Title

In vivo Magnetic Resonance Imaging at 11.7 Tesla Visualized the Effects of Neonatal

Transection of Infraorbital Nerve upon Primary and Secondary Trigeminal Pathways in Rats

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Abbreviations: 5Gn, trigeminal ganglion; 7n, facial nerve; Cbc, cerebellar cortex; cbm, cerebellar medulla; CSF, cerebrospinal fluid; eml, external medullary lamina; Gd-DTPA, Gadolinium diethylenetriamine penta-acetic acid; ic, internal capsule; ml, medial lemniscus; ION, infraorbital nerve; P0, postnatal day 0; P10, postnatal day 10; Pr5, trigeminal principal sensory nucleus; py, pyramidal tract; Rt, thalamic reticular nucleus; sp5t, spinal trigeminal tract; SPFPC, subparafascicular thalamic nucleus, parvicellular part; str, superior thalamic radiation; T1, longitudinal relaxation time; T2, transverse relaxation time; TE, echo time; TR, repetition time; V1, ophthalmic nerve; V2, maxillary nerve; V3, mandibular nerve; VP, ventral posterior nucleus of thalamus; VPL, ventral posterolateral nucleus of thalamus; VPM, ventral posteromedial nucleus of thalamus.

Abstract

Using 11.7 T ultra high-field T2-weighted MRI, the present study aimed to investigate pathological changes of primary and secondary trigeminal pathways following neonatal transection of infraorbital nerve in rats. The trigeminal pathways consist of spinal trigeminal tract, trigeminal sensory nuclear complex, medial lemniscus, ventromedial portion of external medullary lamina and ventral posterior nucleus of thalamus. By selecting optimum parameters of MRI such as repetition time, echo time, and slice orientation, this study visualized the trigeminal pathways in rats without any contrast agents. Pathological changes due to the nerve transection were found at 8 weeks of age as a marked reduction of the areas of the trigeminal pathways connecting from the injured nerve. In addition, T2-weighted MR images of the trigeminal nerve trunk and the spinal trigeminal tract suggest a communication of CSF through the trigeminal nerve between the inside and outside of the brain stem. These results support the utility of ultra high-field MRI system for noninvasive assessment of effects of trigeminal nerve injury upon the trigeminal pathways.

Keywords: ultra high-field T2-weighted MR imaging, neonatal nerve transection, trigeminal pathways, critical period

1. Introduction

In recent years, a remarkable progress of MRI techniques has made possible to visualize fine morphology of nervous system at the sub-millimeter scale (Jahnke et al., 2007; Wu et al., 2010). Those studies revealed that the trigeminal nerve consists of three components in rats (Does and Gore, 2002), nerve tracts of the sciatic nerve were visualized with a contrast agent in rats (Chen et al., 2011) and the volume of dorsal root ganglia decreased after peripheral nerve injury (West et al., 2007). MRI has also several limitations even at present. MR images are constructed from different tissue variables like proton density and relaxation times (longitudinal relaxation time (T1) and transverse relaxation time (T2)) for protons. Since the differences in T1 and T2 between the white and gray matters are small, MR image contrast is poor and therefore the contrast agents have been often necessary in the MRI studies of brain (Inui-Yamamoto et al., 2010; Eschenko et al., 2012). As a result, the nervous tissue can be classified into only three groups, white matter, gray matter and cerebrospinal fluid (CSF) using ordinary MR images. It means that MRI is difficult to definitely discriminate such important structures as cortical layers and thalamus nuclei. In spite of those difficulties, it is expected that an optimum selection of MRI parameters such as repetition time (TR), echo time (TE) and slice orientation enhances the image contrast and discriminates neighboring tissues of nervous system without contrast agents. The purpose of the present study is to show

the possibility written above and further to examine pathological changes of the primary and secondary trigeminal pathways following neonatal transection of infraorbital nerve (ION), one of the branches of maxillary division of the trigeminal nerve, using an 11.7 T ultra high-field MRI system.

To visualize the pathways connecting from the trigeminal nerve to the central nervous system, it is necessary to discriminate between external medullary lamina (eml) and internal capsule (ic) both of which are white matter. There is a thin (<0.4 mm) layer of gray matter called thalamic reticular nucleus (Rt) between them (Paxinos and Watson, 2004). Since Rt receives input from thalamocortical and corticothalamic projections (Carman et al., 1964; Jones, 1975; Pinault et al., 1995a; Pinault et al., 1995b; Parent and Carpenter, 1996; Alexander and Godwin, 2006; Zikopoulos and Barbas, 2006), then Rt has nerve fibers as many as the white matter like eml and ic has, which reduces the MR signal contrast among eml, Rt and ic. Considering this fact as well as thinness of Rt, it is supposed difficult for MRI to discriminate eml from ic. However the spatial resolution of MRI increases with the static magnetic field strength (Magee et al., 2003), therefore 11.7 T is expected sufficient for discriminating among eml, ic and Rt. Another difficulty in visualizing the trigeminal pathway is to distinguish ventral posteromedial thalamic nucleus (VPM) and trigeminal principal sensory nucleus (Pr5) from the surrounding tissue, because they are all gray matters.

Actually, there have been no studies to visualize them using MRI without any contrast agents. However, sensory neurons in VPM and Pr5 are in barrel-like arrangements (Ma, 1991; Land et al., 1995) with a hollow center of lesser cell density surrounded by a circle of higher cell density. Since the hollows are filled with fibers, there should be a certain difference in T2 between VPM/Pr5 and the surrounding tissue. Further in the present study, T2-weighted MRI was employed to observe pathological changes of cerebral tissues following ION transection, since it is suitable to visualize pathological changes of living tissues (Donald et al., 2003; Payne et al., 2011).

A number of anatomical studies have provided the evidences that the effects of peripheral nerve injury on the somatosensory system essentially depend on the time of suffering from injury. In the sensory system during development, several studies have suggested that an intact afferent input is crucial for survival of central sensory neurons (Levi-Montalcini, 1949; Cook et al., 1951; Matthews and Powell, 1962; Powell and Erulkar, 1962; Kupfer and Palmer, 1964; Waite, 1984; Born and Rubel, 1985; Finlay et al., 1986). In the neonatal period, the peripheral nerve injury induces apoptosis in the contralateral thalamus (Baldi et al., 2000). In the adult on the other hand, the peripheral nerve injury induces relatively minor morphological changes in the dorsal root ganglion (Sugimoto and Gobel, 1982). Thus in the nervous system, a critical period with high plasticity is considered to exist

early in life. Several studies estimate the critical period of central nervous system within 7 days of age in rats (Belford and Killackey, 1980; Baldi et al., 2000; Harris et al., 2008). The present study investigated the effects of ION transection in rats at postnatal day 0 (P0).

2. Results

2.1. The influence of nerve transection on trigeminal ganglion

Figure 1 (a) shows the coronal section of rat brain obtained by T2-weighted MRI, the longitudinal position of which is immediately after the mandibular (V3) nerve leaving the trigeminal ganglion (5Gn). The regions encircled by dashed lines in Fig. 1 (a) indicate the left and right bundles of the ophthalmic (V1) and maxillary (V2) nerves. The difference in their sizes demonstrates developmental failure of the 5Gn ipsilateral to the neonatal left ION transection at P0. Figure 1 shows consecutive coronal slices from posterior (a) to anterior (d), where the slice positions of (b), (c) and (d) are 1, 1.5 and 2 mm anterior to (a). They show that the bundles of V1 and V2 are running along the cranial base. Several high intensity spots are observed (especially in (c)) in the right trigeminal nerve. They can be interpreted as the structures containing free water in the trigeminal nerve trunk. High intensity spots were also seen in the normal control rats (data not shown). Figures 1 (a')-(d') show the similar results to Figs. 1 (a)-(d) except that the nerve transection was done at postnatal day 10 (P10). In these figures, there were not any differences in size between left and right bundles. High intensity spots were also observed in the left and right trigeminal nerves.

2.2. The influence of nerve transection on external medullary lamina

Figure 2 shows the coronal section of rat brain whose longitudinal position is at the level of confluence of eml and medial lemniscus (ml) (approximately 3.24 mm posterior to Bregma). Arrows in Fig. 2 indicate the ventral posterior nucleus (VP) of thalamus, the eml merging into ml, Rt, and ic. The area of ventromedial portion of eml contralateral to ION transection is smaller than the area of ipsilateral counterpart as seen from Fig. 2(a). Figure 2(b) is an enlarged sectional view of Fig. 2(a), where the regions encircled by dashed lines indicate the areas covering eml and ml. An anatomical map (c) (Paxinos and Watson, 2004) is shown for comparison. Figure 2 (a) shows the MR image taken at TR (ms)/TE (ms) = 5000/22.5, because the structures could be observed more clearly as compared with the MR image with TR (ms)/TE (ms) = 5000/40.

2.3. The influence of nerve transection on medial lemniscus

MR images (TR (ms)/TE (ms) = 5000/22.5) at the level of separation of eml and ml (approximately 4.44 mm posterior to Bregma) are shown in Fig. 3. Figure 3 (a) also indicates a partial loss in the medial portion of ml contralateral to ION transection. Figure 3(b) is an enlarged sectional view of Fig. 3(a), where the regions encircled by dashed lines indicate ml. An anatomical map (c) (Paxinos and Watson, 2004) is shown in order to better recognize the faint structure of nervous tissue. It is noted that ml could be recognized more clearly with shorter TE (22.5 ms) than 40 ms at this level of coronal slice as well.

2.4. The influence of nerve transection on spinal trigeminal tract and trigeminal principal sensory nucleus

Figure 4 shows the coronal sections taken at the level of anterior part of the trigeminal sensory nuclear complex (approximately 9.96 mm posterior to Bregma). Arrows in Fig. 4 indicate the cerebellar cortex (Cbc), the cerebellar medulla (cbm), spinal trigeminal tract (sp5t), Pr5, the pyramidal tract (py), and the facial nerve (7n). Figure 4(a) shows the MR image taken at TR (ms)/TE (ms) = 5000/40. On the contrary to Figs. 2 and 3, sp5t and Pr5 can be recognized better in longer TE (40 ms). That is, TE of 40 ms is more suitable for visualizing these structures. As observed in Fig. 4(a), the ventral-dorsal length of sp5t ipsilateral to ION transection is shorter than that of the contralateral counterpart, and the area of ventral part of Pr5 ipsilateral to ION transection is smaller than the contralateral counterpart. An anatomical map (c) (Paxinos and Watson, 2004) is shown in the same manner as Figs. 2 and 3.

2.5. Area changes of trigeminal pathways in nerve-transected rats

Cross-sectional areas encircled by dashed lines in Figs. 1(a), 2(c), 3(b) and 4(b) are shown in Fig. 5, where eml+ml represents the sum of areas of external medullary lamina and medial lemniscus in Fig. 2, ml represents the area of medial lemniscus in Fig. 3, sp5t represents the area of the spinal trigeminal tract in Fig. 4, Pr5 represents the area of the trigeminal principal

sensory nucleus in Fig. 4, and V1+V2 represents the area of bundles of the ophthalmic and maxillary nerves in Fig. 1. We selected 4 cross sections for the area comparison along the trigeminal pathway as representative ones according to the visibility of the respective tissues on MR images. Each pair of bar graphs in the figure compares the cross-sectional areas of those structures between contralateral and ipsilateral regions to neonatal ION transection.

Each bar graph represents the mean value $\pm$ standard deviation (SD) of area for the corresponding brain structure. The area of eml+ml in the contralateral side ($0.42 \pm 0.16 \text{ mm}^2$) is significantly (** $P < 0.001$) smaller than that of ipsilateral side ($0.72 \pm 0.20 \text{ mm}^2$). The area of ml in the contralateral side ($0.28 \pm 0.06 \text{ mm}^2$) is significantly (* $P < 0.01$) smaller than that of ipsilateral side ($0.52 \pm 0.20 \text{ mm}^2$). The area of sp5t in the contralateral side ($0.70 \pm 0.11 \text{ mm}^2$) is significantly (*** $P < 0.0001$) larger than that of ipsilateral side ($0.39 \pm 0.09 \text{ mm}^2$). The area of Pr5 in the contralateral side ($0.92 \pm 0.32 \text{ mm}^2$) is significantly (** $P < 0.001$) larger than that of ipsilateral side ($0.41 \pm 0.13 \text{ mm}^2$). The area of V1+V2 in the contralateral side ($2.26 \pm 0.05 \text{ mm}^2$) is significantly (*** $P < 0.0001$) larger than that of ipsilateral side ($0.96 \pm 0.05 \text{ mm}^2$).

These results clearly show that the volume of the primary and secondary trigeminal pathways was significantly reduced in the side associated with the trigeminal sensory system affected by neonatal ION transection.

3. Discussion

Several studies have been conducted to visualize the spinal cord pathways of sciatic nerve in rats by MRI with contrast agents such as Gadolinium diethylenetriamine penta-acetic acid (Gd-DTPA) (Chen et al., 2011) and manganese (Matsuda et al., 2010). The contrast agents are applied in order to increase the sensitivity of MRI for detecting the nervous tissues.

However, the above-mentioned studies required an invasive surgery such as exposure of the nerve ending or introduction of infusion cannula into specific fiber bundles of those nerves.

Further, those may cause serious side effects in the target animals like allergic reaction,

blood vessel irritation and skin reactions including hives, and facial edema (Elster and

Burdette, 2001). Therefore it is better not to use contrast agents especially for clinical

applications. The present study is the first one demonstrating that the 11.7 T ultra high-field

MRI can noninvasively provide extensive details of anatomical structure of the trigeminal

pathways without any contrast agents. Using this technique, we could find that neonatal ION

transection caused developmental failure of the trigeminal pathways in rats. In addition, the

trigeminal nerve trunk was found to have structures containing free water.

One of the key factors to visualize the brain structures composing the secondary trigeminal pathway such as eml and ml is the slice orientation of MRI. As shown in Fig. 2(a), 77 degrees anteriorly upward from the cranial base was the best angle for imaging eml, Rt,

and ic, among the variety of angles tried. It means that there is a specific slice orientation for discriminating between neighboring structures of nervous system such as eml, Rt, and ic, since the morphology of those structures is thin and folded.

The area reduction caused by ION transection was observed in the medial part of ml and the ventromedial part of eml contralateral to the nerve transection compared with the ipsilateral side. Their locations coincided with the secondary trigeminal pathway associated with the transected ION. It was found in contrast that the bundle of V1 and V2 as well as the ventral-dorsal length of sp5t were reduced in size in the ipsilateral side to the nerve transection. The area reduction also occurred in the ventral part of Pr5 ipsilateral to the nerve transection. As illustrated in Fig. 6, this result is coincident with the fact that the sensory information from ION is predominantly conveyed to the ventral half of the ipsilateral trigeminal nuclei through the ipsilateral sp5t (Hayashi, 1985; Miller et al., 1991). The information from the trigeminal nuclei crosses to the contralateral side, ascends in the medial part of the contralateral ml and ventromedial part of the contralateral eml, and terminates in the contralateral VPM. Thus, the experimental results combined with the above consideration indicate that the neonatal ION transection caused developmental failure of the primary and secondary neurons associated with the injured ION fibers. Such changes following ION transection would be further confirmed by retrograde tracing with horse radish peroxidase

(Chiaia et al., 1992). These results suggest that the tactile stimulation from whiskers plays an important role for development of the primary and secondary neurons. However, ION transection at postnatal day 10 had no effects on the structure of Pr5, sp5t and 5Gn (as shown in Fig. 1 (a'-d')). As described in Introduction, the similar results have been reported by conventional *in vitro* observations (Levi-Montalcini, 1949; Cook et al., 1951; Matthews and Powell, 1962; Powell and Erulkar, 1962; Kupfer and Palmer, 1964; Sugimoto and Gobel, 1982; Waite, 1984; Born and Rubel, 1985; Finlay et al., 1986; Chiaia et al., 1992; Baldi et al., 2000). These observations indicate that, there is a critical period with high plasticity early in life in the nervous system. Further, programmed cell death in Pr5 has been reported to begin at embryonic day 19 and continue until P10 during normal development (Ashwell and Waite, 1991; Miller and al-Ghoul, 1993). The critical period of plasticity is probably in close relation to the programmed cell death. Therefore the critical period (within 10 days) estimated for Pr5, sp5t and 5Gn in the present study agrees with these reports.

As the mobility of water molecules becomes higher in the tissue, T2 value becomes longer, yielding a higher signal intensity in T2-weighted MRI used in the present study. Namely the intensity in the T2-weighted MRI can be used as an indicator of the mobility of water molecules. Considering T2 values described in Methods, CSF has the highest signal intensity, followed in order by gray matter and white matter. Although sp5t is white matter,

its signal intensity is as high as that of Cbc which is gray matter, as shown in Fig. 4 (a). This result indicates that sp5t contains free water as much as Cbc. In addition, high intensity spots, which are indicative of free water, are also observed in the trigeminal nerve trunk (Figs. 1(b), (c), (d)). It has not been reported yet by MRI studies that peripheral nerves such as trigeminal nerves contain free water. The peripheral nerve trunk is composed of four connective tissue sheaths. From the outside to inward these are the mesoneurium, epineurium, perineurium, and endoneurium enveloping individual nerve fibers. Since the subarachnoid space filled with CSF is connected with the endoneurium (Haller et al., 1972), CSF is a probable source of the free water in the trigeminal nerve. Considering this in conjunction with the presence of free water in sp5t, it is expected that there is a communication of CSF through the trigeminal nerve between inside and outside of the brain stem. As shown in this example, MRI has a potential for monitoring noninvasively the motion of water molecules in living tissues which is certainly difficult to measure by the other techniques. On the basis of this finding, it is expected that sp5t can be traced by T2-weighted MRI from 5Gn to the other trigeminal nerve nuclei, motor nucleus and mesencephalic nucleus medial to Pr5.

In conclusion, 11.7 T ultra high-field T2-weighted MRI was found capable of visualizing pathological changes in the primary and secondary trigeminal pathways *in vivo* in

rats. This technique may offer many possibilities for understanding the mechanisms of formation and progression of neural pathophysiology after nerve injury.

4. Experimental procedures

4.1. Animals

Animal Experiments were performed in accordance with Guidelines for Animal Experiments of Osaka University and were approved by the Osaka University Animal Care and Use Committee. In total, 7 male Wistar rats (CLEA Japan, Osaka, Japan) were used. Six rats were used for ION transection at P0 and 1 was used for transection at P10. We referred to rat brain atlas to determine the location of the nervous tissue (Paxinos and Watson, 2004).

4.2. Infraorbital nerve transection

For anaesthesia, rats were wrapped with a piece of cloth and put on ice. After 3 min, the rats were put on an ice-cold plate. After the rats became motionless, the skin was incised between the eye and the whisker pad. Then the left ION, which innervates the left whisker, was exposed and transected. Immediately after the transection, the wound was closed by sutures.

4.3. MRI analysis

The rats were anesthetized by urethane (1.25 g/kg) on the day 8 weeks after the nerve transection. Magnetic resonance imaging was performed on the rats with an 11.7 T vertical wide bore scanner system (AVANCE 500 WB; Bruker BioSpin, Ettlingen, Germany). The effects of ION transection on the morphology of trigeminal pathways were examined using the MR images obtained by T2-weighted rapid acquisition with refocused echo MRI

(T2-RARE). Field of view = $2.5 \times 2.5 \text{ cm}^2$; matrix size = 256×256 ; number of acquisitions = 16; number of slices = 36; slice thickness = 0.5 mm; TR = 5000 ms; TE = 22.5 or 40 ms. Total imaging time was about 42 min. The slice plane of MRI was adjusted so that it was perpendicular to the midsagittal plane and at 77 degrees anteriorly upward from the cranial base. Using ImageJ software package (NIH, Bethesda, MD, USA), lower and upper threshold values were interactively set for discriminating the structures given below whose positions were determined with the aid of a rat brain atlas (Paxinos and Watson, 2004). The areas corresponding to 5Gn, Pr5, sp5t, ml, and eml were analyzed on the coronal images using different threshold values optimally chosen for each structure. In the present study, cranial base, a plane consisting of presphenoids and sphenoid bones, is used as the horizontal reference plane in adult rats in place of the bregma and lamda point, since those structures cannot be observed by MRI.

To enhance the contrast of MR images for the trigeminal pathways, two parameters, TR and TE, were selected in relation to T1 and T2 values as follows. Typical values of T1 at 11.7 T MRI are 1350 ms (white matter), 1580 ms (gray matter), and 2430 ms (CSF) (Chuang and Koretsky, 2006). The corresponding T2 values are 70, 80 and 180 ms, respectively. The recovery of longitudinal magnetization follows an exponential curve characterized by a tissue-specific time constant T1. To minimize the effects of insufficient relaxation, TR should

be at least 2-3 times as long as T1 (Hashemi et al., 2010). In the present study, TR of 5000 ms was adopted so that the longitudinal magnetization of white and gray matters might nearly recover at every repetition to minimize the effects of T1 relaxation. In the second place, the optimum TE was determined in relation to the time constant T2. The contrast of T2-weighted MR images is more enhanced with using longer TE. However the signal intensity becomes smaller with longer TE. The signal intensity decays to 37% of its original value at TE=T2. Considering these factors, TE was selected between 20 ms and T2 of the gray matter.

The areas of the structures considered in this study such as eml, ml and so on were expressed as the mean $\pm$ standard deviation (SD) in Fig. 5. Statistical comparisons of the areas between the ipsi- and contralateral sides were performed using paired Student's t-test (statistical significance was indicated by *, ** and *** for $P<0.01$, $P<0.001$ and $P<0.0001$, respectively).

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Figure Legends

Fig. 1. (a) MR image (TR ms/TE ms, 5000/40) of rat brain coronally sliced at the level of the confluence of the ophthalmic and maxillary nerves at 8 weeks after neonatal left infraorbital nerve transection. (b-d) Three consecutive coronal slices at 1, 1.5 and 2 mm anterior to (a). (a-d) ION transection at postnatal day 0, (a'-d') ION transection at postnatal day 10. V1, ophthalmic nerve; V2, maxillary nerve; V3, mandibular nerve.

Fig. 2. (a) T2-weighted MRI (TR ms/TE ms = 5000/22.5) showing a coronal slice of rat brain at the level of confluence of external medullary lamina and medial lemniscus (approximately 3.24 mm posterior to Bregma). (b) Enlarged sectional view of external medullary lamina in (a). (c) Rat brain atlas corresponding to (a) (Paxinos and Watson, 2004). eml, external medullary lamina; ic, internal capsule; ml, medial lemniscus; Rt, thalamic reticular nucleus; VP, ventral posterior nucleus of thalamus; VPL, ventral posterolateral nucleus of thalamus; VPM, ventral posteromedial nucleus of thalamus.

Fig. 3. (a) T2-weighted MRI (TR ms/TE ms = 5000/22.5) of rat brain sliced at the level of separation of external medullary lamina and medial lemniscus (approximately 4.44 mm posterior to Bregma). (b) Enlarged sectional view of medial lemniscus in (a). (c) Rat brain

atlas corresponding to (a) (Paxinos and Watson, 2004). ml, medial lemniscus; SPFPC, subparafascicular thalamic nucleus, parvicellular part; str, superior thalamic radiation; VP, ventral posterior nucleus of thalamus; VPL, ventral posterolateral nucleus of thalamus; VPM, ventral posteromedial nucleus of thalamus.

Fig. 4. (a) T2-weighted MRIs (TR ms/TE ms = 5000/40) of rat brain sliced at the level of anterior part of trigeminal sensory nuclear complex (approximately 9.96 mm posterior to Bregma). (b) Enlarged sectional view of spinal trigeminal tract and trigeminal principal sensory nucleus in (a). (c) Rat brain atlas corresponding to (a) (Paxinos and Watson, 2004). 7n, facial nerve; Cbc, cerebellar cortex; cbm, cerebellar medulla; Pr5, trigeminal principal sensory nucleus; py, pyramidal tract; sp5t, spinal trigeminal tract.

Fig. 5. Comparison of cross-sectional areas of trigeminal pathway at 5 locations between contralateral (white bars) and ipsilateral regions (gray bars) to neonatal infraorbital nerve transection. Each pair of bars is arranged from left to right as the location goes away from the central nervous system as illustrated in Fig. 6. The asterisks indicate the significant difference between contralateral and ipsilateral regions (* $P < 0.01$, ** $P < 0.001$ and *** $P < 0.0001$, as determined by paired Student's *t* test). The error bars represent standard

deviation (SD) where n=6 per group. eml, external medullary lamina; ml, medial lemniscus; Pr5, trigeminal principal sensory nucleus; sp5t, spinal trigeminal tract; V1, ophthalmic nerve; V2, maxillary nerve.

Fig. 6. Diagrammatic representation of primary and secondary trigeminal pathways.

The trigeminal nerve has three major branches: the ophthalmic division (V1), the maxillary division (V2), and the mandibular division (V3). The nerve enters the brainstem from the trigeminal ganglion (5Gn) through the spinal trigeminal tract (sp5t) to the ipsilateral trigeminal nuclei: the trigeminal principal sensory nucleus (Pr5) and the spinal trigeminal nucleus. In the trigeminal nuclei, V1, V2 including infraorbital nerve, and V3 were located ventrally, medially, and dorsally, respectively. The secondary trigeminal fibers except for the small area concerning proprioception cross to the contralateral side, ascend in the medial part of the contralateral medial lemniscus (ml) and ventromedial portion of the contralateral external medullary lamina (eml), and terminate in the contralateral ventral posteromedial nucleus (VPM) of the thalamus. The thalamic reticular nucleus (Rt) that intervenes between the internal capsule (ic) and eml is a thin sheet of neurons. The two-way arrows indicate the positions of MRI slices shown in Figs. 1-4. VP, ventral posterior nucleus; VPL, ventral posterolateral nucleus.

Fig 1

Fig 2

Fig 3

Fig 4

TE=40ms

Fig 5

Fig 6