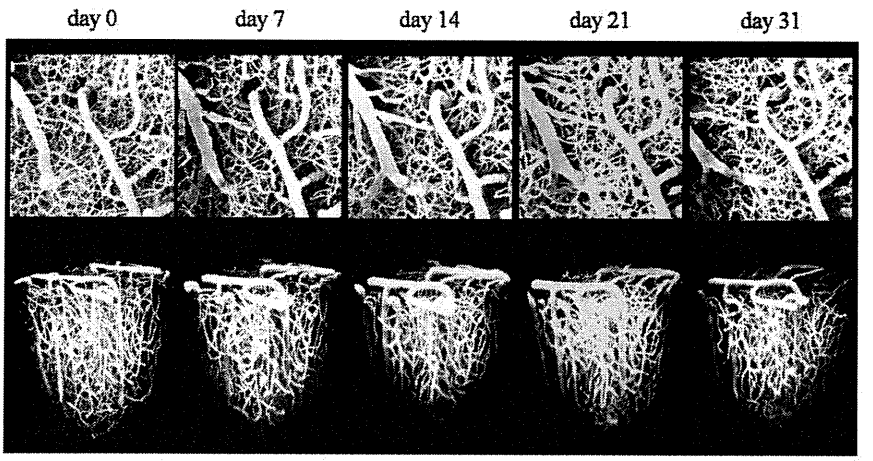

Figure 2. The 3D imaging of cortical microvasculature with two-photon microscopy. Volume images ($0.46\times0.46\times0.80$ mm^3) were obtained from day 0 (start of exposure to 10% oxygen) to day 31 for the identical region within the cortex. The upper panels show the maximum intensity projected images in the x-y plane (0.46×0.46 mm^2), and the lower panels show the 3D reconstructed images.

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Review Article

History of International Society for Cerebral Blood Flow and Metabolism

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Interest in the brain's circulation dates back more than a century and has been steadily growing. Quantitative methods for measurements of cerebral blood flow (CBF) and energy metabolism became available in the middle of the 20th century and gave a new boost to the research. Scientific meetings dealing with CBF and metabolism were arranged, and the fast growing research led to a demand for a specialized journal. In this scientific environment, the *International Society for Cerebral Blood Flow and Metabolism* (ISCBFM) and its official *Journal of Cerebral Metabolism* were established in 1981 and has since then been a major success. The development of new brain imaging methods has had a major impact. Regulation of CBF and ischemia has been the main topics at the meetings. A new field of brain mapping research emerged and has now its own society and meetings. Brain emission tomography research has grown within the society and is now an integrated part. The ISCBFM is a sound society, and support of young scientists is among its goals. Several awards have been established. Other activities including summer schools, courses, satellite meetings, and Gordon conferences have contributed to the success of the society and strengthened the research.
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Introduction and early history of cerebral blood flow

Interest in cerebral blood flow (CBF) and metabolism dates back to at least the 19th century. For many years, studies on CBF were indirect and based first on observations of pressure changes in the brain tissue, direct observation of diameters of cerebral blood vessels, etc. (e.g., Fog, 1938; Roy and Sherrington, 1890), and then later on changes in local tissue temperatures or cerebral arteriovenous oxygen differences (Wolf, 1936; Sokoloff, 1959). The first truly quantitative method for measuring CBF and metabolism was the bubble-flow meter method of Dumke and Schmidt (1943), but this method required such extensive surgical interventions in the cerebral vasculature that it was used only in monkeys under general anesthesia. The field was entirely revolutionized when Kety and Schmidt (1945, 1948) published their nitrous oxide method for the quantitative determination of rates of CBF and metabolic rates in unanesthetized, conscious human subjects.

The nitrous oxide method contributed enormously to knowledge of the physiology, pharmacology, and pathophysiology of the overall cerebral circulation and energy metabolism, but it failed to show any relationships between normal functional activities of the brain and its blood flow and energy metabolism. This failure was believed because the method measured only average blood flow and metabolism in the brain as a whole, whereas functional activities were localized to specific regions of the brain. What was needed were methods to measure blood flow and metabolism in localized regions of the brain.

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Kety (1951) published his theory of inert gas exchange between blood and tissues. He had previously applied these principles to measure local blood flow in muscle on the basis of the time course of the clearance of ^{24}NaCl after its injection directly into the tissue (Kety, 1949). Tissue clearance of ^{24}NaCl could not, however, be used for measuring CBF because Na^+ does not freely diffuse across the blood–brain barrier The same principles, however, were, subsequently, applied to develop a method to measure local CBF (Landau *et al*, 1955). A freely diffusible, chemically inert, radioactive gas, [^{131}I]trifluoroiodomethane, was used as the tracer, and quantitative autoradiography was used to determine local tracer concentrations in the brain from which, together with the history of the tracer's arterial concentration, local blood flow could be calculated. Applications of this method in cats showed that local blood flow in the brain did indeed change with altered local functional activity, and its autoradiographic images provided the first demonstrations of functional brain imaging on the basis of local blood flow (Sokoloff, 1961). The [^{131}I]trifluoroiodomethane method was, however, not widely used because the tracer is a gas and, therefore, difficult with which to carry out autoradiography, and also it was not commercially available and required frequent organic synthesis within the laboratory. These limitations were, subsequently, overcome first by Reivich *et al* (1969) who adapted the method for use with the nonvolatile tracer [^{14}C]antipyrine, and later by Sakurada *et al* (1978) who used the more diffusible [^{14}C]iodoantipyrine.

In the 1950s, Niels Lassen had modified the Kety–Schmidt nitrous oxide method for use with the radioactive inert gases, ^{85}Kr or ^{133}Xe. Subsequently in the 1960s, in collaboration with David Ingvar and others, Lassen adapted the ^{24}NaCl clearance technique (Kety, 1949) for measurement of regional CBF (rCBF) in humans by labeling the brain via direct injection of the ^{85}Kr or ^{133}Xe into the internal carotid artery and then recording its clearance from various regions of the brain by means of a battery of critically positioned scintillation counters (Lassen and Ingvar, 1961; Hoedt-Rasmussen *et al*, 1967). This method was the first to achieve measurements of regional CBF in humans and was widely used by Lassen *et al* (1978) to study the local changes in CBF in a variety of normal and pathological functional states. Obrist *et al* (1967), subsequently, modified this method by replacing the intraarterial injection with inhalation of ^{133}Xe, a modification preferred by many investigators.

The regional cerebral blood flow meetings

The availability of the ^{133}Xe methods led to a flood of studies of regional CBF throughout the world, which stimulated demand for international meetings where new developments and ideas could be presented and discussed. The initiators of these methods, Lassen in Copenhagen and Ingvar in Lund, had very active programs in rCBF research at home and in a network of collaborations internationally. They together provided the driving force to organize meetings, named rCBF Symposia, in which research in the field could be presented. The first one was held in Lund in 1965, and the next one in Lund and Copenhagen in 1968.

Thereafter, the rCBF Symposia were held, at first annually and then biennially, at various places in the world (Table 1). Because the rCBF methods were limited to measurements of CBF, the contents of the rCBF meetings were mainly about the cerebral circulation. The field was, however, greatly expanded from its focus on CBF to include cerebral metabolism after the development in the 1970s of the [^{14}C]deoxyglucose method for measuring local rates of cerebral glucose utilization in animals with autoradiography (Sokoloff *et al*, 1977) and its subsequent adaptations for use in humans with [^{18}F]fluorodeoxyglucose, first with single-photon imaging by Reivich *et al* (1979) and then with positron emission tomography (PET) by Phelps *et al* (1979). The availability of these methods was soon followed by a further proliferation of studies, now not only on the blood flow but also on the energy metabolism of the brain.

Origin of the Journal of Cerebral Blood Flow and Metabolism and the International Society for Cerebral Blood Flow and Metabolism

The idea to establish a society specifically devoted to the field of CBF and metabolism evolved from some casual conversations between Bo K Siesjö and Louis Sokoloff. Siesjö thought that, in view of the explosion of studies in the field, there was need for a more specific forum through which research in this area could be published. The relevant literature in this field was then scattered in a number of journals dedicated to the basic sciences as well as to clinical journals. He, therefore, sought Sokoloff's advice on how to establish such a journal. Sokoloff had previously been Chief Editor of the *Journal of Neurochemistry* and, subsequently, Chairman of the Publications Committee of that journal's parent society, the *International Society for Neurochemistry*, when this committee negotiated a change in publishers of its journal from Pergamon Press to Raven Press, and Siesjö thought this experience would be helpful in establishing a new journal. Sokoloff agreed that there was a need for the proposed journal, but only if it could be the official organ of a professional society directly related to the subject matter of the journal. No such society existed, but as mentioned above, there had been the series of rCBF meetings described

Table 1 The 25 CBF/Brain meeting from 1965 to 2011

Year	*Meeting nr. and location*			*Local main organizer*	*Program Committee Chairman*
1965	1	rCBF	Lund	David H Ingvar and Niels A Lassen	
1968	2	rCBF	Lund-Copenhagen	David H Ingvar, Niels A Lassen, Bo K Siesjö and Erik Skinhøj	
1969	3	rCBF	Mainz	Mario Brock	
1970	4	rCBF	London	John Marshall and Lindsay Symon	
1971	5	rCBF	Rome/Sciena	Cesare Fieschi	
1973	6	rCBF	Philadelphia	Thomas Langfitt, Lawerence McHenry, Martin Reivich, Harry Wollman	
1975	7	rCBF	Aviemore	A Murray Harper	
1977	8	rCBF	Copenhagen	Niels A Lassen	
1979	9	rCBF	Tokyo	Fumio Gotoh	
1981	10	rCBF	St Louis	Marcus Raichle	
1983	11	ISCBFM	Paris	Eric T MacKenzie, Jacques Seylaz, Andre Bes	David H Ingvar
1985	12	Brain'85	Ronneby, Sweden	David Ingvar, Christer Owman and Bo K Siesjö	
1987	13	Brain'87	Montreal	Antoine Hakim	
1989	14	Brain'89	Bologna	Cesare Fieschi and Gian-Luigi Lenzi	Eric MacKenzie
1991	15	Brain'91	Miami	Myron Ginsberg	Eric MacKenzie
1993	16	Brain'93	Sendai	Kyuya Kogure and Takashi Yoshimoto	Olaf B Paulson
1995	17	Brain'95	Cologne	Konstantin-A Hossmann and Wolf-Dieter Heiss	Michael A Moskowitz
1997	18	Brain'97	Baltimore	Richard J Traystman	Wolfgang Kuschinsky
1999	19	Brain'99	Copenhagen	Olaf B Paulson	Frank Welsh
2001	20	Brain'01	Taipei	Tony Lee and Shin-Zonn Lin	Antony Strong
2003	21	Brain'03	Calgary	Roland N Auer	Joel Greenberg
2005	22	Brain'05	Amsterdam	Adriaan Lammertsma	Ulrich Dirnagl
2007	24	Brain'07	Osaka	Koji Abe Hidehiro Iida	Constantino Iadecola
2009	24	Brain'09	Chicago	Dale A Pelligrino	Joseph LaManna
2011	25	Brain'11	Barcelona	Anna M Planas	Edith Hamel

ISCBFM, International Society for Cerebral Blood Flow and Metabolism; rCBF, regional cerebral blood flow.

above and listed in Table 1. These rCBF meetings seemed to provide a foundation on which the new society could be built.

The informal discussions between Siesjö and Sokoloff about the new society and its journal culminated in March 1980 at a scientific meeting in Paris where plans to organize their establishment were formulated. A steering committee, serving as a provisional council, was selected to implement these plans. The committee, chaired by Louis Sokoloff, included Bo K Siesjö, Cesare Fieschi, Konstantin-A Hossman, David H Ingvar, Igor Klatzo, Niels A Lassen, Eric MacKenzie, Marcus Raichle, Martin Reivich, and Fred Plum. Fred Plum accepted the responsibility of consulting lawyers and formulating the By-Laws for the proposed ISCBFM. The committee chose Murray Harper to be the first Editor-in-Chief of the Society's new journal, *The Journal of Cerebral Blood Flow and Metabolism*, as well as an editorial board selected from scientists throughout the world. Raven Press was solicited and accepted to publish the journal on behalf of the proposed society. The goal was to finalize the establishment of the society at the next rCBF Symposium scheduled to be held in St Louis in 1981.

The ISCBFM was formally established at the meeting in St Louis. The provisional council appointed Seymour Kety as honorary President for Life and Sokoloff as president for a 2-year term. The decision was also made to hold the Society's first official organizational meeting in Paris in 1983 and their international meetings biennially thereafter.

The Society's first meeting in Paris was notable for a number of reasons. First of all, the By-Laws, which define the Society's rules and procedures and are essential for legal incorporation, were ratified by those attending the business meeting. Second, the choice of Raven Press to publish the Journal on behalf of the Society was approved. Third, the meeting was the first one for which the number and diversity of abstracts required parallel scientific sessions to be held, reflecting the growth of the research field. Finally, a new president had to be elected because Sokoloff's term was expiring at the end of the 1983 meeting. Because there had not previously been any By-Laws, procedures for election of a president had not been defined before the meeting, and an *ad hoc* procedure had to be implemented. A committee chaired by Seymour Kety was appointed and charged with the task of nominating a candidate to be presented for ratification by the attendees at the meeting. The committee could not decide and nominated both Niels Lassen and David Ingvar to serve as joint presidents, and their election was ratified by a vote of the attendees. The meeting was a spectacular success, not only scientifically, but also socially. The banquet was held in the Conciergerie where Marie Antoinette had been held prisoner before her execution during the French Revolution.

The second meeting of ISCBFM (12th rCBF meeting) was held in 1985 in Ronneby, Sweden. It was at this meeting that David Ingvar, Chairman of the Local Organizing Committee, introduced the term **'Brain'85'** for the meeting, and it was celebrated by good 'Brain Brew' (Figure 1). This became a tradition, and all meetings since then have been called Brain'XX (Table 1). A new president was elected, now in full accordance with the By-Laws of the society and since then a new distinguished scientist has been elected president to take over at the biennial meetings for a 2-year term (Table 2). A ballot system send out to all member of ISCBFM before the biennial meetings is used for the election of both president and other officers of the board of directors.

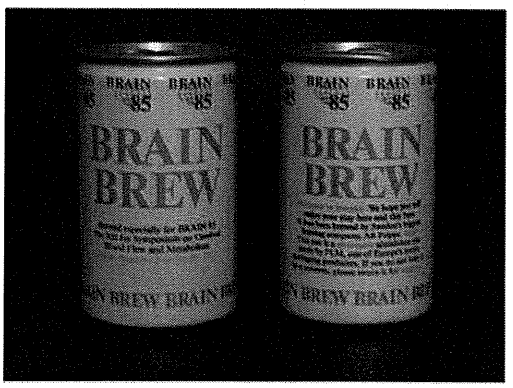

Figure 1 Brain brew. A happening with Swedish beer prepared for the Brain'85 meeting in Ronneby, Sweden. It was the first time the International Society for Cerebral Blood Flow and Metabolism (ISCBFM) meeting was called Brain'XX and the name has remained for all subsequent meetings.

The International Society for Cerebral Blood Flow and Metabolism

Since 1981, seventeen distinguished scientists have served as president, seven as secretary, and six as treasurer of the ISCBFM (Table 2). The society is financially sound. Most meetings have had a balanced budget or produced a small surplus, the journal has provided a steady income, and capital has accumulated. This has allowed the society to support activities related to its meetings and educational courses, including travel grants for young scientist attending the Brain' and BrainPET meetings (the latter are described below).

Special features, which permeated research meetings in the field of CBF from its earliest origins, are commitments to develop young investigators and to maintain an interactive social program in our biennial meetings (and Summer Schools, satellite meetings, and Gordon Conferences). Promotion of informal interactions between senior and junior researchers at our meetings with the involvement of young investigators (via their own committee) in the affairs of the society has been a distinctive feature of our meetings for decades and will be maintained in the decades to come.

The Organ, newsletter from 1990

The Newsletter of the ISCBFM was created in 1990 to promote communication among the membership under the editorship of James McCulloch. The Newsletter takes its name, 'Complex Heterogeneous Organ,' from the opening line of a paper by the first president of the society (Sokoloff *et al*, 1977). From 2005 on, it has just been called 'The Organ'.

Table 2 ISCBFM presidents, secretaries, and treasures

Year	*President*	*Secretary*	*Treasurer*
1981–1983	Louis Sokoloff	Bo K Siesjö	Konstantin-A Hossmann
1983–1985	Niels A Ladssen and David H Ingvar	Marcus Raichle	Konstantin-A Hossmann
1985–1987	Bo K Siesjö	James McCulloch	William Pulsinelli
1987–1989	Cesare Fieschi	James McCulloch	William Pulsinelli
1989–1991	Fred Plum	James McCulloch	Akira Tamura
1991–1993	Marcus Raichle	James McCulloch	Akira Tamura
1993–1995	Konstantin-A Hossmann	James McCulloch	Akira Tamura
1995–1997	Kyoya Kogure	Lorris Betz	Eric MacKenzie
1997–1999	Martin Reivich	Lorris Betz	Eric MacKenzie
1999–2001	Olaf B Paulson	Lorris Betz	Eric MacKenzie
2001–2003	Michael Moskowitz	Gitte Moos Knudsen	Wolfgang Kuschinsky
2003–2005	Iwao Kanno	Gitte Moos Knudsen	Wolfgang Kuschinsky
2005–2007	James McCulloch	Dale A Pelligrino	Wolfgang Kuschinsky
2007–2009	Joel H Greenberg	Dale A Pelligrino	Peter Herscovitch
2009–2011	Koji Abe	Dale A Pelligrino	Peter Herscovitch
2011–2013	Richard J Traystman	Joseph C LaManna	Peter Herscovitch

ISCBFM, International Society for Cerebral Blood Flow and Metabolism.
The term of the president and secretary is from biannual to biannual meeting.
The term of the treasure is shifted and starts with the year after the biennial meeting.

Summer schools

Summer schools or educational satellite courses in connection with the biennial meetings were established in 1994 and have since been a regular event.

Lifetime achievement awards

This award was established in 1997 and is awarded to a scientist for his outstanding contributions to the field of CBF and metabolism and the ISCBFM. The recipient of the Life Time Achievement Award receives a medal at the award ceremony at the opening of the biennial meeting. The recipients are listed in Table 3.

Lassen award

The Lassen award was established in 1999 in memory of Niels A Lassen (1926 to 1997) whose influence, not only generally on the field of CBF and metabolism but also on the young investigators, was so profound. The award recognizes an outstanding scientific contribution made by a young scientist. The recipients have been selected based on an abstract submitted for presentation at the biennial meeting of the Society. In 2005, the procedure was modified, and a shortlist of the top ranked abstracts was selected for oral presentation in a special session. Based on the presentations, a final decision on the recipient was then made. The award consists of a bursary to partially defray the expense of attending the Brain' meeting, a certificate of recognition, and a small cash prize. The recipients of the Lassen Award are listed in Table 4.

Table 3 Lifetime achievement award

1997	Niels A Lassen and David H Ingvar
1999	Seymour S Kety and Louis Sokoloff
2001	Fumio Gotoh
2003	Fred Plum and Bo K Siesjö
2005	Martin Reivich
2007	Konstantin-A Hossmann
2009	Cesare Fieschi, Takaaki Kirino, and Akira Tamura
2011	Marcus Raichle

Technological advances and the research fields

In the last three decades of the 20th century, new brain imaging methods were developed, for example, PET, single-photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI), which became important tools in the investigation of CBF and metabolism and exerted major impact on the field. The earliest demonstration of a relationship between regional cerebral glucose utilization and functional activity in the brain was achieved with the autoradiographic [^{14}C]deoxyglucose method in animals (Kennedy *et al*, 1975) and, subsequently, in the human brain with PET and [^{18}F]fluorodeoxyglucose (Phelps and Mazziotta, 1985). After Lassen's and Ingvar's demonstrations in the 1970's of changing landscapes of regional CBF with altered functional activities in the working human brain (Lassen *et al*, 1978), two milestones have contributed enormously to the development of functional imaging. Fox and Raichle (1986) showed a mismatch between CBF and cerebral oxygen metabolism under normal physiological brain activation which surprised the neuroscience community. Then, Ogawa *et al* (1990) showed that the blood oxygenation level-dependent signals in MRI could be used to localize neural responses during functional activation. It was soon realized that the blood oxygenation level-dependent effect reflected the relatively greater increase in CBF than in oxygen utilization during neuronal activation. The discovery of the blood oxygenation level-dependent effect stimulated a new flood of investigations into the mechanism behind it. Many of these focused on physiological variables like blood flow, blood volume, hematocrit, blood oxygenation, oxygen consumption, energy balance, etc. Understanding the mechanisms of how blood flow and

Table 4 Lassen award recipients

	Name	*Title of presentation*
1999	Matthias Endres	A novel role of DNA methyltransferase during murine cerebral ischemia
2001	Sylvain Doré	Amyloid precursor protein (APP) renders neurons more vulnerable to oxidative stress
2003	Fahmeed Hyder	Probing neural function with fMRI via changes in oxidative energy consumption
2005	Kirsten Caesar	Activity-dependent oxygen transients in rat cerebellar cortex are blocked by synaptic inhibition
2007	Kazuhiko Hayashi	Cerebral blood flow, oxygen consumption, and glucose utilization in humans: a stereotaxic correlation studied with PET and SPM
2009	Alyson A Miller	Excessive superoxide production and endothelial dysfunction in cerebral arteries of atherosclerotic mice are due to enhanced activity of NOX2-containing NADPH oxidase
2011	Virginia Newcombe	Serial diffusion tensor imaging suggest progressive pathophysiology for weeks after traumatic brain injury, and white matter repair month after injury

fMRI, functional magnetic resonance imaging; PET, positron emission tomography; SPM, statistical parametric mapping.

oxygen supply are regulated in response to neuronal stimulation and inhibition, spiking, and field potentials, and the role of glial cells remain some of the main interests of the ISCBFM.

Recent investigations of neurovascular coupling have used a variety of new modern techniques. One example is two-photon laser microscopy. By using fluorescent dyes and endogenous fluorescent transgenic mouse models, one can visualize the 3-dimensional network structure of vessels, glia, neurons, fibers, and various molecular behaviors. Many other topics and goals of current research, including, for example, molecular and genetic aspects, are also presented at the brain meetings.

Positron emission tomography, SPECT, and MR have also had a broader impact on the field. Positron emission tomography and SPECT have made possible molecular mapping and mapping of neurotransmitter and receptor binding, and MR spectroscopy has enabled measurements of regional metabolite concentrations in the brain.

The development of focal CBF techniques underpinned the tremendous expansion of brain ischemia research throughout the 1980s and 1990s. The biochemical cascades that lead to ischemic cell death were elucidated in animal models together with mechanisms (physiologic, pharmacologic, and genetically) by which they could be manipulated. Notable examples of this research are Tamura's studies on a focal ischemia model (Tamura *et al*, 1981), the Ginsberg group demonstration of the impact of small temperature changes on brain damage (Busto *et al*, 1987), and Siesjö and Bengtsson's review (1998) on mechanisms of ischemic damage.

In addition to the methodological developments, CBF regulation, cerebral ischemia, and brain mapping have throughout the years remained major topics at the Brain meetings. Important subtopics contributing to the highlights of the meetings have been control of cerebral blood vessels, mechanisms of injury, neuroprotection, neurobehavior, model development, and role of genetics, for example, in injury.

Satellite meetings

In 1973, Fred Plum unofficially organized a satellite meeting on severe hypoxia and brain oxygen consumption to be held in New York after the rCBF meeting in Philadelphia. Officially sanctioned satellite meetings began with a satellite meeting on the blood–brain barrier, organized in Copenhagen by Olaf B Paulson and co-workers, after the biennial meeting of the ISCBFM in Ronneby, Sweden in 1985. Since then, satellite meetings have been held in association with the biennial meetings and have been arranged to be held in the vicinity (bus transportation) of the main meeting. They emerged from the interest of scientists who had the desire of organizing smaller symposia focusing on subtopics of special interest, preferentially for researchers who attended the main meeting. There is a strong preference that such satellites are held after and not before the main meeting to not distract the attention from the main event.

The BrainPET' meetings

The number of participants and topics at the main Brain meetings grew rapidly and thus left limited time for detailed discussion on some methodological issues. This led to a desire for more communications, particularly about PET studies. As a consequence, in Miami in 1991, Terry Jones, Jim Holden, Jean-Claude Baron, and Iwao Kanno agreed to organize a meeting focused on PET methodology and quantification. That meeting, the first BrainPET, was held in Akita as a satellite of the main Brain meeting in Sendai in 1993. Under the title 'Quantification of Brain Function using PET', the meeting aimed not only to improve the exchange of ideas, but also more ambitiously strove to set new and higher standards for PET research. It was a success, and since then BrainPET meetings have been continued. As many would like to attend both the Brain meeting and the BrainPET satellite, and as the number of international meetings was increasing rapidly, discussions were undertaken by the BrainPET organizers of the Bethesda Brain PET satellite meeting in Brain'97 and the organizers of Brain'99, especially Gitte Moos Knudsen. The decision was made to include the BrainPET sessions as an integral part of the next biennial ISCBFM meetings to be held in Copenhagen. Brain'99 was thus called 'Brain'99 and BrainPET'99'. It proved to be a success, and since then, BrainPET has been an integral part of all subsequent Brain'XX meetings.

In response to developments in neuroscience PET research, the main topics of the BrainPET meeting have expanded from studies of CBF and metabolism to include quantification of molecular biochemical processes (e.g., neurotransmission, neurodegeneration, and neuroinflammation).

The organization for human brain mapping

That local CBF is increased by local functional activation was first demonstrated and visualized in cats by means of the autoradiographic [^{131}I]trifluoroiodomethane method (Sokoloff, 1961). Lassen *et al* (1978) were the first to show with their ^{133}Xe methods that measurement of rCBF could also be used for mapping local brain functions in the human brain. The subsequent development of SPECT, PET, and functional MRI methods for mapping local changes in cerebral glucose utilization or blood flow in human subjects led to an enormous proliferation

of studies on the mapping of functional activities in the human brain. In the early 1980s, <1% of brain research involved PET, MRI, and SPECT, and, reflected only a minor part of the activities in the ISCBFM when the society was established in 1981. Since then, human brain mapping has steadily grown, and now PET and MRI studies account for just above 20% of all brain research (PubMed search using the queries 'positron emission tomography (MeSH Terms) OR magnetic resonance imaging (MeSH Terms) AND brain (MeSH Terms) AND year (dp)' and 'brain (MeSH Terms) AND year (dp)'). This led in the late 1980s and the early 1990s to discussions by the Board of Directors of the ISCBM about whether the names of the society and its journal should have added to them the term 'and Function', for example, 'ISCBFM' and 'Journal of Cerebral Blood Flow, Metabolism, and Function.' Many were reluctant to such a name change because it might create confusion and move the scope of the journal away from its original orientation toward more broad-based science related to CBF and metabolism. Some expressed the opinion that molecular biology was just as important as or maybe even more so than function reflected by blood flow and energy metabolism. No changes were, therefore, made in the names of the society and journal.

In 1995, after the ISCBFM meeting in Cologne, a satellite meeting on human brain mapping was held in Paris. There it was decided to create a new society, the *Organization for Human Brain Mapping*, and two new journals were born, that is, 'Neuroimage' and 'Human Brain Mapping.' It was also decided to hold annual Human Brain Mapping meetings. At first, some thought that it was a pity to split the activity of the ISCBFM into two independent meetings and societies. At the ISCBFM meeting held in Copenhagen in June 1999, the organizers tried to keep the two meetings back-to-back by moving Brain'99 1 week to allow people coming from overseas to attend both meetings, including the Human Brain Mapping meeting being held in Düsseldorf. Since then, coordination has not been tried, and Human Brain Mapping meetings are completely independent of the ISCBFM meetings. In the long term, it seems best to separate these two activities. Human Brain Mapping now attracts several thousand scientists whose main interest appears to be to exploit phenomena that reflect changes in blood flow for mapping psychological brain functions and brain connectivity rather than on the normal physiology and pathophysiology of the cerebral circulation and metabolism, the main focus of the ISCBFM.

Gordon conferences

The Gordon conferences on CBF and metabolism have taken place during the last decade and represent one of the major activities of the ISCBFM. The initiative came from Martin Lauritzen, who proposed it to the Board of ISCBFM Directors after the Brain-BrainPET'01 meeting, and the first Gordon Conference was held in 2004. These meeting have taken place biennially since then in the years between the biennial Brain/BrainPET meetings. They are independent on the ISCBFM, but organized by members of the Society and also receive the Society's financial support. The primary topic of the biennial Gordon Conference on CBF and metabolism has been the basic physiological aspects of CBF and metabolism regulation. The main Brain meetings are too big and often cannot provide an atmosphere where researchers can discuss their results in depth and at length. Under these circumstances, the Gordon Research Conferences provide an excellent platform where cutting edge results can be presented and discussed in depth in a relatively informal manner.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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Early and progressive impairment of spinal blood flow–glucose metabolism coupling in motor neuron degeneration of ALS model mice

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The exact mechanism of selective motor neuron death in amyotrophic lateral sclerosis (ALS) remains still unclear. In the present study, we performed *in vivo* capillary imaging, directly measured spinal blood flow (SBF) and glucose metabolism, and analyzed whether if a possible flow–metabolism coupling is disturbed in motor neuron degeneration of ALS model mice. *In vivo* capillary imaging showed progressive decrease of capillary diameter, capillary density, and red blood cell speed during the disease course. Spinal blood flow was progressively decreased in the anterior gray matter (GM) from presymptomatic stage to 0.80-fold of wild-type (WT) mice, 0.61 at early-symptomatic, and 0.49 at end stage of the disease. Local spinal glucose utilization (LSGU) was transiently increased to 1.19-fold in anterior GM at presymptomatic stage, which in turn progressively decreased to 0.84 and 0.60 at early-symptomatic and end stage of the disease. The LSGU/SBF ratio representing flow–metabolism uncoupling (FMU) preceded the sequential pathological changes in the spinal cord of ALS mice and was preferentially found in the affected region of ALS. The present study suggests that this early and progressive FMU could profoundly involve in the whole disease process as a vascular factor of ALS pathology, and could also be a potential target for therapeutic intervention of ALS.
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Keywords: ALS; flow–metabolism uncoupling (FMU); G93A; local spinal glucose utilization (LSGU); spinal blood flow (SBF)

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal disease that is caused by the selective death of motor neurons. Approximately 5% to 10% of patients have a genetically inherited form known as familial ALS. About 15% to 20% of familial ALS cases are associated with missense mutations or small deletions in the gene that encodes Cu/Zn-superoxide dismutase 1 (SOD1) (Aoki *et al*, 1993; Rosen *et al*, 1993). Transgenic (Tg) mice that carry mutant SOD1 genes have been generated to elucidate how mutations in the SOD1 gene cause motor neuron death (Gurney *et al*, 1994; Murakami *et al*, 2007). Although the underlying mechanism of ALS has not yet been fully clarified, several reports have demonstrated noncell autonomous death of motor neurons (Boillee *et al*, 2006; Clement *et al*, 2003; Llinas *et al*, 2004; Nagai *et al*, 2007; Pramatarova *et al*, 2001; Wang *et al*, 2005).

During physiological conditions, there was a close relationship between blood flow and glucose metabolism (flow–metabolism coupling; FMC) (Leybaert, 2005; Weir *et al*, 2002), and this coupling was tightly controlled through functional regulation of neurovascular unit (NVU) (del Zoppo, 2010). Structural and functional abnormalities in this NVU have not been fully elucidated under pathological conditions (Lo and Rosenberg, 2009) especially in ALS. Several reports have demonstrated the reductions of blood

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flow and glucose metabolism in the cerebral cortex and the spinal cord of both ALS patients and the animal model (Dupuis *et al*, 2011; Guo *et al*, 2000; Waldemar *et al*, 1992; Zhong *et al*, 2008). Furthermore, we have very recently found that the damage in NVU was observed before motor neuron degeneration (Miyazaki *et al*, 2011), which may be an important pathologic pathway in human and animal model of ALS.

It remains still unclear whether if a possible FMC abnormality is present in ALS, and if such a FMC abnormality is related to motor neuron degeneration. In the present study, therefore, we performed *in vivo* capillary imaging and directly analyzed the FMC with spinal circulation and glucose metabolism.

Materials and methods

Animal Models

During the experiment, the animals were treated in accordance with the declaration of Helsinki and the guiding principles in the care and use of animals. Also, all experimental and animal care procedures were approved by the Animal Care and Use Committee of the Graduate School of Medicine, Dentistry, and Pharmaceutical Science of Okayama University. A Tg mouse line with the G93A human *SOD1* mutation (G1H/+) was obtained from Jackson Laboratories (Bar Harbor, ME, USA) and maintained as hemizygotes by mating Tg males with C57BL/6J females. The offspring were genotyped using a PCR assay with DNA obtained from tail tissue samples. We used 12-, 16-, and 19-week-old (W) G93A mice and age-matched non-Tg C57BL/6J littermates (wild type, WT) as controls. The 12-W Tg mice were considered to be at the presymptomatic stage, the 16-W mice to be at the early-symptomatic stage, and the 19-W mice to be at the end stage of the disease.

In Vivo Imaging of Spinal Capillary Vessels

For *in vivo* imaging, 12, 16, and 19 W of Tg mice ($n=5$, 6, 8 at each W), and 19 W of WT littermates ($n=5$) were used. The animals were initially anesthetized with 2% isoflurane and maintained with 1% during surgical procedures. Rectal temperature was maintained at 37.0°C ± 0.2°C by means of a feed back-controlled warm pad. The back of the animal was shaved, and the midline incision of the skin was made to expose the back musculature. The paravertebral muscles were carefully removed from the vertebral column, and laminectomy of the lumber vertebrae at the level of L4–5 was performed. Bleeding was controlled either by coating bone wax or by using small pieces of gelatin sponges (Astellas Pharma, Tokyo, Japan). For visualization of the spinal vasculature, a bolus of Qdot605 (2 μmol/L in 50 μL buffered solution; Invitrogen, Carlsbad, CA, USA) was injected from tail vein. Mice were put in lateral decubitus position, and the spinal column was stabilized by placing two of the spinal clamps along the anterior–posterior axis using stabilized devise as described in previous report (Davalos *et al*, 2008) with some modifications. A small well of Gelseal (GE HealthCare, Milwaukee, WI, USA) was built around the exposed spinal cord and filled with physiological saline, followed by immersion of ×20 objective lens (0.5 Na; Leica Microsystems, Wetzlar, Germany). The spinal vasculature was visualized with multiphoton excitation fluorescent microscope (TSC SP5; Leica Microsystems) at 900-nm excitation (Mai Tai HP; Spectra-Physics, Santa Clara, CA, USA) with an emission band-pass filter of 655/50 nm. Images were captured toward anterior horn (AH) region up to 500 μm depth from the spinal surface with a step size of 0.01 mm in the *z*-direction. A single capillary was defined as the single vessel having crosssectional thickness < 8 μm and for both edges continued to two new vessels as a branching, and the capillary diameter was measured using LAS AF software (Leica Microsystems). Capillary density was measured as percentage of area using ImageJ software (National Institutes of Health, Bethesda, MD, USA). For creation of Figures 1A–1D, each image was converted to TIFF format using LAS AF software and three continuous images were merged using photoshop software (Adobe, San Jose, CA, USA). The red blood cell (RBC) velocity was measured by tracking RBC that appeared as dark segment against brilliant plasma background through single capillary. Time-lapse images were obtained by line scan in each single flame, and image processing and calculation of average RBC velocity in each capillary were performed as described (Autio *et al*, 2011).

Histological Pathology

For histological pathology, 12, 16, and 19 W Tg mice ($n=5$ at each W), and 19 W WT littermates ($n=5$) were used. Each mouse was deeply anesthetized and transcardially perfused with heparinized saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The lumbar spinal cord spanning L4–5 was removed and further fixed by immersion in the same fixative for 4 hours and then frozen after cryoprotection with a series of phosphate-buffered sucrose solutions of increasing concentration (10%, 15%, and 20%). Transverse sections of 12 μm thickness were cut through the lumbar cord with a cryostat following cresyl violet staining (Nissl stain).

Measurement of Spinal Blood Flow

For the measurement of spinal blood flow (SBF), 12, 16, and 19 W of Tg mice ($n=6$, 5, 5 at each W), and age-matched WT littermates ($n=5$ at each W) were used. Prior to experiment, HR (heart rate) and blood pressure (SBP (systolic blood pressure), MBP (mean blood pressure), and DBP (diastolic blood pressure)) were measured by tail-cuff method in each mouse (PB-98A; Softron, Tokyo, Japan). Spinal blood flow was determined by the standard autoradiographic ^{14}C-iodoantipyrine (IAP) method as described (Sakurada *et al*, 1978) modified for mice (Jay *et al*, 1988). Briefly, the animals were initially anesthetized with 2% isoflurane and maintained with 1% in 69% N_2O/30% O_2, and polyethylene catheters (Neuroscience,

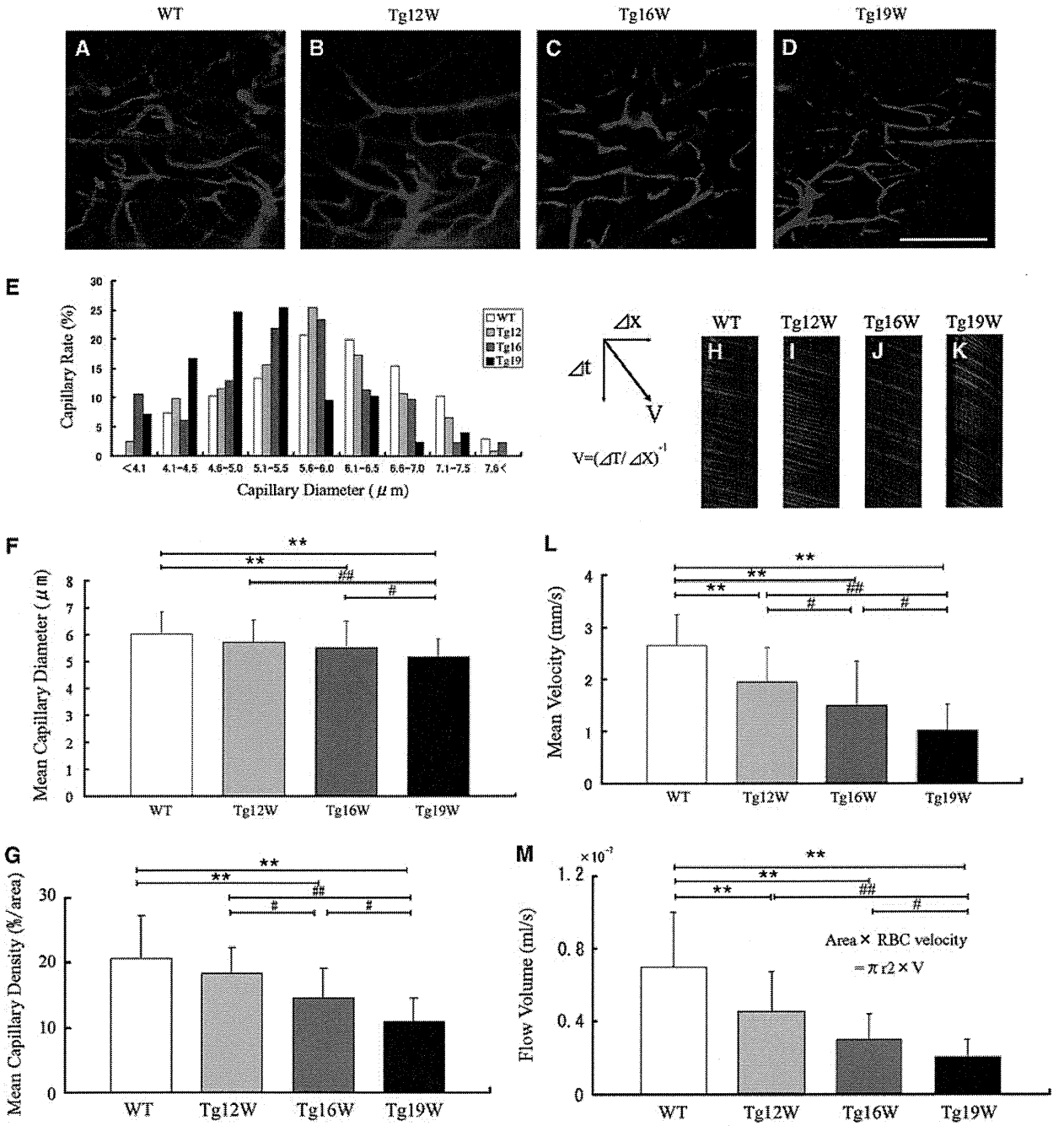

Figure 1 *In vivo* capillary vessel imaging in the anterior horn (AH) of lumbar cord in wild-type (WT; 19 W; **A**) and G93A-transgenic (Tg) mice at 12, 16, and 19 W (**B–D**). Scale bar = 100 μm. Frequency distribution histograms of the number of capillaries (percent in total counted capillaries) according to diameter (**E**), mean capillary diameter (**F**), and mean capillary density (**G**) during the disease course. Line scans of microvessels in the lumbar AH (**H–K**). Red blood cells (RBCs) appeared as dark segment against brilliant plasma background through single capillary. Mean RBC velocity (**L**). Flow volume calculated from RBC velocity and diameter in each capillary (**M**). $^{**}P < 0.01$ versus WT mice. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus Tg mice in different W.

Tokyo, Japan) were inserted into the left femoral artery and vein. Arterial blood was collected, and physiological parameters such as Hct (hematocrit), Hb (hemoglobin), pH, PO_2, PCO_2, and HCO_3 were measured (iSTAT300F, Fuso Pharmaceutical Industries, Osaka, Japan). ^{14}C-IAP (5 μCi in 100 μL of physiological saline; Perkin-Elmer Life and Analytical Sciences, Boston, MA, USA) was injected continuously via the femoral venous catheter. During injection, timed arterial blood was collected in preweighed tubes, and volume was calculated. Blood samples were resolved in tissue solubilizer (Perkin-Elmer), reacted with hydrogen peroxide to reduce quenching, added 3 mL of

scintillation fluid (Clear-Sol II, Nacalai tesque, Kyoto, Japan), and ^{14}C-IAP concentration was determined using liquid scintilation counter (TRICARB; Packard Instrument, Downers Grove, IL, USA). Precisely 1 minute after the IAP injection, mice were decapitated. The spinal cords were rapidly removed, frozen on a powdered dry ice, cut into 20 μm sections on a cryostat, and dried on a hot plate at 55°C. The sections were subject to autoradiography with ^{14}C-standards on an imaging plate (BAS-SR, Fujifilm, Tokyo, Japan) for 7 days, and autoradiograms were captured by image reader (FLA-7000, Fujifilm). Local tissue ^{14}C-concentrations were determined according to standards using densitometric analysis software (Multi Gauge Ver3.0, Fujifilm). The regions of interest were placed on five regions in gray matter (GM) (right and left AH, middle region of AH, right and left dorsal horn (DH)), and four regions in white matter (WM) (anterior funiculus, right and left lateral funiculus, and dorsal funiculus). Local SBF was calculated from the tissue concentration of ^{14}C-IAP and timed tracer concentration in the arterial blood according to the operational equation as described (Sakurada *et al*, 1978). Mean SBF for each region was determined by three contiguous sections in each regions of interest.

Measurement of Local Spinal Glucose Utilization

With a different set of mice group, 12, 16, and 19 W of Tg mice ($n=6$, 5, 5 at each W, and age-matched WT ($n=5$ at each W), were used for the measurement of local spinal glucose utilization (LSGU). Local spinal glucose utilization was determined by the standard autoradiographic ^{14}C-2-deoxyglucose (DG) method as described (Sokoloff, 1977). Briefly, the animals were inserted catheter under isoflurane condition. ^{14}C-2DG (3 μCi in 50 μL of physiological saline; Perkin-Elmer) was intravenously injected, and timed arterial blood was collected. The blood samples were centrifuged, and plasma glucose (Glucose analyzer, Pilot) and tracer ^{14}C-concentrations (Liquid Scintillaqtion Counter, Packard Instrument) were determined. After 45 minutes experimental period, spinal cords were removed, sectioned, and autoradiography was performed as described above. The setting of regions of interest and densitometric methodology were as same as SBF measurement described as above. Local spinal glucose utility was calculated from the tissue concentration of ^{14}C-2DG, timed tracer concentration and glucose concentration in the arterial plasma according to the operational equation with a lumped constant for rat as described (Sokoloff, 1977). Mean LSGU for each region was determined by three contiguous sections in each regions of interest.

Statistical Analysis

For the *in vivo* imaging analysis, differences among the each group were evaluated with one-way analysis of variance with normal distribution followed by the Tukey–Kramer test. Statistical differences in the physiological parameters, SBF, and LSGU analyses between the age-matched WT and Tg mice were evaluated by Student's *t*-test with normal distribution. A probability value <0.05 was regarded as statistically significant. Parametric data were presented as mean ± s.d.

Results

Physiological Parameters

There was no significant difference in physiological parameters (HR, SBP, MBP, DBP, Hct, Hb, pH, PO_2, PCO_2, and HCO_3) in Tg mice compared with age-matched WT mice (Table 1).

In Vivo Imaging of Spinal Capillary Vessels

There were considerable amount of blood vessel capillaries in the lumbar spinal GM of the WT with a diameter <8 μm (Figure 1A), but less dense in the (WM) (data not shown). The diameter of capillary vessels apparently became smaller in Tg mice with disease progression, and density of the capillary vessels progressively became lower (Figures 1B–1D).

Table 1 Physiological parameter

	12 W		*16 W*		*19 W*	
	WT (n = 5)	*Tg (n = 6)*	*WT (n = 5)*	*Tg (n = 5)*	*WT (n = 5)*	*Tg (n = 5)*
HR (b.p.m.)	679 ± 12	664 ± 15	682 ± 33	688 ± 40	692 ± 32	665 ± 85
SBP (mm Hg)	104 ± 3	110 ± 3	118 ± 10	112 ± 9	112 ± 8	112 ± 5
MBP (mm Hg)	81 ± 2	82 ± 2	85 ± 6	80 ± 7	81 ± 6	84 ± 4
DBP (mm Hg)	70 ± 1	68 ± 2	69 ± 7	64 ± 7	65 ± 7	71 ± 6
Hct (%)	38.4 ± 5.5	39.1 ± 7.8	37.6 ± 6.9	39.2 ± 6.1	40.2 ± 5.0	39.0 ± 6.2
Hb (g/dL)	13.2 ± 1.9	13.2 ± 2.5	13.0 ± 1.3	11.3 ± 4.8	13.7 ± 1.7	13.5 ± 1.9
pH	7.32 ± 0.07	7.34 ± 0.08	7.33 ± 0.06	7.33 ± 0.08	7.33 ± 0.06	7.29 ± 0.06
PO_2 (mm Hg)	123 ± 17	141 ± 20	114 ± 18	110 ± 28	106 ± 29	131 ± 33
PCO_2 (mm Hg)	38 ± 6	40 ± 7	32 ± 9	41 ± 5	35 ± 10	42 ± 7
HCO_3 (mmol/L)	20.8 ± 2.8	22.0 ± 2.3	20.6 ± 2.0	22.7 ± 3.6	20.9 ± 3.3	22.5 ± 4.0

DBP, diastolic blood pressure; Hb, hemoglobin; Hct, hematocrit; HR, heart rate; MBP, mean blood pressure; SBP, systolic blood pressure; Tg, transgenic; WT, wild type.

The histograms of capillary diameter gradually shifted from the right (large) to the left (small) with age in Tg mice (Figure 1E, gray to black bars), with the average of capillary diameter of progressive smaller among WT, Tg12, Tg16, and Tg19 W of $6.01 \pm 0.84\,\mu m$, $5.72 \pm 0.81\,\mu m$, $5.50 \pm 0.99\,\mu m$ ($^{**}P<0.01$ versus WT), and $5.16 \pm 0.68\,\mu m$ ($^{**}P<0.01$ versus WT, $^{\#\#}P<0.01$ versus Tg12, $^{\#}P<0.05$ versus Tg16) (Figure 1F). Mean capillary density measured as percentage of area showed progressive decrease from 20.7% in WT mice to 18.3% in Tg12 W, 14.6% in Tg16 W ($^{**}P<0.01$ versus WT, $^{\#}P<0.05$ versus Tg12), and 10.8% in Tg19 W ($^{**}P<0.01$ versus WT, $^{\#\#}P<0.01$ versus Tg12, and $^{\#}P<0.05$ versus Tg16) (Figure 1G). The mean RBC speed in a single capillary significantly decreased with disease progression (Figures 1H–1L), from 2.66 ± 0.59 mm/s of WT to 1.95 ± 0.65 mm/s of Tg12 W ($^{**}P<0.01$ versus WT), 1.50 ± 0.85 mm/s of Tg16 W ($^{**}P<0.01$ versus WT, $^{\#}P<0.05$ versus Tg12), and 1.01 ± 0.51 mm/s of Tg19 W ($^{**}P<0.01$ versus WT, $^{\#\#}P<0.01$ versus Tg12, and $^{\#}P<0.05$ versus Tg16), respectively. Flow volume was calculated from RBC velocity and diameter in each capillary, and showed significant decrease with disease progression in $0.70 \pm 0.30\ 10^{-7}$ mL/s in WT, $0.46 \pm 0.21\ 10^{-7}$ mL/s in Tg12 W ($^{**}P<0.01$ versus WT), $0.30 \pm 0.14 \times 10^{-7}$ mL/s in Tg16 W ($^{**}P<0.01$ versus WT), and $0.20 \pm 0.10\ 10^{-7}$ mL/s in Tg19 W ($^{**}P<0.01$ versus WT, $^{\#\#}P<0.01$ versus Tg12, and $^{\#}P<0.05$ versus Tg16), respectively (Figure 1M).

Histological Analysis

Nissl staining of the lumbar cord revealed that there were a number of large motor neurons in the AH of WT mice (Figure 2A). Although the number of motor neuron of Tg mice was similar to that of WT mice at 12 W (Figure 2B), it decreased progressively to ~78% of WT level at 16 W ($^{*}P<0.05$), and 35% at 19 W ($^{**}P<0.01$) (Figures 2C and 2D).

Spinal Blood Flow

In WT mice, SBF of GM was much higher than that of WM, and was slightly increased with normal aging from 12 to 19 W (Table 2; Figures 2E, 2I, 2M, and 3A–3I). Anterior horn showed a trend toward higher SBF than DH in cervical, thoracic, and lumbar regions (Table 2). As compared with age-matched WT mice, significant reduction of SBF was found in GM of Tg mice as early as 12 W, especially AH of cervical (−20%, $^{**}P<0.01$ in AH; −15%, $^{**}P<0.01$ in DH), thoracic (−16%, $^{*}P<0.05$ in AH), and lumbar cord (−24%, $^{**}P<0.01$ in AH; −18%, $^{**}P<0.01$ in DH), except for dorsal region of thoracic cord (Table 2; Figures 2F, 2J, 2N, 3A, 3D, and 3G). In contrast, there were no significant changes of SBF in any region of WM at 12 W. At 16 W of Tg mice, the reduction of SBF was progressively exacerbated in GM, and the reduction rate of SBF was prominent in AH (−39%, −32%, and −45% in cervical, thoracic, and lumbar cord, respectively, $^{**}P<0.01$ in each), and not so prominent in DH (−23%, −20%, and −32% in cervical, thoracic, and lumbar cord, respectively, $^{**}P<0.01$ in each), resulting in dissociative reduction of SBF between AH and DH (Table 2; Figures 2G, 2K, 2O, 3B, 3E, and 3H). There was no significant difference of SBF in WM too at this 16 W. At 19 W of Tg mice, the reduction of SBF in GM was further aggravated in cervical (−52%, $^{**}P<0.01$ in AH; −48%, $^{**}P<0.01$ in DH), thoracic (−41%, $^{**}P<0.01$ in AH; −35%, $^{**}P<0.01$ in DH), and lumbar cord (−56%, $^{**}P<0.01$ in AH; −50%, $^{**}P<0.01$ in DH) (Table 2; Figures 2H, 2L, 2P, 3C, 3F, and 3I). At this 19 W, SBF was only slightly reduced in WM especially lateral funiculus of cervical (−22%, $^{**}P<0.01$) and lumbar cord (−23%, $^{**}P<0.01$).

Local Spinal Glucose Utility

In WT mice, there were higher levels of LSGU in GM than WM of cervical, thoracic, and lumbar cords (Table 3; Figure 2Q, 2U, 2Y, 3J–R). The LSGU of AH had higher tendency than that of DH in each part of spinal cord, and LSGU were slightly decreased with normal aging from 12 to 19 W (Table 3). Transgenic mice at 12 W showed significant increases of LSGU in GMs of cervical (+8%, $^{*}P<0.05$ in AH) and lumbar cord (+18%, $^{**}P<0.01$ in AH; +16%, $^{*}P<0.05$ in DH) (Table 3; Figures 2R, 2V, 2Z, 3J, 3M, and 3P). No significant difference of LSGU was observed in GM of thoracic cord, or WM of all spinal cord regions at 12 W. In Tg mice at 16 W, significant reductions of LSGU were observed in GM regions of cervical (−23%, $^{**}P<0.01$ in AH; −35%, $^{**}P<0.01$ in DH) and lumbar spinal cord (−28%, $^{**}P<0.01$ in AH; −33%, $^{**}P<0.01$ in DH) (Table 3; Figures 2S, 2W, 2AA, 3T, 3W, and 3Z). There was no significant change in GM of thoracic cord and WM of all levels of spinal cord. In Tg mice at 19 W, the LSGU further decreased in GMs of cervical (−42%, $^{**}P<0.01$ in AH; −45%, $^{**}P<0.01$ in DH), thoracic cord (−45%, $^{**}P<0.01$ in AH; −45%, $^{**}P<0.01$ in DH), and lumbar cord (−40%, $^{**}P<0.01$ in AH; −35%, $^{**}P<0.01$ in DH) (Table 3; Figure 2T, 2X, 2AB, 3L, 3O, and 3R). Local spinal glucose utilization were slightly reduced in funiculus at this 19 W in cervical (−35%, $^{**}P<0.01$ in lateral funiculus; −21%, $^{*}P<0.05$ in dorsal funiculus), thoracic (−29%, $^{*}P<0.05$ in anterior funiculus; −30%, $^{**}P<0.01$ in lateral funiculus), and lumbar cord (−35%, $^{**}P<0.05$ in lateral funiculus).

Ratio of Local Spinal Glucose Utilization to Spinal Blood Flow

In WT mice, LSGU/SBF ratio of WM was slightly higher than that of GM, which slightly decreased

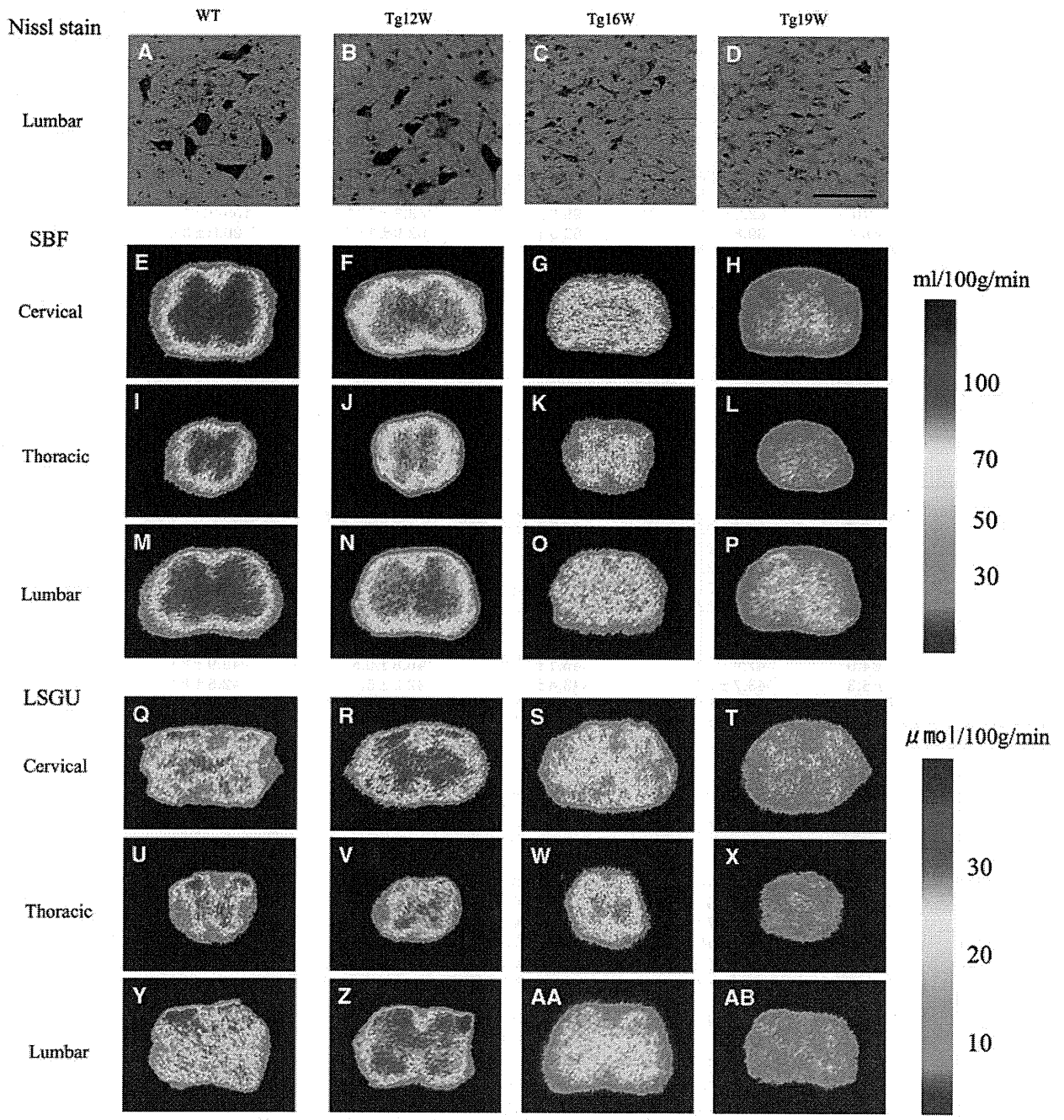

Figure 2 Nissl staining in anterior horn (AH) of lumbar cord in wild-type (WT) and transgenic (Tg) mice (**A–D**). Scale bar = 100 μm. Autoradiograms of spinal blood flow (SBF) in the cervical, thoracic, and lumber cord of WT and G93A-Tg mice (**E–P**). Autoradiograms of local spinal glucose utilization (LSGU) in the cervical, thoracic, and lumber cord of WT and Tg mice (**Q–AB**).

with normal aging from 12 to 19 W. Although the LSGU/SBF ratio remained at about 0.30 in most GM and WM of each spinal level, GM of lumbar cord showed the lowest ratio (0.27 ± 0.02, 0.23 ± 0.02, 0.21 ± 0.01 at 12, 16, and 19 W, respectively) among spinal coronal areas and longitudinal levels. The LSGU/SBF ratio of Tg mice at 12 W were significantly increased in GM of 0.40 ± 0.01 (+29%, $^{**}P<0.01$) in cervical, 0.41 ± 0.02 (+23%, $^{**}P<0.01$) in thoracic, and 0.39 ± 0.04 (+48%, $^{**}P<0.01$) in lumbar cord (Figures 3S, 3V, and 3Y). At 16 W, significant increases of the ratio were also seen in the GM of 0.36 ± 0.01 (+19%, $^{**}P<0.01$) in thoracic and 0.27 ± 0.02 (+15%, $^{*}P<0.05$) in lumbar cord. This increase continued until 19 W in lumbar GM of 0.28 ± 0.02 (+34%, $^{**}P<0.05$), but not seen in cervical and thoracic levels (Figure 3AA). In contrast, there was no significant difference of the LSGU/SBF ratio in WM between WT and Tg mice and no significant change with age in WT and Tg mice at any region (Figures 3S–3AA).

Table 2 Spinal blood flow (SBF)

Cervical	*12 W*		*16 W*		*19 W*	
	WT	*Tg*	*WT*	*Tg*	*WT*	*Tg*
GM						
AH (R)	96.7 ± 12.5	78.0 ± 13.6*	104.3 ± 19.5	64.0 ± 8.7**	104.9 ± 13.4	49.8 ± 5.5**
AH (L)	99.3 ± 12.5	79.8 ± 5.4*	106.0 ± 18.3	64.7 ± 15.9**	107.2 ± 9.4	51.6 ± 5.7**
MH	91.2 ± 10.5	82.3 ± 7.1	99.5 ± 18.4	65.3 ± 13.3**	105.8 ± 14.3	50.1 ± 5.0**
DH (R)	83.5 ± 8.4	69.8 ± 9.9*	82.5 ± 9.5	62.9 ± 11.7*	90.0 ± 3.4	44.9 ± 3.8**
DH (L)	82.4 ± 11.0	71.1 ± 3.3*	81.4 ± 12.5	63.6 ± 15.2	88.0 ± 8.6	48.4 ± 6.4**
WM						
AF	46.5 ± 9.3	53.5 ± 2.9	47.1 ± 6.3	48.9 ± 6.0	43.9 ± 8.0	40.6 ± 2.0
LF (R)	48.0 ± 8.3	48.9 ± 6.5	45.9 ± 7.1	46.2 ± 2.9	46.1 ± 10.3	37.4 ± 3.0
LF (L)	47.4 ± 4.8	54.6 ± 6.6	46.5 ± 7.1	50.9 ± 9.8	50.4 ± 8.6	38.1 ± 5.0*
DF	45.6 ± 6.6	45.4 ± 3.2	46.4 ± 5.8	47.2 ± 7.8	44.2 ± 8.8	35.9 ± 6.0
Thoracic	*12 W*		*16 W*		*19 W*	
	WT	*Tg*	*WT*	*Tg*	*WT*	*Tg*
GM						
AH (R)	79.4 ± 9.4	67.6 ± 6.2*	88.1 ± 1.9	59.9 ± 9.4**	80.7 ± 6.7	46.9 ± 4.7**
AH (L)	79.0 ± 11.3	66.0 ± 2.7*	85.8 ± 3.7	58.4 ± 10.0**	79.8 ± 7.3	47.1 ± 5.5**
MH	77.5 ± 9.4	67.0 ± 3.7*	78.5 ± 2.2	56.6 ± 5.2**	75.0 ± 10.5	45.4 ± 5.2**
DH (R)	68.2 ± 8.1	60.5 ± 6.7	68.4 ± 1.8	56.5 ± 8.5*	66.0 ± 3.8	44.3 ± 5.5**
DH (L)	68.2 ± 12.4	62.2 ± 2.7	66.8 ± 3.0	52.2 ± 4.0**	71.7 ± 9.1	45.3 ± 7.9**
WM						
AF	47.7 ± 4.0	47.9 ± 5.8	45.1 ± 1.7	46.8 ± 9.5	43.9 ± 8.9	37.7 ± 3.8
LF (R)	43.2 ± 5.3	43.7 ± 5.8	43.4 ± 1.3	42.2 ± 5.3	42.5 ± 8.9	37.7 ± 5.5
LF (L)	39.7 ± 4.8	46.1 ± 5.8	44.6 ± 1.9	38.9 ± 3.6	43.8 ± 8.6	35.6 ± 6.4
DF	44.6 ± 3.7	46.2 ± 4.7	43.1 ± 2.0	47.8 ± 8.4	43.0 ± 6.9	38.5 ± 6.5
Lumbar	*12 W*		*16 W*		*19 W*	
	WT	*Tg*	*WT*	*Tg*	*WT*	*Tg*
GM						
AH (R)	103.1 ± 14.1	74.3 ± 14.0**	119.9 ± 20.8	62.4 ± 7.3**	116.1 ± 22.3	49.0 ± 5.6**
AH (L)	103.0 ± 4.6	81.9 ± 9.1**	115.3 ± 19.8	66.3 ± 14.3**	114.2 ± 20.7	51.4 ± 7.7**
MH	104.0 ± 11.7	80.8 ± 7.9**	112.5 ± 24.1	60.7 ± 8.0**	120.2 ± 50.8	49.2 ± 6.9*
DH (R)	88.7 ± 13.7	67.7 ± 10.3*	87.0 ± 11.3	58.3 ± 5.4**	96.0 ± 10.2	45.8 ± 3.8**
DH (L)	86.9 ± 10.5	76.5 ± 8.7	85.6 ± 10.3	58.4 ± 8.2**	96.4 ± 9.6	50.5 ± 7.7**
WM						
AF	50.2 ± 4.2	51.1 ± 4.3	48.6 ± 5.9	50.3 ± 6.5	50.8 ± 4.5	40.9 ± 4.6*
LF (R)	45.8 ± 6.7	47.4 ± 3.7	46.4 ± 6.6	45.6 ± 5.8	45.9 ± 10.1	39.3 ± 3.6
LF (L)	49.7 ± 7.9	54.9 ± 8.4	45.4 ± 7.3	46.7 ± 4.1	54.8 ± 7.2	38.6 ± 3.8**
DF	47.6 ± 4.2	50.2 ± 4.4	46.1 ± 5.1	46.2 ± 8.2	48.7 ± 4.4	40.4 ± 3.8*

AF, anterior funiculus; AH, anterior horn; DF, dorsal funiculus; DH, dorsal horn; GM, gray matter; LF, lateral funiculus; MH, middle region of AH; Tg, transgenic; WM, white matter; WT, wild type.
The values are expressed as mean values ± s.d.
*$P < 0.05$, **$P < 0.01$ versus age-matched WT mice.

Figure 3 Regional spinal blood flow (SBF) in anterior horn (AH), dorsal horn (DH), anterior and dorsal funiculus (AF + DF), and lateral funiculus (LF) of cervical (**A–C**), thoracic (**D–F**), and lumber cord (**G–I**) of 12, 16, and 19 W in wild-type (WT) ($n = 5$ at each W) and transgenic (Tg) mice ($n = 6, 5, 5$ at each W). Local spinal glucose utilization (LSGU) in AH, DH, AF + DF, and LF of cervical (**J–L**), thoracic (**M–O**), and lumber cord (**P–R**) of 12, 16, and 19 W in WT ($n = 5$ at each W) and Tg mice ($n = 6, 5, 5$ at each W). LSGU/SBF ratio in AH, DH, AF + DF, and LF of cervical (**S–U**), thoracic (**V–X**), and lumber cord (**Y–AA**) of 12, 16, and 19 W in WT and Tg mice. *$P < 0.05$, **$P < 0.01$ versus age-matched WT mice.

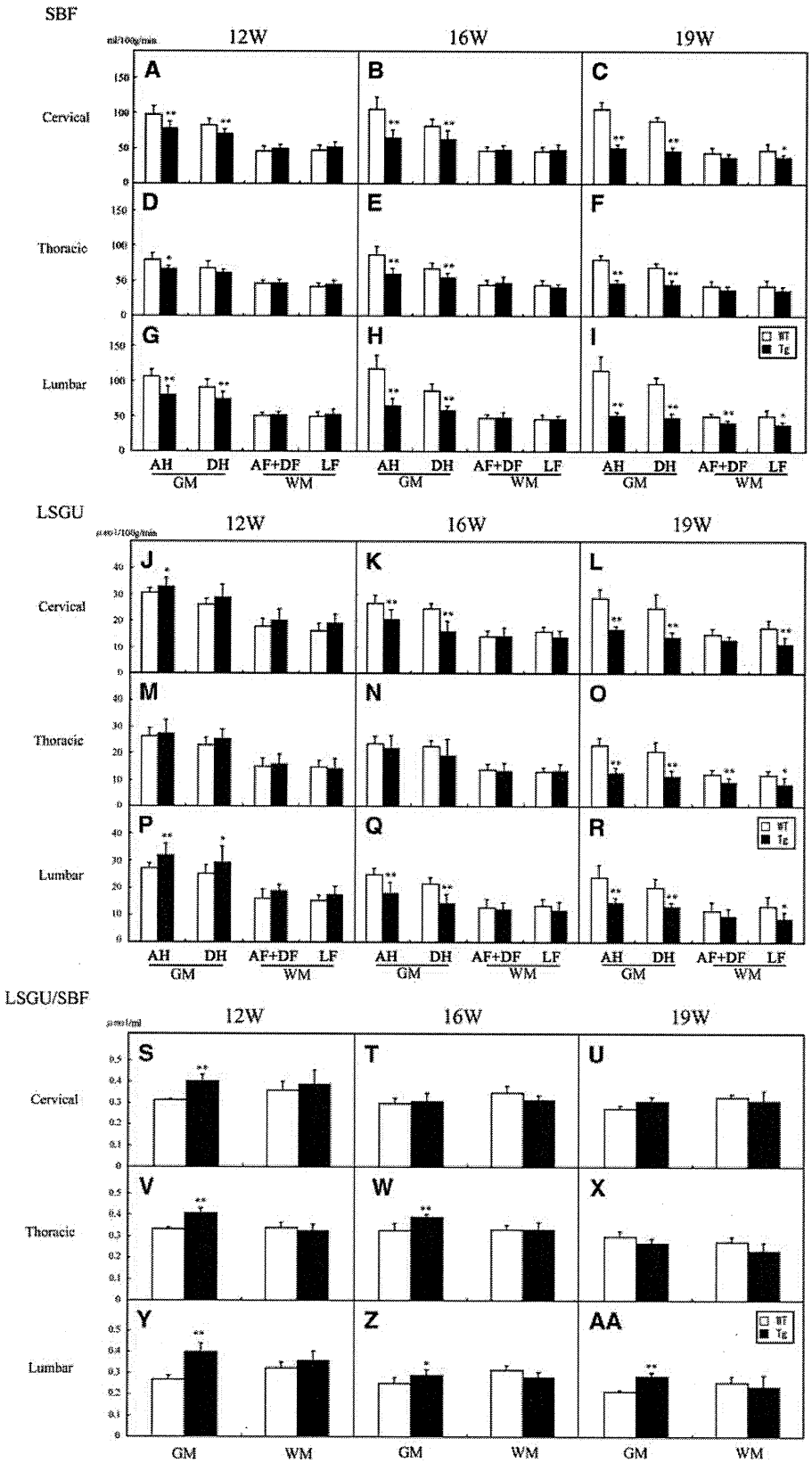
SBF
12W
16W
19W
Cervical
Thoracic
Lumbar
A
B
C
D
E
F
G
H
I
WT
Tg
AH
DH
AF+DF
LF
GM
WM
LSGU
J
K
L
M
N
O
P
Q
R
LSGU/SBF
S
T
U
V
W
X
Y
Z
AA

Table 3 Local spinal glucose utilization (LSGU)

Cervical	*12 W*		*16 W*		*19 W*	
	WT	*Tg*	*WT*	*Tg*	*WT*	*Tg*
GM						
AH (R)	30.9 ± 1.8	33.6 ± 3.6**	26.1 ± 3.1	20.0 ± 4.5*	27.1 ± 1.2	16.3 ± 1.0**
AH (L)	30.2 ± 1.7	32.1 ± 3.4	26.9 ± 3.6	20.9 ± 3.3*	30.1 ± 4.3	16.9 ± 2.0**
MH	28.5 ± 4.1	30.9 ± 4.3	26.2 ± 2.5	18.4 ± 4.1**	29.6 ± 5.7	15.6 ± 3.2**
DH (R)	26.3 ± 2.4	30.3 ± 3.7*	24.3 ± 2.3	16.2 ± 4.4**	23.3 ± 2.6	13.6 ± 1.7**
DH (L)	26.0 ± 2.3	27.0 ± 5.9	24.6 ± 2.3	15.5 ± 4.1**	26.0 ± 7.8	13.5 ± 2.8*
WM						
AF	16.8 ± 2.8	19.3 ± 3.6	14.8 ± 1.6	14.8 ± 3.9	14.6 ± 2.4	13.4 ± 1.5
LF (R)	17.4 ± 3.7	21.0 ± 2.7	16.4 ± 1.4	14.1 ± 3.2	16.1 ± 2.1	12.9 ± 2.1*
LF (L)	14.7 ± 1.5	17.0 ± 3.0	15.2 ± 2.6	13.4 ± 1.7	18.4 ± 3.4	9.4 ± 2.2**
DF	18.7 ± 3.1	20.7 ± 5.3	13.3 ± 2.8	13.5 ± 2.1	15.0 ± 2.3	11.8 ± 1.5*
Thoracic	*12 W*		*16 W*		*19 W*	
	WT	*Tg*	*WT*	*Tg*	*WT*	*Tg*
GM						
AH (R)	25.9 ± 3.8	26.2 ± 5.4	23.1 ± 1.9	22.5 ± 4.9	21.1 ± 1.2	12.0 ± 2.4**
AH (L)	26.8 ± 2.0	28.5 ± 5.1	23.8 ± 3.7	20.6 ± 5.5	24.8 ± 2.9	13.3 ± 1.3**
MH	25.3 ± 1.8	27.6 ± 4.5	23.6 ± 2.2	20.4 ± 4.8	24.4 ± 6.6	13.8 ± 2.8*
DH (R)	23.8 ± 3.9	25.9 ± 3.6	21.8 ± 1.8	19.3 ± 5.2	18.4 ± 1.6	11.7 ± 2.0**
DH (L)	22.2 ± 1.1	24.4 ± 4.3	22.8 ± 3.0	18.9 ± 7.4	23.0 ± 3.3	11.2 ± 2.7**
WM						
AF	15.8 ± 2.8	16.1 ± 2.9	15.2 ± 1.7	14.2 ± 3.1	13.5 ± 0.1	9.6 ± 2.3*
LF (R)	15.0 ± 3.1	16.1 ± 3.5	12.8 ± 1.3	13.2 ± 2.3	11.5 ± 1.2	10.1 ± 1.9
LF (L)	14.6 ± 1.7	12.3 ± 3.4	13.3 ± 1.9	13.2 ± 3.7	12.1 ± 2.4	6.4 ± 2.7*
DF	14.2 ± 4.0	15.5 ± 4.7	12.6 ± 2.0	12.5 ± 3.2	10.9 ± 1.9	8.7 ± 1.3
Lumbar	*12 W*		*16 W*		*19 W*	
	WT	*Tg*	*WT*	*Tg*	*WT*	*Tg*
GM						
AH (R)	27.8 ± 2.2	33.1 ± 3.6**	24.6 ± 2.3	19.0 ± 4.2*	23.8 ± 1.4	14.1 ± 2.3**
AH (L)	26.3 ± 1.4	30.5 ± 5.0*	26.5 ± 2.4	17.9 ± 4.7**	25.2 ± 7.0	15.6 ± 1.5*
MH	25.8 ± 1.7	29.1 ± 5.4	23.9 ± 1.9	15.8 ± 4.2**	24.8 ± 3.8	13.0 ± 2.7**
DH (R)	23.9 ± 4.0	30.0 ± 5.3*	21.9 ± 2.4	15.6 ± 2.8**	20.5 ± 2.3	13.8 ± 1.3**
DH (L)	26.3 ± 1.9	28.6 ± 6.8	22.3 ± 2.8	13.7 ± 4.2**	20.9 ± 4.9	13.2 ± 2.0*
WM						
AF	17.0 ± 2.6	19.5 ± 2.4	14.2 ± 1.9	13.1 ± 3.0	12.8 ± 2.6	11.9 ± 0.7
LF (R)	16.1 ± 2.2	18.9 ± 3.3	14.1 ± 3.0	13.1 ± 3.8	13.5 ± 3.5	10.9 ± 1.5
LF (L)	14.4 ± 1.8	16.2 ± 2.5	13.6 ± 2.4	10.8 ± 2.8	13.9 ± 4.6	6.8 ± 1.8*
DF	15.2 ± 3.9	18.0 ± 2.2	11.8 ± 4.1	11.6 ± 2.7	11.1 ± 3.9	8.1 ± 3.1

AF, anterior funiculus; AH, anterior horn; DF, dorsal funiculus; DH, dorsal horn; GM, gray matter; LF, lateral funiculus; MH, middle region of AH; Tg, transgenic; WM, white matter; WT, wild type.
The values are expressed as mean values ± s.d.
*$P < 0.05$, **$P < 0.01$ versus age-matched WT mice.

Discussion

In the present study, we first performed an *in vivo* capillary imaging of lumbar spinal cord using two-photon microscope. In Tg mice, capillary diameter in AH of lumbar cord became progressively smaller (Figures 1A–1F), capillary density became lower (Figure 1G), and mean RBC speed progressively decreased during the disease course (Figures 1H–1L), resulting in progressive decrease of flow volume only in Tg mice (Figure 1M). We then analyzed SBF and LSGU of cervical, thoracic, and lumbar cord using standard autoradiographic technique. In Tg mice, SBF was significantly decreased in GM, especially AH, before motor neuron loss at 12 W, and then progressively decreased with disease progression from 16 to 19 W (Table 2; Figures 2E–2P and Figures 3A–3I). Contrary to the results of SBF,

Tg mice initially showed a significant increase of LSGU at 12 W in GM of cervical and lumbar cords (Table 3; Figures 2Q–AB and Figures 3J–3R). However, LSGU now turned a progressive decrease from 16 to 19 W (Figures 3J–3R, black bars). The LSGU/SBF ratio showed a significant and continuous increase in GM of cervical, thoracic, and lumbar cords from 12 to 19 W of Tg mice (Figures 3S–AA).

Zhong *et al* (2008) showed a reduction of SBF in the cervical and lumbar cord as a whole in the same mice model of ours, but did not examine detailed regional differences and throughout the course before and after the disease onset. Decrease of cerebral blood flow was reported in ALS patients (Abe *et al*, 1997; Ishikawa *et al*, 2007; Waldemar *et al*, 1992), but that of SBF has not been reported in the ALS patients. Capillary diameter, density, and RBC velocity are important parameters of SBF. Our *in vivo* optical study strongly suggests that such an SBF reduction (Table 2; Figures 2E–2P) was closely related to the decrease of capillary diameter, density, and RBC velocity (Figure 1), and the early SBF reduction from the presymptomatic stage at 12 W might provide chronic and progressive ischemic stress to the affected spinal cord as implicated by early increase of Hif-1α and vascular endothelial growth factor (Murakami *et al*, 2003; Xu *et al*, 2011).

Several reports have shown weight loss, hypermetabolism, and hyperlipidemia in ALS patients and the animal model, suggesting a disturbance of energy metabolism (Dupuis *et al*, 2011; Guo *et al*, 2000; Hatazawa *et al*, 1988). Amyotrophic lateral sclerosis patients showed a reduced glucose metabolism in their cerebral cortex (Dalakas *et al*, 1987; Ludolph *et al*, 1992; Waldemar *et al*, 1992). Amyotrophic lateral sclerosis model mice showed a slight but nonsignificant increase of LSGU in the spinal cord at presymptomatic stage with a significant reduction at the end stage (Browne *et al*, 2006). Blood flow and glucose metabolism are well coupled under physiological conditions in both the brain and spinal cord FMC, where blood flow increases in response to an increased glucose metabolism (Krafft *et al*, 2000; Lenz *et al*, 1999; Leybaert, 2005; Leybaert *et al*, 2007; Sokoloff, 1977; Weir *et al*, 2002; Zivin *et al*, 1982).

In the present study, we found four important aspects of flow–metabolism uncoupling (FMU) in ALS spinal cord. (1) Spinal blood flow was progressively reduced beginning before the disease onset, and did not couple to the increasing LSGU at 12 W (Figures 2D,2E and Figures 3A–3I). (2) The initial LSGU increase at presymptomatic stage was followed by the LSGU reduction after the disease onset, when SBF reduction seemed to be partially coupled (Figures 2Q–2AB and Figure 3J–3R). (3) The LSGU/SBF ratio, a good indicator of FMC, showed that a high LSGU with uncoupled SBF continued from presymptomatic to the end stage of ALS (Figures 3S–AA). (4) Such an FMU was found only in Tg mice, preferentially in GM (AH > DH) and LF (Figures 2 and 3), where pathological changes are the most prominent in this ALS mice. The increase of LSGU at 12 W might be compensatory mechanism against early depletion of ATP (Browne *et al*, 2006) probably accompanied by mitochondrial deficit and hyperactivation of synaptic terminal, both of which were observed before apparent motor neuron degeneration (Gordon *et al*, 2010; Sasaki *et al*, 2004). The initial high LSGU with SBF reduction could cause not only an absolute ischemia (absolute SBF reduction) but also a relative hypoxia (regardless of LSGU increase or decrease) in the spinal motor neurons, resulting in a strong hypoxic/oxidative damage and reactive inflammatory responses. Such an initial presymptomatic event to spinal motor neurons and the surrounding inflammatory responses could bring secondary and continuous damage to motor neurons after the disease onset (Figure 3).

Recent reports have demonstrated a damage of blood spinal cord barrier components (Garbuzova-Davis *et al*, 2007; Henkel *et al*, 2009; Ishikawa *et al*, 2007) of both ALS patients and the model mice. Vascular endothelium, neurons, and glial cells form a functional unit together, called NVU (del Zoppo, 2010; Zlokovic, 2008), and blood flow is regulated by modulating blood vessel diameter to couple to the demand of glucose metabolism depending on local neuronal activity (Dirnagl, 1997; Harder *et al*, 1998; Kuschinsky, 1997). We have previously shown that a disruption of NVU enhanced an acute ischemic brain damage (Yamashita *et al*, 2009) and aggravated ALS pathology in the model mouse (Miyazaki *et al*, 2011). We found that significant changes of SBF and LSGU were largely restricted to GM, where damage of NVU was also prominent. The constant increase of LSGU/SBF ratio (Figures 3S–3AA) regardless of initial increase of LSGU and the later decrease (Figures 3J–3R) strongly suggests a larger decline of SBF than LSGU increase/decrease (Figures 3S–3AA). Such a large decline of SBF could be accounted for this NVU disruption (Miyazaki *et al*, 2011), as well as decreasing capillary diameter and density cooperatively affecting the neurodegenerative process of ALS. We have previously shown that motor function was decreased after 15 W using rotarod test in the same line of this model mouse (Ohta *et al*, in press). Thus, aberrant changes of SBF and LSGU persuaded the initiation of motor function reduction, and then progressively decreased with disease progression.

In the present study, we first showed a progressive impairment of FMU in the spinal cord of ALS mice, preceding the sequential changes of the disease, which strongly correlated with the affected regions of ALS, and is strongly related to decreasing capillary diameter and density and to NVU disruption. Although the reason why the presence of SOD1 caused initial SBF reduction and LSGU increase has to be dissolved by further analysis, we conclude that this early FMU could profoundly involve in the whole disease process as a vascular factor of ALS pathology, and could be a potential target for therapeutic intervention of ALS.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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