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ORGANIZATION OF ELECTRICALLY AND CHEMICALLY EVOKED DEFENSIVE BEHAVIORS WITHIN THE DEEPER COLLICULAR LAYERS AS COMPARED TO THE PERIAQUEDUCTAL GRAY MATTER OF THE RAT

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Abstract—Stimulation of the periaqueductal gray matter (PAG) and the deeper layers of superior colliculus (SC) produces both freezing (tense immobility) and flight (trotting, galloping and jumping) behaviors along with exophthalmus (fully opened bulging eyes) and, less often, micturition and defecation. The topography of these behaviors within the distinct layers of SC remains unclear. Therefore, this study compared the defensive repertoire of intermediate (ILSC) and deep (DLSC) layers of SC to those of dorsolateral periaqueductal gray matter (DLPAG) and lateral periaqueductal gray matter (LPAG) [Neuroscience 125 (2004) 71]. Electrical stimulation was carried out through intensity- (0–70 μA) and frequency-varying (0–130 Hz) pulses. Chemical stimulation employed a slow microinfusion of *N*-methyl-D-aspartic acid (NMDA, 0–2.3 nmol, 0.5 nmol/min). Probability curves of intensity-, frequency- and NMDA-evoked behaviors, as well as the unbiased estimates of median stimuli, were obtained by threshold logistic analysis. Compared with the PAG, the most important differences were the lack of frequency-evoked jumping in both layers of SC and the lack of NMDA-evoked galloping in the ILSC. Moreover, although galloping and jumping were also elicited by NMDA stimulation of DLSC, effective doses were about three times higher than those of DLPAG, suggesting the spreading of the injectate to the latter structure. In contrast, exophthalmus, immobility and trotting were evoked throughout the tectum structures. However, whatever the response and kind of stimulus, the lowest thresholds were always found in the DLPAG and the highest ones in the ILSC. Besides, neither the appetitive, nor the offensive, muricide or male reproductive behaviors were produced by any kind of stimulus in the presence of appropriate targets. Accordingly, the present data suggest that the deeper layers of SC are most likely involved in the increased attentiveness (exophthalmus, immobility) or restlessness (trotting) behaviors that herald a full-blown flight reaction (galloping, jumping) mediated in the PAG. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: periaqueductal gray matter, superior colliculus, defense, flight, freezing, *N*-methyl-D-aspartic acid.

The major visual organ of lower vertebrates is the optic tectum, a midbrain structure that contributes to the generation of motivated behaviors through both recognition of key-stimuli and execution of appropriate motor commands (Ewert et al., 1985, 1999; Patton and Grobstein, 1998). The emergence of cerebral cortex in mammals did not suppress the visual functions of the tectum. Instead, as the superior colliculus (SC), the tectum retained a prominent role in most, if not all, visually guided behaviors. On the other hand, the SC has been also suggested to be involved in both the defensive behaviors of rodents (Blanchard et al., 1981; Sahibzada et al., 1986; Dean et al., 1988b; Keay et al., 1990; Sudré et al., 1993; Vargas et al., 2000) and in the recognition of fearful expressions in humans (de Gelder et al., 1999; Morris et al., 1999, 2001, 2002; Vuilleumier et al., 2003). However, whereas some authors suggested that the SC is directly involved in the production of rat defensive behaviors (Sahibzada et al., 1986; Dean et al., 1988b), others argued that it participates solely as a relay of visual threatening stimuli (Blanchard et al., 1981). Therefore, experiments were carried out to appraise the contribution of SC in the production of rat defensive behaviors, namely, its presumptive role as a defense command system. The repertoire and threshold curves of SC-evoked defensive responses were then compared with those of periaqueductal gray matter (PAG) described in an earlier study (Bittencourt et al., 2004).

The SC is divided in seven layers, namely, zonal layer (lamina I), superficial gray layer (lamina II), optic layer (lamina III), intermediate gray layer (lamina IV), intermediate white layer (lamina V), deep gray layer (lamina VI), and deep white layer (lamina VII) (Kanaseki and Sprague, 1974; Huerta and Harting, 1984). Usually, layers above and below the intermediate gray layer are referred to as the ‘superficial’ (I–III) and ‘deeper’ (IV–VII) layers of SC, respectively. However, because the SC becomes progressively less laminated as one moves from the most superficial to the deepest layer, Edwards (1980) pointed out many years ago that the distinct reticular properties of deeper collicular layers sharply distinguish them from the

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Abbreviations: CnF, cuneiform nucleus; DLPAG, dorsolateral periaqueductal gray matter; DLSC, deep layers of superior colliculus; ED_{50}, median effective dose; F_{50}, median effective frequency; I_{50}, median effective intensity; ILSC, intermediate layers of superior colliculus; LPAG, lateral periaqueductal gray; NMDA, *N*-methyl-D-aspartic acid; o.d., outer diameter; PAG, periaqueductal gray matter; R, responsiveness; R4, receptor response-related responsiveness; r.m.s., root mean square; SC, superior colliculus; μ_{50}, median effective stimuli; 95% C.I., 95% confidence interval.

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superficial ones whose structural and functional characteristics are more those of a sensory structure. More important to the present study, Holstege (1991) suggested that the deep gray layer of SC and the dorsal PAG should be considered as one single structure split up by the thick tract of fibers of the deep white layer bordering the PAG.

The functional organization of the SC follows a general plan in vertebrate species (Stein, 1981; Stein and Gaither, 1983). Superficial layers receive massive visual inputs from retina, primary visual cortex and parabigeminal nuclei. In turn, they project to the deeper collicular layers, thalamus and dorsolateral periaqueductal gray matter (DLPAG) (Linden and Perry, 1983; Mooney et al., 1988; Rhoades et al., 1989; Harvey and Worthington, 1990; Kasper et al., 1994; Lane et al., 1993; Serizawa et al., 1994; Lee et al., 1997; Stepniewska et al., 2000; Doubell et al., 2003). In contrast, visual inputs of intermediate layers arise predominantly from the secondary visual cortices 18 and 18a (Harvey and Worthington, 1990; Serizawa et al., 1994). The deeper layers are also the recipient of auditory and somatosensory inputs (Stein et al., 1975; Edwards et al., 1979; Cadusseau and Roger, 1985; Telford et al., 1996; Wallace et al., 1996; Tsumori et al., 1997; Sterbing et al., 2002; Grunwerg and Krauthamer, 1990; Wang and Redgrave, 1997; Veinante and Deschenes, 1999).

Retinotectal and corticotectal projections, as well as the auditory and somatosensory collicular maps, are long believed to lie in spatial register across the collicular layers (Stein et al., 1975; Dräger and Hubel, 1975; Stein and Clamann, 1981). Neurons of deeper layers are thus in a favorable position for processing the sensory synthesis that plays a prominent role in both the orienting reflexes and attentive behaviors that are universal components of defensive behaviors. Indeed, neurons of these layers were shown to perform 'multisensory integration,' i.e. a nonlinear enhancement (or inhibition) of responses from two sensory modalities (Stein, 1998; Stein et al., 1995; Wallace et al., 1996, 1998). Yet, multisensory neurons are, quite often, motor units. In fact, besides the ascending projections of deeper collicular layers (Coizet et al., 2003; Comoli et al., 2003), these layers are the source of tecto-reticulo-spinal, tecto-cuneiform and tecto-pontine motor systems (Huerta and Harting, 1984; Redgrave et al., 1986, 1987, 1988; Nudo et al., 1993; King et al., 1996). The tecto-reticulo-spinal pathway is a crossed projection classically related to the control of rapid eye movements (saccades) and head/body turns (orienting reflex) that shift the line-of-sight (gaze) onto a visual target (Sparks, 1978, 1999). In turn, the tecto-cuneiform pathway is a largely uncrossed projection to the cuneiform nucleus (CnF), a wedge-like region ventral to the inferior colliculus (Huerta and Harting, 1984; Mitchell et al, 1988; Redgrave et al., 1987, 1988) that is part of the so-called mesencephalic locomotor region (Shik and Orlovsky, 1976; Armstrong, 1988; Garcia-Rill and Skinner, 1988; Grillner and Wallén, 2002). However, whereas the somata of tecto-reticulo-spinal pathway are found in the lateral districts of deeper collicular layers, neurons of tecto-cuneiform pathway are concentrated in its medial sectors (Redgrave et al., 1986, 1987, 1988). Consequently, because lateral and medial sectors of SC are known to process visual and auditory information of lower and upper visual fields, respectively (Lane et al., 1971, 1973; King and Hutchings, 1987; Nudo et al., 1993; Sterbing et al., 2002), it has been proposed that the crossed pathway is primarily involved with events in the lower visual field, e.g. foraging behavior, whereas the tecto-cuneiform pathway is most likely dedicated to events in the upper visual field such as the sight of a distant predator (Redgrave and Dean, 1991; Redgrave et al., 1986, 1987, 1988). On the other hand, the tecto-pontine pathway is an ipsilateral projection to the ventrolateral pons that appears to be involved in circling behavior (DiChiara et al., 1982; Dean et al., 1988a; Buckenham and Yeomans, 1993). The deeper collicular layers also control pinnae movements (Henkel and Edwards, 1978; Henkel, 1981; Stein and Clamann, 1981) and relay information from the orofacial/vibrissae motor cortex to the motor neurons of facial nucleus (Miyashita et al., 1994; Miyashita and Mori, 1995; Tsumori et al., 1997). Pathways controlling circling behavior, pinnae movements and orofacial motor reflexes are likely to play an important role in the defensive behaviors. Thus, whereas the fast circling is most often a behavior of a cornered prey, pinnae movements are of paramount importance for sound localization in both predator and prey. Furthermore, SC-mediated projections to the facial motor nucleus may be a key component of defensive biting to the tactile stimulation of vibrissae.

Nonetheless, whereas the decisive role of PAG in the expression of defensive behaviors was long established by the pioneering study of Fernandez de Molina and Hunsperger (1962), conclusive evidences on the participation of SC in these behaviors had to await 20 years until the publication of Blanchard's influent studies on defensive behaviors of wild rats (Blanchard et al., 1981). Besides showing that wild rats are 'tamed' by lesions of PAG, these authors showed that lesions of SC produce marked deficits in the defensive reaction to an approaching experimenter, while having no effect in the vigor of the escape to the tactile stimulation of the dorsum or vibrissae. These studies strongly suggested that the SC was involved in the visual detection of threatening stimuli, but not in the expression of defensive behaviors properly. However, lesion studies do not tell us about the specific role of lesioned structures. For instance, behavior attenuation following a lesion of a given structure could be the result of a disruption of sensory (relay), motivational (drive), executive (motor), modulatory (cognitive) or some combination of these mechanisms. Moreover, a wealth of evidence from brain stimulation studies suggests that the SC may play an active role as a command system of defensive behaviors. Thus, electrical and chemical stimulation of the SC were shown to produce defensive behaviors in the rat (Sahibzada et al., 1986; Dean et al., 1988a,b; Keay et al., 1990; Schenberg et al., 1990; Sudré et al., 1993; Vargas et al., 2000). The question then arises whether the SC is a mere relay of sensory information conveyed to the PAG or if it has the inherent capability of producing the rat defensive behaviors (Redgrave and Dean, 1991).