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PROJECTIONS OF THE CENTRAL MEDIAL NUCLEUS OF THE THALAMUS IN THE RAT: NODE IN CORTICAL, STRIATAL AND LIMBIC FOREBRAIN CIRCUITRY

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Abbreviations: AAA, anterior amygdaloid area; ac, anterior commissure; AC, anterior cingulate cortex; ACC, nucleus accumbens; AGm, medial agranular (frontal) cortex; AGl, lateral agranular (frontal) cortex; AH, anterior nucleus of hypothalamus; AI,d,p,v, agranular insular cortex, dorsal, posterior and ventral divisions; AM, anteromedial nucleus of thalamus; AON, anterior olfactory nucleus; AUD, auditory cortex; AV, anteroventral nucleus of thalamus; BLA, basolateral nucleus of amygdala; BMA, basomedial nucleus of amygdala; BST, bed nucleus of stria terminalis; CEA, central nucleus of amygdala; CL, central lateral nucleus of thalamus; CLA, claustrum; CM,c,r, central medial nucleus of thalamus, caudal division, rostral division; COA,a,p, cortical nucleus of amygdala, anterior, posterior division; C-P, caudate-putamen, striatum; DLO, dorsolateral orbital cortex; DM, delayed matching to sample/position task; DMh, dorsomedial nucleus of hypothalamus; DNM, delayed non-matching to sample/position task; EC, entorhinal cortex; ECT, ectorhinal cortex; EP, endopiriform nucleus; GP, globus pallidus; GU, gustatory cortex; IAM, interanteromedial nucleus of thalamus; IL, infralimbic cortex; ILt, intralaminar nuclei of thalamus; IMD, intermediodorsal nucleus of thalamus; LA, lateral nucleus of amygdala; LD, lateral dorsal nucleus of thalamus; LH, lateral habenula; LHy, lateral hypothalamus; LO, lateral orbital cortex; LP, lateral posterior nucleus of thalamus; LPO, lateral preoptic area; LS, lateral septum; MA, magnocellular preoptic nucleus; MD, mediodorsal nucleus of thalamus; MO, medial orbital cortex; mPFC, medial prefrontal cortex; MPO, medial preoptic area; MS, medial septum; mt, mammillothalamic tract; OT, olfactory tubercle; PB, phosphate buffer; PC, paracentral nucleus of thalamus; PFC, prefrontal cortex; PH, posterior hypothalamus; PHA-L, *Phaseolus vulgaris*-leucoagglutinin; PIR, piriform cortex; PL, prelimbic cortex; PO, posterior nucleus of thalamus; PRC, perirhinal cortex; PT, paratenial nucleus of thalamus; PTL, posterior parietal cortex; PV, paraventricular nucleus of thalamus; RE, nucleus reuniens of thalamus; RF, reticular formation; RH, rhomboid nucleus of thalamus; RSC, retrosplenial cortex; RT, reticular nucleus of thalamus; SF, septofimbrial nucleus; SI, substantia innominata; SMT, submedial nucleus of thalamus; SSI, primary somatosensory cortex; SSII, secondary somatosensory cortex; TBS, Tris-buffered saline; TEA, temporal association area; TR, postpiriform transition area; VAL, ventral anterior-lateral nucleus of thalamus; VB, ventral basal nucleus of thalamus; VISC, visceral cortex; VLO, ventrolateral orbital cortex; VM, ventral medial nucleus of thalamus; VMh, ventromedial nucleus of hypothalamus; VO, ventral orbital cortex; ZI, zona incerta; 3V, third ventricle.

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Abstract—The central medial nucleus (CM) of thalamus is a prominent cell group of the rostral intralaminar complex of the thalamus. No previous report in the rat has comprehensively described the projections of CM. Using the anterograde anatomical tracer, *Phaseolus vulgaris* leucoagglutinin, we examined the efferent projections of CM, comparing projections from rostral (CMr) and caudal (CMc) regions of CM. We showed that the central medial nucleus distributes substantially to several cortical sites and to a limited number of subcortical structures. The primary CM targets were anterior and posterior regions of cortex, the claustrum, the caudate-putamen, the nucleus accumbens (ACC), the olfactory tubercle, and the amygdala. CMr and CMc distribute to several of the same structures but essentially to different parts of these structures. By comparison, CMr more strongly targets limbic structures, CMc more heavily sensorimotor structures. Main CMr projection sites were the medial agranular, anterior cingulate, prelimbic, dorsolateral orbital and dorsal agranular insular cortices, the dorsal striatum, the ACC, and the basolateral nucleus of the amygdala. Main CMc cortical projection sites were the ventrolateral, lateral and dorsolateral orbital cortices, dorsal, ventral and posterior agranular insular cortices, visceral cortex, primary somatosensory and motor cortices, and perirhinal cortex. Main CMc subcortical projection sites were the dorsal striatum and the lateral, central, anterior cortical, and basomedial nuclei of amygdala. The largely complementary output of CMr and CMc to diverse areas of cortex and to regions of the striatum and amygdala suggest a combined CM influence over a widespread area of the anterior cortex and throughout the dorsal and ventral striatum and the amygdala. CM projections to limbic and sensorimotor structures of the rostral forebrain suggest that CM may serve to integrate affective, cognitive and sensorimotor functions for goal-directed behavior. © 2012 Published by Elsevier Ltd. on behalf of IBRO.

Key words: medial prefrontal cortex, insular cortex, nucleus accumbens, striatum, basolateral nucleus of amygdala, working memory, limbic thalamus.

INTRODUCTION

The central medial nucleus (CM) is a prominent cell group of the rostral intralaminar complex of the thalamus which also includes the paracentral (PC) and central lateral nuclei. CM extends rostrocaudally over a considerable length of the thalamus (Swanson, 2004).

The midline and intralaminar (ILt) nuclei receive a diverse and widespread set of afferent projections (Krout

et al., 2002; Van der Werf et al., 2002; Vertes, 2002; McKenna and Vertes, 2004). Specifically, CM receives a relatively vast array of projections, primarily from the brainstem and caudal diencephalon. Significant among them are afferents from the brainstem reticular formation (RF). Several early reports in the rat demonstrated that the intralaminar nuclei, together with the zona incerta, were the major forebrain targets of RF projections (Jones and Yang, 1985; Vertes et al., 1986; Vertes and Martin, 1988). These connections constitute a principal component of the ascending RF activating system – or a reticular-ILt-cortical circuit responsible for behavioral and cortical EEG arousal of waking and REM sleep (Steriade and Glenn, 1982; Glenn and Steriade, 1982; Steriade et al., 1982; Kinomura et al., 1996). Damage to this system profoundly alters states of consciousness (Castaigne et al., 1981; Mair, 1994; Schiff, 2008), leading to the view that the midline/ILt thalamus (including CM) is critically involved in processes of arousal and attention (Paus, 2000; Van der Werf et al., 2002; Schiff, 2008).

Whereas CM receives widespread afferents, the output of CM is relatively restricted and mainly directed to rostral regions of the cortex, or to the orbitomedial prefrontal cortex (PFC) (Berendse and Groenewegen, 1991; Van der Werf et al., 2002). This suggests a role for CM in PFC functions such as decision-making and goal-directed behaviors (Dalley et al., 2004; Kolb et al., 2004; Sul et al., 2010).

Specifically, CM/ILt lesions have been shown to produce impairments in working memory as assessed by delayed matching (DM) and non-matching (DNM) to sample/position tasks in rats (Burk and Mair, 1998; Mair et al., 1998; Bailey and Mair, 2005; Newman and Burk, 2005; Mitchell and Dalrymple-Alford, 2005, 2006; Mair and Hembrook, 2008). For instance, Mair and Hembrook (2008) demonstrated that pharmacologically or electrically elicited activation of ILt improved performance on a DM to position task, while ILt suppression impaired performance on the task.

On the human level, Van der Werf et al. (1999) described severe impairments in declarative memory in a patient with damage to the right side of the intralaminar thalamus which were attributed to ''impaired use of mental flexibility''. They postulated that this dysfunction ''arose from the loss of cortical activation, caused by deafferentation of the prefrontal cortex through damage to the intralaminar nuclei'' (Van der Werf et al., 2003).

The foregoing indicates, then, a critical role for CM in arousal/attention and cognition and hence the need to understand the pattern of CM projections. While earlier reports in rats have described CM projections to some structures (Berendse and Groenewegen, 1990, 1991; Van der Werf et al., 2002), no previous study has comprehensively examined the output of CM. The present report, then, sought to fully describe the pattern of CM projections throughout the brain.

EXPERIMENTAL PROCEDURES

Single injections of *Phaseolus vulgaris*-leuccoagglutinin (PHA-L) were made into the rostral central medial (CMr) or caudal central medial (CMc) nucleus of the intralaminar thalamus in 42 male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 300–420 g. The experiments were approved by the Florida Atlantic University IACUC committee and conform to all Federal regulations and National Institutes of Health Guidelines for the care and use of laboratory animals.

PHA-L procedures

Powdered lectin from PHA-L was reconstituted to 4–5% in 0.05 M sodium phosphate buffer (PB), pH 7.4. The PHA-L solution was iontophoretically deposited in the brains of anesthetized rats by means of a glass micropipette with an outside tip diameter of 40–50 μm. Rats were anesthetized for surgery using an 80 mg/kg dose of Ketamine (100 mg/ml) and 10 mg/kg dose of Xylazine (20 mg/ml). Positive direct current (7–12 μA) was applied through a Grass stimulator (Model 88) coupled with a high-voltage stimulator (Frederick Haer Co., Bowdoin ME) at 2-s ''on''/2-s ''off'' intervals for 40–50 min. After a survival time of 7–10 days, rats were deeply anesthetized with Ketamine/Xylazine (150 mg/kg and 50 mg/kg, respectively), and perfused transcardially with a heparinized buffered saline wash (50–75 ml/animal) followed by a fixative (4% paraformaldehyde, 0.2–0.5% glutaraldehyde in 0.1 M PB, pH 7.4) (300–500 ml/animal). The brains were removed and postfixed overnight in 4% paraformaldehyde 0.1 M PB at 4 °C, pH 7.4. Following postfixing, the brains were transferred to 30% sucrose in 0.1 M PB solution for 2 days at 4 °C. Following sucrose cryoprotection, 50-μm frozen sections were collected in phosphate-buffered saline (PBS, 0.9% sodium chloride in 0.01 M sodium phosphate buffer, pH 7.4) using a freezing microtome. Six series of sections were taken. A complete series of sections was treated with 1% sodium borohydride in 0.1 M PB for 30 min to remove excess reactive aldehydes. Following this, sections were thoroughly rinsed in 0.1 M PB, and then incubated for 60 min at room temperature in 0.5% bovine serum albumin in Tris-buffered saline (TBS) to minimize nonspecific labeling. The sections were then incubated in the primary antiserum directed against PHA-L [biotinylated goat (IgG) anti-PHA-L, Vector Labs, Burlingame, CA] and diluent (0.1% bovine serum albumin in TBS containing 0.25% Triton X-100) at a concentration 1:500 overnight. Sections were then washed in 0.1 M PB (6 × 4 min) and placed in a 1:500 concentration of biotinylated rabbit anti-goat immunoglobulin (IgG) and diluent for 2 h. Sections were washed and then incubated in a 1:100 concentration of peroxidase–avidin complex from the Elite kit (Vector Labs) and diluent for 1 hour. Following another 0.1 M PB wash, the peroxidase reaction product was visualized by incubation in a solution containing 0.022% 3,3′ diaminobenzidine (DAB, Aldrich, Milwaukee, WI) and 0.003% H_2O_2 in TBS for 6 min. Sections were then rinsed again in 0.1 M PB (3 × 1 min) and mounted onto chrome–alum gelatin-coated slides. An adjacent series of sections from each rat was stained with cresyl violet for anatomical reference. Sections were examined using light and darkfield optics. Injection sites, cells and labeled fibers were plotted on representative schematic coronal sections through the brain using sections adapted from the rat atlas of Swanson (2004). Brightfield and darkfield photomicrographs of injection sites and labeled fibers were taken with a QImaging (Q ICAM) camera mounted on a Nikon Eclipse E600 microscope. Digital images were captured and reconstructed using Nikon Elements 3.0 imaging software (Melville, NY), and adjusted for brightness and contrast using Adobe PhotoShop 7.0 (Mountain View, CA). Sections that were reacted without either the primary or secondary antibodies did not show immunoreactivity (data not shown).

RESULTS

The patterns of distribution of labeled fibers throughout the brain following PHA-L injections in the rostral (CMr) and the caudal (CMc) central medial nucleus of the rostral ILt are described. Fig. 1 depicts the sites of injection in