Complementary distribution of glutamatergic cerebellar and GABAergic basal ganglia afferents to the rat motor thalamic nuclei

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Abstract

Motor thalamic nuclei, ventral anterior (VA), ventral lateral (VL) and ventral medial (VM) nuclei, receive massive glutamatergic and GABAergic afferents from the cerebellum and basal ganglia, respectively. In the present study, these afferents were characterized with immunoreactivities for glutamic acid decarboxylase of 67 kDa (GAD67) and vesicular glutamate transporter (VGluT)2, and examined by combining immunocytochemistry with the anterograde axonal labeling and neuronal depletion methods in the rat brain. VGluT2 immunoreactivity was intense in the caudodorsal portion of the VA–VL, whereas GAD67 immunoreactivity was abundant in the VM and rostroventral portion of the VA–VL. The rostroventral VA–VL and VM contained two types of GAD67-immunonegative varicosities (large and small), but the caudodorsal VA–VL comprised small ones alone. VGluT2-immunonegative varicosities were much larger in the caudodorsal VA–VL than those in the rostroventral VA–VL and VM. When anterograde tracers were injected into the basal ganglia output nuclei, the ventral tegmental area, the majority of labeled axon varicosities were large and distributed in the rostroventral VA–VL and VM, showing immunonegativity for GAD67, but not for VGluT2. Only the large GAD67-immunonegative varicosities were mostly abolished by kainic acid depletion of substantia nigra neurons. In contrast, large to giant axon varicosities derived from the deep cerebellar nuclei were distributed mostly in the caudodorsal VA–VL, displaying VGluT2 immunoreactivity. The VGluT2-positive varicosities disappeared from the core portion of the caudodorsal VA–VL by depletion of cerebellar nucleus neurons. Thus, complementary distributions of large VGluT2- and GAD67-positive terminals in the motor thalamic nuclei are considered to reflect glutamatergic cerebellar and GABAergic basal ganglia afferents, respectively.

Introduction

Motor thalamic nuclei, composed of ventral anterior (VA), ventral lateral (VL) and ventral medial (VM) nuclei, receive massive inhibitory and excitatory afferents from the basal ganglia and cerebellum, respectively (for review, see Groenewegen & Witter, 2004; Jones, 2007). The basal ganglia afferents, emitted by the internal segment of the globus pallidus (GPI) (entopeduncular nucleus) and substantia nigra pars reticulata (SNr), have been suggested to be GABAergic by several lines of evidence. The destruction of the GPi/SNr has been reported to cause the reduction of GABA content and glutamic acid decarboxylase activity in the motor thalamic nuclei (Di Chiara et al., 1979; Kilpatrick et al., 1980; Penney & Young, 1981), nigrothalamic transmission is blocked by GABA antagonist (MacLeod et al., 1980), and GABA immunoreactivity has frequently been observed in the axon terminals derived from the GPi/SNr (Ilinsky et al., 1997; Bodor et al., 2008).

On the other hand, the cerebellothalamic projection, originating from the deep cerebellar nuclei (DCN), has been supposed to be glutamatergic, because anterogradely labeled terminals derived from the DCN often showed glutamate immunoreactivity (Schwarz & Schmitz, 1997). However, because glutamate is a general metabolic substrate or serves as the precursor of GABA, glutamate immunoreactivity is not specific to glutamatergic neurons or terminals. Thus, a more specific marker for glutamatergic terminals is necessary for the characterization of cerebellothalamic transmission. Recently, immunoreactivities for vesicular glutamate transporter (VGluT)1 and VGluT2 have been widely used as markers for the vast majority of glutamatergic axon terminals in the central nervous system. Interestingly, VGluT1 is predominantly expressed by telencephalic/cortical neurons, whereas VGluT2 is mainly produced by neurons in diencephalic and lower brainstem regions (for review, see Kaneko & Fujiyama, 2002; Kaneko et al., 2002; Fremeau et al., 2004). As thalamic nuclei receive excitatory inputs from the cerebral cortex as well as from subcortical regions, VGluT1 and VGluT2 immunoreactivities may be used as markers for cortical and subcortical glutamatergic afferents, respectively, in the thalamic nuclei.

In rat brain, the VA and VL constitute a single nuclear mass as the VA–VL complex (Groenewegen & Witter, 2004; Jones, 2007).
Recently, however, we found that the rat VA-VL was segregated into two portions based on differential distributions of immunoreactivities for VGluT2 and glutamic acid decarboxylase of 67 kDa (GAD67) (Kuramoto et al., 2009), suggesting functional compartmentalization of the VA-VL. To understand the function of those compartments, we need to know the major origins of these VGluT2-immunopositive glutamatergic afferents and GAD67-positive GABAergic afferents in the motor thalamic nuclei. In the present study, we first, in rat VA-VL and VM, examined the distributions of glutamatergic inputs immunopositive for VGluT1 and VGluT2 and of GABAergic inputs labeled with antibodies not only to GAD67 but also to glutamic acid decarboxylase of 65 kDa (GAD65) and vesicular GABA transporter (VGAT). We then tried to reveal the main origins of these glutamatergic and GABAergic terminals in the motor thalamic nuclei by using an anterograde axonal labeling technique and neuronal depletion method with kainic acid.

Materials and methods

Animals

In the present study, we used 38 adult male Wistar rats (350–400 g body weight; Shizuoka Laboratory Animal Center, Shizuoka, Japan). Experiments were conducted in accordance with the guidelines on animal care of the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University.

Injection of anterograde tracers and kainic acid, and fixation

For labeling of axon fibers derived from the DCN, SNr and GPi, we used anterograde tracers, 10% (w/v) biotinylated dextran amine (BDA3k) (Invitrogen, Eugene, OR, USA) in 5 mM sodium phosphate (pH 7.4)-buffered 0.9% (w/v) saline (PBS), and 2.5% (w/v) Phaseolus vulgaris leucoagglutinin (PHA-L4) (J-OIL MILLS Inc., Tokyo, Japan) in 0.1 M sodium phosphate buffer (pH 7.4). After rats were anesthetized by intraperitoneal injection of chloral hydrate (35 mg/100 g body weight), BDA3k solution (0.2–0.5 μL) was injected into the DCN of five rats (2.5 mm posterior to the interaural, 2.5 mm lateral to the midline and 4.0 mm deep from the brain surface) by pressure through a glass micropipette attached to a Picospritzer II (General Valve Corporation, East Hanover, NJ, USA). BDA3k or PHA-L4 was electro-osmotically delivered into the SNr (6.2 mm posterior to the bregma, 2.4 mm lateral to the midline, and 7.8 mm deep from the brain surface) of six rats or into the GPi (3.0 mm posterior to the bregma, 3.0 mm lateral to the midline, and 7.2 mm deep from the brain surface) of four rats by passing positive 2-μA current pulses at 7-s intervals for 30 min through a glass micropipette to obtain a more restricted injection site. For neuronal depletion of the DCN and substantia nigra (SN), five or 10 rats were anesthetized and received a pressure injection of 0.2–0.5 μL of 0.5% (w/v) kainic acid in 0.1 M sodium phosphate buffer (pH 7.4) into the DCN or SN, respectively. The rats survived for 1 week after the injection of BDA3k and kainic acid, and for 2 weeks after the PHA-L4 injection.

The 30 treated rats and eight untreated rats were deeply anesthetized with chloral hydrate (70 mg/100 g), and perfused transcardially with 200 mL of PBS, followed by 200 mL of 3% (w/v) formaldehyde, 75% (v/v)-saturated picric acid and 0.1 M Na₂HPO₄ (adjusted with NaOH to pH 7.0). The brains were then removed, and postfixed for 4 h at room temperature (20–25 °C) with the same fixative. After cryoprotection with 30% (w/w) sucrose in PBS, the brains of untreated and tracer-injected rats were cut into 40-μm-thick parasagittal or coronal sections, and those of kainic acid-injected rats were cut into 40-μm-thick coronal sections on a freezing microtome, and the sections were collected serially in PBS. Some sections were stained for Nissl with cresyl violet to determine cytoarchitectonic areas according to the rat brain atlas of Paxinos & Watson (2007).

Immunoperoxidase staining

The sections obtained from untreated rats, kainic acid-injected rats and PHA-L4-injected rats were incubated overnight with one of the following antibodies: 1 μg/mL mouse monoclonal anti-GAD67 IgGₐ (MAB5406, Millipore, Billerica, MA, USA), 1 μg/mL mouse monoclonal anti-GAD65 IgGₐ (MAB351; Millipore), 1 μg/mL affinity-purified rabbit anti-VGAT antibody (131 003; Synaptic Systems, Göttingen, Germany), 1 μg/mL affinity-purified rabbit anti-VGluT1 antibody (Hioki et al., 2003), 1 μg/mL affinity-purified guinea pig anti-VGlut2 antibody (Fujiyama et al., 2001), 1 μg/mL mouse monoclonal anti-calbindin D28k IgG₁ (C9848; Sigma, St Louis, MO, USA) or 4 μg/mL rabbit anti-PHA-L4 antibody (AL-1801-2; E-Y Laboratories, Inc., San Mateo, CA, USA) in PBS containing 0.3% (v/v) Triton X-100, 0.12% (w/v) lambda-carrageenan, 0.02% (w/v) sodium azide and 1% (v/v) normal donkey serum. After several washes in PBS containing 0.3% (v/v) Triton X-100 (PBS-X), these sections were incubated for 2 h with 10 μg/mL biotinylated antibody to mouse IgG (AP192B; Millipore), rabbit IgG (BA-1000; Vector Laboratories, Burlingame, CA, USA) or guinea pig IgG (BA-7000; Vector Laboratories) in PBS containing 0.3% (v/v) Triton X-100, 0.12% (w/v) lambda-carrageenan, 0.02% (w/v) sodium azide and 1% (v/v) normal donkey serum. After several washes in PBS-X, the sections were further incubated for 1 h with avidin-biotinylated peroxidase complex (1 : 100, ABC-Elite; Vector Laboratories) in PBS-X. After a rinse with PBS, the bound peroxidase was developed brown by reaction for 30–60 min with 0.02% (w/v) 3,3'-diaminobenzidine Shizuoka Laboratory Animal Center (Dojindo, Kumamoto, Japan) and 0.003% (v/v) H₂O₂ in 50 mM Tris-HCl pH 7.6. The sections obtained from BDA3k-injected rats were simply incubated overnight with ABC-Elite (1 : 100; Vector Laboratories) in PBS-X, and the bound peroxidase was developed brown as described above. All of the above incubations and reactions were performed at room temperature. The stained sections were thoroughly washed in PBS, mounted onto gelatinized glass slides, dried up, dehydrated in an ethanol series, cleared in xylene, and coverslipped.

Digital images were captured under a microscope (FXA or ECLIPSE E1000M, Nikon, Tokyo, Japan) using a QICAM FAST digital monochrome camera (QImaging, Surrey, BC, Canada). All of the digital images were arranged and modified (<30% contrast enhancement) using software CANVAS X (ACD Systems International Inc., Victoria, Canada) and saved as TIFF files. To measure the size of GAD67- and VGlut2-immunopositive axon varicosities in the VM, the rostroventral VA-VL and caudodorsal VA-VL of normal rats, six coronal sections that were rostrocaudally spaced at regular intervals (120 μm for the rostroventral VA-VL and 200 μm for the VM and caudodorsal VA-VL), were selected from each subnucleus. In the center of the subnucleus contained in the selected sections, 300, 3'-diaminobenzidine-labeled axon varicosities (50 varicosities per section) were randomly sampled under the FXA light microscope with an oil-immersion × 100 objective lens (PlanApo 100; numerical aperture 1.4; Nikon). The sampling of varicosities in SN-damaged rats will be described in the Results. Immunoreactive varicosities were frequently independent and round to oval structures with a minimum diameter of more than 0.4 μm and occasionally large swellings associated with fibrous immunoreactive structures. After
taking images with the digital camera, the maximum and minimum diameters and cross-sectional area of the varicosity were measured by fitting an ellipse to each varicosity on the images using the software CANVAS X. The histogram of the cross-sectional area of axon varicosities was fitted with log-normal curves, as reported previously (Ahmed et al., 1997), by the maximum likelihood estimation using a program written in software IGOR Pro 5.0 (Wavemetrics Inc., Lake Oswego, OR, USA). A unimodal or bimodal fitting curve was selected on the basis of the Bayesian information criterion for parameters (Schwarz, 1978).

Double immunofluorescence staining

The sections obtained from untreated rats were incubated overnight with one of the following mixtures in PBS containing 0.3% (v/v) Triton X-100, 0.12% (w/v) lambda-carrageenan, 0.02% (w/v) sodium azide and 1% (v/v) normal donkey serum: (i) 2 μg/mL mouse anti-GAD67 IgG2a and 3 μg/mL rabbit anti-VGAT antibody; (ii) 2 μg/mL mouse anti-GAD67 IgG2a and 2 μg/mL rabbit anti-VGluT1 antibody; (iii) 2 μg/mL mouse anti-GAD67 IgG2a and 2 μg/mL guinea pig anti-VGluT2 antibody; (iv) 3 μg/mL rabbit anti-VGAT antibody and 2 μg/mL mouse anti-GAD65 IgG2a, and (v) 2 μg/mL rabbit anti-VGluT1 antibody and 2 μg/mL guinea pig anti-VGluT2 antibody. The sections obtained from PHA-L-injected rats were incubated with a mixture of 4 μg/mL rabbit anti-PHA-L antibody and either 2 μg/mL mouse anti-GAD67 IgG2a or 2 μg/mL guinea pig anti-VGluT2 antibody. The sections obtained from BDA3k-injected rats were incubated with 2 μg/mL mouse anti-GAD67 IgG2a or 2 μg/mL guinea pig anti-VGluT2 antibody.

After several washes with PBS-X, the sections derived from untreated rats and PHA-L-injected rats were incubated for 2 h in PBS containing 0.3% (v/v) Triton X-100, 0.12% (w/v) lambda-carrageenan, 0.02% (w/v) sodium azide and 1% (v/v) normal donkey serum with 5 μg/mL Alexa Fluor 488-conjugated antibody to mouse or rabbit IgG (A-11029, A-11034; Invitrogen), and then for 2 h with 5 μg/mL Alexa Fluor 594-conjugated antibody to rabbit or guinea pig IgG (A-11037, A-11076; Invitrogen) in the presence of 10% (v/v) normal donkey or rabbit serum. The sections derived from BDA3k-injected rats were incubated with 5 μg/mL Alexa Fluor 594-conjugated antibody to mouse or rabbit IgG and with Alexa Fluor 488-conjugated streptavidin (S-32354; Invitrogen).

All of the sections were washed thoroughly in PBS, mounted onto gelatinized glass slides, air dried, and coverslipped with 90% (v/v) glycerol and 2.5% (w/v) triethylenediamine (antifading agent) in 20 mM Tris-HCl, pH 7.6. The sections were observed under an epifluorescence microscope Axioskop (Carl Zeiss, Overkochen, Germany) with appropriate filter sets for Alexa Fluor 488 (excitation 450–490 nm, emission 514–565 nm) and Alexa Fluor 594 (excitation 530–585 nm, emission ≥615 nm), or under a confocal laser-scanning microscope (SP2; Leica, Heidelberg, Germany) with appropriate sets of laser beams and emission windows for Alexa Fluor 488 (excitation 488 nm, emission 505–530 nm) and Alexa Fluor 594 (excitation 594 nm, emission 630–800 nm). For the analysis of colocalization of chemical markers (Fig. 3), digital images were captured under the confocal microscope with an oil-immersion ×63 objective lens (HCX PL APO, numerical aperture 1.4; Leica), pinhole of 1.0 Airy unit, and zoom factor of 10. For the analysis of GAD67 and VGluT2 immunoreactivities on anterograde tracer-positive axon varicosities (Fig. 5), digital images were obtained under the fluorescence microscope with an oil-immersion ×100 objective lens (Plan-NEOFLUAR, numerical aperture 1.3; Zeiss) using the QICAM FAST digital monochrome camera.

Results

Immunoreactivities for GABAergic and glutamatergic markers in the rat motor thalamic nuclei

In rat, a distinction between the VA and VL was difficult to make in cytoarchitecture, as the VA and VL were similarly cell sparse with relatively large neurons (Figs 1A and B, and 2A, B, F, G, K and L) (Jones, 2007; Paxinos & Watson, 2007). However, the distributions of some chemical markers were heterogeneous in the rat VA-VL as reported previously (Paxinos et al., 1999; Bodor et al., 2008; Kuramoto et al., 2009). Neuronal immunoreactivity for GAD67 was more intense in the rostroventral portion of the VA-VL than in the caudal dorsal portion, whereas neuronal immunoreactivity for VGluT2, a glutamatergic axon terminal marker of subcortical inputs, was more abundant in the caudal dorsal portion but sparse in the rostroventral portion of the VA-VL (Figs 1C, D, I and J, and 2C, D, H, I, M and N). In the VM, neuronal immunoreactivities for GAD67 and VGluT2 were intense and weak, respectively, being similar to those in the rostroventral VA-VL, although VGluT2 immunoreactivity in the VM was slightly more intense than in the rostroventral VA-VL (Figs 1C, D, I and J, and 2C, D, H, I, M and N). Thus, the distributions of GAD67 and VGluT2 immunoreactivities were mostly complementary in intensity throughout the motor thalamic nuclei (Fig. 2P–R). It was further found that the distributions of immunoreactivities for GAD65 and VGAT, other GABAergic markers, resembled that for GAD67 in the motor thalamic nuclei (Fig. 1E–H), although the rostrocaudal or dorsoventral difference in GAD65 and VGAT immunoreactivities was less clear than that in GAD67 immunoreactivity (compare Fig. 1D, F and H). In addition, although it was reported that the distribution of calbindin D28k-immunoreactive neuropil and neuronal cell bodies was similar to that of intensely GAD67-positive neuropil throughout the motor thalamic nuclei (Kuramoto et al., 2009), the distribution of calbindin immunoreactivity was slightly more restricted than that of GAD67 immunoreactivity (Fig. 2D, E, I, J and N–R). In contrast, neuronal immunoreactivity for VGluT1 was intense and mostly homogeneous not only in the motor thalamic nuclei but also throughout the thalamus except the parafascicular nucleus (Fig. 1K and L). As few, if any, mRNA signals for VGluT1 were expressed in subcortical regions projecting to the thalamic nuclei (Ni et al., 1994, 1995; Fremeau et al., 2001; Herzog et al., 2001), the thalamic VGluT1 immunoreactivity might be derived principally from corticothalamic neurons expressing VGluT1 mRNA strongly.

Double-immunofluorescence confocal laser-scanning microscopic study revealed that GAD67 and VGAT immunoreactivities as well as GAD65 and VGAT immunoreactivities were almost completely colocalized in varicose structures of the motor thalamic nuclei (white color in Fig. 3A–F). Because VGAT immunoreactivity was located at synaptic vesicles of GABAergic axon terminals (Chaudhry et al., 1998; Dumoulin et al., 1999), these results indicated that the distributions of GAD67 and GAD65 immunoreactivities were restricted to axon terminals in the motor thalamic nuclei, although not only neurpil but also cell bodies showed glutamic acid decarboxylase immunoreactivity in the cortex and thalamic reticular nucleus. However, neither VGluT1 nor VGluT2 immunoreactivity was observed in GAD67-immunoreactive varicosities (Fig. 3G–L), suggesting that glutamatergic and GABAergic axon terminals were almost completely segregated from each other. VGluT1 and VGluT2 immunoreactivities were not colocalized in single varicosities (Fig. 3M–O).

The microscopic study of higher magnification further revealed the presence of at least two types of GABAergic varicosities (large and
small) in the motor thalamic nuclei (Fig. 3). The large GABAergic varicosities were mostly found in the VM and rostroventral portion of the VA-VL (Fig. 3A, B, D, E, G, H, J and K), whereas the small ones were distributed throughout the motor thalamic nuclei including the caudodorsal VA-VL (Fig. 3A–L). It was also found that VGluT2-immunoreactive varicosities in the motor thalamic nuclei were classified into two groups in terms of their size (Fig. 3J–O). Large to giant VGluT2-positive varicosities were confined to the caudodorsal VA-VL (Fig. 3L and O), whereas small ones were restrictively located in the VM and rostroventral VA-VL (Fig. 3J, K, M and N). In contrast to VGluT2-positive varicosities, VGluT1-positive varicosities were very small and numerous throughout the VA-VL and VM (Fig. 3G–I and M–O).

To confirm these results quantitatively, we measured morphological parameters of VGluT2- and GAD67-positive axon varicosities in the motor thalamic nuclei of normal rats by fitting an ellipsoid to each varicosity. When the number of GAD67-immunopositive varicosities was plotted against the cross-sectional area in the caudodorsal VA-VL, the histogram was well fitted with a log-normal distribution, as reported previously (Ahmed et al., 1997), showing a peak of 1.00 $\mu m^2$ (Fig. 6U). The median and interquartile range of the maximum diameter were 1.30 and 1.07–1.57 $\mu m$, respectively (Table 1). The aspect ratio (ratio of the maximum diameter to the minimum diameter) of the varicosities was 1.38 ± 0.34 (mean ± SD).

However, the histogram was clearly bimodal in the VM and rostroventral VA-VL (Fig. 6Q and S), and better fitted with a mixture of two log-normal distributions than a single distribution or a mixture of three distributions. The peaks of the bimodal fitting curve were 1.10 and 9.13 $\mu m^2$ in the VM and 0.97 and 7.76 $\mu m^2$ in the rostroventral VA-VL, indicating that these regions contained at least two types of GAD67-positive axon varicosities and the small varicosities were similar in size to those in the caudodorsal VA-VL. After dividing the axon varicosities into small and large at the line of equal probability of the two distributions (arrowheads in Fig. 6Q and S), the maximum diameters of the small and large varicosities in the VM were in a similar range to those in the rostroventral VA-VL (Table 1). The mean aspect ratios of the small and large GAD67-positive varicosities were 1.45 ± 0.34 and 1.96 ± 0.59 in the VM, and 1.39 ± 0.30 and 1.90 ± 0.60 in the rostroventral VA-VL, indicating that large varicosities were more elongated than small ones (Table 1).

In contrast to the GAD67-positive varicosities, the size histogram of VGluT2-positive varicosities was unimodal and well fitted with a single log-normal distribution in any subregion of the motor thalamic nuclei (Supporting Information Fig. S1; Table 1). The peaks of cross-sectional areas were 2.39, 2.36 and 17.39 $\mu m^2$ in the VM, rostroventral VA-VL, and caudodorsal VA-VL, respectively. Thus, the sizes of varicosities were in the following order from the smallest: (i) small-type GAD67-positive varicosities in all of the motor subnuclei (peak at cross-sectional area of 0.96–1.10 $\mu m^2$); (ii) VGluT2-positive varicosities in the VM and rostroventral VA-VL (2.36–2.39 $\mu m^2$); (iii) large-type GAD67-positive varicosities in the VM and rostro-
ventral VA-VL (7.76–9.13 \( \mu \text{m}^2 \)); and (iv) VGluT2-positive varicosities in the caudodorsal VA-VL (17.4 \( \mu \text{m}^2 \)). As the aspect ratio of large VGluT2-positive and GAD67-positive varicosities in the motor thalamic nuclei was larger than that of small VGluT2-positive and GAD67-positive varicosities (Table 1), large varicosities tended to be more elongated than small ones. Finally, it was noted that large to giant glutamatergic terminals and large GABAergic terminals were distributed in a complementary manner within the motor thalamic nuclei.

Anterograde labeling and chemical characterization of nigrothalamic, pallidothalamic and cerebellothalamic projections

Motor thalamic nuclei were suggested to mainly receive GABAergic inputs from the thalamic reticular nucleus and SNr/GPi, and to principally accept glutamatergic subcortical inputs from the DCN (for review, see Groenewegen & Witter, 2004; Jones, 2007). Thus, we injected anterograde tracers into the SNr (Figs 4A and D, and 5A and E), GPi (Fig. 5M) and DCN (Figs 4G and 5R) to anterogradely label...
basal ganglia and cerebellar afferents to the motor thalamic nuclei. We here used two anterograde tracers, BDA3k and PHA-L, but noted almost no difference between the two tracers except that retrogradely labeled neuronal cell bodies were often found outside the thalamic nuclei in the cases of BDA3k injection. Thus, the results of BDA3k and PHA-L injections are mostly described together in the following paragraphs.

In the coronal brain sections of the rats injected with the tracer into the SNr, intense anterograde labeling was distributed ipsilaterally in the VM and the rostroventral portion of the VA-VL, which showed intense GAD67 immunoreactivity (Fig. 4B, C, E and F). When the tracer was injected into the ventral part of the SNr, anterograde labelings were observed massively in the rostroventral portion of the VA-VL (Fig. 4A and B for coronal sections; Fig. 5A and B for sagittal sections), whereas the labeling was found chiefly in the VM after tracer injection into the dorsal part of the SNr (Figs 4D and E, and 5E, F and I). In all of the cases of SNr injection, no anterogradely labeled axon varicosities were observed in the intensely VGluT2-positive caudodorsal portion of the VA-VL, but a few varicosities were found in the lateral portion of the mediodorsal thalamic nuclei including the central lateral, paracentral and parafascicular nuclei. Outside the dorsal thalamus, anterogradely labeled fibers were observed consistently in the superior colliculus and red nucleus, and less frequently in the thalamic reticular nucleus, zona incerta and GPi. In the subthalamic nucleus and pedunculopontine tegmental nucleus, there were not only anterogradely labeled axon varicosities, but also retrogradely labeled cell bodies in the cases of BDA3k injection. Retrogradely labeled neurons were also found in the neostriatum and external segment of the globus pallidus.

Fig. 3. Double-immunofluorescence labeling for GABAergic and glutamatergic chemical markers in the motor thalamic nuclei. Almost all axon varicosities, which showed immunoreactivity for VGAT, were immunopositive for GAD67 (A–C) and GAD65 (D–F) in the motor thalamic nuclei. The rostroventral VA-VL and VM contained two types of GABAergic marker-positive varicosities (large and small) (A, B, D and E), whereas the caudodorsal VA-VL contained small ones alone (C and F). Almost no GAD67-immunopositive varicosities showed immunoreactivities for glutamatergic terminal markers, VGluT1 (G–I) or VGluT2 (J–L). In the caudodorsal VA-VL, VGluT2-positive varicosities were much larger than those in the rostroventral VA-VL and VM. Immunoreactivities for VGluT1 and VGluT2 were not colocalized in the motor thalamic nuclei (M–O). Scale bar in O applies to A–O.
In contrast, when the tracer injection was made into the DCN (Figs 4G and 5R), the labeled axon fibers were always distributed sparsely in the caudodorsal V A-VL (Fig. 5S), but only slightly in the VM and rostroventral V A-VL of the contralateral thalamus. The moderate anterograde labeling was observed in the paracentral and central medial nuclei, and weak labeling was found in the posterior nuclei, laterodorsal nucleus and parafascicular nucleus of the contralateral thalamus. In addition, the labeled axon fibers were frequently observed in the red nucleus, anterior pretectal nucleus, zona incerta and superior colliculus, and less frequently in the broad regions including the pontine nuclei, inferior olivary complex, vestibular nuclei, spinogeminal nucleus and brainstem reticular formation.

We then performed double immunofluorescent labeling to examine the chemical nature of the anterogradely labeled axon varicosities. After injecting the tracer into the ventral part of the SNr, the labeling in the rostroventral VA-VL formed large axon varicosities that were immunopositive for GAD67, but not for VGluT2 (Fig. 5A–D). Of 100 anterogradely labeled axon varicosities that were randomly selected, 100 varicosities showed GAD67 immunoreactivity. In the cases with the tracer injection into the dorsal part of the SNr, large anterogradely labeled varicosities were distributed mainly in the VM and partly in the mediodorsal thalamic nucleus, and immunopositive for GAD67 alone (100 of 100 labeled varicosities) (Fig. 5E–L).

Of four cases where the injection was made into the GPi, tracer deposits of two cases were located at the caudal portion of the GPi (Fig. 5M). The anterogradely labeled axon varicosities were sparsely observed in the rostroventral VA-VL and VM of the injection side with the densest distribution in the most caudalateral portion of the rostroventral VA-VL (Fig. 5N and O). Although these injection sites might not include a GPi portion that was crucial for dense VA-VL projection, the two injections covered more than two-thirds of the caudal one-third of the GPi, which was known to send axon fibers to the VA-VL (van der Kooy & Carter, 1981; Takada & Hattori, 1987). In any case, the labeled axons were almost always equipped with large varicosities showing GAD67 immunoreactivity alone (99 of 100 labeled varicosities; Fig. 5P and Q) as the varicosities derived from the SNr. When the injection of the other two cases was made into the more rostral part of the GPi, almost no axon fibers were observed in the motor thalamic nuclei. In contrast, in all of the four cases of GPi injection, labeled axon fibers were observed densely in the lateral habenular nucleus, moderately in the parafascicular thalamic nucleus, subthalamic nucleus and zona incerta, and infrequently in the superior colliculus. In the SNr and external segment of the globus pallidus, not only anterogradely labeled axon fibers, but also retrogradely labeled cell bodies were observed in the cases of BDA3k injection. Retrogradely labeled neurons were found in the cerebral cortex and neostriatum.

When the anterograde tracer was injected into the DCN, very large axon varicosities were observed to be labeled in the caudodorsal portion of the VA-VL, and they showed clear immunoreactivity for VGluT2 (99 of 100 labeled varicosities), but not for GAD67 (Fig. 5R–U). Furthermore, when the anterogradely labeled fibers were found in the rostroventral VA-VL or VM, their axon varicosities were invariably small to medium-sized but positive for VGluT2. These results revealed that at least a part of the large GAD67-immunopositive axon varicosities in the rostroventral VA-VL and VM were derived from the SNr and GPi. Similarly, at least some large to giant VGluT2-positive axon varicosities in the caudodorsal VA-VL were emitted from the DCN.

### Table 1. The size of immunoreactive varicosities in the motor thalamic nuclei

<table>
<thead>
<tr>
<th>GAD67</th>
<th>Cross-sectional area (μm²)</th>
<th>Maximum diameter (μm)</th>
<th>Aspect ratio*</th>
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<tr>
<td></td>
<td>Small*</td>
<td>Large*</td>
<td>Small</td>
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<tr>
<td>VM</td>
<td>1.05†</td>
<td>9.21</td>
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<td>5.23–12.1</td>
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<tr>
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<td>1.30</td>
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<tr>
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* When the histogram was fitted with a mixture of two log-normal curves (Fig. 6Q and S), the data were calculated after dividing the varicosities into small and large types at the point of equal probability of the two fitting curves. †Median. ‡Mean. §25–75% interquartile range. **SD.

In each motor thalamic subnucleus, 300 immunoreactive varicosities were analyzed. *Aspect ratio is the ratio of the maximum diameter to the minimum diameter. Kainic acid injection into the substantia nigra

To reveal principal sources of the GAD67-immunopositive axon varicosities in the VA-VL and VM, we largely depleted SN neurons by the kainic acid injection in two rats (Fig. 6A–D). These injections spared GPi neurons from the injury. In the thalamus ipsilateral to the lesion, large GAD67-positive varicosities disappeared almost completely from the VM (Fig. 6L) and a large part of the rostroventral VA-VL (Fig. 6N and P) except the most caudalateral portion of the rostroventral VA-VL. A significant reduction of large GAD67-positive varicosities was also observed in the central lateral and paracentral complex, vestibular nuclei, spinal trigeminal nucleus and brainstem reticular formation.
intralaminar thalamic nuclei and lateral portion of the mediodorsal thalamic nucleus (Fig. 6J). In contrast, small GAD67-positive varicosities remained resistant to the chemical damage (Fig. 6P), suggesting that the small varicosities were derived from other sources than the SNr. No VGluT2-immunopositive varicosities in the VA-VL or VM were decreased by the neuronal depletion of the SN (Fig. 6F and H). When the kainic acid injection was made in the GPi, the neuronal depletion was always observed significantly in the thalamic reticular nucleus and thalamic relay nuclei. It is well known that neurons of the thalamic reticular nucleus are all GABAergic and send axons to the thalamic relay nuclei (for review, see Groenewegen & Witter, 2004). When a reduction of GAD67-positive varicosities in the thalamic nuclei was observed after the kainic acid lesion involving the reticular nucleus as well as the GPi, it was not judged whether the origin of the lost GAD67-positive varicosities was the GPi or reticular nucleus. Further, by the neuronal depletion of the thalamic relay nuclei including motor nuclei, the thalamotectal afferents lost postsynaptic targets, and were likely to change their shape. Thus, we did not further study the change of GAD67-immunopositive varicosities after the kainic acid injection into the GPi.

To characterize the change of GAD67-positive varicosities quantitatively, we measured the cross-sectional area of the varicosities in the affected regions of the motor thalamic nuclei, and plotted the frequency against the cross-sectional area (Fig. 6R, T and V). As the affected region was widely distributed in the VM and rostroventral VA-VL, we selected six sections, which were spaced at regular intervals within each subnucleus, and sampled 300 varicosities (50 per section) in the center of the affected region contained in the section. In the affected regions, axon varicosities with a cross-sectional area of more than 4.0 \( \mu m^2 \) were almost completely abolished by the SN neuronal depletion (Fig. 6R and T). These histograms were well fitted with a unimodal log-normal distribution, and the peaks of the fitted distributions were 0.98 \( \mu m^2 \) in the VM and 0.96 \( \mu m^2 \) in the rostroventral VA-VL, being similar to the first peaks of GAD67-positive varicosities in normal brains (1.10 and 0.97 \( \mu m^2 \) in the VM and rostroventral VA-VL, respectively). In contrast, no significant change was produced by the SN lesion in the histogram of the caudodorsal VA-VL, which did not have GAD67-positive axon varicosities larger than 4.0 \( \mu m^2 \) even in the normal brain (Fig. 6U and V). These results indicate that the vast majority of large GAD67-positive axon varicosities in the VA-VL and VM were derived from SN neurons, although the GPi sent a small number of large varicosities to the VA-VL (Fig. 5M–Q) and VM.

Fig. 4. The distributions of anterogradely labeled afferents from the SNr and DCN in the coronal sections. When BDA3k was injected into the ventral (arrow in A) and dorsal (D) portions of the SNr, labeled varicosities were observed mainly in the rostroventral VA-VL (B) and VM (E), respectively. The labeling was mostly restricted to inside the intensely glutamic acid decarboxylase-immunopositive regions (C and F). In contrast, after the BDA3k injection into the DCN (G), the labeled varicosities were distributed within the dorsolateral VA-VL that showed intense VGluT2 immunoreactivity (H and I). The broken lines indicating the range of the VA-VL or VM were determined using adjacent Nissl-stained sections. Arrowheads in E indicate weak labeling found in the lateral portion of the mediodorsal thalamic nucleus (MDl). Scale bar in G applies to A, D and G; scale bar in I applies to B, C, E, F, H and I.
Kainic acid injection into the deep cerebellar nuclei

Finally, to elucidate the major source of the VGluT2-immunopositive varicosities in the motor thalamic nuclei, we injected kainic acid into the DCN (Fig. 7A–C and H1–H3). When a small amount of kainic acid injection was made in the DCN, neuronal depletion was observed in an area involving the interposed nucleus (Fig. 7A and C). By this treatment, large VGluT2-positive varicosities were significantly reduced in a part of the caudodorsal VA-VL contralateral to the injection site (Fig. 7D–F). However, neuropil immunoreactivity for GAD67 remained almost unchanged throughout the motor thalamic nuclei including the region where VGluT2-positive varicosities were depleted (Fig. 7G).

When a large volume of kainic acid solution was injected into the DCN, almost all neurons were abolished from the DCN of the injection side (Fig. 7H1–H3). In this case, large VGluT2-positive varicosities were almost completely abolished from a large part of the caudodorsal VA-VL contralateral to the injection site (Fig. 7D–F). However, neuropil immunoreactivity for GAD67 remained almost unchanged throughout the motor thalamic nuclei including the region where VGluT2-positive varicosities were depleted (Fig. 7G).

Discussion

The present results indicated that the motor thalamic nuclei, VM and VA-VL, contained large and small GABAergic axon varicosities and large to giant and small to medium-sized VGluT2-equipped varicosities. The vast majority of large GABAergic axon varicosities, which were highly depleted from a part of the posterior nuclei that was located close to the border between the VA-VL and posterior nuclei (Fig. 7l3), it seems likely that the VA-VL had an extension into the posterior nuclei beyond the border that is delineated in the atlas of Paxinos & Watson (2007). In contrast to an extensive depletion of large varicosities, small to medium-sized VGluT2-positive varicosities were preserved in the rostroventral VA-VL and VM even by the entire neuronal depletion of the DCN. These results clearly indicate that neurons of the DCN are the major source of large VGluT2-positive varicosities in the caudodorsal VA-VL, but not that of small to medium-sized varicosities in the rostroventral VA-VL and VM.
were distributed in the VM and rostroventral portion of the VA-VL, were derived from the SN and, to a lesser extent, from the GPi. In contrast, large to giant VGluT2-positive axon varicosities, which were restricted to the caudodorsal portion of the VA-VL, mostly originated from the DCN (Fig. 8).

In primate motor thalamic nuclei, it has been reported that the distributions of afferents from the cerebellar nuclei and basal ganglia output nuclei were almost segregated from each other in the VA, VM, and other thalamic nuclei. The segregation of these afferents is thought to play a crucial role in the functional organization of the motor thalamus.
oral and caudal parts of the VL, and oral part of the ventral posterolateral nucleus (for review, see Jones, 2007). Roughly speaking, the subdivisions of the primate motor thalamus, VA/VM, oral part of the VL and caudal part of the VL/oral part of the ventral posterolateral nucleus, receive afferents from the SNr, GPi and DCN, respectively, and these afferents overlap little, if at all, with one another. The afferents from the basal ganglia and DCN have been reported to show only a little overlap in the rat brain (Deniau et al., 1992), and this is supported by the present results. In addition, neurons in the dorsal and ventral portions of the SNr send axons to the VM and rostroventral VA-VL, respectively (Deniau & Chevalier, 1992; Sakai et al., 1998), and GPi neurons project to the crescent-shaped arc within the VA-VL as well as to the VM (Sakai & Bruce, 2004). Thus, the rostroventral VA-VL of the rat thalamus may be equivalent to the VA and oral part of the VL of the monkey thalamus, and the rat caudodorsal VA-VL to the monkey caudal part of the VL/oral part of the ventral posterolateral nucleus.

The target thalamic regions of the basal ganglia afferents, the afferents from the SNr and GPi, were not well segregated from each other, as shown by the previous reports in rats (Carter & Fibiger, 1978; Deniau et al., 1992; Sakai et al., 1998; Sakai & Grofova, 2002; Sakai & Bruce, 2004). In the present study, when we damaged SN neurons with the kainic acid injection, the large GAD67-positive axon varicosities were almost completely lost from the rostroventral VA-VL and VM (Fig. 6R and T) except the most caudal lateral portion of the rostroventral VA-VL. Although a considerable reduction of large varicosities was observed even in the most caudal lateral portion of the rostroventral VA-VL, some large GAD67-positive varicosities were preserved, suggesting that these unaffected varicosities were derived from the GPi and intermingled with the varicosities from the SNr. In contrast, in the remaining large part of the VA-VL as well as in the VM, the large GAD67-positive varicosities almost completely disappeared after the depletion of SN neurons. This indicates that, in terms of the amount of axon varicosities, the motor thalamic nuclei chiefly receive the basal ganglia information from the SNr in the rat.

Ventral medial thalamic nucleus and rostroventral portion of ventral anterior-ventral lateral thalamic nuclei

The VM of the rat thalamus was, in the present study, similar to the rostroventral VA-VL in immunostaining for GAD65, GAD67, VGAT, VGlut2, VGlut1 and calbindin as well as in Nissl staining. This was further confirmed quantitatively by the size measurement of varicosities immunopositive for GAD67 and VGlut2. GAD67-positive varicosities in both the VM and rostroventral VA-VL showed a bimodal size distribution with similar peaks and extents (Fig. 6). The size of the VGlut2-positive varicosities in the VM was similar to that in the rostroventral VA-VL, but much smaller than that in the caudodorsal VA-VL. When the anterograde tracer was injected into the SNr or GPi, anterograde labeling was observed in both the VM and rostroventral VA-VL, although more projections to the VM were found after the injection into the dorsal part than into the ventral part of the SNr as reported previously (Deniau & Chevalier, 1992; Nishimura et al., 1997; Sakai et al., 1998). By the chemical depletion of SN neurons, large GABAergic axon varicosities with a cross-sectional area of ≥ 4 μm² (Fig. 6) were mostly eliminated not only from the rostroventral VA-VL but also from the VM. In the previous electron-microscopic studies, nigrothalamic afferents have been reported to form large terminals in both the rostroventral VA-VL and VM with a maximum diameter of 2–4 μm (Sakai et al., 1998; Bodor et al., 2008), although the size was slightly smaller than that of GAD67-positive varicosities in the present study (median of the maximum diameter, 4.3–4.7 μm; Table 1). Thus, no clear difference was observed between afferents to the VM and those to the rostroventral VA-VL.

Glutamatergic cerebellar inputs to the thalamus

Glutamatergic terminals of cerebellar afferent axons to the VA-VL have been suggested to express VGlut2 in the monkey (Bodor et al., 2008) and rat (Kuramoto et al., 2009). The present results clearly showed that the varicosities of cerebellar afferent axons were immunopositive for VGlut2 in the rat. As no VGlut1 immunoreactivity was colocalized in VGlut2-positive varicosities in the VA-VL, the cerebellar afferent terminals were thought to be equipped with VGlut2 alone for glutamate uptake. This is supported by the fact that DCN neurons are negative for VGlut1 mRNA signals, but positive for VGlut2 signals (Hisano et al., 2002; Hioki et al., 2003). Furthermore, in a large part of the caudodorsal VA-VL, VGlut2-positive terminals were almost abolished by the nearly complete lesion of the DCN (Fig. 7). This suggests that neurons of
the caudodorsal VA-VL are driven principally by the cerebellar inputs.

The highly VGluT2-depleted region produced by the damage to DCN neurons extended into the posterior nuclei beyond the posterior border of the VA-VL. This result raises the question of the border between the VA-VL and posterior nuclei. Actually, although the posterior nuclei appear at Bregma –2.52 mm in rostrocaudally arranged frontal sections of the rat atlas used here (Paxinos & Watson, 2007), the nuclei do not appear up to Bregma –2.80 mm in a different atlas (Swanson, 2004), suggesting uncertainty about the border. The posterior border of the VA-VL might be more caudal because of the following reasons. (i) The highly VGluT2-depleted region was continuous from the VA-VL to the rostralmost part of the posterior nuclei in the present study. (ii) When the anterograde tracer was injected into the DCN, labeling was observed in the rostralmost part of the posterior nuclei (Donoghue et al., 1979). (iii) In the course of the single-cell labeling study of VA-VL neurons with viral vectors (Kuramoto et al., 2009), we encountered a neuron that was located at the rostralmost portion of the posterior nuclei. The neuron, like caudodorsal VA-VL neurons, projected mainly to the middle layers of

Fig. 7. Effects of neuronal depletion of the DCN. When a small amount of kainic acid was injected into the DCN, the region encircled with a dashed line (A) was affected and neuronal cell bodies were depleted from the region (C; cf. control side B). In the VA-VL of the contralateral hemisphere, VGluT2-immunoreactive varicosities were partly decreased (arrowheads in D and E). Small arrows in D indicate the sites in which images E and F were captured. In contrast, GAD67-immunoreactive varicosities in the next section displayed no significant difference between the ipsilateral and contralateral motor thalamic nuclei (G). When almost all of the neurons were lost from the DCN of one side (hatched area in H1–H3) by the injection of a larger amount of kainic acid, VGluT2-immunoreactive varicosities were highly (black filled area (I1–I6); E) and partially (stippled area) depleted from the caudodorsal VA-VL (VA-VLcd) and its surroundings, respectively (I1–I6). Int, interposed cerebellar nucleus; Lat, lateral cerebellar nucleus; LVe, lateral vestibular nucleus; Med, medial cerebellar nucleus; VeCb, vestibulocerebellar nucleus; see the legends of Figs 1 and 2 for the other abbreviations. Scale bar in C applies to B and C; scale bar in F applies to E and F; scale bar in G applies to D and G.
The origin of small GABAergic and small to medium-sized glutamatergic afferents in the rat motor thalamic nuclei

In the present study, both the cerebellothalamic and nigro-/pallidothalamic afferent fibers selectively formed large or giant, often elongated varicosities in the motor thalamic nuclei, supporting the previous reports in rat thalamus (Aumann et al., 1994; Aumann & Horne, 1996; Schwarz & Schmitz, 1997; Sakai et al., 1998; Bodor et al., 2008). Hence, the small GABAergic or small to medium-sized VGluT2-equipped axon varicosities are not likely to originate from the SNr/GPi or DCN. Small varicosities immunoreactive for GAD67, GAD65 and VGAT were distributed throughout the motor thalamic nuclei. The distribution of small GABAergic varicosities did not show a significant change after the neuronal depletion of the SN or DCN. It is known that GABAergic neurons in the thalamic reticular nucleus (for review, see Pinault, 2004), anterior pretectal nucleus (Bokor et al., 2005; Wanaverbecq et al., 2008) and zona incerta (for review, see Mitrofanis, 2005) projected to the thalamic nuclei. However, neurons in the anterior pretectal nucleus are known to project mainly to the higher-order sensory nuclei (Bokor et al., 2005) with large axon varicosities (Wanaverbecq et al., 2008), and neurons in the zona incerta also send axon fibers to the higher-order/association and intralaminar nuclei (Mitrofanis, 2005) with large terminals (Barthó et al., 2002). Thus, the main source of the small GABAergic terminals in the motor thalamic nuclei is likely to be the thalamic reticular nucleus (Fig. 8). This speculation is supported by the fact that the maximum diameter of axon varicosities derived from thalamic reticular nucleus neurons was rather small compared with GABAergic terminals of different origins, such as the anterior pretectal nucleus (Pinault & Deschênes, 1998; Wanaverbecq et al., 2008).

The origin of small to medium-sized VGluT2-positive axon varicosities in the rostroventral VA-VL and VM is unknown. When the anterograde tracer was injected into the DCN in the present study, a small number of axon varicosities were observed in the VM and rostroventral VA-VL as reported previously in the VM (Deniau et al., 1992; Aumann et al., 1994). However, as no significant changes were noticed in VGluT2-positive varicosities of the rostroventral VA-VL or VM after an extensive depletion of DCN neurons, DCN neurons may not be the major origin of those small to medium-sized VGluT2-positive axon varicosities. Some retrograde labeling studies have shown that the deep layers of the superior colliculus and subnucleus reticularis dorsalis of the medulla send projections to the VM (Herkenham, 1979; Villanueva et al., 1998; Desbois & Villanueva, 2001). Because many neurons in these regions expressed mRNA for VGluT2 (Fremeau et al., 2001; Herzog et al., 2001; Pang et al., 2009), the regions might be candidates for the sources of VGluT2-positive axon varicosities in the VM.

It is well known that most thalamocortical neurons are driven by large excitatory terminals of subcortical or cortical afferents, which are often called ‘driver inputs’ (Sherman & Guillery, 2001). However, as the main afferents to the rostroventral VA-VL and VM are inhibitory terminals derived from the basal ganglia, it is unknown what ‘drives’ the thalamocortical neurons receiving the basal ganglia inhibitory inputs. Although one candidate might be small cortical excitatory terminals as shown with VGluT1 immunoreactivity in the present study (Fig. 3), they are known to serve as ‘modulators’ but not as ‘drivers’ in thalamic nuclei other than the motor nuclei (Sherman & Guillery, 2001). We further noticed, in the present study, that VGluT1-containing varicosities in the rostroventral VA-VL and VM were as numerous and small as those in the caudodorsal VA-VL or other thalamic nuclei (Figs 1 and 3). It is thus unlikely that VGluT1-containing cortical afferents might work as ‘driver inputs’ in the VM and rostroventral VA-VL. In conclusion, the remaining candidate for the ‘drivers’ is small to medium-sized VGluT2-positive varicosities in the rostroventral VA-VL and VM, although their density was not so high in those subnuclei.

Supporting Information

Additional supporting information may be found in the online version of this article:
Fig. S1. The size distributions of VGluT2-immunopositive axon varicosities in the motor thalamic nuclei. In the VM (A), rostroventral VA-VL (B) and caudodorsal VA-VL (C) of normal rats, 300 VGluT2-positive axon varicosities were each selected as described for GAD67-positive varicosities in the Materials and Methods section, and the number of varicosities was plotted against the cross sectional area of the varicosities. Arrows indicate peak values after the histograms were well fitted with a single lognormal distribution. Please note: As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset by Wiley-Blackwell. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.


Supplementary Figure 1. The size distributions of VGluT2-immunopositive axon varicosities in the motor thalamic nuclei. In the VM, rostroventral VA-VL and caudodorsal VA-VL of normal rats, 300 VGluT2-positive axon varicosities were each selected as described for GAD67-positive varicosities in the Materials and Methods section, and the number of varicosities was plotted against the cross sectional area of the varicosities. Arrows indicate peak values after the histograms were well fitted with a single lognormal distribution.

Supplementary Fig. 1 (for online)
Kuramoto et al.,
Complementary distribution of glutamatergic cerebellar and GABAergic basal ganglia afferents to the rat motor thalamic nuclei