Neuron Glia Biology, 2011, 7(1), 55–66. © Cambridge University Press, 2011
doi:10.1017/S1740925X11000135

Using comparative anatomy in the axotomy model to identify distinct roles for microglia and astrocytes in synaptic stripping

SHOZO JINNO[1] AND JUN YAMADA[2]

The synaptic terminals' withdrawal from the somata and proximal dendrites of injured motoneuron by the processes of glial cells following facial nerve axotomy has been the subject of research for many years. This phenomenon is referred to as synaptic stripping, which is assumed to help survival and regeneration of neurons via reduction of synaptic inputs. Because there is no disruption of the blood–brain barrier or infiltration of macrophages, the axotomy paradigm has the advantage of being able to selectively investigate the roles of resident glial cells in the brain. Although there have been numerous studies of synaptic stripping, the detailed mechanisms are still under debate. Here we suggest that the species and strain differences that are often present in previous work might be related to the current controversies of axotomy studies. For instance, the survival ratios of axotomized neurons were generally found to be higher in rats than in mice. However, some studies have used the axotomy paradigm to follow the glial reactions and did not assess variations in neuronal viability. In the first part of this article, we summarize and discuss the current knowledge on species and strain differences in neuronal survival, glial augmentation and synaptic stripping. In the second part, we focus on our recent findings, which show the differential involvement of microglia and astrocytes in synaptic stripping and neuronal survival. This article suggests that the comparative study of the axotomy paradigm across various species and strains may provide many important and unexpected discoveries on the multifaceted roles of microglia and astrocytes in injury and repair.

Keywords: Axotomy, comparative anatomy, glial augmentation, neuronal survival, synaptic stripping

INTRODUCTION

Microglia and astrocytes play central roles in maintaining brain homeostasis (Graeber and Streit, 2010; Halassa and Haydon, 2010). Both types of glial cells secrete a myriad of bioactive substances, and regulate the activity of neurons by physical and humoral factors (Banati *et al.*, 1993; Raivich *et al.*, 1999; Schwartz et al, 2006). For instance, astrocytes provide structural and metabolic support for neurons and control synaptic transmission (Haydon, 2001; Horner and Palmer, 2003; Perea *et al.*, 2009). Microglial cells continuously survey the local microenvironment and work as housekeepers in the brain (Davalos *et al.*, 2005; Nimmerjahn *et al.*, 2005). In addition to these beneficial functions, glial cells mediate detrimental effects on neurons under specific circumstances. Namely, reactive astrocytes impede neuronal survival and regeneration by forming scar tissue and synthesizing neurotoxic molecules, i.e., nitric oxide and arachidonic acid metabolites (Marchetti and Abbracchio, 2005). Activated microglia kill and phagocytose damaged neurons following excessive and uncontrolled adverse stimulation (van Rossum and Hanisch, 2004; Cardona *et al.*, 2006). Altogether, the evidence to date suggests that glial cells may act as a double-edged sword with protective features predominating in the healthy nervous system and destructive properties in certain pathological conditions (Biber *et al.*, 2007).

Corresponding author:
Shozo Jinno
Email: sjnno@med.kyushu-u.ac.jp

The axotomy paradigm offers systematic and detailed study of glial reactions and neuronal cell death in the central nervous system. Because there is no disruption of the blood–brain barrier or infiltration of macrophages, the axotomy model has the advantage of selectively stimulating the reactions of resident glial cells (Moran and Graeber, 2004). The changes in neuron–glia interactions are one of the most extensively studied events occurring after axotomy. The seminal work by Blinzinger and Kreutzberg (1968) has indicated that perineuronal microglia might actively engage in the displacement of synaptic boutons from the surface of regenerating motoneurons in the axotomized rat facial nucleus. This phenomenon is referred to as synaptic stripping (Graeber *et al.*, 1993), which has been considered to promote survival and regeneration of axotomized motoneurons. Although a number of studies have aimed to elucidate the mechanisms underlying synaptic stripping following axotomy, the roles of glial cells in this phenomenon are still under debate.

Here we suggest that species and strain differences that are often ignored in previous papers might be related to the current controversies surrounding the interpretation of axotomy studies. For instance, the survival ratios of axotomized neurons were higher in rats than in mice (Kiryu-Seo *et al.*, 2005). However, the majority of studies were devoted only to glial reactions and synaptic stripping, without accounting for differences in neuronal survival between species. In the first part of this article, we summarize and discuss the current knowledge on species and strain differences in neuronal survival, glial augmentation and synaptic

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

stripping. In the second part, we focus on our recent studies using rats and mice, which unveil the differential involvement of microglia and astrocytes in synaptic stripping and neuronal survival after axotomy (Yamada *et al.*, 2008, 2011; Yamada and Jinno, 2011). Ultimately, we consider the species and strain differences seen in the axotomy paradigm to offer novel and important insights into the glial involvement in synaptic stripping, neuronal regeneration and apoptotic cell death.

SPECIES AND STRAIN DIFFERENCES IN THE AXOTOMY PARADIGM: AN OVERVIEW OF PAST AND PRESENT STUDIES

Neuronal viability

Retrograde changes in neuronal cell bodies after peripheral nerve transection were first recognized more than 100 years ago (Nissl, 1894). After peripheral nerve injury, affected neuronal somata undergo a series of metabolic and structural changes (Snider *et al.*, 1992). Central chromatolysis occurs almost exclusively in response to axon injury and is present in many but not all axotomized neurons, though transiently and acutely (Barron, 2004). It is marked by rounding and enlargement of the soma and nucleus, disaggregation and peripheral dispersion of the somal ribonucleoprotein aggregates and clearing of the cytocentrum. The basic degenerative and regenerative responses of axotomized neurons were well documented at the light microscopic level by the early part of the 20th century (Cajal, 1928; Sofroniew, 1999). In the late 20th century, several studies examined the responses of neurons to axotomy by electron microscopy. For instance, Hornung *et al.* (1989) observed the formation of cytoplasmic vacuoles in the soma and dendrites of dying neurons in the developing chick isthmo-optic nucleus after mimicking axotomy via colchicine injections. Intravascularly injected horseradish peroxidase labeled vacuoles but not the free cytoplasm, indicating that the uptake occurred by endocytosis rather than by leakage through holes in the membrane.

Up to the present time, a number of studies have reported the fate of axotomized neurons (Table 1). At 10–12 weeks after facial nerve axotomy of adult Sprague–Dawley (SD) rats, the survival ratios of motoneurons were 80% in males and 70% in females (Yu, 1988). In the dorsal motor nucleus of the vagus (DMV) and in the hypoglossal nucleus of the SD rat, 70 and 100% of neurons survived 28 days after axotomy (D28), respectively (Aldskogius *et al.*, 1980). At D28 and 2 months after hypoglossal nerve injury, almost all motoneurons survived in the Wistar rat (Kiryu-Seo *et al.*, 2005; Yamada *et al.*, 2008). Age dependency in the neuronal survival after axotomy has also been reported. After crush lesion of the facial nerve in the albino rat, the survival ratio of motoneurons was about 100% in adult animals whereas the ratio was 10% in newborn animals (Søreide, 1981a). On the other hand, axotomy-induced cell death is probably more severe in mice than in rats. Oh *et al.* (1994) reported that only 35% of axotomized facial motoneurons survived at D21 in C57BL/6J mice. At 8 weeks after the hypoglossal nerve axotomy, 20% of motoneurons persisted in C57BL/6 mice (Kiryu-Seo *et al.*, 2005). In the DMV, 25–35% of lesioned motoneurons survived at D28 in C57BL/6N mice (Yamada *et al.*, 2008). Several strain differences in neuronal viability were found in mice. At D35 of hypoglossal nerve axotomy, the survival ratio of motoneurons in ICR mice was 80%, whereas that in C57BL/6 mice was 50% (Kiryu-Seo *et al.*, 2006). Facial nerve axotomy in 5-month-old Balb/C mice led to a severe loss of motoneurons, with only 40% of the neurons surviving compared with the nonlesioned side at D30 (Hottinger *et al.*, 2000). It might also be noted that the axonal regeneration after dorsal root crush in C57BL/6J mice was only one-fifth of that in the A/J mouse (Xin *et al.*, 1990). Variable degrees of motoneuron death following axotomy have also been reported in other species. For instance, in the adult cat DMV and hypoglossal nucleus, 72 and 85% of lesioned neurons survived, respectively, at D80 (Cova and Aldskogius, 1986). In the adult cat spinal cord, axotomized motor neurons survived for long term after peripheral nerve injury (Carlson *et al.*, 1979). In the adult rabbit, 90% of injured hypoglossal neurons survived at D28 (Sjöstrand, 1971), whereas 65% of DMV neurons disappeared 18 days following unilateral cervical vagotomy (Aldskogius, 1978). In the axotomized human facial nucleus, there was no indication of cell death (Graeber *et al.*, 1993). These results might indicate an important aspect of the axotomy paradigm: experiments using some regions in specific species and strains will work as models for neuronal regeneration, but other animal models will work best for studying neuronal cell death.

Glial augmentation

Franz Nissl (1894) was the first to report augmentation of glial cell numbers in the rabbit facial nerve nucleus following peripheral axonal injury. The origin and structural characteristics of proliferating cells after axotomy has been an object of study for a long time (Sjöstrand, 1971). Incorporation of isotope-labeled thymidine and thymidine analogs into newly synthesized DNA has been used to identify proliferating cells. In the axotomized hypoglossal nucleus of adult A/J mice, ^{3}H-thymidine-labeled cells were considered to be microglia by the ultrastructural features (Adrian *et al.*, 1978). Schelper and Adrian (1980) examined whether axotomy-induced proliferating cells exhibited the diffuse cytoplasmic activity of sodium fluoride (NaF)-sensitive non-specific esterase (NSE), an enzyme characteristic for monocytes. The authors observed that no ^{3}H-thymidine-labeled cells showed NaF-sensitive NSE activity in the axotomized hypoglossal nucleus of A/J mice. They thus concluded that the origin of proliferating cells were intrinsic microglia in the hypoglossal nucleus but not hematogenous monocytes. The identity of ^{3}H-thymidine-labeled mitotic cells as microglia was also confirmed by electron microscopy in the lesioned facial nerve nucleus of Wistar rats (Graeber *et al.*, 1988).

To date, expansion of intrinsic microglial population after axotomy is widely accepted, and the degree of proliferation has been estimated in several species and strains (Table 2). In the SD rat, the number of microglial cells dramatically increased (more than ten times) by hypoglossal axotomy at D4 (Svensson and Aldskogius, 1993). Approximately three- to four-fold increase in microglial cells occurred after facial nerve axotomy in the albino rats (Søreide, 1981b). In the rat hypoglossal nucleus, microglial hyperplasia occurred within a few days after axotomy, but it waned rapidly (Barron *et al.*, 1990). Comparable degrees of axotomy-induced increase in microglial cells were found in mice. For instance,

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

Table 1. Species and strain differences in survival rates of neurons after axotomy.

Species	Strain	Region	Age	Survival rates of axotomized neurons	References
Rat	SD	Facial	3–6 weeks	82–90% (male, D70–84), 70–75% (female, D70–84)	Yu (1988)
		Facial	Adult	72% (D28)	Mattsson *et al.* (1999)
		Vagal	Adult	71–77% (D28), 22–34% (D164)	Aldskogius *et al.* (1980)
		Hypoglossal	Adult	90–109% (D28), 75% (D164)	Aldskogius *et al.* (1980)
		Hypoglossal	Adult	89–95% (D120)	Arvidsson and Aldskogius (1982)
	Wistar	Facial	Adult (6 months), aged (22–24 months)	75% (D84, adult), 60% (D84, aged)	Johnson and Duberley (1998)
		Vagal	Juvenile (16–20 days)	90–100% (D28)	Yamada *et al.* (2008)
		Hypoglossal	7 weeks	100% (D56)	Kiryu-Seo *et al.* (2005)
		Hypoglossal	6 weeks	90–100% (D14)	Namikawa *et al.* (2000)
		Hypoglossal	8–11 weeks	90–100% (D28)	Yamada *et al.* (2011)
	Fischer344	Facial	Adult (6 months), aged (22–24 months)	83% (D84, adult), 77% (D84, aged)	Johnson and Duberley (1998)
	(Albino)	Facial	Adult, new bom (8–11 days)	100% (adult), 10% (new born)	Søreide (1981a)
Mouse	C57BL/6J	Facial	7–9 weeks	35% (D21): lower than A/J mice	Oh *et al.* (1994)
		Hypoglossal	8–11 weeks	32% (D28)	Yamada and Jinno (2011)
		Dorsal root ganglion	9 weeks	Axonal regeneration is only one-fifth of A/J mice	Xin *et al.* (1990)
	C57BL/6N	Vagal	Juvenile	25–35% (D28)	Yamada *et al.* (2008)
		Hypoglossal	8–11 weeks	30% (D28)	Yamada *et al.* (2011)
	C57BL/6	Facial	2–3 months	63% (D25): lower than 129/SvJ mice	Ferri *et al.* (2002)
		Hypoglossal	7 weeks	50% (D35), 20% (D56): lower than ICR mice	Kiryu-Seo *et al.* (2005)
	ICR	Facial	7 weeks	86% (D28), 60% (D70)	Serpe *et al.* (2000)
		Hypoglossal	7 weeks	80% (D35), 60% (D56): higher than C57BL/6 mice	Kiryu-Seo *et al.* (2006)
	A/J	Facial	7–9 weeks	90% (D2): higher than C57BL/6 mice	Oh *et al.* (1994)
	Balb/c	Facial	4 months	45% (D30)	Hottinger *et al.* (2000)
		Facial	Adult	72% (D21)	Kou *et al.* (1995)
		Facial	2–3 months	65% (D25)	Ferri *et al.* (2002)
	129/SvJ	Facial	2–3 months	75% (D25): higher than C57BL/6 mice	Ferri *et al.* (2002)
		Facial	2 months	60% (D42)	de Bilbao *et al.* (2000)
	(Albino)	Facial	60–75 days	50–45% (D30), 25–35% (D60)	Torvik and Skjörten (1971a)
Cat		Vagal	Adult	72% (D80)	Cova and Aldskogius (1986)
		Hypoglossal	Adult	85% (D80)	Cova and Aldskogius (1986)
		Spinal cord	Adult	Minimal induction of cell death	Carlson *et al.* (1979)
Rabbit		Vagal	Adult	55% (D18), 30% (D90)	Aldskogius (1978)
		Hypoglossal	Adult	90% (D28)	Sjöstrand (1971)
Guinea pig		Vagal	1–2 months	82% (D28), 27% (D84)	Laiwand *et al.* (1987)
Human		Facial	77 years old	No induction of cell death	Graeber *et al.* (1993)

the number of microglial cells drastically increased (about 15 times) by hypoglossal axotomy at D7 in the C57BL/6J mouse (Yamada and Jinno, 2011). In the lesioned facial nucleus of B6C3Fe mice, microglia showed a 3.5-fold increase at D7 (Raivich *et al.*, 1994). Similarly, in the injured DMV and hypoglossal nucleus of adult cats, the microglial cell densities increased by three- to four-fold at D35 (Cova and Aldskogius, 1986). In the lesioned DMV of adult rabbits, the numbers of microglia showed two- to ten-fold increase at D3–30 (Aldskogius, 1982). Interestingly, activated microglial cells were located more remotely from the neuronal cell bodies in rabbits, whereas these microglia were closely associated with lesioned neurons in rats and mice (Sjöstrand, 1971). At any rate, the mitotic responses of microglia to peripheral nerve injury seem to commonly occur in rats, mice, cats and rabbits, and the degrees of proliferation are rather similar among these species.

It has been reported that astrocytes proliferate in response to brain damage, such as stab wounds and ibotenic acid injection (Latov *et al.*, 1979; Smith *et al.*, 1987). However, some studies have demonstrated that peripheral nerve injury does not lead to mitotic response by astrocytes. For instance, there was no increase in the number of astrocytes in the SD rat following hypoglossal nerve transection (Svensson and Aldskogius, 1993). In the facial nucleus of Wistar rat, no increase of astrocytes was observed after axotomy either (Graeber *et al.*, 1988). Counter to these studies, a small degree of astrocytic hyperplasia was seen during D1–10 of

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

Table 2. Species and strain differences in microglial and astrocyte proliferation after axotomy.

Species	Strain	Region	Age	Microglial proliferation	Astrocyte proliferation	References
Rat	SD	Facial	Adult		GFAP ↑	Tetzlaff *et al.* (1988)
	SD	Hypoglossal	Adult	>× 10 (D4)[a]	–	Svensson and Aldskogius (1993)
		Hypoglossal	Adult	+	–	Svensson *et al.* (1994)
	Wistar	Facial	8 weeks	+	–	Graeber *et al.* (1988)
	(Albino)	Hypoglossal	3 months		+	Watson (1972)
		Facial	Adult	×3–4 (D4–10)		Søreide (1981b)
Mouse	C57BL/6J	Hypoglossal	8–11 weeks	×12 (D3), ×15 (D7–14), ×2 (D28)	–	Yamada and Jinno (2011)
		Spinal cord	6–8 weeks		GFAP ↑: weaker than A/J mice	Emirandetti *et al.* (2006)
	C57BL/6	Facial	8–12 weeks	×3.5 (D14)		Petitto *et al.* (2003)
		Hypoglossal	Adult	×3–4 (D3)[b]		Gowing *et al.* (2006)
	B6C3Fe	Facial	3 months	×3.5 (D7)		Raivich *et al.* (1994)
	A/J	Spinal cord	6–8 weeks		GFAP ↑↑: stronger than C57BL/6 mice	Emerandetti *et al.* (2006)
Cat		Vagal	Adult	×2–3 (D10–35): lesser extent than rats and rabbits	–	Cova and Aldskogius (1986)
		Hypoglossal	Adult	×2–3 (D10–35): lesser extent than rats and rabbits	–	Cova and Aldskogius (1986)
Rabbit		Vagal	Adult	×2–10 (D4–16)	×1–2 (D4–16)	Aldskogius (1982)
		Facial		+		Nissl (1894)
		Hypoglossal	11–13 weeks	×10 (D2–D4)		Sjöstrand (1965)

[a]The data were calculated from Fig. 2A of the article by Svensson and Aldskogius (1993).
[b]The data were calculated from Fig. 2G of the article by Gowing *et al.* (2006).

hypoglossal axotomy in the albino rat (Watson, 1972). Enhanced expression of glial fibrillary acidic protein (GFAP) was also seen in the rat facial nucleus after axotomy (Tetzlaff *et al.*, 1988). GFAP is a major constituent of glial intermediate filament proteins, and the upregulation of GFAP by astrocytes is a well-established hallmark of reactive astrocytes and gliosis (Pekny and Nilsson, 2005). When axon regeneration was prevented or delayed by placing a metal clip on the proximal nerve stump, increased GFAP expression lasted longer (up to 1 year) in the axotomized facial nucleus in CD rats (Laskawi and Wolff, 1996). Mouse inbred strain differences in astrocytic response were reported in the spinal cord after sciatic nerve transection (Emirandetti *et al.*, 2006). The degree of GFAP increase after axotomy was significantly higher in A/J mice than in C57BL/6J mice. In the adult cat, the density of astrocytes was not affected by hypoglossal or DMV nerve transection (Cova and Aldskogius, 1986). In the adult rabbit, astrocytes increased to some extent at a later postoperative stage of DMV axotomy (Aldskogius, 1982). The author suggested that astrocytic hyperplasia was likely accompanying a necrobiotic neuronal response. Altogether, the mitotic response of astrocytes to the peripheral nerve transection seems weaker than that of microglia. Future comparative studies are required to determine the degree of proliferation of astrocyte after axotomy.

Synaptic stripping

The intrusion of glial processes into synaptic space, so-called synaptic stripping, has been considered to be involved in the protection of lesioned neurons via physical denervation. For decades, many neuroscientists have been attracted to this phenomenon (Table 3). Early work using electron microscopy provided essential structural information on what happens during synaptic stripping. For instance, Sumner and Sutherland (1973) described the time course of synaptic stripping after rat hypoglossal axotomy. At D1, no microglial wrapping was seen in the hypoglossal nuclei. At D7, microglial wrapping of neurons and dendrites had begun. At 2 weeks after the transection, neuron perikarya were completely surrounded by microglial processes. Similar synaptic detachments were seen after facial nerve lesion in adult albino mice (Torvik and Søreide, 1975). Ultrastructural measurements of synaptic elimination after axotomy were performed in the sciatic motor nuclei using three isogenic mouse strains (Sabha *et al.*, 2008). The authors showed that C57BL/6J mice exhibited a significantly larger preservation of synaptic boutons when compared to A/J mice and Balb/cJ mice. By contrast, an apparent separation of synaptic boutons by microglia was not observed in the lesioned spinal cord of adult cats (Chen, 1978). These results indicate the possible existence of species and strain differences in the involvement of microglial cells in synaptic stripping.

Recent studies emphasize that astrocytes play various roles in the stabilization and maintenance of synapse by providing a proper microenvironment surrounding the active synapses (Araque and Perea, 2004), limiting the diffusion of ions and neurotransmitters, and releasing neurotransmitters (Walz, 1989; Castonguay *et al.*, 2001). In addition to microglial cells, astrocytes are also involved in synaptic stripping. In close association with microglial process, lamellar astrocytic processes were observed around the axotomized neurons in the hypoglossal nucleus of albino rats (Sumner and Sutherland, 1973). Thin layers of astrocyte processes were often located between microglial process and neuron perikarya, or between microglial process and boutons in the lesioned hypoglossal nucleus of Wistar rats (Reisert *et al.*, 1984). In comparison with the volume and surface contribution of astrocytic processes, the presumed microglial component was minimal. The authors thus hypothesized that

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

Table 3. Species and strain differences in microglial and astrocytic synaptic stripping after axotomy.

Species	Strain	Region	Age	Microglial stripping	Astrocyte stripping	References
Rat	SD	Hypoglossal	Adult	+		Svensson and Aldskogius (1993)
	Wistar	Facial	6–9 days	+		Schiefer *et al.* (1999)
		Hypoglossal	Adult	Microglial component is fewer than astrocyte component	Astrocyte processes interpose between neuron and microglial processes	Reisert *et al.* (1984)
		Vagal	Juvenile	+	Extracellular nucleotides, such as ATP and adenosine from astrocytes, transiently inhibit the release of glutamate and/or GABA	Yamada *et al.* (2008)
	Long–Evans	Facial	Adult	+		Blinzinger and Kreutzberg (1968)
	Osborn–Mendel	Hypoglossal	10–21 days	+	Astrocyte slips often intervene between neuron and microglia	Borke (1982)
	(Albino)	Facial	Adult		Microglial ensheathment is less pronounced than the astrocytic reaction	Søreide (1981b)
		Hypoglossal	3 months	+	+	Sumner and Sutherland (1973), Sumner (1979)
Mouse	C57BL/6J	Spinal cord	6–8 weeks	Synaptic elimination is weaker than A/J and Balb/c mice		Oliveira *et al.* (2008), Sabha *et al.* (2008)
	C57BL/6N	Hypoglossal	8–11 weeks	Intrusion of microglial processes into synaptic space is more extensive in C57BL/6 mice than in Wistar rats	+	Yamada *et al.* (2011)
	A/J	Spinal cord	6–8 weeks	Synaptic elimination is stronger than C57BL/6 mice		Sabha *et al.* (2008)
	Balb/c	Spinal cord	6–8 weeks	Synaptic elimination is stronger than C57BL/6 mice		Sabha *et al.* (2008)
	(Albino)	Facial	Adult	+	+	Torvik and Søreide (1975)
Cat		Spinal cord	Adult	–	Astrocyte processes ensheath neuronal surface membrane	Chen (1978)
Rabbit		Vagal	Adult	Microglial cell processes more or less completely envelope neurons	Astrocytic processes are sometimes intermingled with processes from microglial cells around neurons showing signs of degeneration	Aldskogius (1982)
Human		Facial	77 years	+	+	Graeber *et al.* (1993)

the microglial contribution to the regeneration of hypoglossal nerves might be negligible in rats. As opposed to the rat model, astrocytes did not show any clear tendency to enclose or encircle degenerating nerve cells in the mouse (Torvik and Skjörten, 1971b). In the rabbit DMV, astrocytic process intrusion appeared only at a later postoperative stage (Aldskogius, 1982). Around neurons showing signs of degeneration, astroglial cell processes were sometimes intermingled with process from microglial cells. In the adult cat, many layers of astrocytic processes ran parallel to each other and ensheathed the motoneuronal surface membrane at D15 after the transection of ventral root (Chen, 1978). The astrocytic responses coincided with the loss of synapses, whereas microglial cell processes covered only a small portion of lesioned neuronal surface in adult cats. In the human facial nucleus, wrapping of a number of neurons by newly formed GFAP-positive astrocytic cell process were identified in the regenerating facial motor nucleus (Graeber *et al.*, 1993).

To date, several studies have suggested plausible molecular mechanisms behind the different synaptic stripping responses in different animal strains/species after axotomy. For instance, MHC class I molecules, which traditionally have been linked with immunological mechanisms, are crucial for a selective maintenance of synapses during the synaptic removal process in the axotomy paradigm (Oliveira *et al.*, 2004). After lesion of peripheral nerves of motoneurons, MHC

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

I was upregulated in microglia surrounding axotomized motoneurons (Lindå *et al.*, 1998; Lidman *et al.*, 1999), and might be induced in astrocytes both *in vitro* and *in vivo* (Jarosinski and Massa, 2002). The strain differences in MHC I expression level after the sciatic nerve axotomy were examined in inbred mice (Sabha *et al.*, 2008). C57BL/6J mice, that had a comparatively poor regenerative potential, displayed a lower upregulation of MHC I, coupled with a slower synaptic stripping. On the other hand, A/J mice, which had a stronger axonal regrowth potential, showed a clear upregulation of MHC I and a sharp acute loss of afferents. These findings indicate that upregulation of MHC I may positively influence the regenerative outcome associated with a more effective axonal regrowth.

Neurotransmission

A single motoneuron is contacted by as many as 100,000 synaptic boutons containing a variety of different neurotransmitters (Ulfhake and Cullheim, 1988). Among numerous substances, glutamate is one of the most important excitatory neurotransmitters. In the adult rat superior cervical ganglion cells, the excitatory postsynaptic potentials (EPSPs) recorded *in vitro* with intracellular microelectrodes showed a decline to about 50% of their control values at D7–10 of axotomy (Sánchez-Vives and Gallego, 1993). In the lesioned adult cat dorsal root motoneurons, the amplitude of unit EPSPs recorded *in vivo* was significantly reduced (Kuno and Llinás, 1970). The authors have further suggested a lack of the excitatory synaptic input to the cell body and the proximal dendrites of the axotomized spinal motor neurons on the basis of Rall's compartment model (Rall, 1964). Within 72 h of axon interruption of superior cervical ganglion cells in the adult guinea pig, the amplitude of EPSPs recorded *in vitro* in response to maximal preganglionic stimulation were reduced by more than 70% (Purves, 1975). Such reduction of EPSPs is presumed to promote neuronal survival. For instance, blockade of the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor rescued spinal motoneurons after peripheral nerve transection in the neonatal rat (Mentis *et al.*, 1993; Greensmith *et al.*, 1994). Intramedullary axotomy resulted in a preferential elimination of glutamatergic inputs to lesioned motoneurons in the adult cat, which may support the survival of motoneurons through reducing glutamate excitotoxicity on these neurons (Lindå *et al.*, 2000). In summary, excitatory synaptic transmission might be modulated in response to peripheral nerve injury, although the mechanisms underlying such alteration remain to be determined. On the other hand, few studies have electrophysiologically examined the species and strain differences in alterations of synaptic transmission following axotomy. Novel hints about the structural and humoral factors regulating synaptic transmission after axotomy have been gained from our recent comparative studies on the vagal and hypoglossal motoneurons between Wistar rats and C57BL/6N mice at juvenile and adult ages (see the next section).

DIFFERENTIAL INVOLVEMENT OF ASTROCYTES AND MICROGLIA IN THE AXOTOMY PARADIGM: FROM OUR RECENT FINDINGS

Alterations in neurotransmission and synaptic stripping in the DMV after axotomy in rats and mice

We examined whether alterations in synaptic transmission after nerve injury were correlated with synaptic detachment from injured DMV neurons using Wistar rats and C57BL/6N mice at juvenile ages (Yamada *et al.*, 2008). Nerve transection was performed on 16- to 20-day-old animals, and electrophysiological recordings were made in acute slice preparations from 17- to 30-day-old animals. The mean frequency of spontaneous EPSCs in injured DMV neurons in rats was significantly decreased to about one-third of that of control neurons at D2 (Fig. 1A). This significant decline in the mean frequency of spontaneous EPSCs in injured DMV neurons lasted until D7 in rats. There was no significant change in the amplitude of spontaneous EPSCs recorded from injured DMV neurons in rats. By contrast, the mean frequency of spontaneous EPSCs in injured DMV neurons in mice showed no significant decrease from D3 to D7, although we encountered a transient increase at D1.

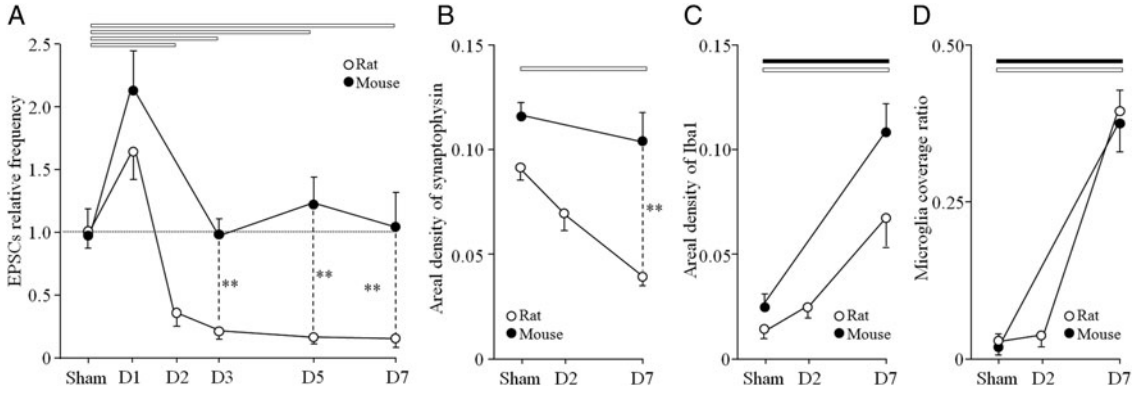


Fig. 1. Species differences in synaptic transmission and microglial reaction after DMV nerve axotomy. (A) The spontaneous EPSCs recorded from the lesioned DMV neurons in rats (open circles) and in mice (filled circles). (B) The areal density of synaptophysin-positive puncta in the lesioned DMV in rats and in mice. (C) The areal density of Iba-1-positive microglial processes in the lesioned DMV in rats and in mice. (D) The microglial coverage ratio of perikarya in the lesioned DMV in rats and in mice. Horizontal bars (open bar = rat; filled bar = mouse) at the upper-level of each graph show statistical significances between sham-operated and axotomized animals. Asterisks show statistically significant differences between rats and mice, $^{**}P < 0.01$. Modified and reproduced from Yamada *et al.* (2008), with permission of the publisher.

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

Similar to rats, there was no significant change in the amplitude of spontaneous EPSCs in mice. These findings indicate that the suppression of synaptic transmission in the injured DMV neurons might occur in rats but not in mice during the early phase after axotomy.

The possible contribution of synaptic detachment to suppression of synaptic transmission in the injured DMV neurons was comparatively estimated in rats and mice. In the rat injured DMV, the areal density of synaptophysin-positive puncta was significantly lower at D7 versus sham treatment (Fig. 1B), whereas no significant difference was found at D2. In the mouse injured DMV, the areal density of synaptophysin-positive puncta showed no significant decrease from control to D7. The synaptophysin density in the lesioned DMV was significantly higher in mice than in rats at D7. In contrast, the areal density of Iba1-positive microglia processes in the lesioned DMV markedly increased at D7 versus sham in both in rats and mice (Fig. 1C). In the rat DMV, there was no statistically significant difference in the Iba1 density between sham and D2 animals. The microglia coverage ratio (percentage of perimeter surface covered by microglia) was significantly increased at D7 in both species versus sham (Fig. 1D). There was no difference in the microglia coverage ratio between mice and rats. It is noteworthy that the decline in synaptic boutons and the increase in microglial coverage first become apparent at D7 in rats, although the suppression of spontaneous EPSCs in the injured DMV occurred at D2, as mentioned above. In addition, the species differences between rats and mice suggest that the reduction of synaptic boutons and the increase in microglial intrusion might occur independently in the injured DMV during the early phase after axotomy.

Involvement of astrocyte-derived nucleotides in neurotransmission in the DMV at early stage post-axotomy in rats

We then carried out additional pharmacological experiments to elucidate the mechanisms underlying decline in spontaneous EPSCs preceding synaptic stripping in the injured DMV of Wistar rats. All recordings were made in juvenile (17- to 30-day-old) animals. The main results are summarized as follows. (1) Stimulation with high-K^+ solution, which enhances the release of neurotransmitters from the presynaptic terminals, restored the frequency of miniature EPSCs and inhibitory postsynaptic currents (IPSCs) of injured DMV neurons to the control levels. (2) Application of a non-selective P2 purinergic receptor antagonist, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS), recovered the frequency of miniature EPSCs recorded from injured DMV neurons on D2 to the control levels. (3) Application of a selective A1-adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), recovered the frequency of both the miniature EPSCs and IPSCs recorded from injured DMV neurons on D2 to the control levels. (4) There was no significant change in either the miniature EPSCs or IPSCs recorded from mechanically dissociated DMV neurons on D3 and D7. (5) High-K^+ stimulation failed to restore the mean frequency of either the miniature EPSCs or IPSCs of injured DMV neurons on D7. (6) Neither PPADS nor DPCPX was able to increase mean frequency of miniature EPSCs and IPSCs on D7.

Adenosine triphosphate (ATP), the energy currency of the cell, has been shown to control the synaptic activity in the central nervous system. For instance, ATP inhibits glutamate release from the presynaptic terminal through P2Y receptors (Koizumi and Inoue, 1997; Koizumi *et al.*, 2003; Zhang *et al.*, 2003). Adenosine also suppresses the release of glutamate and/or GABA through A1 receptors (Oliet and Poulain, 1999). Interestingly, the most likely source of ATP and adenosine is astrocytes (Koizumi *et al.*, 2003; Martín, 2007). Recent studies show that astrocytic modulation of basal synaptic transmission is mediated by the release of purines and the activation of presynaptic P2A receptors by adenosine (Panatier *et al.*, 2011). Therefore, we hypothesize that extracellular nucleotides, such as ATP and adenosine from astrocytes, transiently inhibit the release of glutamate and/or GABA via activation of P2Y and A1 receptors of presynaptic terminals in the rat injured DMV during the early phase of axotomy (e.g. D2). After the stage of humoral suppression by astrocytes (e.g. D7), microglial cells could start suppression of neurotransmission through synaptic stripping.

Responses of perineuronal astrocytes and microglia in the hypoglossal nucleus after axotomy in rats and mice

It is generally accepted that reactive glial cells can be either detrimental or protective depending on the context (Vilhardt, 2005; Schwartz *et al.*, 2006; Biber *et al.*, 2007). However, the majority of axotomy studies have focused on the beneficial contribution of glial cells to neuronal survival, and little attention has been given to the possible multifaceted roles of glial cells. Consequently, we examined the structural alterations of perineuronal glial cells in the lesioned hypoglossal nucleus using Wistar rats and C57BL/6N mice to identify a possible unfavorable role of microglia and astrocytes in synaptic stripping (Yamada *et al.*, 2011).

Gradual neuronal death occurred in C57BL/6N mice but not in Wistar rats at D28 after the hypoglossal transection. Based on immunofluorescent quadruple labeling for synaptophysin, Iba1, glutamate-aspartate transporter (GLAST; marker of astrocyte) and TOTO-3 (for counter staining), we classified the glial and synaptic coverage patterns of perikarya into three types (Fig. 2A): S-type, the profile closest to the soma was a synaptophysin-positive puncta; A-type, the closest profile was a GLAST-positive astrocytic process; and M-type, the closest profile was an Iba1-positive microglial process. A significant decline in S-type was observed after axotomy in both species (Fig. 2B). Axotomy-induced intrusion of astrocytic processes into the synaptic space (A-type) was significantly more extensive in rats than in mice, whereas microglial intrusion into the synaptic space (M-type) was significantly more severe in mice than in rats (Fig. 2C,D).

We also examined perisomal areas lacking synaptophysin-positive puncta, which were defined as the representation of the extrasynaptic environment. The patterns of coverage of perikarya in the extrasynaptic environment were classified into three categories (Fig. 2E): EA-type, the profile closest to the soma was an astrocytic process; EM-type, the closest profile was a microglial process; and unclassified, no apparent profiles were detected. The fractions of unclassified type were very low in both species (Fig. 2F). In the extrasynaptic

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

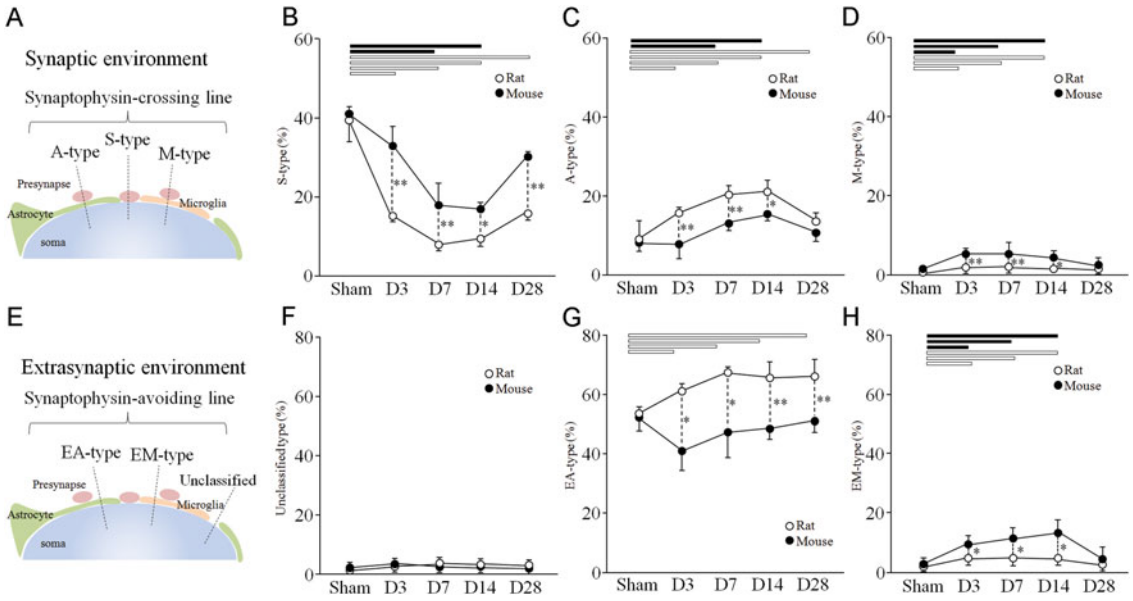


Fig. 2. Species differences in glial reactions in the synaptic and extrasynaptic environment after hypoglossal nerve axotomy. (A) Synaptophysin crossing lines are classified into S-type, A-type and M-type. (B–D) Axotomy-induced alterations in the fractions of S-type (B), A-type (C) and M-type (D) in rats (open circles) and in mice (filled circles). (E) Synaptophysin-avoiding lines are classified into EA-type, EM-type and unclassified. (F–G) Axotomy-induced alterations in the fractions of unclassified type (F), EA-type (G) and EM-type (H) in rats and mice. Horizontal bars (open bar = rat; filled bar = mouse) at the upper-level of each graph show statistical significances between sham-operated and axotomized animals. Asterisks show statistically significant differences between rats and mice, $^{*}P < 0.05$, $^{**}P < 0.01$. Modified and reproduced from Yamada *et al.* (2011), with permission of the publisher.

environment, the prevalence of contact between astrocytic processes and lesioned motoneurons (EA-type) was significantly increased in rats, whereas no significant axotomy-induced alterations in astrocytic contact were observed in mice (Fig. 2G). Microglial intrusion into the extrasynaptic space (EM-type) was significantly more severe in mice than in rats (Fig. 2H).

Astrocyte and microglia may play distinct roles in the axotomy paradigm

The main findings of our second study can be summarized as follows: (1) In the healthy hypoglossal nucleus, motoneurons were mainly covered with synaptic terminals and astrocytic processes. (2) Axotomy caused slow motoneuron death in mice but not in rats. (3) In rats, axotomy induced the extensive astrocytic interposition and the removal of synaptic terminals. (4) By contrast, in mice, prominent microglial intrusion into synapse was induced after axotomy. Together, these observations give rise to a novel hypothesis regarding the double-edged sword nature of glial cells in the axotomy paradigm: the astrocytic interposition may primarily mediate anti-apoptotic effects through reduction of synaptic inputs, whereas the microglial intrusion might be involved in slow apoptotic cell death of motoneurons.

Our results also give some insight into the neuroprotective role of astrocytes via glutamates clearance in the extrasynaptic space. Glutamate transporter is abundantly expressed in astrocytes, which regulate synaptic and extrasynaptic glutamate by importing glutamate through transporters (Huang and Bergles, 2004). Inhibition of astrocytic glutamate transporters with pharmacological blockers, antisense oligonucleotides or by transgenic knockout, produce elevated extracellular glutamate levels and neuronal death. Recent studies showed that increased expression of astrocytic glutamate transporters rescued degradation of motoneurons in ALS animals (Lepore *et al.*, 2008; Blackburn *et al.*, 2009). Interestingly, the prevalence of contact between astrocytic processes and lesioned motoneurons in extrasynaptic sites was significantly increased in rats after axotomy, whereas there were no significant alterations in mice. We thus hypothesize that the enlargement of astrocytic processes in the extrasynaptic environment seen in rats might help neuronal survival via the increased uptake of excessive glutamate or suppression of synaptic inputs. This idea seems to be supported by several axotomy studies of different species. In the axotomized adult cat spinal cord, most areas of neuronal membrane bared of synaptic contact were occupied by astrocytic processes, whereas microglia covered only a small portion of it (Chen, 1978). In line with our prediction, cat spinal motor neurons survived for long term after peripheral axotomy (Carlson *et al.*, 1979). In the rabbit DMV, rapid and remarkable nerve cell loss occurred after nerve transection (Aldskogius, 1978). Interestingly, in the rabbit DMV after vagal nerve lesion, microglial cell processes more or less completely enveloped neurons, whereas astrocytic processes were sometimes intermingled with processes from microglial cells around neurons showing signs of degeneration (Aldskogius, 1982). It should also be noted that, in the human facial nucleus, Graeber *et al.* (1993) found no indication of cell death but wrapping of axotomized motoneurons by astrocytic processes in the facial nucleus.

CONCLUDING REMARKS

Despite a large amount of studies with the axotomy model, the mechanisms underlying synaptic stripping remain to be determined. We now know the results obtained from peripheral nerve injury vary substantially among species and strains. Here we suggest that using the comparative anatomy in the

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

axotomy paradigm offers a unique experimental model to study glial involvement in synaptic stripping. Indeed, our recent studies using Wistar rats and C57BL/6N mice uncovered the differential roles of microglia and astrocytes in the axotomy paradigm. Contrary to past belief, which regarded microglia to be dominant player in synaptic stripping, the new hypothesis suggested by our findings regards astrocytes as an essential and major element in synaptic suppression and neuronal protection. Previous reports using other species, such as cats, rabbits and humans, could support the neuroprotective role of astrocytes after axotomy. Future studies utilizing the comparative anatomy in the axotomy paradigm will provide further important and unexpected discoveries on the multifaceted roles of microglia and astrocytes in injury and repair.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Hiroshi Nakanishi for his support to this work. The authors also thank Dr. Eric Bushong for his constructive comments and grammatical corrections on earlier versions of the manuscript. This study was partially supported by Grant-in-Aid for Research Activity Start-up (22890130 to J.Y.) and Grant-in-Aid for Research (C) (21500328 to S.J.) from the Ministry of Education, Science and Culture, Japan, Uehara Memorial Foundation, Kaibara Morikazu Medical Science Promotion Foundation, Takeda Science Foundation, and Kobayashi Magobe Memorial Medical Foundation.

Statement of interest

There is no conflict of interest.

REFERENCES

Adrian E.K. Jr., Williams M.G. and George F.C. (1978) Fine structure of reactive cells in injured nervous tissue labeled with 3H-thymidine injected before injury. *Journal of Comparative Neurology* 180, 815–839.

Aldskogius H. (1978) Lipid accumulation in axotomized adult rabbit vagal neurons. Electron microscopical observations. *Brain Research* 140, 349–353.

Aldskogius H. (1982) Glial cell responses in the adult rabbit dorsal motor vagal nucleus during axon reaction. *Neuropathology and Applied Neurobiology* 8, 341–349.

Aldskogius H., Barron K.D. and Regal R. (1980) Axon reaction in dorsal motor vagal and hypoglossal neurons of the adult rat. Light microscopy and RNA-cytochemistry. *Journal of Comparative Neurology* 193, 165–177.

Araque A. and Perea G. (2004) Glial modulation of synaptic transmission in culture. *Glia* 47, 241–248.

Arvidsson J. and Aldskogius H. (1982) Effect of repeated hypoglossal nerve lesions on the number of neurons in the hypoglossal nucleus of adult rats. *Experimental Neurology* 75, 520–524.

Banati R.B., Gehrmann J., Schubert P. and Kreutzberg G.W. (1993) Cytotoxicity of microglia. *Glia* 7, 111–118.

Barron K.D. (2004) The axotomy response. *Journal of Neurological Science* 220, 119–121.

Barron K.D., Marciano F.F., Amundson R. and Mankes R. (1990) Perineuronal glial responses after axotomy of central and peripheral axons. A comparison. *Brain Research* 523, 219–229.

Biber K., Neumann H., Inoue K. and Boddeke H.W. (2007) Neuronal 'On' and 'Off' signals control microglia. *Trends in Neurosciences* 30, 596–602.

Blinzinger K. and Kreutzberg G. (1968) Displacement of synaptic terminals from regenerating motoneurons by microglial cells. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 85 145–157.

Blackburn D., Sargsyan S., Monk P.N. and Shaw P.J. (2009) Astrocyte function and role in motor neuron disease: a future therapeutic target? *Glia* 57, 1251–1264.

Borke R.C. (1982) Perisomatic changes in the maturing hypoglossal nucleus after axon injury. *Journal of Neurocytology* 11, 463–485.

Cajal Y.R. (1928) Cajal's degeneration and regeneration of the nervous system. In DeFilipe J. & Jones E.G. (eds) *History of Neuroscience No. 5*. New York: Oxford University Press, 1991.

Cardona A.E., Pioro E.P., Sasse M.E., Kostenko V., Cardona S.M., Dijkstra I.M. *et al.* (2006) Control of microglial neurotoxicity by the fractalkine receptor. *Nature Neuroscience* 9, 917–924.

Carlson J., Lais A.C. and Dyck P.J. (1979) Axonal atrophy from permanent peripheral axotomy in adult cat. *Journal of Neuropathology and Experimental Neurology* 38, 579–585.

Castonguay A., Lévesque S. and Robitaille R. (2001) Glial cells as active partners in synaptic functions. *Progress in Brain Research* 132, 227–240.

Chen D.H. (1978) Qualitative and quantitative study of synaptic displacement in chromatolyzed spinal motoneurons of the cat. *Journal of Comparative Neurology* 177, 635–664.

Cova J.L. and Aldskogius H. (1986) Effect of axotomy on perineuronal glial cells in the hypoglossal and dorsal motor vagal nuclei of the cat. *Experimental Neurology* 93, 662–667.

de Bilbao F., Giannakopoulos P., Srinivasan A. and Dubois-Dauphin M. (2000) In vivo study of motoneuron death induced by nerve injury in mice deficient in the caspase 1/ interleukin-1 beta-converting enzyme. *Neuroscience* 98, 573–583.

Davalos D., Grutzendler J., Yang G., Kim J.V., Zuo Y., Jung S. *et al.* (2005) ATP mediates rapid microglial response to local brain injury in vivo. *Nature Neuroscience* 8, 752–758.

Emirandetti A., Graciele Zanon R., Sabha M. Jr. and de Oliveira A.L. (2006) Astrocyte reactivity influences the number of presynaptic terminals apposed to spinal motoneurons after axotomy. *Brain Research* 1095, 35–42.

Ferri C.C., Ghasemlou N., Bisby M.A. and Kawaja M.D. (2002) Nerve growth factor alters p75 neurotrophin receptor-induced effects in mouse facial motoneurons following axotomy. *Brain Research* 950, 180–185.

Gowing G., Vallières L. and Julien J.P. (2006) Mouse model for ablation of proliferating microglia in acute CNS injuries. *Glia* 53, 331–337.

Graeber M.B., Tetzlaff W., Streit W.J. and Kreutzberg G.W. (1988) Microglial cells but not astrocytes undergo mitosis following rat facial nerve axotomy. *Neuroscience Letters* 85, 317–321.

Graeber M.B., Bise K. and Mehraein P. (1993) Synaptic stripping in the human facial nucleus. *Acta Neuropathologica* 86, 179–181.

Graeber M.B. and Streit W.J. (2010) Microglia: biology and pathology. *Acta Neuropathologica* 119, 89–105.

Greensmith L., Mentis G.Z. and Vrbová G. (1994) Blockade of N-methyl-D-aspartate receptors by MK-801 (dizocilpine maleate) rescues motoneurones in developing rats. *Developmental Brain Research* 81, 162–170.

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

Halassa M.M. and Haydon P.G. (2010) Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annual Review of Physiology* 72, 335–355.

Haydon P.G. (2001) GLIA: listening and talking to the synapse. *Nature Reviews Neuroscience* 2, 185–193.

Horner P.J. and Palmer T.D. (2003) New roles for astrocytes: the nightlife of an 'astrocyte'. La vida loca! *Trends in Neurosciences* 26, 597–603.

Hornung J.P., Koppel H. and Clarke P.G. (1989) Endocytosis and autophagy in dying neurons: an ultrastructural study in chick embryos. *Journal of Comparative Neurology* 283, 425–437.

Hottinger A.F., Azzouz M., Déglon N., Aebischer P. and Zurn A.D. (2000) Complete and long-term rescue of lesioned adult motoneurons by lentiviral-mediated expression of glial cell line-derived neurotrophic factor in the facial nucleus. *Journal of Neuroscience* 20, 5587–5593.

Huang Y.H. and Bergles D.E. (2004) Glutamate transporters bring competition to the synapse. *Current Opinion in Neurobiology* 14, 346–352.

Jarosinski K.W. and Massa P.T. (2002) Interferon regulatory factor-1 is required for interferon-gamma-induced MHC class I genes in astrocytes. *Journal of Neuroimmunology* 122, 74–84.

Johnson I.P. and Duberley R.M. (1998) Motoneuron survival and expression of neuropeptides and neurotrophic factor receptors following axotomy in adult and ageing rats. *Neuroscience* 84, 141–150.

Kiryu-Seo S., Hirayama T., Kato R. and Kiyama H. (2005) Noxa is a critical mediator of p53-dependent motor neuron death after nerve injury in adult mouse. *Journal of Neuroscience* 25, 1442–1447.

Kiryu-Seo S., Gamo K., Tachibana T., Tanaka K. and Kiyama H. (2006) Unique anti-apoptotic activity of EAAC1 in injured motor neurons. *EMBO Journal* 25, 3411–3421.

Kou S.Y., Chiu A.Y. and Patterson P.H. (1995) Differential regulation of motor neuron survival and choline acetyltransferase expression following axotomy. *Journal of Neurobiology* 27, 561–572.

Koizumi S. and Inoue K. (1997) Inhibition by ATP of calcium oscillations in rat cultured hippocampal neurons. *British Journal of Pharmacology* 122, 51–58.

Koizumi S., Fujishita K., Tsuda M., Shigemoto-Mogami Y. and Inoue K. (2003). Dynamic inhibition of excitatory synaptic transmission by astrocyte-derived ATP in hippocampal cultures. *Proceedings of the National Academy of Sciences of the U.S.A.* 100, 11023–11028.

Kuno M. and Llinás R. (1970) Alterations of synaptic action in chromatolysed motoneurones of the cat. *Journal of Physiology* 210, 823–838.

Laiwand R., Werman R. and Yarom Y. (1987) Time course and distribution of motoneuronal loss in the dorsal motor vagal nucleus of guinea pig after cervical vagotomy. *Journal of Comparative Neurology* 256, 527–537.

Laskawi R. and Wolff J.R. (1996) Changes in glial fibrillary acidic protein immunoreactivity in the rat facial nucleus following various types of nerve lesions. *European Archives of Oto-Rhino-Laryngology* 253, 475–480.

Latov N., Nilaver G., Zimmerman E.A., Johnson W.G., Silverman A.J., Defendini R. *et al.* (1979) Fibrillary astrocytes proliferate in response to brain injury: a study combining immunoperoxidase technique for glial fibrillary acidic protein and radioautography of tritiated thymidine. *Developmental Biology* 72, 381–384.

Lepore A.C., Rauck B., Dejea C., Pardo A.C., Rao M.S., Rothstein J.D. *et al.* (2008) Focal transplantation-based astrocyte replacement is neuroprotective in a model of motor neuron disease. *Nature Neuroscience* 11, 1294–1301.

Lindå H., Hammarberg H., Cullheim S., Levinovitz A., Khademi M. and Olsson T. (1998) Expression of MHC class I and beta2-microglobulin in rat spinal motoneurons: regulatory influences by IFN-gamma and axotomy. *Experimental Neurology* 150, 282–295.

Lindå H., Shupliakov O., Ornung G., Ottersen O.P., Storm-Mathisen J., Risling M. *et al.* (2000) Ultrastructural evidence for a preferential elimination of glutamate-immunoreactive synaptic terminals from spinal motoneurons after intramedullary axotomy. *Journal of Comparative Neurology* 425, 10–23.

Lidman O., Olsson T. and Piehl F. (1999) Expression of nonclassical MHC class I (RT1-U) in certain neuronal populations of the central nervous system. *European Journal of Neuroscience* 11, 4468–4472.

Marchetti B. and Abbracchio M.P. (2005) To be or not to be (inflamed) – is that the question in anti-inflammatory drug therapy of neurodegenerative disorders? *Trends in Pharmacological Sciences* 26, 517–525.

Martín E.D., Fernández M., Perea G., Pascual O., Haydon P.G., Araque A. *et al.* (2007) Adenosine released by astrocytes contributes to hypoxia-induced modulation of synaptic transmission. *Glia* 55, 36–45.

Mattsson P., Meijer B. and Svensson M. (1999) Extensive neuronal cell death following intracranial transection of the facial nerve in the adult rat. *Brain Research Bulletin* 49, 333–341.

Mentis G.Z., Greensmith L. and Vrbová G. (1993) Motoneurons destined to die are rescued by blocking N-methyl-D-aspartate receptors by MK-801. *Neuroscience* 54, 283–285.

Moran L.B. and Graeber M.B. (2004) The facial nerve axotomy model. *Brain Research Reviews* 44, 154–178.

Namikawa K., Honma M., Abe K., Takeda M., Mansur K., Obata T. *et al.* (2000) Akt/protein kinase B prevents injury-induced motoneuron death and accelerates axonal regeneration. *Journal of Neuroscience* 20, 2875–2886.

Nimmerjahn A., Kirchhoff F. and Helmchen F. (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 308, 1314–1318.

Nissl F. (1894) Uber eine neue Untersuchungsmethode des Centralorgans speziell zur Feststellung der Lokalisation der Nervenzellen. *Zentralbl Nervenheilkd Psychiatr* 17, 337–344.

Oh C., Murray B., Bhattacharya N., Holland D. and Tatton W.G. (1994) (-)-Deprenyl alters the survival of adult murine facial motoneurons after axotomy: increases in vulnerable C57BL strain but decreases in motor neuron degeneration mutants. *Journal of Neuroscience Research* 38, 64–74.

Oliet S.H. and Poulain D.A. (1999) Adenosine-induced presynaptic inhibition of IPSCs and EPSCs in rat hypothalamic supraoptic nucleus neurones. *Journal of Physiology* 520, 815–825.

Oliveira A.L., Thams S., Lidman O., Piehl F., Hökfelt T., Kärre K. *et al.* (2004) A role for MHC class I molecules in synaptic plasticity and regeneration of neurons after axotomy. *Proceedings of the National Academy of Sciences of the U.S.A.* 101, 17843–17848.

Panatier A., Vallée J., Haber M., Murai K.K., Lacaille J.C. and Robitaille R. (2011) Astrocytes are endogenous regulators of basal transmission at central synapses. *Cell* 146, 785–798.

Pekny M. and Nilsson M. (2005) Astrocyte activation and reactive gliosis. *Glia* 50, 427–434.

Perea G., Navarrete M. and Araque A. (2009) Tripartite synapses: astrocytes process and control synaptic information. *Trends in Neurosciences* 32, 421–431.

Petitto J.M., Huang Z., Lo J. and Streit W.J. (2003) IL-2 gene knockout affects T lymphocyte trafficking and the microglial response to regenerating facial motor neurons. *Journal of Neuroimmunology* 134, 95–103.

Purves D. (1975) Functional and structural changes in mammalian sympathetic neurones following interruption of their axons. *Journal of Physiology* 252, 429–463.

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

Raivich G., Moreno-Flores M.T., Möller J.C. and Kreutzberg G.W. (1994) Inhibition of posttraumatic microglial proliferation in a genetic model of macrophage colony-stimulating factor deficiency in the mouse. *European Journal of Neuroscience* 6, 1615–1618.

Raivich G., Bohatschek M., Kloss C.U., Werner A., Jones L.L. and Kreutzberg G.W. (1999) Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function. *Brain Research Reviews* 30, 77–105.

Rall W. (1964) Theoretical significance of dendritic trees for neuronal input output relations. In Reiss R. (ed.) *Neural Theory and Modeling*. Stanford: Stanford University Press.

Reisert I., Wildemann G., Grab D. and Pilgrim C. (1984) The glial reaction in the course of axon regeneration: a stereological study of the rat hypoglossal nucleus. *Journal of Comparative Neurology* 229, 121–128.

Sabha M. Jr., Emirandetti A. Cullheim S. and De Oliveira A.L. (2008) MHC I expression and synaptic plasticity in different mice strains after axotomy. *Synapse* 62, 137–148.

Sánchez-Vives M.V. and Gallego R. (1993) Effects of axotomy or target atrophy on membrane properties of rat sympathetic ganglion cells. *Journal of Physiology* 471, 801–815.

Schelper R.L. and Adrian E.K. Jr. (1980) Non-specific esterase activity in reactive cells in injured nervous tissue labeled with 3H-thymidine or 125iododeoxyuridine injected before injury. *Journal of Comparative Neurology* 194, 829–844.

Schiefer J., Kampe K., Dodt H.U., Zieglgänsberger W. and Kreutzberg G.W. (1999) Microglial motility in the rat facial nucleus following peripheral axotomy. *Journal of Neurocytology* 28, 439–453.

Schwartz M., Butovsky O., Brück W. and Hanisch U.K. (2006) Microglial phenotype: is the commitment reversible? *Trends in Neurosciences* 29, 68–74.

Serpe C.J., Sanders V.M. and Jones K.J. (2000) Kinetics of facial motoneuron loss following facial nerve transection in severe combined immunodeficient mice. *Journal of Neuroscience Research* 62, 273–278.

Sjöstrand J. (1965) Proliferative changes in glial cells during nerve regeneration. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 68, 481–493.

Sjöstrand J. (1971) Neuroglial proliferation in the hypoglossal nucleus after nerve injury. *Experimental Neurology* 30, 178–189.

Smith M.E., Gibbs M.A., Forno L.S. and Eng L.F. (1987) [3H]thymidine labeling of astrocytes in experimental allergic encephalomyelitis. *Journal of Neuroimmunology* 15, 309–321.

Snider W.D., Elliott J.L. and Yan Q. (1992) Axotomy-induced neuronal death during development. *Journal of Neurobiology* 23, 1231–1246.

Sofroniew M.V. (1999) Neuronal responses to axotomy. In Tuszynski M.H. & Kordower J. (eds) *CNS Regeneration: Basic Science and Clinical Advances*. New York: Academic Press.

Søreide A.J. (1981a) Variations in the axon reaction in animals of different ages. A light microscopic study on the facial nucleus of the rat. *Acta Anatomica (Basel)* 110, 40–47.

Søreide A.J. (1981b) Variations in the perineuronal glial changes after different types of nerve lesion: light and electron microscopic investigations on the facial nucleus of the rat. *Neuropathology and Applied Neurobiology* 7, 195–204.

Sumner B.E. and Sutherland F.I. (1973) Quantitative electron microscopy on the injured hypoglossal nucleus in the rat. *Journal of Neurocytology* 2, 315–328.

Sumner B.E. (1979) Ultrastructural data, with special reference to bouton/glial relationships, from the hypoglossal nucleus after a second axotomy of the hypoglossal nerve. *Experimental Brain Research* 36, 107–118.

Svensson M. and Aldskogius H. (1993) Infusion of cytosine-arabinoside into the cerebrospinal fluid of the rat brain inhibits the microglial cell proliferation after hypoglossal nerve injury. *Glia* 7, 286–298.

Svensson M., Mattsson P. and Aldskogius H. (1994) A bromodeoxyuridine labelling study of proliferating cells in the brainstem following hypoglossal nerve transection. *Journal of Anatomy* 185, 537–542.

Tetzlaff W., Graeber M.B., Bisby M.A. and Kreutzberg G.W. (1988) Increased glial fibrillary acidic protein synthesis in astrocytes during retrograde reaction of the rat facial nucleus. *Glia* 1, 90–95.

Torvik A. and Skjörten F. (1971a) Electron microscopic observations on nerve cell regeneration and degeneration after axon lesions. I. Changes in the nerve cell cytoplasm. *Acta Neuropathologica* 17, 248–264.

Torvik A. and Skjörten F. (1971b) Electron microscopic observations on nerve cell regeneration and degeneration after axon lesions. II. Changes in the glial cells. *Acta Neuropathologica* 17, 265–282.

Torvik A. and Søreide A.J. (1975) The perineuronal glial reaction after axotomy. *Brain Research* 95, 519–529.

Ulfhake B. and Cullheim S. (1988) Postnatal development of cat hind limb motoneurons. II: In vivo morphology of dendritic growth cones and the maturation of dendrite morphology. *Journal of Comparative Neurology* 278, 88–102.

van Rossum D. and Hanisch U.K. (2004) Microglia. *Metabolic Brain Disease* 19, 393–411.

Vilhardt F. (2005) Microglia: phagocyte and glia cell. *International Journal of Biochemistry and Cell Biology* 37, 17–21.

Walz W. (1989) Role of glial cells in the regulation of the brain ion microenvironment. *Progress in Neurobiology* 33, 309–333.

Watson W.E. (1972) Some quantitative observations upon the responses of neuroglial cells which follow axotomy of adjacent neurones. *Journal of Physiology* 225, 415–435.

Xin L., Richardson P.M., Gervais F. and Skamene E. (1990) A deficiency of axonal regeneration in C57BL/6J mice. *Brain Research* 510, 144–146.

Yamada J. and Jinno S. (2011) Alterations in neuronal survival and glial reactions after axotomy by ceftriaxone and minocycline in the mouse hypoglossal nucleus. *Neuroscience Letters* 504, 295–300.

Yamada J., Hayashi Y., Jinno S., Wu Z., Inoue K., Kohsaka S. *et al.* (2008) Reduced synaptic activity precedes synaptic stripping in vagal motoneurons after axotomy. *Glia* 56, 1448–1462.

Yamada J., Nakanishi H. and Jinno S. (2011) Differential involvement of perineuronal astrocytes and microglia in synaptic stripping after hypoglossal axotomy. *Neuroscience* 182, 1–10.

Yu W.H. (1988) Sex difference in neuronal loss induced by axotomy in the rat brain stem motor nuclei. *Experimental Neurology* 102, 230–235.

Zhang J.M., Wang H.K., Ye C.Q., Ge W., Chen Y., Jiang Z.l. *et al.* (2003) ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron* 40, 971–982.

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press

AUTHORS' ADDRESSES

[1] Department of Developmental Molecular Anatomy, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

[2] Department of Anatomy and Neurobiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Correspondence should be addressed to:
Shozo Jinno
Department of Developmental Molecular Anatomy
Graduate School of Medical Sciences
Kyushu University
3-1-1 Maidashi, Higashi-ku
Fukuoka 812-8582
Japan
phone: 81-92-642-6051
fax: 81-92-642-6202
email: sjnno@med.kyushu-u.ac.jp

https://doi.org/10.1017/S1740925X11000135 Published online by Cambridge University Press