Neural Plasticity of Neonatal Hypoglossal Nerve for Effective Suckling

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ABSTRACT

The adaptive movement of the tongue after unilateral lesion of the hypoglossal (XII) nerve during the early postnatal days is essential for recovery of milk intake. The present study investigated the basic mechanisms underlying such adaptation, focusing on the neural plasticity that allows effective suckling. After resection of the ipsilateral XII nerve on P1, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlolate (DiI), a postmortem neuronal tracer, was applied to the contralateral uninjured XII nerve on P4 and P7. DiI-labeled fibers were successfully traced within the tongue and showed gradually increased extension over the XII nerve-injured side in the central core portion of the denervated tongue between P4 and P7. Systematic neuroanatomical experiments revealed that contralateral axonal sprouting occurred as early as 1 day after nerve injury (P2), and that such axonal sprouting occurred exclusively from the medial branch of the XII nerve responsible for tongue protrudor, an essential movement for suckling. These findings provide direct evidence of functional neural plasticity that allows effective suckling in XII nerve-injured newborns with suckling disturbance.

Key words: breastfeeding; tongue movement; DiI; axonal sprouting; neonatal rat

INTRODUCTION

Newborn mammals cannot survive without effective suckling. Recently, we reported the essential roles of the tongue in suckling in hypoglossal (XII) nerve-injured newborn rats (Fujita et al., 2006; Fukuyama et al., 2006). The XII nerve controls tongue movements and has two functionally different nerve branches: the medial branch related to protrusion of the tongue and the lateral branch related to its retraction (Lowe, 1978; Dobbins and Feldman, 1995; Fuller et al., 1999; Mu and Sanders, 1999; McClung and Goldberg, 2000). We showed that postnatal day (P) 1 pups with bilateral resection of either of the XII nerve components (main trunk: XII-trunk; medial branch: XII-med; lateral branch: XII-lat) failed to suckle milk and did not survive. Pups with unilateral XII nerve injury also showed disturbed suckling capability and lowered survival rates, except for those with unilateral XII-lat nerve injury that achieved sufficient milk intake and high survival rates. Some of the pups with unilateral XII-trunk or XII-med nerve injury were unsuccessful in suckling milk and died, whereas others in the same experimental groups gradually acquired suckling capability and survived. The surviving pups with unilateral XII-trunk or XII-med nerve injury on P1 showed marked increases in milk intake between P4 and P7, strongly suggesting adaptive tongue movements for effective suckling (Fujita et al., 2006).

Regarding the basic mechanisms for adaptation, we consider that neural control must be newly established during development on the nerve-injured side of the tongue. Neural plasticity ipsilaterally from the injured XII nerve seems unlikely to have occurred considering the vulnerability of neonatal motor neurons to axon injuries (Kuzis et al., 1999; Koyama et al., 2003) and our recent data showing that axotomized neonatal motor neurons had not extended regenerated fibers through the nerve-resected site 8 weeks after nerve injury (Higashiyama et al., 2005). Therefore, neural plasticity of the contralateral uninjured XII nerve seems more likely to have been involved.

To demonstrate neural plasticity from the uninjured XII nerve, we used 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlolate (DiI), a postmortem neuronal tracer, which has been proven to be useful in visualizing fiber connections in the immature nervous system (Godement et al., 1987; O'Leary and Terashima, 1988; Vanselow et al., 1989; Loeliger et al., 2000; Krimm et al., 2001; Zhang and Ashwell, 2001). Applying DiI to the XII nerve contralateral to the nerve-injured side, we demonstrated direct evidence of the neural plasticity of the uninjured XII nerve in developing pups with XII nerve injuries during the neonatal stage.

MATERIALS AND METHODS

Animals

Newborn rat pups of the Wistar strain (Japan SLC Inc., Hamamatsu, Shizuoka, Japan) were used in this study. P0 refers to the first 24 h after birth. All procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and protocols were approved by our Institutional Animal Care and Use Committee. Every effort was made to minimize animal suffering and pain as well as the number of animals used necessary to produce reliable scientific date.

Before starting the experiments on P1 pups, we observed the dam's maternal behavior (Jirik-Babb et al., 1984; Yeo and Keverne, 1986; Kinsley et al., 1994) and the pup's gastric milk. If the dam did not show maternal behavior and the pups did not contain milk in their stomachs, the animals were excluded from the experiments as described in our previous reports (Fukushima et al., 2006; Fujita et al., 2006; Fukuyama et al., 2006). Thus a total of 49 newborn pups from 6 pregnant dams were used in the present study.

Surgical procedures

Surgical manipulations of neonatal pups were performed on P1 under general anesthesia with hypothermia (-20°C, 15 min). Under a surgical microscope, the right

XII-trunk nerve was exposed and a length of about 1.5 mm was resected (n = 21). In a subset of the pups, resection of the right XII-med (n = 4) or XII-lat (n = 4) nerve was performed in the same manner. In the control pups, the right XII nerve was exposed and left intact (n = 20). Great care was taken to expose the nerve itself and not to damage the surrounding tissues, including the tongue.

After these surgical procedures, pups were subcutaneously injected with acetated Ringer's solution with 5% glucose (50 mL/kg) to increase their survival rates, and returned to the dam. Acetated Ringer's solution with glucose has the following ionic content (mEq/L): Na 130, K 4, Ca 3, Mg 1, Cl 109, and acetate 28. The dams and pups were kept in a single cage (26 cm x 42 cm x 18 cm) under standard laboratory conditions with a 12 h light/dark cycle, and room temperature of 22±1 °C. Food and water were supplied *ad libitum*. Acetated Ringer's solution with glucose was injected once a day until the weights of the pups increased. The pups were housed with the dam until P4 or P7 at the time of tracer application.

Postmortem neuronal tracing

After resection of the right XII nerve components on P1, the animals were deeply anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and perfused through the

heart with 10 ml of 4% paraformaldehyde in 0.1 M phosphate buffer on P4 or P7. The uninjured left XII-trunk nerve was exposed and resected under a surgical microscope. Then DiI (Molecular Probes, Eugene, OR) crystals were placed on the distal stump of the nerve for tracing of the nerve extension to the tongue. DiI applications were always performed at the XII-trunk nerve throughout the experiments. Namely, to trace fibers derived from the XII-med or XII-lat nerve, DiI was placed on the transected XII-trunk nerve after postmortem resection of the XII-lat or XII-med nerve, respectively. In the control rats, DiI labeling was performed in a similar way. The details of the nerve resections and DiI applications are shown in Figure 1.

To know the earliest date of axonal sprouting from the uninjured XII-trunk nerve, part of the unilateral (right) XII-trunk nerve-resected P1 pups (n = 5) received transcardial perfusion on P2 (n = 3) or P3 (n = 2), and DiI crystals were placed on the distal stump of the contralateral (left) XII-trunk nerve in the same manner as described above.

The pups were incubated in 0.4% paraformaldehyde in 0.1 M phosphate buffer at 37°C in the dark for three months for postmortem anterograde neuronal tracing to the tongue. After the incubation period, the pup's tongue was removed and cut into serial sections 200 μm thick along the frontal plane using a vibratome. Sections were

collected at 1-mm intervals (8-9 sections per animal), mounted in 50% glycerol, and examined for the extent of DiI-labeled nerve fibers.

Retrograde neuronal tracing

Adult Wistar rats (female, 11-12 weeks old, 150-170 g, *n* = 12) were used to examine the possibility of contralateral axonal sprouting following unilateral XII-trunk nerve injury at the adult stage. All surgical manipulations were done under general anesthesia with sodium pentobarbital (50 mg/kg, i.p.). First, as the preliminary experiments, the rats (*n* = 3) received different volumes of 3 injections of 2% Fluoro-Gold (FG) (Fluorochrome, Denver, CO), a fluorescent retrograde neuronal tracer, into the center of the right tongue rostrocaudally at the level of its anterior two-thirds (*n* = 1: 0.1 μl x 3; n = 1: 0.2 μl x 3; *n* = 1: 0.3 μl x 3). The animals were allowed to survive for 4 days, and perfused through the heart with 300-400 ml of 4% paraformaldehyde in 0.1 M phosphate buffer. The tongue and the brainstem were removed, postfixed overnight in the same fixative, and soaked in 30% sucrose for 2 days. They were cut into serial sections 30 μm (brainstem; transverse plane) and 100 μm (tongue; frontal plane) thick using a freezing microtome. These sections were observed with a fluorescence microscope. Injections of 0.5 μl of FG consistently produced massive

diffusion of the tracer contralaterally to the left tongue, and resulted in bilateral neuronal labeling in the XII nucleus. In case of 0.2 μl of FG injections, the tracer sometimes slightly diffused contralaterally to the left tongue, and caused rare neuronal labeling in the contralateral XII nucleus. However, 0.1 μl of the injected FG was always confined within the right tongue, and no neuronal labeling occurred in the contralateral XII nucleus. Based on these results, each injection volume of the tracer was determined as 0.1 μl in the following experiments.

After exposing the right XII-trunk nerve, nerve resection was made in a length of about 1.5 mm (n = 6). In the control rats, the right XII nerve was exposed and left intact (n = 3). They were allowed to survive for 4 (control: n = 1; resected: n = 2), 7 (control: n = 1; resected: n = 2), and 14 (control: n = 1; resected: n = 2) days. The control and nerve-injured rats received 0.1 μl of 7 injections of FG into the center of the right tongue in a rostrocaudal sequence at the level of its anterior two-thirds. Four days later, the rats were perfused transcardially with a fixative, and frozen sections of the tongue and the brainstem were examined with a fluorescence microscope in the same manner.

RESULTS

Postnatal growth and survival

The control pups showed a steady increase in body weight between P1 and P7, whereas all pups with unilateral XII nerve injury on P1 showed decreased body weight on P2. The pups with unilateral XII-trunk nerve injury began to show increased body weight between P2 and P3 (50%), between P3 and P4 (25%) or between P4 and P5 (25%) in the surviving cases. Only four pups in this group showed continual decreases in body weight and died by P5. Increases in body weight occurred between P2 and P3 in pups with unilateral XII-lat nerve injury (100%), and later between P3 and P4 in those with unilateral XII-med nerve injury (100%). Compared to control pups, the body weights of pups with unilateral XII-trunk, XII-med and XII-lat nerve injury were reduced by 73%, 75% and 95% on P4, and 63%, 66% and 90% on P7, respectively. Survival rates on P4 were reduced by 81% (13/16) in the XII-trunk nerve-injured group, 100% (4/4) in the XII-med nerve-injured group and 100% (4/4) in the XII-lat nerve-injured group.

DiI-labeled fibers traced from XII-trunk nerve on P4 and P7

In the DiI neuronal labeling, we failed to trace the XII nerve in 35% (7/20) of the control group and 25% (5/20) of the nerve-injured group. In such failed cases, DiI

labeling occurred only in thick fiber bundles in the posterior one-third of the left tongue, a region close to the site of DiI application. In the successful cases of the control pups, DiI-labeled fibers derived from the left XII-trunk nerve were distributed throughout the left tongue including its apex in the antero-posterior axis, and from the lateral to the midline in the medio-lateral axis on P4 (Fig. 1 (1) and Fig. 2A) and P7 (Fig. 1 (1) and Fig. 2C).

In pups with XII-trunk nerve injury, DiI-labeled fibers derived from the left XII-trunk nerve showed distribution patterns similar to those fibers in the controls, with the additional interesting finding that DiI-labeled fibers extended across the midline to the right tongue on the nerve-injured side. Serial tongue sections demonstrated that the contralateral fiber extension occurred in the middle one-third of the right tongue and increased in length between P4 (Fig. 1 (4) and Fig. 2B) and P7 (Fig. 1 (4) and Fig. 2D). The maximal lengths of fiber elongation from the midline were 350 μm on P4 and 600 μm on P7.

DiI-labeled fibers traced from XII-med and XII-lat nerves on P4 and P7

In the control pups, DiI-labeled fibers derived from the left XII-med nerve were localized in the central core portion of the left tongue, leaving its outer peripheral

portion unlabeled by DiI on P4 and P7 (Fig. 1 (2) and Fig. 3A). In contrast, those fibers derived from the left XII-lat nerve occupied the outer peripheral portion of the left tongue on P4 and P7 (Fig. 1 (3) and Fig. 3C).

In pups with XII-trunk nerve injury, DiI-labeled fibers derived from the left XII-med nerve showed distribution patterns similar to those fibers in the controls. Contralateral fibers crossing beyond the midline were traced from the left XII-med nerve on P4, and had extended further on P7 (Fig. 1 (5) and Fig. 3B). DiI-labeled fibers derived from the left XII-lat nerve also showed distribution patterns similar to those fibers in the controls, but there were no contralateral fibers crossing from the left XII-lat nerve to the right tongue detected on either P4 or P7 (Fig. 1 (6) and Fig. 3D).

DiI-labeled fibers in combination with different nerve branch injuries on P7

After right XII-med nerve injury on P1, DiI-labeled fibers were traced from the left XII-med (Fig. 1 (7) and Fig. 3E) or XII-lat (Fig. 1 (8) and Fig. 3G) nerve on P7. In addition, similar fiber tracings from the left XII-med (Fig. 1 (9) and Fig. 3F) or XII-lat (Fig. 1 (10) and Fig. 3H) nerve were observed following right XII-lat nerve injury on P1. Out of the four types of combined experiments with nerve injuries and DiI applications on different nerve branches, contralateral fiber extension to the right tongue occurred

only from the left XII-med nerve in pups with right XII-med nerve injury (Fig. 3E).

DiI-labeled fibers traced from XII-trunk nerve on P2 and P3

Contralateral fiber extension from the left XII-trunk nerve occurred as early as on P2 (1 day after nerve injury) in one case (1/3) (Fig. 3I), and on P3 (2 days after nerve injury) in two cases (2/2) of the unilateral (right) XII-trunk nerve-resected P1 pups. The experimental animals and DiI-labeled fibers extending across the midline are summarized in Table I.

FG-positive XII motor neurons

Careful analyses of serial sections of all injection sites of FG showed that the tracer was well confined within the right tongue and did not diffuse into the left tongue at each injection site of the control and nerve-injured rats (Fig. 4A). In the control rats, there were a substantial number of FG-positive neurons in the XII nucleus on the right side, but not on the left side. FG-positive neurons at different levels of the XII nucleus are shown in Figures 4B-4D. In the unilaterally XII-trunk nerve-injured rats, FG-positive neurons never occurred in the XII nucleus on both sides at any survival periods of 4, 7, and 14 days after nerve insult (Fig. 4E).

DISCUSSION

DiI-labeled fibers in the tongue traced from XII nerve

Postmortem fiber tracing with carbocyanine DiI, a lipophilic tracer, has been widely used for anterograde and retrograde labeling to investigate fiber tracts of the cranial nerves (Godement et al., 1987; Vanselow et al., 1989; Loeliger et al., 2000; Krimm et al., 2001; Zhang and Ashwell, 2001). To our knowledge, however, there are no data available concerning anterograde fiber tracing of the XII nerve to the tongue. We have revealed in the anterogradely DiI-labeled tongue that the XII-med and XII-lat nerves showed completely different distribution patterns in the tongue with no apparent overlapping. DiI-labeled fibers derived from the XII-med nerve innervated the central core portion of the tongue, which seems to correspond to the tongue protrudor muscles, including the extrinsic genioglossus muscle, and intrinsic vertical and transverse muscles. On the other hand, those fibers derived from the XII-lat nerve innervated its outer peripheral portion, which seems to correspond to the tongue retractor muscles, including the extrinsic styloglossus and hyoglossus muscles, and intrinsic superior and inferior longitudinal muscles (O'Reilly and FitzGerald, 1990; Mu and Sanders, 1999; McClung and Goldberg, 2000; Sokoloff, 2000).

Axonal sprouting by contralateral uninjured XII nerve

It is known for very long time that in the peripheral nervous system, axonal sprouting occurs after denervation. However, since few organs are not bilateral, it is true that there are very few examples of axonal sprouting across the midline in the peripheral nervous system (Kinnman and Aldskogius, 1988). The present study confirmed that the XII nerve provided strictly ipsilateral innervation to the tongue under normal conditions, and demonstrated that contralateral axonal sprouting was induced to penetrate into the denervated tongue following unilateral XII nerve injury. Additional experiments using a retrograde tracer in adult rats denied the possibility of such contralateral axonal sprouting at the adult stage. Competitive axonal sprouting by ipsilateral and contralateral nerve fibers following unilateral nerve injury is a matter of great concern in neuroplasticity. Blake-Bruzzini et al. (1997), using a retrograde tracing method, reported occurrence of ipsilateral regeneration 14 days after unilateral crush or transection injuries on the XII nerve in rats of different postnatal ages (P10, P14 and P21). According to Kinnman and Aldskogius (1988), ipsilataral regeneration was induced by the lingual nerve 4-12 weeks after unilateral transection of the chorda tympani nerve, while contralateral regeneration was induced by the lingual nerve 8-12

weeks after unilateral transection of the lingual and chorda tympani nerves in adult rats. Differences between our results and those of these authors were ages of animals at nerve insult and types of nerve injuries. Nerve injuries at the neonatal stage, regardless of their types (crush, transection or resection), are well known to cause a substantial number of neuronal cell death in motoneurons (Kuzis et al., 1999; Koyama et al., 2003), and nerve resections used in our study did not seem to permit ipsilateral regeneration, as shown by no retrograde neuronal labeling ipsilaterally from the resected XII nerve even in adult rats. Taken all together, it is considered that complete unilateral lesions of the XII nerve induced contralateral axonal sprouting in neonates, but not in adults.

Functional neuroplasticity

Numerous studies demonstrating axonal sprouting have been published in the peripheral as well as central nervous systems (Murakami et al., 1992; Wilson and Kitchener, 1996; Ding et al., 2005; Buchli and Schwab, 2005; Wieloch and Nikolich, 2006). However, evidence for neuroplasticity closely associated with neural function appears to remain limited. Two factors are considered to be important for functional recovery (milk-suckling) in the unilaterally XII nerve-injured pups with suckling disturbance. One is onset of axonal sprouting, and the other is nature of reinnervated

tongue muscle.

Contralateral axonal sprouting occurred as early as 1 day (P2) after nerve injury, and proceeded further until 6 days (P7) after nerve injury. It seems surprising that only one or two days are needed for axonal sprouting. Early onset of nerve reinnervation is of considerable significance for survival of neonatal pups, because the XII nerve-injured pups without milk intake mostly died 2-3 (P3-P4) days after nerve injury (Fujita et al., 2006; Fukuyama et al., 2006). At the later stages, advances in axonal sprouting between P4 and P7 corresponded well with the timing of marked increment in milk intake in pups with unilateral XII-trunk (P4: 30% of the control value; P7: 52%) or XII-med (P4: 36%; P7: 71%) nerve injury (Fujita et al., 2006).

We have demonstrated the important roles of tongue protrudor muscles in suckling by showing differences in milk intake, postnatal growth and survival rates between the unilateral XII-med and XII-lat nerve-injured neonatal pups (Fujita et al., 2006; Fukuyama et al., 2006). Our systematic neuroanatomical studies revealed that neural plasticity occurred exclusively from the XII-med nerve, and sprouting fibers were confined to the central core portion of the denervated tongue, a presumed region of XII-med nerve innervation. The establishment of the original relationships between the XII-med nerve and the tongue protrudor muscles by sprouting fibers seems to have

contributed substantially to the functional recovery of tongue movements during milk suckling. Tongue muscles are characterized by their bilateral symmetric movements during suckling, and thus unilateral injury to the XII-trunk or XII-med nerve did cause serious suckling disturbance (Fujita et al., 2006; Fukuyama et al., 2006), but axonal sprouting from the contralateral uninjured XII-med nerve may have helped to achieve bilateral protruding functioning of the unilaterally denervated tongue.

Another concern is whether contralateral axonal sprouting depended on a factor of distance or of specificity. The former factor seems unlikely, because the XII-lat nerve that did not show axonal sprouting also reached the midline of the tongue, and the distance to the denervated side was just about the same as the XII-med nerve. It may be some underlying molecular mechanisms relating to specific nerve-muscle interactions representing myotopic organization, but we can not provide a reasonable explanation for the absence of neuroplasticity of the XII-lat nerve.

Extension of post-lesion neuroplasticity during time-course is an important issue to be discussed. Sprouting fibers innervated the restricted area (middle one-third) in the central core portions of the denervated tongue on P4 and P7. It is of particular interest whether axonal sprouting thereafter spreads widely within the denervated tongue, but we could not determine this because of difficulty in DiI staining of fibers at

the later developmental stage (P11) (unpublished data). Nerve maturation, a factor unfavorable for labeling by DiI, seems to occur in the XII nerve between P7 and P11, which corresponds to the myelination stage (Fukuyama et al., 2006). Further studies using anterograde tracing methods other than DiI will be needed to solve this.

Survival

Survival of newborn rats with unilateral XII nerve injury was greatly improved as shown by the survival rates in the previous (XII-trunk: 20%-38%; XII-med: 24%-28%; XII-lat: 92%-93%) (Fujita et al., 2006; Fukuyama et al., 2006) and present (XII-trunk: 80%; XII-med: 100%; XII-lat: 100%) papers. Artificial nutrition with acetated Ringer's solution containing glucose substantially contributed to the higher survival rates of those pups. The XII nerve-injured pups could survive longer due to artificial nutrition while neural plasticity allowed gradual reinnervation, then the animals could suckle efficiently and their survival rates were elevated. We consider that neural plasticity is a common phenomenon and inducible within a few days in this experimental model, and thus one or two days' longer survival seems enough to acquire prolonged survival of newborn pups with XII nerve injury.

In conclusion, we demonstrated neural plasticity of the uninjured XII nerve

fibers extending beyond the midline of the tongue in pups with unilateral injuries to the XII-trunk or XII-med nerve at the neonatal stage (P1). Neural plasticity was only apparent in the XII-med nerve responsible for tongue protrusion, an essential movement for milk suckling. Neural plasticity was demonstrated on P2 (1 day after nerve injury), and increased between P4 and P7 when pups with XII nerve injury showed greatly increased milk intake.

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FIGURE LEGENDS

Fig. 1. A schematic drawing (**upper left, 1**) shows bilaterally exposed XII nerves. A XII-trunk nerve (double arrows) bifurcates into XII-med (arrowhead) and XII-lat (asterisk) nerves. Experimental designs (**1-10**) show nerve resections and DiI applications. Resections of XII nerve components were made on the right side on P1. Dashed lines indicate the sites of postmortem nerve resections on the left side on P4 or P7 for specific DiI labeling from XII nerve components. DiI was applied to XII-trunk nerves on the left side. **1-3:** Control pups. DiI was applied to trace XII-trunk (**1**), XII-med (**2**) and XII-lat (**3**) nerves. **4-6:** After resections of right XII-trunk nerves, DiI was applied to trace XII-trunk (**4**), XII-med (**5**) and XII-lat (**6**) nerves. **7, 8:** After resections of right XII-med nerves, DiI was applied to trace XII-med (**7**) and XII-lat (**8**) nerves. **9, 10:** After resections of right XII-lat nerves, DiI was applied to trace XII-med (**9**) and XII-lat (**10**) nerves.

Fig. 2. DiI-labeled fibers traced from left XII-trunk nerves of control pups on P4 (**A**) and P7 (**C**). After resections of right XII-trunk nerves on P1, DiI-labeled fibers traced from left XII-trunk nerves can be seen to extend over the right side on P4 (**B**) and P7 (**D**). Note the advanced extension of DiI-labeled fibers on P7. Arrowheads point to

midlines of tongues. The numbers between brackets correspond to the experiments described in figure 1. Scale bar, 1 mm.

Fig. 3. **A, C:** Control P7 pups. DiI-labeled fibers traced from left XII-med (**A**) and XII-lat (**C**) nerves. **B, D:** P7 pups with resections of right XII-trunk nerves on P1. DiI-labeled fibers traced from a left XII-med nerve (**B**) extend over the right side with no such extension by DiI-labeled fibers derived from a left XII-lat nerve (**D**). **E, G:** P7 pups with resections of right XII-med nerves on P1. Note the occurrence of axonal sprouting contalaterally from a left XII-med nerve (**E**), but not from a XII-lat nerve (**G**). **F, H:** P7 pups with resections of right XII-lat nerves on P1. Note the absence of axonal sprouting contralaterally from left XII-med (**F**) and XII-lat (**H**) nerves. **I:** P2 pup with resection of a right XII-trunk nerve on P1. Contralaterally directed DiI-labeled fibers traced from a left XII-trunk nerve. Arrowheads point to a midline of a tongue. The numbers between brackets correspond to the experiments described in figure 1. Scale bar, 1 mm.

Fig. 4. **A:** FG-injected tongue in a control adult rat. Note that the tracer is localized in the right tongue with no diffusion to the contralateral side. Scale bar, 1 mm. **B-D:**

FG-positive neurons retrogradely labeled from the right tongue in a control adult rat. Photomicrographs are taken from serial sections at different levels (**B,** rostral; **C,** middle; **D,** caudal) of the XII nucleus. Note neuronal labeling only in the ipsilateral XII nucleus. **E:** Fourteen days after resection of the right XII-trunk nerve in an adult rat. No FG-positive neurons are found in the XII nucleus on both sides. Scale bar, 500 μm.

TABLE I. Summary of DiI-labeled Fibers Extending across the Midline of the Tongue in Surviving Pups

DiI application		Nerve resection site (P1)	DiI labeling		Fiber extension across the midline
Age	Site		Successful	Failed	
P2	XII-trunk	XII-trunk	3	0	1
P3	XII-trunk	XII-trunk	2	0	2
P4	XII-trunk	XII-trunk	1	1	1
P4	XII-med	XII-trunk	2	0	2
P4	XII-lat	XII-trunk	1	1	0
P7	XII-trunk	XII-trunk	1	1	1
P7	XII-med	XII-trunk	1	1	1
P7	XII-lat	XII-trunk	1	1	0
P7	XII-med	XII-med	2	0	2
P7	XII-lat	XII-med	2	0	0
P7	XII-med	XII-lat	2	0	0
P7	XII-lat	XII-lat	2	0	0

Fig. 1

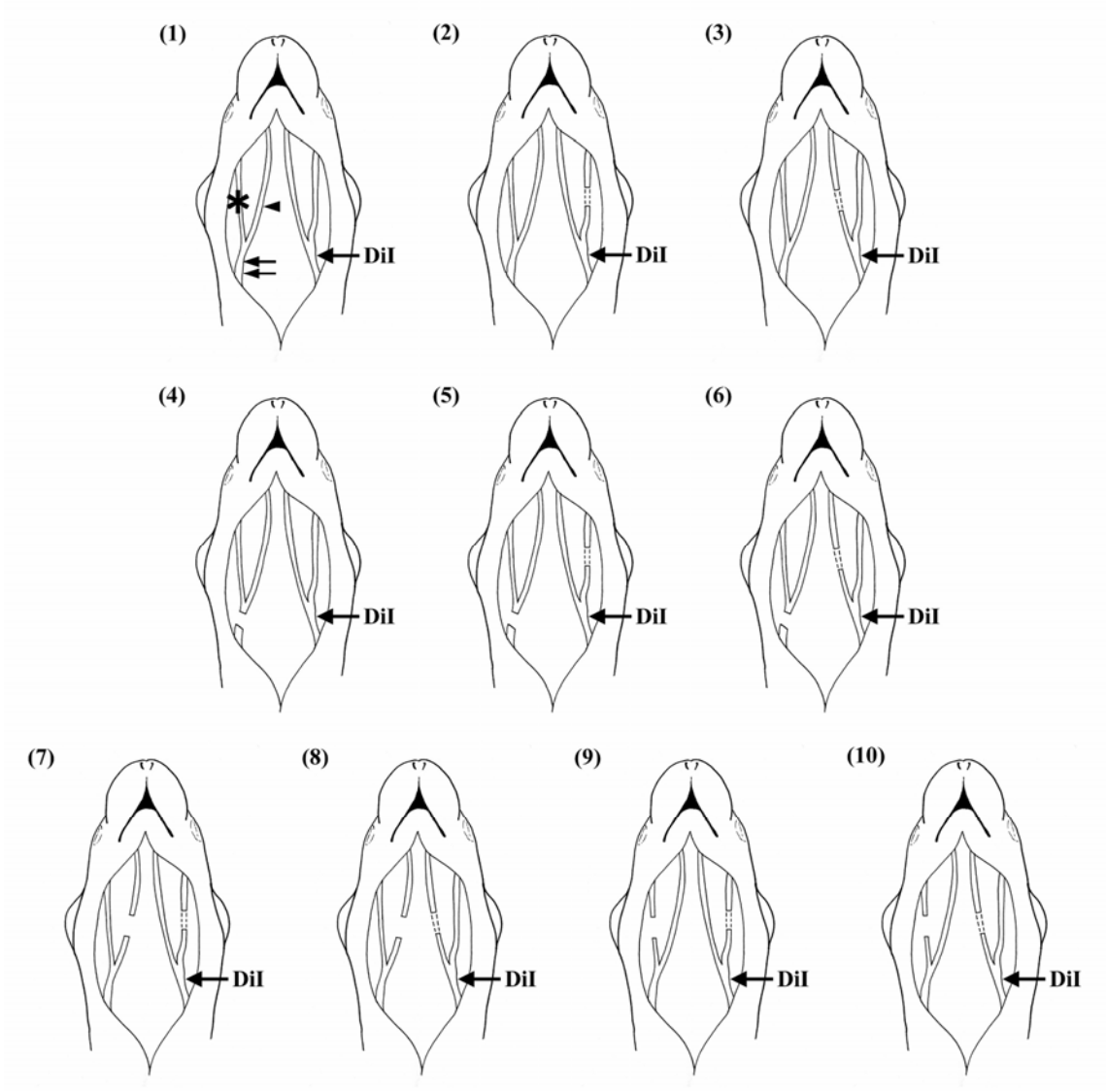

Fig. 2

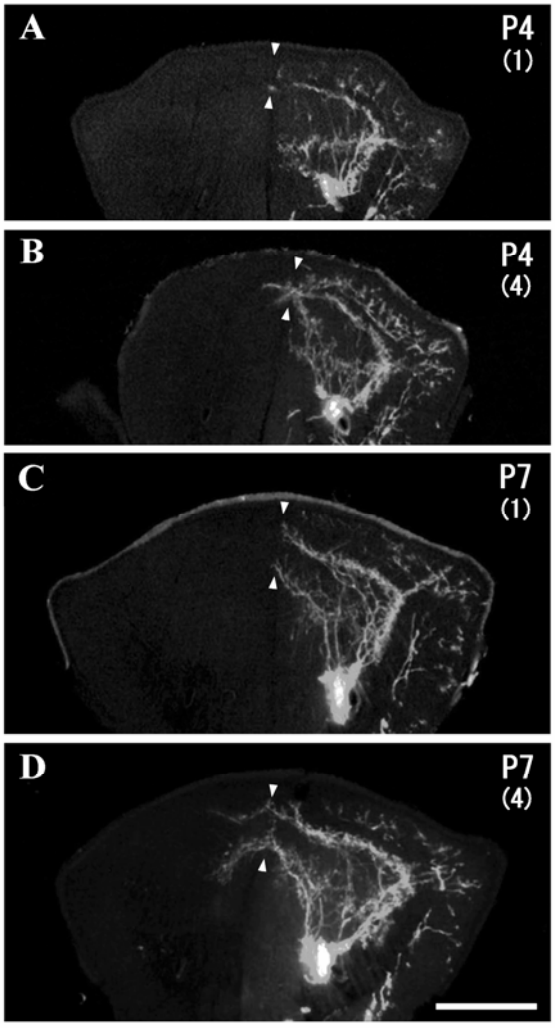

Fig. 3

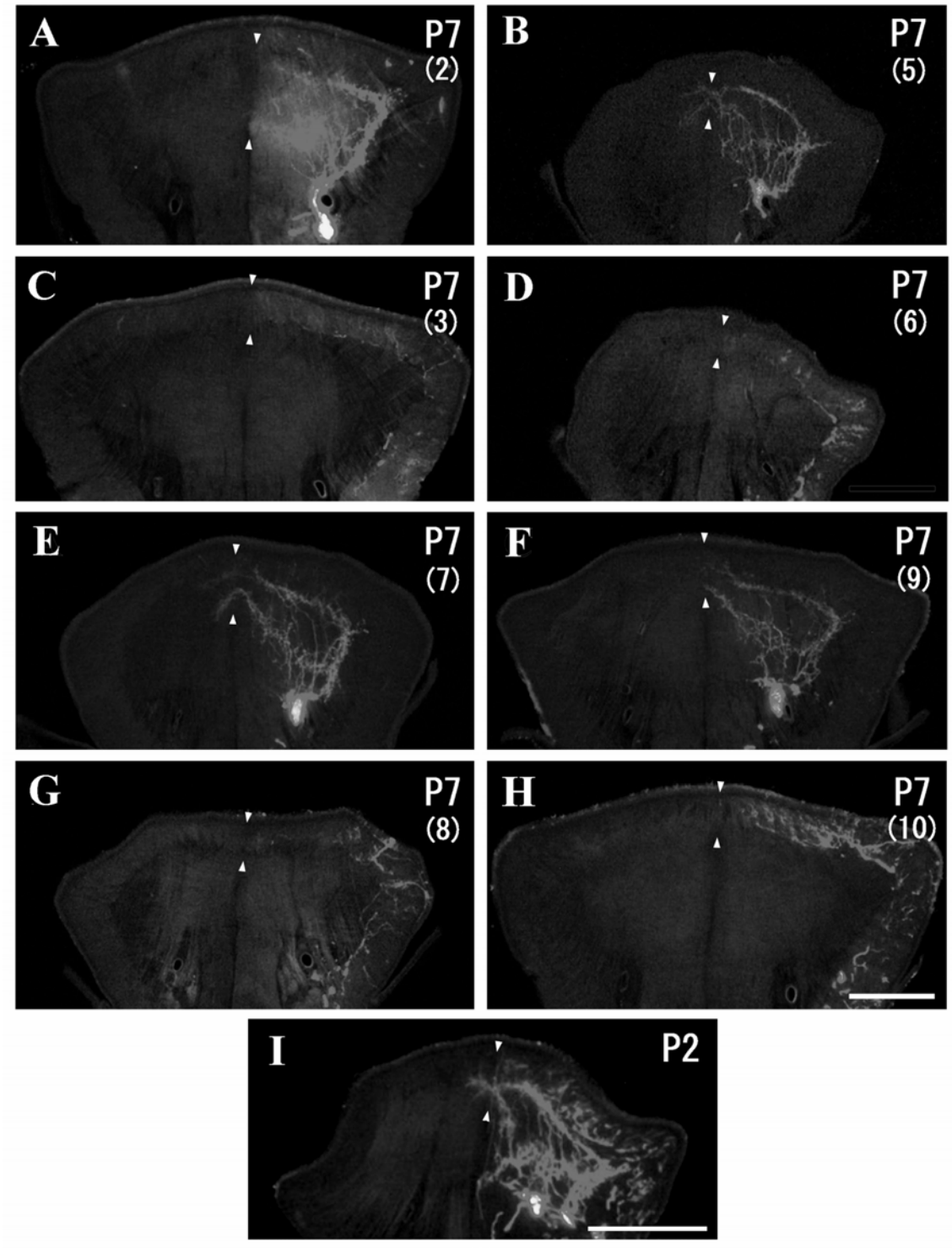

Fig. 4