Korean J Biol Sci 6: 159-165, 2002

Projections from the Prefrontal Cortex to the Dorsal Raphe Nucleus of the Rat

Hyun S. Lee* and Myung A Kim

Department of Premedical Science, School of Medicine, Konkuk University, Chungbuk 380-701, Korea

Key Words:
Prefrontal cortex
Dorsal raphe nucleus
Anterograde tracing
Retrograde tracing

Projections from the prefrontal cortex to subdivisions of the dorsal raphe nucleus were investigated in the rat using retrograde and anterograde tracing methods. A retrograde tracer, gold-conjugated horseradish peroxidase (WGA-apo-HRP-gold), was injected into each subdivision of the dorsal raphe including lateral wing, dorsomedial, and ventromedial areas. The majority of retrogradely labeled cells were located in the prelimbic, infralimbic, and dorsal peduncular areas of the medial prefrontal cortex. A few cells were also identified in the cingulate, various regions of the orbital, and agranular insular cortices. Secondly, an anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHA-L), was injected into the medial prefrontal cortex involving the prelimbic or infralimbic areas. Axonal fibers with varicosities were identified in all subdivisions of the DR including the lateral wing, dorsomedial, and ventromedial areas. Projections were bilateral, with ipsilateral predominance. Axonal fibers were observed at the lateral border of medial longitudinal fasciculus or in the interfascicular region at the midline. The present findings demonstrate that both the midline and lateral wing regions of the dorsal raphe nucleus receive excitatory input from cognitive and emotional centers of the cerebral cortex.

Selective prefrontal cortical projections to the midbrain raphe regions have been described in the rat (Aghajanian and Wang, 1977; Beckstead, 1979; Dalsass et al., 1981) and the monkey (Arnsten and Goldman-Rakic, 1984). In the rat, the origin of this projection was described to be localized predominantly in the ventral medial prefrontal cortex including infralimbic and dorsal peduncular cortices (Hajos et al., 1998). In the monkey, the dorsal prefrontal cortex including the dorsolateral and dorsomedial regions may be the only cortical area to have direct influence on the raphe nuclei (Arnsten and Goldman-Rakic, 1984).

Anatomically, the dorsal raphe nucleus (DR) is one of at least nine regions of the mammalian brainstem that contains large numbers of serotonin (5-hydroxy tryptamine, 5-HT)-containing cells (Lidov and Molliver, 1982). In the rat, the DR extends for 2.6 mm from the caudal midbrain through the pons and into the rostral portion of the medulla. In the coronal plane it lies just beneath the cerebral aqueduct and fourth ventricle and extends ventrally between the medial longitudinal fasciculi. While the nucleus is not exclusively composed of 5-HT neurons, it is frequently defined anatomically by four major clusters of 5-HT cells within its borders (Diaz-Cintra et al., 1981; Steinbusch, 1981; Agnati et al., 1982). Accordingly, there are dorsomedial (beneath the aqueduct), ventromedial (between the medial longitudinal fasciculi) and two bilateral groupings (lateral wings) of 5HT-positive cells running rostrocaudally through the nucleus.

There has been preliminary evidence that the midline portion of the DR involving the dorsomedial and ventromedial subdivisions projects to cortical and sub-cortical sites, whereas the lateral wings project exclusively to sub-cortical locations (O'Hearn and Molliver, 1984; Waterhouse et al., 1986; 1993). Thus, the goal of the present study was to investigate the possibility of differential cortical regulation of lateral wing versus midline regions of the DR. We reasoned that small deposits of the retrograde tracer, gold-conjugated horseradish peroxidase (WGA-apo-HRP-gold, WG), would produce highly localized, non-overlapping injection sites within the nucleus and, thus, be capable of revealing differences in the afferent innervation of dorsomedial, ventromedial, or lateral wing regions of the DR. Secondly, an anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHA-L), was injected into the medial prefrontal cortex and the distribution of fiber terminals within each subregion of the DR was investigated. Based on these observations, it may become clear whether both midline and lateral wing regions of the DR receive excitatory input from the cognitive and emotional centers of the cerebral cortex.

* To whom correspondence should be addressed.
Tel: 82-43-840-3723, Fax: 82-43-851-9329
E-mail: hyunsook.lee@kku.ac.kr

Materials and Methods

A total of forty-five Sprague-Dawley rats including both sexes and ranging in weight from 340 to 380 g, were used in this study. Prior to surgery, each rat was anesthetized with an intraperitoneal injection of chloral hydrate (3.6% in distilled water, 1 ml/100 g body weight). All animals used in this study were treated according to guidelines approved by the institutional animal care and use committee and conformed to the NIH guidelines on Care and Use of Animals in Research.

WGA-apo-HRP-gold (WG) injection

The WG was synthesized using inactivated WGA-HRP (Sigma) and 10 nm colloidal gold (Sigma), as described in Basbaum and Menetrey (1987). The skull around lambdoid suture was removed and a double-wire needle was inserted beneath the superior sagittal sinus to make sutures. Angiovasectomy was performed in order to expose the brain structures above the DR. At the rostrocaudal dimension, injections into subdivisions of the DR were targeted for sites between 0-1.0 mm caudal to the bony (false) lambda. The dorsomedial and ventromedial subdivisions were approached vertically from the midline at the depths of 5.3 and 5.7 mm, respectively. The lateral wing subdivision was approached from 0.3 mm lateral to the midline at the depth of 5.4 mm. The injection apparatus consisted of a glass micropipette (10-20 µm tip diameter) hydraulically linked to a 2.0 µl Hamilton syringe. A total volume of 0.15-0.20 µl of WG was injected into a single site within the DR over a thirty-min period.

Silver enhancement reaction

After 48-72 h, the animals were perfused using 150 mL of saline followed by a fixative containing 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS, pH 7.4). The brain was removed and stored in PBS containing 30% sucrose overnight. A series of 40 µm sections were made using cryostat and every 5th section was collected. The WG was detected using a commercial silver intensification kit (Sigma), as described in L-Smith et al. (1992).

5-Hydroxytryptamine (5-HT, serotonin) immunocytochemistry

The boundary of lateral wing injection site of the DR was determined using 5-HT immunocytochemistry. The conventional peroxidase-antiperoxidase (PAP) method of Sternberger (1986) was used. Briefly, sections were washed with 0.05 M tris-buffered saline (TBS, pH 7.4) and incubated in 0.5% hydrogen peroxide for 20 min to inhibit endogenous peroxidase activity. After rinses, sections were incubated in 10% normal goat serum (Vector, S-1000) for 1 h to block non-specific binding. Free-floating sections were then incubated for 24-48 h at 4℃ in 1:1200 dilution of rabbit antibodies raised against 5-HT (Diasorin). They were then incubated in 1:300 dilution of goat anti-rabbit immunoglobulin (Chemicon). After rinses, sections were incubated in 1:600 dilution of rabbit PAP (Chemicon). After rinses with Tris buffer (TB, pH 7.4), sections were reacted with the peroxidase substrate kit (Vector) for 3, 3'-diaminobenzidine (DAB) for 2-3 min at 4℃.

PHA-L injection and immunocytochemistry

In a second series of experiments, PHA-L was injected within prelimbic or infralimbic regions of the medial prefrontal cortex and orthogradely-labeled terminals at DR sub-regions were investigated. The location of the cortical injection site was determined from the atlas of Paxinos and Watson (1998). A glass micropipette containing 2.5% PHA-L (tip diameter, 10-15 µm) was stereotaxically lowered over the prefrontal cortex. The tracer was then ejected iontophoretically with 5 µA pulsed current on a 5-sec duty cycle for 20 min. Post-injection survival time ranged from 7 to 10 d. Tissue blocks containing the prefrontal cortex or the DR were sectioned transversely on a vibratome or cryostat. Every 5th section was processed for the visualization of PHA-L according to the protocol described by Peyron et al. (1998).

Results

Representative injections of WG at lateral wing (Fig. 1A), dorsomedial (Fig. 1B), ventromedial (Fig. 1C), and midline (Fig. 1D) subregions of the DR are depicted in Fig. 1. The confinement of the tracer within lateral wing subregion was insured by the examination following 5-HT immunostaining (Fig. 1A). Injections into the dorsomedial (Fig. 1B), ventromedial (Fig. 1C), and midline (Fig. 1D) involved both sides of the DR, whereas those into the lateral wing subdivision were unilateral (Fig. 1A). The amount (0.15-0.20 µl) of injected tracer produced an injection site whose medio-lateral dimension was in the range of 200-300 µm. Cases with involvement of the surrounding periaqueductal gray (PAG) were not included in the analysis.

Following WG injections into each DR subregion, the total number of retrogradely-labeled neurons at various regions of the prefrontal cortex were counted for representative injection cases (Table 1). For midline injection cases (rat #'s 20, 30, 31, 39 and 42), the sum total of labeled neurons located at left and right sides of the prefrontal cortex were counted. In contrast, cells were counted separately for lateral wing injection cases (rat #'s 27 and 43). Based on the latter cases, the projections from several prefrontal cortical regions to the DR were bilateral, with ipsilateral predominance. Based on the analysis of lateral wing, dorsomedial, ventromedial, and midline injection cases, the majority of retrogradely-labeled cells were identified at the prelimbic or infralimbic areas of the prefrontal

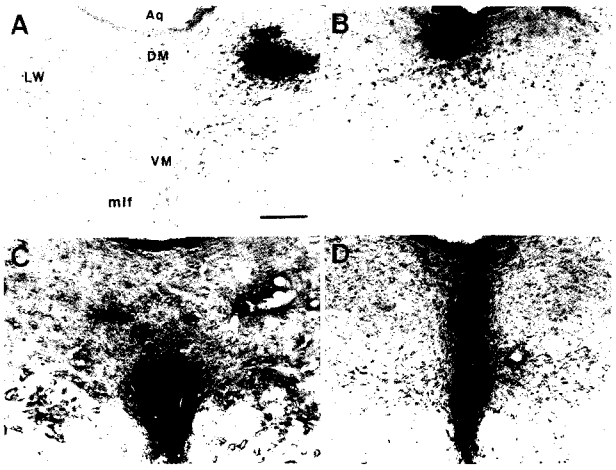

Fig. 1. Representative examples of injection sites in lateral wing (A; rat #27), dorsomedial (B; rat #30), ventromedial (C; rat #31), or midline (D; rat #39) subdivisions of the DR. Immunocytochemistry reveals clusters of 5-HT containing neurons that help define the lateral wing regions (A). Aq, cerebral aqueduct; DM, dorsomedial subdivision; LW, lateral wing; mlf, medial longitudinal fasciculus; VM, ventromedial subdivision. Scale bar = 200 μm.

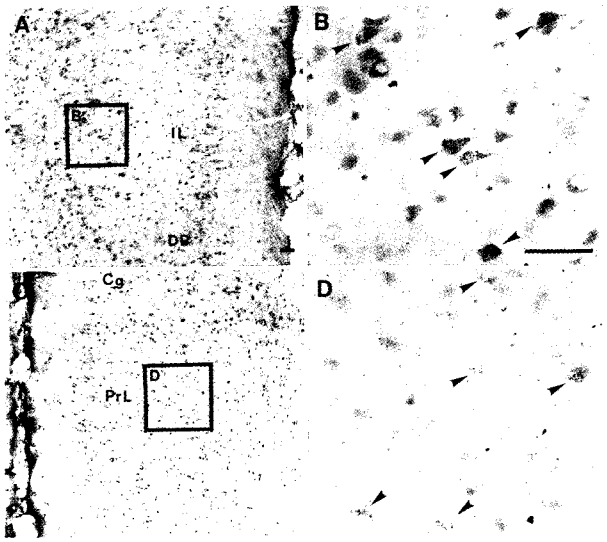

Fig. 2. Retrogradely labeled cells (arrowheads) within infralimbic (A and B) and prelimbic (C and D) regions of the medial prefrontal cortex. Cg, cingulate cortex; DP, dorsal peduncular cortex; IL, infralimbic cortex; PrL, prelimbic cortex. Scale bars = 50 μm.

cortex. The dorsal peduncular area in the medial prefrontal cortex also contained a number of retrogradely labeled cells. Labeled neurons in the cingulate, various regions of the orbital, and agranular insular cortices were minimal.

Examples of retrogradely labeled neurons at various regions of the prefrontal cortex are depicted after counterstaining with neutral red (Figs. 2-4). WG-labeled cells were often observed at various stages along the thickness of the section. Cells located close to the slide glass were well-counterstained with neutral red (Fig. 2B), whereas those located close to the cover glass were outlined only by black WG granules (Fig. 2D). As indicated in Table 1, the majority of retrogradely-labeled cells were located in the infralimbic (Fig. 2A and B) or the prelimbic (Fig. 2C and D) areas of the medial prefrontal cortex. These neurons were pyramidal cells at layers 5 and 6 and the size was in the range of 10-15 μm (Fig. 2B and D). Retrogradely-labeled cells were also observed at various areas of the orbital cortex including medial, lateral (Fig. 3B and C), and ventral (Fig. 3D) subregions. The morphology of labeled neurons in these orbital cortices was distinctively spherical, whereas the size was in the range of 10-15 μm (Fig. 3B-D). A few cells were also observed in the agranular insular cortex (Fig. 4). The density of WG granules within these neurons was relatively low compared with that within neurons located in the medial prefrontal (Fig. 2B and D) or orbital (Fig. 3B-D) cortices and the morphology of these neurons was mostly multipolar (Fig. 4B and C).

In a second series of experiment, PHA-L was iontophoretically injected into prelimbic (Fig. 5A) or infralimbic (Fig. 5B) areas of the medial prefrontal cortex. The injection produced a circumscribed injection site whose medio-lateral dimension was in the range of 200-300 μm (Fig. 5A and B). At the injection site, a large number of PHA-L filled somata were distinguished

Table 1. Distribution of retrogradely labeled cells at various regions of the prefrontal cortex in representative dorsal raphe (DR) injection cases

Cases	Rat #20	Rat # 27		Rat # 30	Rat # 31	Rat # 39	Rat # 42	Rat # 43	
DR injection site	Rostral 1/2 DM midline	Entire LW unilateral		Caudal 1/2 DM midline	Rostral 1/2 IF + VM midline	Caudal 1/2 DM + VM midline	Rostal 1/2 DM + VM midline	Mid 1/2 LW unilateral	
Cortex	total	ipsi	contra	total	total	total	total	ipsi	contra
Cingulate	1	9	0	0	0	6	1	1	1
Prelimbic	22	63	24	17	47	74	24	5	2
Infralimbic	10	30	24	12	28	63	15	12	3
Dorsal peduncular	8	11	8	9	5	38	13	8	2
Medial orbital	1	5	4	2	1	0	2	2	2
Ventral orbital	0	4	2	1	1	3	2	3	0
Lateral orbital	0	4	0	1	2	2	0	1	1
Agranular insular	0	4	0	0	0	1	0	0	0

DM, dorsomedial subdivision of the DR; IF, interfascicular region; LW, lateral wing subdivision; VM, ventromedial subdivision

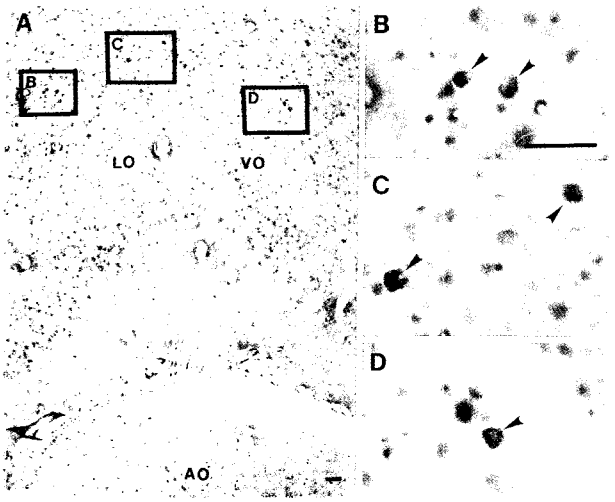

Fig. 3. WGA-apo-HRP-gold labeled neurons (arrowheads) within lateral orbital (B and C) and ventral orbital (D) cortices. AO, anterior olfactory nucleus; LO, lateral orbital cortex; VO, ventral orbital cortex. Scale bars = 50 μm.

at layers 5 and 6. Anterogradely-labeled, parallel axonal fibers arranged perpendicular to the midline were often observed at layers 1 to 4 (Fig. 5A). Following the cortical PHA-L injection, anterogradely labeled axonal fibers with dot-shaped varicosities (arrows in Fig. 5D and F) were evident at subdivisions of the DR including lateral wing, dorsomedial, and ventromedial subregions (Fig. 5C-F). In addition to these terminal fibers, a large number of axonal fibers without varicosities were often observed at the lateral border of medial longitudinal fasciculus or in the fascicular region at the midline.

The distribution of axonal fibers with varicosities within DR subregions was depicted at a rostro-caudal series of DR transverse sections (Fig. 6). Following

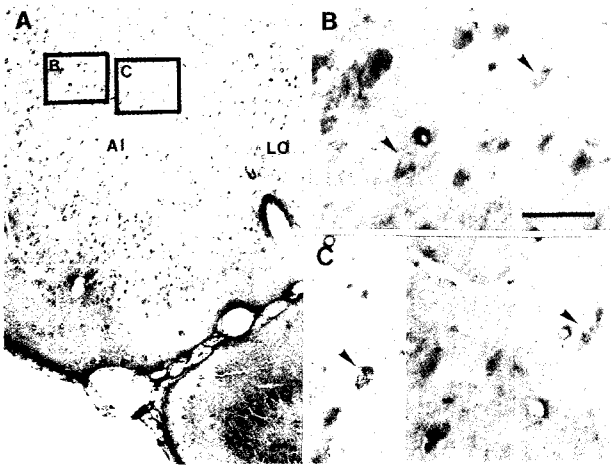

Fig. 4. Retrogradely labeled cells (arrowheads) within agranular insular cortex (A-C). AI, agranular insular cortex; AO, anterior olfactory nucleus; LO, lateral orbital cortex. Scale bars = 50 μm.

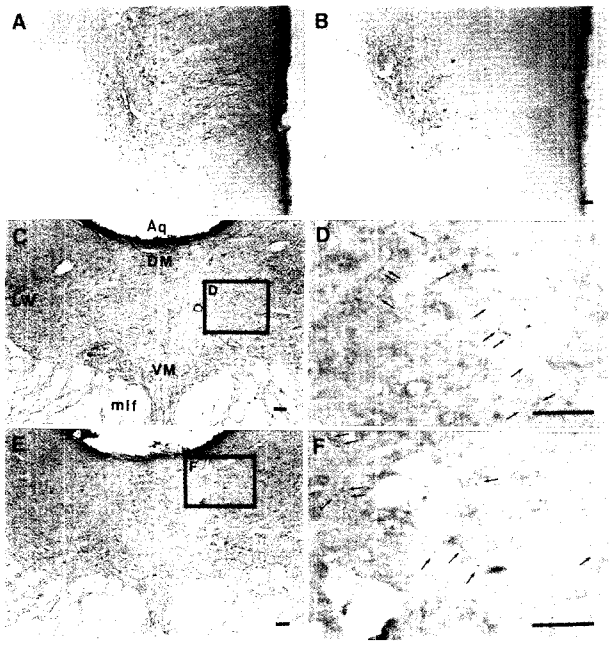

Fig. 5. PHA-L injection sites within prelimbic (A) or infralimbic (B) cortices and terminal fiber labeling in various areas of the dorsal raphe nucleus (C-F). Micrographs in the right column (D and F) are higher magnification views of those in the left column (C and E). Arrows represent PHA-L labeled varicosities along the axonal fibers. Aq, cerebral aqueduct; DM, dorsomedial subdivision of the dorsal raphe nucleus; LW, lateral wing subdivision; mlf, medial longitudinal fasciculus; VM, ventromedial subdivision. Scale bars = 50 μm.

unilateral injection of PHA-L into the prelimbic cortex (Fig. 5A), the distribution of anterogradely labeled axonal fibers was bilaterally located within each subregion of the DR, with ipsilateral predominance (Fig. 6). They were relatively homogeneously distributed along all the rostro-caudal series of sections (Fig. 6, sections 1-6). A few elongated PHA-L labeled axonal fibers could be traced in transverse sections for more than 300 μm. Most of axonal fibers, however, were in the range of 50-100 μm (Fig. 5D and F). It should be noted that axonal fibers with varicosities were also observed outside of the DR, i.e., ventrolateral PAG regions.

Discussion

It has been reported that 5-HT containing DR neurons as well as norepinephrine (NE)-containing locus coeruleus (LC) neurons provide the most extensive, efferent projections to multiple cortical targets and, thus, exert modulatory influences on arousal, autonomic, emotional, and sensorimotor activities of the animal (Jones and Moore, 1977; Steinbusch, 1981). Despite the broad influence of DR or LC neurons upon widespread areas of the cerebral cortex, descending cortical projections to these brainstem regions originate only from the prefrontal cortex (Arnsten and Goldman-Rakic, 1984).

An electrophysiological study demonstrated that in the rat stimulation of the ventral medial prefrontal

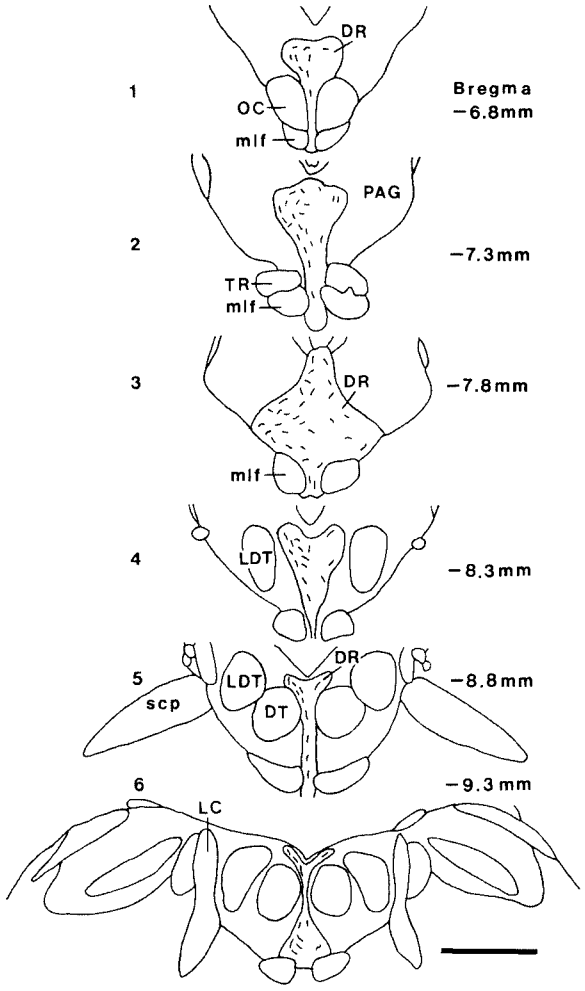

Fig. 6. The distribution of terminal axonal fibers within the dorsal raphe nucleus (DR) at a rostro-caudal series of transverse sections (1-6). DT, dorsal tegmental nucleus; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; mlf, medial longitudinal fasciculus; OC, oculomotor nucleus; PAG, periaqueductal gray; scp, superior cerebellar peduncle; TR, trochlear nucleus. Scale bar = 1.0 mm.

cortex causes marked post-stimulus inhibition in 5-HT containing DR neurons (Hajos et al., 1998). It is well-known that changes in 5-HT function might be associated with affective disorders and the DR neurons are targets for antidepressant treatments (Blier and de Montigny, 1994; Heninger et al., 1996). Recent observations indicated that dysfunction within the medial prefrontal cortex and its connected areas is linked with the pathophysiology of schizophrenia (Damasio et al., 1990). Neuropsychological and neuroimaging studies provide converging evidence that medial prefrontal cortex might be related with the clinical symptoms of affective disorders (Rolls et al., 1994).

Previous anatomical studies indicated that the DR is topographically organized with respect to its efferent targets, i.e., the midline region of the DR involving dorsomedial and ventromedial subdivisions projects to cortical as well as sub-cortical sites, whereas the lateral wings project only to sub-cortical sites (Steinbusch, 1981; O'Hearn and Molliver, 1984; Villar et al., 1988; Waterhouse et al., 1993; Janusonis et al., 1999). Thus, the present study was designed to determine whether there exists any differential distribution of neurons within the prefrontal cortex which project either to midline or to lateral wing subdivisions of the DR.

In the first series of experiment, small deposits of the retrograde tracer, WG, were made into each subdivision of the DR and the distribution of retrogradely-labeled cells was examined at various regions of the prefrontal cortex. Midline injections including the dorsomedial and ventromedial subregions were performed in a bilateral manner, whereas the lateral wing was injected unilaterally (Fig. 1). Based on the number of labeled cells in the prefrontal cortex, ipsilateral projection was more prominent than contralateral one for the lateral wing injection case (Table 1; rat #'s 27 and 43). Several areas of the medial prefrontal cortex involving prelimbic, infralimbic, and dorsal peduncular regions provided major inputs to each DR subregion, whereas projections from cingulate, various regions of the orbital, and agranular insular cortices to DR subregions were minimal (Table 1). The labeling pattern in the prefrontal cortex is consistent with the previous study indicating that the DR receives extensive inputs from various regions of the medial prefrontal cortex (Peyron et al., 1998). They, however, reported that the DR receives extensive inputs also from the cingulate, orbital, and insular cortices. Such discrepancies between the results of the two studies might be explained by the fact that WG injections in our material were highly restricted in comparison to cholera toxin B (CTB) injection sites in their study (cf. Fig. 1 in Peyron et al., 1998). Since CTB is a very sensitive tracer, any spillage of the tracer into the surrounding PAG could produce artifactual labeling, since the DR and the PAG often have many afferent inputs in common (Mehler, 1980; Norita and Kawamura, 1980).

Medial and orbital prefrontal cortices have been suggested to be two functionally different systems and lesions to these areas have produced distinct behavioral deficits consistent with the dissimilarity in their afferent and efferent projections (Kupfermann, 1991). The medial prefrontal cortex is mainly concerned with the strategic planning of higher motor actions, including cognitive tasks. This area of the prefrontal cortex has been implicated in the monkey as one of afferent sources which can convey the highly processed information concerning the relevance of complex sensory events and situations (Arnsten and Goldman-Rakic, 1984).

On the other hand, the orbitofrontal cortex has been known to be mainly involved in emotional behavior of the animal. The projection from medial and lateral orbital cortices to the DR might play a role in obsessive compulsive disorders of depression (MacLean,

1989). The orbitofrontal cortex is a part of limbic association cortex, which has direct connection with limbic structures, such as amygdala. Based on anterograde and retrograde labeling studies, it has been demonstrated that descending projections from the amygdala to the DR are minimal and they originate mainly from the central and medial amygdaloid nuclei (Lee and Waterhouse, 2000). It is consistent with the electrophysiological study indicating that only very few cortical stimuli induce modification of the regular and pacemaker-like activity of serotonergic neurons during wakefulness (Hurley et al., 1991).

The present study also indicated minor inputs from the cingulate or agranular insular cortices to the DR (Table 1). Various visceral, somatic, and behavioral responses can be obtained by electrical stimulation of the anterior cingulate cortex and the orbital-insular-temporal cortex. Recent studies reported that the cingulate gyrus along with the orbitofrontal cortex is concerned with emotional behavior of the animal, whereas the insular cortex is known to receive nociceptive and viscerosensory input (MacLean, 1989). Based on the present observations, it can be concluded that there is an extensive projection from the medial prefrontal cortex to the DR, which is concerned with strategic planning of future actions. On the other hand, the projection from the cingulate, various regions of the orbital, and insular cortices to the DR is minimal, which is involved in emotional behavior of the animal.

In the second series of experiments, an anterograde tracer, PHA-L, was injected into prelimbic or infralimbic regions of the medial prefrontal cortex and terminal axonal fibers were examined within each subdivision of the DR. Axonal fibers with varicosities existed along almost all rostro-caudal series of the DR sections (Fig. 6). The distribution of terminal axonal fibers was bilaterally located within each subdivision of the DR, with ipsilateral predominance. It requires further investigation such as a double-labeling study using two different retrograde tracers to determine whether a single neuron in the prefrontal cortex send collateral fibers to different subdivisions of the DR or different neurons in the same cortical region send efferent fibers into each different sub-region of the DR. The complete path of these descending fibers from the prefrontal cortex to the DR has not been determined. They, however, seemed to reach the DR via axonal fiber bundles located at the lateral border of the medial longitudinal fasciculus or at the interfascicular region in the midline.

Based on the present investigation, we conclude that the midline structures of the DR receive inputs mainly from the medial prefrontal cortex involving the prelimbic, infralimbic, and dorsal peduncular cortices, whereas less amounts of inputs from the cingulate, various regions of the orbital, and insular cortices. In addition, the lateral wings receive a major cortical information from the medial prefrontal cortex as well as a minor input from the cingulate, orbital, and insular cortices, despite the fact that they project only to sub-cortical targets. Thus, it can be postulated that both the midline and lateral wing regions of the DR receive major excitatory inputs from cognitive centers of the brain in addition to a minor input from emotional centers of the brain.

Acknowledgements

This work was supported by grant No. R04-2001-000-00010-0 from the Korea Science & Engineering Foundation.

References

Aghajanian GK and Wang RY (1977) Habenular and other midbrain raphe afferents demonstrated by a modified retrograde tracing technique. *Brain Res* 122: 229-242.

Agnati LF, Fuxe K, Hokfelt T, Benfenati F, Calza L, Johansson O, and DeMey J (1982) Morphometric characterization of transmitter-identified nerve cell groups: analysis of mesencephalic 5-HT nerve cell bodies. *Brain Res Bull* 9: 45-51.

Arnsten AFT and Goldman-Rakic PS (1984) Selective prefrontal cortical projections to the region of the locus coeruleus and raphe nuclei in the rhesus monkey. *Brain Res* 306: 9-18.

Basbaum AI and Menetrey D (1987) WGA-apo-HRP-gold: A new retrograde tracer for light and electron-microscopic single- and double-label studies. *J Comp Neurol* 261: 306-318.

Beckstead RM (1979) An autoradiographic examination of corticocortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. *J Comp Neurol* 184: 43-62.

Blier P and de Montigny C (1994) Current advances and trends in the treatment of depression. *Trends Pharmacol* 15: 220-226.

Dalsass M, Kiser S, Mendershausen M, and German DC (1981) Medial prefrontal cortical projections to the region of the dorsal periventricular catecholamine system. *Neuroscience* 6: 657-665.

Damasio AR, Tranel D, and Damasio H (1990) Individuals with sociopathic behavior caused by frontal damage fail to respond autonomically to social stimuli. *Behav Brain Res* 41: 81-94.

Diaz-Cintra S, Cintra L, Kemper T, Resnick O, and Morgane PJ (1981) Nucleus raphe dorsalis: morphometric Golgi study in rats of three age groups. *Brain Res* 207: 1-16.

Hajos M, Richards CD, Szekely AD, and Sharp T (1998) An electrophysiological and neuroanatomical study of the medial prefrontal cortical projection to the midbrain raphe nuclei in the rat. *Neuroscience* 87: 95-108.

Heninger GR, Delgado PL, and Charney DS (1996) The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry* 29: 2-11.

Hurley KM, Herbert H, Moga MM, and Saper CB (1991) Efferent projections of the infralimbic cortex of the rat. *J Comp Neurol* 308: 249-276.

Janusonis S, Fite KV, and Foote W (1999) Topographic organization of serotonergic dorsal rahe neurons projecting to the superior colliculus in the Mongolian gerbil (*Meriones unguiculatus*). *J Comp Neurol* 413:342-355.

Jones BE and Moore RY (1977) Ascending projections of the locus coeruleus in the rat. II. Autoradiographic study. *Brain Res* 127: 23-53.

Kupfermann I (1991) Localization of higher cognitive and affective functions: the association cortices. In: Kandel ER, Schwartz JH, and Jessell TM (eds), Principles of Neural Science, Elsevier, New York, pp 823-838.

Lee HS and Waterhouse BD (2000) Differential distribution of glutamate-immunoreactive neurons that project to subdivi-

sions of the rat dorsal raphe nucleus. In: The 30th Annual Meeting of the Society for Neuroscience, New Orleans, USA, Abstract 657.4.
Lidov HG and Molliver ME (1982) Immunohistochemical study of the development of serotonergic neurons in the rat CNS. *Brain Res Bull* 9: 559-604.
L-Smith IJ, Pilowsky P, and Mison JB (1992) Retrograde tracers for light and electron microscopy. In: Bolam JP (ed), Experimental Neuroanatomy, Oxford University Press, Oxford, pp 31-59.
MacLean PD (1989) The Triune Brain in Evolution. Plenum Press, New York.
Mehler WR (1980) Subcortical afferent connections of the amygdala in the monkey. *J Comp Neurol* 190: 733-762.
Norita M and Kawamura K (1980) Subcortical afferents to the monkey amygdala: an HRP study. *Brain Res* 190: 225-230.
O'Hearn E and Molliver ME (1984) Organization of raphe-cortical projections in rat: a quantitative retrograde study. *Brain Res Bull* 13: 709-726.
Paxinos G and Watson C (1998) The rat brain in stereotaxic coordinates. Academic Press, New York.
Peyron C, Petit JM, Rampon C, Jouvet M, and Luppi PH (1998) Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. *Neuroscience* 82: 443-468.
Rolls ET, Hornak J, Wade D, and McGrath J (1994) Emotion-related learning in patients with social and emotional changes associated with frontal lobe damage. *J Neurol Neurosurg Psychiat* 57: 1518-1524.
Steinbusch HWM (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat cell bodies and terminals. *Neuroscience* 6: 557-618.
Sternberger LA (1986) Immunocytochemistry. John Wiley & Sons, New York.
Villar MJ, Vitale ML, Hokfelt T, and Verhofstad AA (1988) Dorsal raphe serotonergic branching neurons projecting both to the lateral geniculate body and superior colliculus: a combined retrograde tracing immunohistochemical study in the rat. *J Comp Neurol* 277: 126-140.
Waterhouse BD, Mihailoff GA, Baack JC, and Woodward DJ (1986) Topographical distribution of dorsal and median raphe neurons projecting to motor, sensorimotor and visual cortical areas in the rat. *J Comp Neurol* 249: 460-481.
Waterhouse BD, Border B, Wahl L, and Mihailoff GA (1993) Topographic organization of rat locus coeruleus and dorsal raphe nuclei: distribution of cells projecting to visual system structures. *J Comp Neurol* 336: 345-361.

[Received April 19, 2002; accepted May 24, 2002]