number of Fos+ neurons during REMc in any of the subregions of the POA. In conclusion, within the POA, a selective neuronal activation during NREM sleep was found only in the MnPN. In addition, our data suggest a potential role of the SCN in NREM sleep. Finally, based on the distribution of Fos+ neurons in the entire POA, we conclude that the neuronal network involved in the regulation of NREM sleep is dispersed and intermingled with waking-related neurons.

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Abbreviations: AP, anterior–posterior; AW, alert wakefulness; EEG, electroencephalogram; EMG, electromyogram; EOG, electro-oculogram; H, height; L, lateral; MnPN, median preoptic nucleus; NPO, nucleus pontis oralis; NREM, non-REM sleep; PB, phosphate buffer; PBS, phosphate buffer saline; PGO, ponto-geniculo-occipital; POA, preoptic area of the hypothalamus; QW, quiet wakefulness; REM, rapid eye movements; REMc, REM sleep induced by microinjections of carbachol; SCN, suprachiasmatic nucleus; SCNr, rostral tip of the suprachiasmatic nucleus; VLPO, ventrolateral preoptic area

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1. Introduction

Since the early decades of the 20th century, the hypothalamus has been proposed to participate in the regulation of sleep (von Economo, 1930; McGinty and Szymusiak, 2005; Saper, 2006; Szymusiak et al., 2007; Szymusiak and McGinty, 2008). Electrolytic and neurotoxin lesions of the preoptic area of the hypothalamus (POA), as well as electrical and neurochemical stimulation in this site have confirmed Von Economo's concept that dual, mutually antagonistic sleep and waking centers exist in the hypothalamus, and that the POA is involved in the control of non-REM (NREM) sleep (von Economo, 1930; McGinty and Szymusiak, 2005; Saper, 2006; Szymusiak et al., 2007; Szymusiak and McGinty, 2008).

The POA is comprised of a number of regions that are structurally and functionally distinct. For example, a cluster of neurons in the ventrolateral preoptic area (VLPO) of the rat has been reported to be active, based upon the expression of the early gene *c-fos*, during the light period, when the animals spend most of the time asleep (Sherin et al., 1996). These data were reinforced by unit recordings that revealed an increase in the rate of discharge of cells in this area during NREM sleep compared to wakefulness (Szymusiak et al., 1998). A separate aggregate of neurons expressed Fos immunoreactivity in the median preoptic nucleus (MnPN) during NREM sleep. The neurons within this nucleus have also been reported to exhibit an increase in firing rate during NREM and REM sleep compared to wakefulness (Gong et al., 2000; Suntsova et al., 2002). A third cluster of cells, located dorsally and medially to the VLPO in the rat (named as the extended-VLPO by the authors), were reported to exhibit an increase in Fos expression during REM sleep, but not during NREM sleep (Lu et al., 2002). Although lesions in the extended-VLPO result in minor reductions in NREM sleep, they produce a significant decrease in the amount of REM sleep (Lu et al., 2000).

The Fos protein takes 1 to 2 h to accumulate in the nuclei of cells following their activation (Dragunow and Faull, 1989; Morgan and Curran, 1991; Yamuy et al., 1993; Torterolo et al., 2003). Thus, the use of this protein as an effective marker of neuronal activation, requires long lasting and consolidated periods of sleep and wakefulness which do not occur spontaneously in the rat, wherein there are short and fragmented periods of spontaneous wakefulness, NREM sleep and REM sleep (Cerri et al., 2005). We therefore believe that the cat, in which individual sleep and waking states can be maintained for over an hour, is an ideal species for studying these states using this technology (Dragunow and Faull, 1989; Morgan and Curran, 1991; Yamuy et al., 1993; Torterolo et al., 2003). Consequently, we analyzed the distribution of Fos immunoreactive neurons in sub-regions of the POA in the cat (Fig. 1), during quiet wakefulness (QW), alert wakefulness (AW), NREM sleep and REM sleep induced by microinjection of carbachol (REMc) into the nucleus pontis oralis (NPO) (Yamuy et al., 1993). The principal finding was that within all the sub-regions of the POA, only in the MnPN and the rostral tip of the suprachiasmatic nucleus (SCN) were there clusters of state-dependent neurons, and that these cells were preferentially active only during NREM sleep.

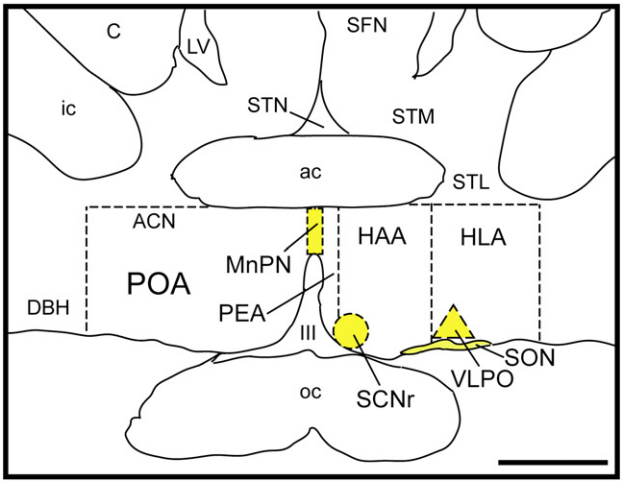

Fig. 1 – POA neuronal counting grids. Diagram of a coronal section of the POA at anterior 14.5 mm rostro-caudal level (Berman and Jones, 1982). The numbers of Fos+ neurons were determined in the SCNr, SON, MnPN, VLPO, PEA, HAA and HLA. Calibration bar, 2 mm. ac, anterior commissure; ACN, nucleus of the anterior commissure; C, caudate nucleus; DBH, nucleus of the diagonal band of Broca, horizontal division; HAA, anterior hypothalamic area; HLA, lateral hypothalamic area; ic, internal capsule; LV, lateral ventricle; MnPN, median preoptic nucleus; oc, optic chiasm; PEA, anterior periventricular nucleus; POA, preoptic area; SCNr, rostral tip of the suprachiasmatic nucleus; SFN, septofimbrial nucleus; SON, supraoptic nucleus; STL, nucleus of the stria terminalis, lateral division; STM, nucleus of the stria terminalis, medial division; STN, triangular septal nucleus; VLPO, ventrolateral preoptic area; III, third ventricle.

2. Results

2.1. *Analysis of sleep and waking states*

Hypnograms of representative animals in each behavioral state are presented in Fig. 2. The QW and AW groups of animals were maintained in their corresponding states for periods of 90–120 min prior to euthanasia (Fig. 2A). The NREM group of animals had consolidated states of NREM sleep following the mild sleep deprivation paradigm (Fig. 2B). These animals spent an average of 85.0±1.8% in NREM sleep during the hour prior to euthanasia. In the REMc group of animals, REM sleep which occurred with a latency of 6.6±2.3 min, had a duration of 81.3±19.5 min as shown in Fig. 2C (see Torterolo et al. 2001b, 2006 for a description of the polysomnographic characteristics of a typical REMc state, and the location of the microinjection site).

2.2. *Characteristic of immunostained neurons*

Fos immunoreactivity was present on the basis of black staining that was restricted to neuronal nuclei. Pyronin-y

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