Spumiform capillary basement membrane swelling

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SPUMIFORM BASEMENT MEMBRANE ABERRATIONS IN THE MICROVASCULATURE OF THE MIDBRAIN PERIAQUEDUCTAL GRAY REGION IN HAMSTER: ROSTRO-CAUDAL PATHOGENESIS?

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Abstract—Spumiform basement membrane degeneration (sbmd) is a specific kind of aberration present in the capillaries of the midbrain periaqueductal gray (PAG) region of the senescent hamster. These capillaries, separated by the ependymal cell layer, are bordering the Sylvian cerebral aqueduct. The aqueduct, connecting the 3rd and 4th ventricle, may be crucial for local homeostatic as well as general autonomic functions of the PAG. Local pressure effects of the flowing and pulsating cerebrospinal fluid on the PAG-vasculature are probably different for the rostral 'entrance' and the caudal 'exit' of the aqueduct. In view of the different functions of the various divisions of the PAG, the frequency and extent of the aberrations in the rostral, intermediate and caudal dl/vlPAG-microvasculature could shed some light on the causal factors involved in the regional distribution of the particular microvascular aberrations found in the PAG during aging. In the present study we investigated the ultrastructure of capillaries in dorsal and ventral subdivisions of anterior and posterior regions of the PAG of young and old female Syrian hamsters. Sbmds were classified into four stages of spumiform severity and for each stage the frequency was determined in the rostral PAG, at two levels in the intermediate PAG and in a dorsal and a ventral part of the caudal PAG.

Results of our quantitative studies showed that in aged hamster PAG various stages of sbmd were present in 91.6 ± 0.6% of all capillaries. No clear evidence was found for regional differentiation between rostral, intermediate and caudal parts of the PAG. Next to sbmd, capillary split basement membrane (sbm) and vacuolization were common features at all five PAG locations. 84.3 ± 2.3% of all screened PAG capillaries displayed sbm. In agreement with our previous findings, several other types of microvascular aberrations were observed in addition to general aspects of aging and some ependymal structural peculiarities. We conclude that the presence of various forms of sbmds in the PAG of senescent hamsters is a phenomenon that appears to be specific to the PAG region, but causal factors for this type of capillary degeneration remain unclear. Sbmds in the PAG may have serious consequences not only for blood–brain barrier functioning, but also for vascular perfusion and blood supply with eventually serious consequences for adequate regulation of the autonomic and motor control functions of the PAG region.

Key words: aging, capillary, blood–brain barrier, basement membrane, (micro)-vascular degeneration, spumiform basement membrane degeneration.

INTRODUCTION

Studies on aging and degenerative diseases related to compromised cognitive status mostly focused on cerebral cortical and hippocampal regions (Shah and Mooradian, 1997; De Jong et al., 1999; de la Torre, 2000, 2005, 2010a,b; Farkas and Luiten, 2001; Kalaria, 2003; Miller et al., 2007; Zlokovic, 2011). Despite the crucial role of the midbrain periaqueductal gray matter (PAG) as a key intermediary structure between higher order cortical regions and brainstem effector systems in the control of a myriad of autonomic and motor functions, there are hardly data available of aging effects on microvascular conditions in regions like the PAG.

The PAG is located in the gray matter of the mesencephalon surrounding the cerebral aqueduct and this position provides unique opportunities for connecting higher brain centers and brainstem effector

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Abbreviations: 3, ocular motor nucleus; BBB, blood–brain barrier; bm, basement membrane; CSF, cerebrospinal fluid; g, gliosis; ISF, interstitial fluid; PAG, periaqueductal gray; sbm, split basement membrane; sbmd, spumiform basement membrane degeneration.

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systems. Cytoarchitectonically, the PAG is not a homogeneous structure and already in 1954, the human PAG was divided into dorsal, medial and ventral subregions (Olszewski and Baxter, 1954). More recently, the cyto- and myeloarchitecture of the PAG has been described and partitioned in more detail (Liu and Hamilton 1980; Beitz, 1985; Beitz and Shepard, 1985; Veening et al., 1991; Gerrits et al., 1993) The PAG has been included in the concept of the 'greater limbic system' (Nieuwenhuys et al., 1988, 2008; Nieuwenhuys, 1996).

Functionally, the PAG is strongly involved in basic functions like the survival of the individual and the species, and plays a role in a variety of behavioral and physiological functions like: aggressive and defensive behaviors (Bandler and Depaulis, 1991; Bandler et al., 1991; Bandler and Shipley, 1994; Bandler and Keay, 1996), pain and analgesia (Giesler and Liebeskind, 1976; Mayer and Price, 1976; Basbaum and Fields, 1978; Fardin et al., 1984a,b; Besson et al., 1991), cardiovascular control mechanisms (Carrive, 1989; Carrive et al., 1989; Bandler et al., 1991; Bandler and Keay, 1996; Lovick, 1996), lordosis (Sakuma and Pfaff, 1979a,b, 1980, 1983) and estrous-cycle-related changes in neuronal responsiveness (Lovick et al., 2005; Lovick, 2006, 2008), and the vocal expression of emotions (Kanai and Wang, 1962; Jurgens and Pratt, 1979; Larson, 1985; Bandler et al., 1991; Jurgens, 1994). This wide-ranging functional involvement makes the PAG an important object to study the occurrence and effects of the vascular condition during aging, since all neural activity is directly dependent on adequate and effective blood supply.

The PAG is located around about the narrowest part of the ventricular system, the cerebral aqueduct (of Sylvius). Because of that special location around a narrow channel, the PAG is subjected to appreciable pressure changes induced by the pulsatile flow of the cerebrospinal fluid (CSF) (Stoquart-ElSankari et al., 2007; Klarica et al., 2009; Bulat and Klarica, 2011). For that reason, we decided to focus our attention on the effects of aging on microvascular integrity and condition in the PAG. Apart from microvascular age-associated changes we unexpectedly discovered capillaries with a new kind of vascular aberration, hitherto not previously reported (Gerrits et al., 2010, 2012b; Veening et al., 2012). Because of the 'foamy' electron lucent character of this particular aberration, we have termed these structures as 'spumiform basement membrane degeneration', (sbmd). In addition, we reported evidence for similar processes in rat and man (Gerrits et al., 2012b). Sbmd in the hamster PAG shares some characteristics with the membranous inclusions observed in the bm in the cerebral cortex (de Jong et al., 1990), and in the dorsal lateral geniculate nucleus of the aged rat brain (Alba et al., 2004), suggesting inter-species similarities. These findings open new ways for studies on capillary bm integrity in rat and mouse and man.

In the present study we performed a quantitative electron microscopical analysis comparing the sbmd aberrations in the rostral, intermediate and caudal PAG-microvasculature in young and aged hamster. We have chosen for this species because of our extensive previous findings on the relationship between the anatomical characteristics of the PAG and its autonomic functions in this mammalian species.

EXPERIMENTAL PROCEDURES

Animals

The experiments were performed on inbred animals obtained from Harlan (strain HsdHan: Aura; Harlan, Boxmeer, The Netherlands). Young (22 weeks, 120–122 g, cases H547, H548, H552, H556) and aged (95 ± 0.5 weeks, 130–140 g, cases H571, H574, H575, and H576) female golden hamsters (*Mesocricetus auratus*) were used for the present study. All protocols, concerning housing and handling of the animals and efforts to minimize animal suffering were in accordance with Dutch legalization and the ethical guidelines approved by the University of Groningen/University Medical Center Groningen (license number DEC 5142A).

Housing and handling

All hamsters were housed separately in clear plastic cages in a 14/10-h reversed light/dark cycle with food and water available *ad libitum*. Room temperature was maintained at 22–24 °C and humidity at 50–70%; wood shaving and straw were used as bedding materials. The animals were under daily monitoring for their general health condition and weighed once a week. Lifespan of hamsters varies considerably from 82–118 weeks depending on sex, strain and housing conditions (Kamino et al., 2001; Oklejewicz and Daan, 2002). Therefore, it was decided to euthanize aging animals at the age of 95–96 weeks, actually at the end of the female hamster lifespan.

Tissue processing

Perfusion. After an overdose of Nembutal (sodium pentobarbital, 50 mg/kg, i.p.; Lundbeck Inc., Deerfield, IL, USA), the animals were transcardially perfused with 20 mL of heparinized phosphate buffer (0.1 M, pH 7.4), containing 0.4% sodium nitrite and 2% polyvinylpyrrolidone (molecular weight 40,000) at 37 °C, followed by 350 mL of fixative containing 0.05% glutaraldehyde, 4% paraformaldehyde, 0.2% picric acid and 2% polyvinyl-pyrrolidone in 0.1 M phosphate buffer, pH 7.4, at room temperature. Following perfusion, the brains were removed and postfixed for one hour in the same fixative at 4 °C.

Electron microscopy. PAG tissue was cut on a vibratome into 60-μm transverse sections and collected in 0.01 M phosphate-buffered saline (PBS) at 4 °C. Every other section was processed for a standard EM protocol: osmicated, dehydrated in a graded series of ethanol and flat-embedded in Epon between dimethyldichlorosilane-coated glass slides. Samples of tissue containing the PAG and brainstem control regions were glued on Epon stubs. After blocking, the tissue was trimmed and cut into 1-μm semithin sections. Finally, 60-nm ultrathin sections from the selected regions were cut with a diamond knife for further electron microscopical analysis.

Control tissue

PAG tissue obtained from the young adult female hamsters was processed in the same way as the aged animals and served as control.

Photomicrography

The rostro-caudal PAG locations (Fig. 1) were determined using a Zeiss Axioplan light microscope (Carl Zeiss Benelux, Trapezium 300, Sliedrecht, The Netherlands) at 10× magnification. Representative PAG sections were photographed by using a DC500 digital camera and a DM4000B photomicroscope connected to a Q550IW computer and QWIN software (Leica Microsystems, Rijswijk, The Netherlands). Drawings of the sections and overlayers were made using Adobe Illustrator 8.0 software (Adobe Systems, Mountain View, CA, USA). Microvasculature and surrounding profiles were photographed at 10,000–20,000× magnification using a Philips CM 100 electron microscope (Philips, Eindhoven, The Netherlands).

Quantitative analysis

Fig. 1 shows cross sections of the rostro-caudal PAG comprising the selected PAG subdivisions. Sixty-nanometer ultrathin PAG sections, taken at different depth intervals of 1 µm, were screened for capillaries. Photomicrographs at 10,000–20,000× of complete transverse sectioned capillaries were randomly taken for further analysis. A total of 1200 capillaries were

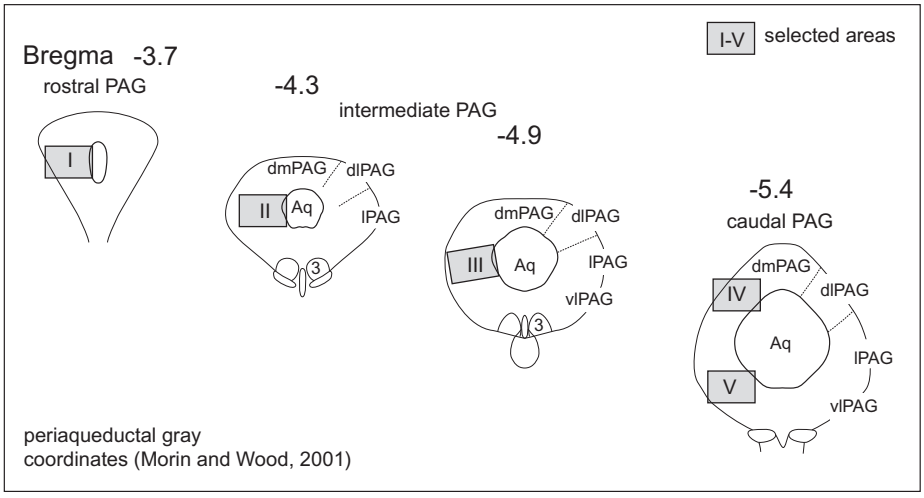

Fig. 1. Schematic overview of the five PAG locations selected for capillary basement membrane analysis. Aq, Sylvian aqueduct; 3, ocular motor nucleus.

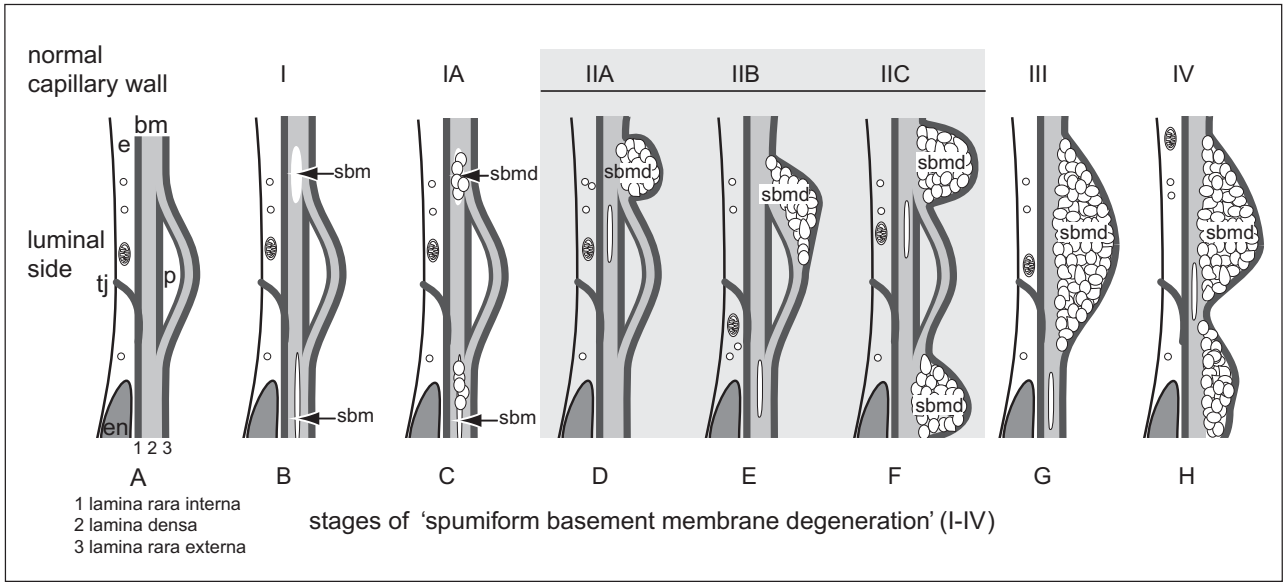

Fig. 2. (A–H) Schematic representation of the stages of spumiform basement membrane degeneration (sbmd). Stage IA depicts a transitional phase between sbm (stage I) and sbmd. The gray boxed area shows three subdivisions of stage II (IIA–C). Note that split basement membrane (sbm) can be found in combination with all other manifestations. *Abbreviations*: e, endothelial cytoplasm; en, endothelial nucleus; bm, basement membrane; sbm, split basement membrane; sbmd, 'spumiform' basement membrane degeneration; l, luminal side; m, mitochondrion; n, nucleus; p, pericyte; tj, tight junction.

studied; 600 capillaries from four young hamsters were compared with 600 capillaries from four aged hamsters. Per PAG location 30 capillaries/animal were studied with emphasis on basement membrane aberrations of the spumiform type. Basement membrane degeneration per location was classified (double blind). Basement membrane degeneration was quantified into four stages ranging from vacuolization (split basement membrane, or sbm, stages I and IA) to extensive spumiform basement membrane degeneration (sbmd), outlining almost the complete capillary bm (stage IV), see Fig. 2A–H. Stage IA was added as a necessary intermediate step to fill the gap between stages I and II as reported earlier (Gerrits et al., 2012b). Differences between aged and young animals were tested for five locations (rostral PAG, bregma −3.7; intermediate PAG, bregma, −4.5 and −4.9; and caudal vl and dlPAG, bregma −5.4) with a two-tailed two sample *t*-test assuming unequal variance between groups. To control for an inflated type I error resulting from 10 *t*-tests, we applied Bonferroni correction, which is very conservative in the scenario of ten comparisons. In aged animals, location effects were tested with a single factor ANOVA.

RESULTS

The sbmds in the PAG showed unique and characteristic 'foamy-like' manifestations of electron lucent vacuoles within the confines of capillary bm and pericytic bm (Fig. 3A, B) and identified as one of four stages as defined above (Fig. 2A–H). Our study suggested a process of steadily increasing 'spumiform' aberrations within the lamina densa of the capillary basement membrane, and we hypothesized that this new form of bm-degeneration most likely begins with local splitting/vacuolization of the lamina rara densa, to develop over time finally into a rim of spumiform degradation products positioned as a cuff around the capillary (Gerrits et al., 2012b). The mesencephalic PAG emerged as the most affected site, compared to other caudal brainstem areas like nucleus pararetroambiguus, commissural nucleus of the solitary tract and the medial tegmental field. Outside

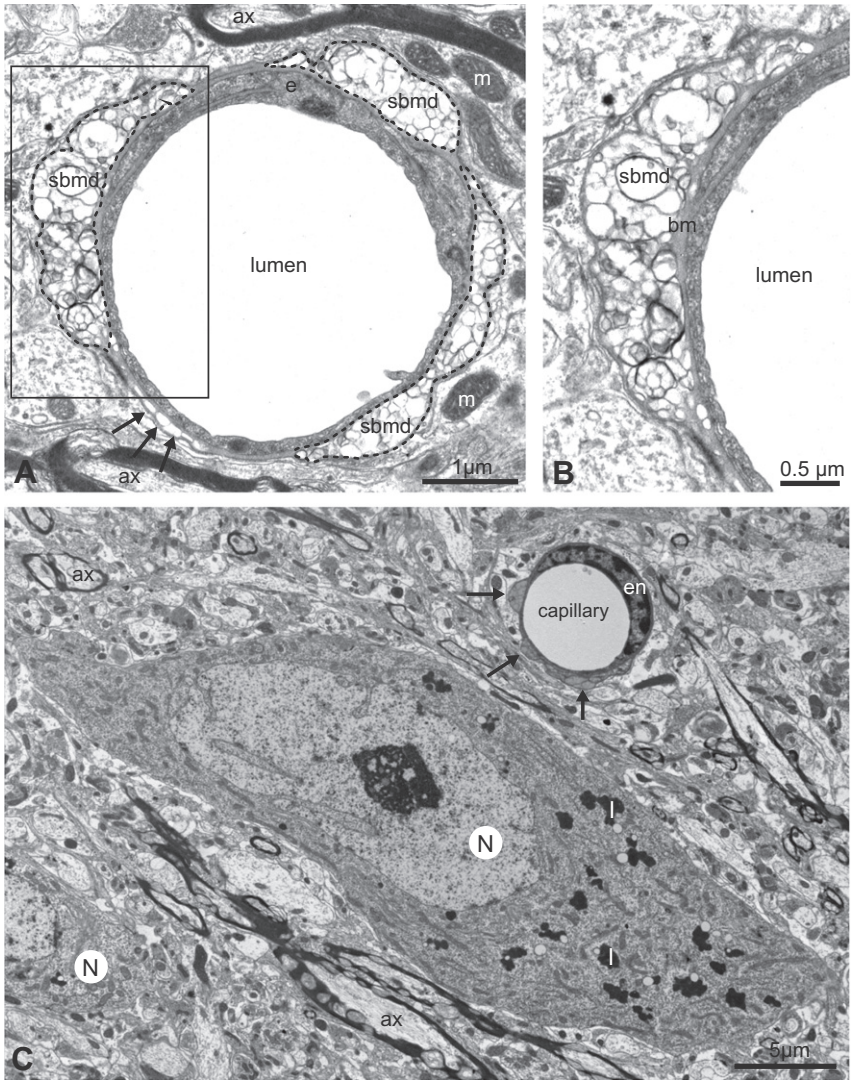

Fig. 3. (A–C) Electron microscopic photomicrographs showing spumiform basement membrane degeneration (sbmd) in the PAG. (A) Extensive form of sbmd indicated by dashed lines. Note the characteristic morphology of the translucent spumiform degeneration present in almost the complete bm. Arrows point to transitional phase between sbm and sbmd. (B) Detail of sbmd as presented in the boxed area in A. (C) Capillary with extensive sbmd (arrows) in close proximity of a PAG neuron with intracytoplasmic lipofuscin (I). Sbmd and neuron are digitally contrasted with a gray overlay.

Table 1. The percentage of PAG capillaries with spumiform basement membrane degeneration at five rostro-caudal PAG levels. Mean percentages ± 1SD. Gray bars show the percentages of stages I–IIC present in these capillaries; white bars the percentages of stages III–IV

Level PAG	Bregma	% SBMD	% BM split 1	Stages of spumiform basement membrane degeneration					
				IA	IIA	IIB	IIC	III	IV
Aged hamsters									
Rostral	−3.7	91.3	83.0	15.7	16.1	2.7	44.9	8.0	3.9
Intermediate 1	−4.3	91.6	82.8	13.9	17.3	1.5	40.6	11.8	6.5
Intermediate 2	−4.9	90.8	86.5	10.8	17.2	3.3	39.7	13.8	6.1
Caudal dl	−5.4	91.8	82.0	15.9	15.6	5.4	41.8	9.1	4.0
Caudal vl	−5.4	92.5	87.0	18.8	19.8	2.5	41.3	7.5	2.7
Average %		91.6	84.3	15.0	17.2	3.1	41.7	10.0	4.6
STDEV		0.6	2.3	2.9	1.6	1.4	2.0	2.7	1.6
Young hamsters									
Rostral	−3.7	3.3	20.0	1.7	1.7	0.0	0.0	0.0	0.0
Intermediate 1	−4.3	5.0	33.4	0.0	0.0	3.3	1.7	0.0	0.0
Intermediate 2	−4.9	5.0	14.1	1.7	3.3	0.0	0.0	0.0	0.0
Caudal dl	−5.4	6.7	20.0	3.3	3.3	0.0	0.0	0.0	0.0
Caudal vl	−5.4	5.0	20.0	3.3	0.0	0.0	1.7	0.0	0.0
Average %		5.0	21.5	2.0	1.7	0.7	0.7	0.0	0.0
STDEV		1.2	7.1	1.4	1.7	1.5	0.9	0.0	0.0

the PAG, hardly any sbmd could be observed (Gerrits et al., 2012b).

Split basement membrane/vacuolization and sbmd in PAG

Fig. 3C, in a low-magnification electron photomicrograph, shows a brain capillary in the vicinity of a PAG neuronal cell body. The capillary located in close proximity to the neuron shows an extensive form of sbmd (stages III–IV; arrows), the neuron itself contains numerous intracytoplasmic lipofuscin granules.

Split basement membrane (sbm) in PAG. Capillary bm splitting and vacuolization (sbm) were common features at all five locations in the aging PAG. In aged animals sbm was observed in 84.3 ± 2.3% (mean ± 1SD) of all screened PAG capillaries. Intermediate forms between sbm (stage I) and sbmd (stage IIA), classified as stage IA, were present in 15.0 ± 2.9% of all capillaries (see Fig. 2C and 3A, Table 1).

The presence of sbm was much more prevalent in aged animals compared to young animals: 84.3 ± 2.3 versus 19.5 ± 5.3% (Table 1, Fig. 4A).

Sbmd in PAG. The analysis of the PAG capillaries in the old animals revealed that various forms of sbmd were present in 91.6 ± 0.6% of all capillaries, whereas in young animals this was only 4.0 ± 1.1% of capillaries (Table 1). Severe sbmd stages III–IV were more frequently observed in the intermediate PAG levels but this proved to be non-significant [$F(2,17) = 3.592$; $p = 0.175$)]. Likewise, sbmd stages IIA–C were more prominently present (Table 1) in the rostral PAG level around the inlet of the aqueduct and in the caudal PAG around the outlet of the aqueduct but this was also non-significant [$F(2,17) = 1.151$; $p = 0.340$].

Overall stages IIA–C of sbmd were most frequently present (62%) followed by stages III and IV (Table 1). In 3.1% of the capillaries sbmd was observed covering the abluminal side of the pericyte (Fig. 5A). About 8% of the capillary cross-sections displayed no sbmd or only minimal splits. In some rare cases (3/600 capillaries) collagen type fibrils displaying its characteristic periodicity were found intermingled with sbmd, similar to what has been described previously in the rat brain (De Jong et al., 1990; Farkas et al., 2001) (see Fig. 6A, B). In the young control animals only 4.0 ± 1.1% of all PAG capillaries displayed sbmd, mainly of category II (Table 1, Fig. 4B). PAG capillaries showing sbmd categories III and IV were not observed.

The mild aberrations (stages IIA–C) were more common in old animals compared to young at all rostro-caudal levels (all $t > 21$, all $p < 0.0002$). The more severe aberrations (stages III–IV) were also more common in old animals at intermediate and caudal levels (both $t > 5.2$, both $p < 0.013$) but this did not apply to the rostral location ($t = 3.052$, $p = 0.554$).

Generalized aberrations in PAG

In addition to these characteristic sbmd, other diffuse (peri)vascular and neurodegenerative changes were observed as well. Concerning capillary aberrations, these changes varied from endothelial malformations, disrupted and/or widened tight junctions, bm thickening (Fig. 5B), and pericyte degeneration to perivascular edema (Fig. 5A) and gliosis. Subependymal edema was frequently observed along the borders of the Sylvian aqueduct (Fig. 5C). Increased levels of neuronal intracytoplasmic lipofuscin (Fig. 3C), however, were less frequently present in these cells. Abnormal (giant) mitochondria, degenerated myelin accumulations, and age-related bodies were present, in similar amounts as reported previously (Gerrits et al., 2009, 2012a,b; Gerrits and Veening, 2012).

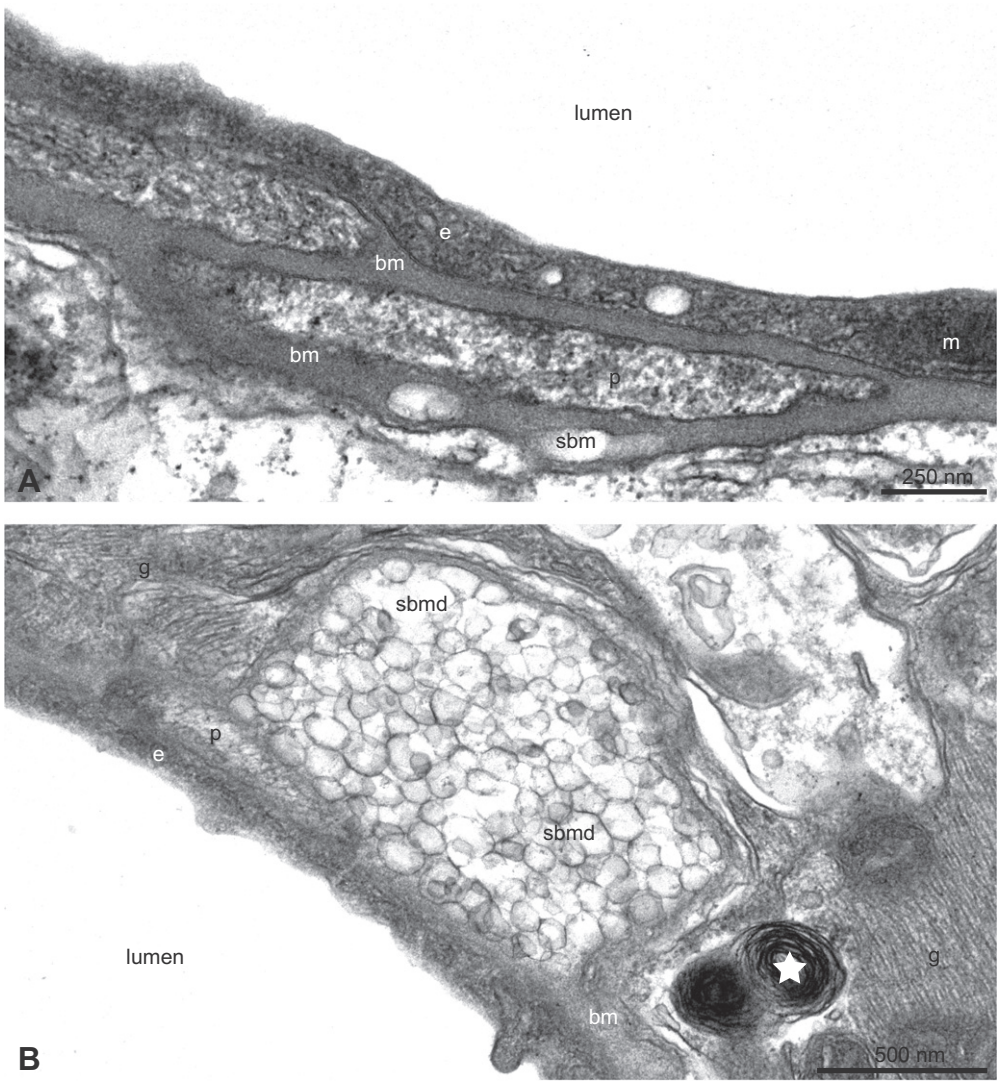

Fig. 4. (A) High magnification electron microscopic photomicrograph of a capillary wall of a young female hamster (H547) showing characteristic sbm in the bm at the abluminal side of a pericytic process. (B) Case H552. Isolated sbmd stage IIA showing characteristic morphology of the translucent spumiform degeneration product in the capillary bm. White asterisk indicates myelin degeneration.

DISCUSSION

Brain microvasculature plays an essential role in the regulation of homeostasis of neural tissues, in particular in the direct supply of glucose and oxygen for the maintenance of an optimal physiological environment. The presence of degenerated parts of the blood–brain barrier (BBB) in aging animals and humans may directly or indirectly lead to less optimal neuronal functioning and is increasingly associated with neurodegenerative mechanisms (Mooradian, 1988; Nishizuka and Pfaff, 1989; Shah and Mooradian, 1997; Ballabh et al., 2004; Zlokovic, 2008, 2011) but also with impaired brain functioning during non pathological aging.

In our investigations of the microvascular integrity of the PAG in the aging hamster, we recently observed a new form of age-related basement membrane degeneration (Gerrits et al., 2012b). Ultrastructural evidence showed this microvascular bm pathology appearing in the hamster PAG as a so far unreported form of bm degeneration, which we termed ‘spumiform basement membrane degeneration’ (sbmd). For the sake of clarity, sbmd is not linked to a particular cell type and should not be confused with the ‘foam cells’ appearing among the first cells during atherosclerotic plaque formation in vessels. The latter type of cells is monocyte-derived macrophages that accumulate cholesterol in atherosclerotic lesions (Ball et al., 1995; Ross, 1999; Kruth, 2001).

We identified a process of steadily increasing ‘spumiform’ degradation products within the vascular lamina densa, leading to extreme capillary bm swelling (Fig. 2). Sbmd is almost exclusively present in the PAG of aged hamsters and virtually absent in PAG of young animals, and as far as we know in other brain regions, suggesting a progressive process of capillary degeneration during aging of the hamster PAG. The precise nature and origin of sbmd is still unknown but we speculate that the membranous component of sbmd originates from collagen IV fibrils of the lamina densa.

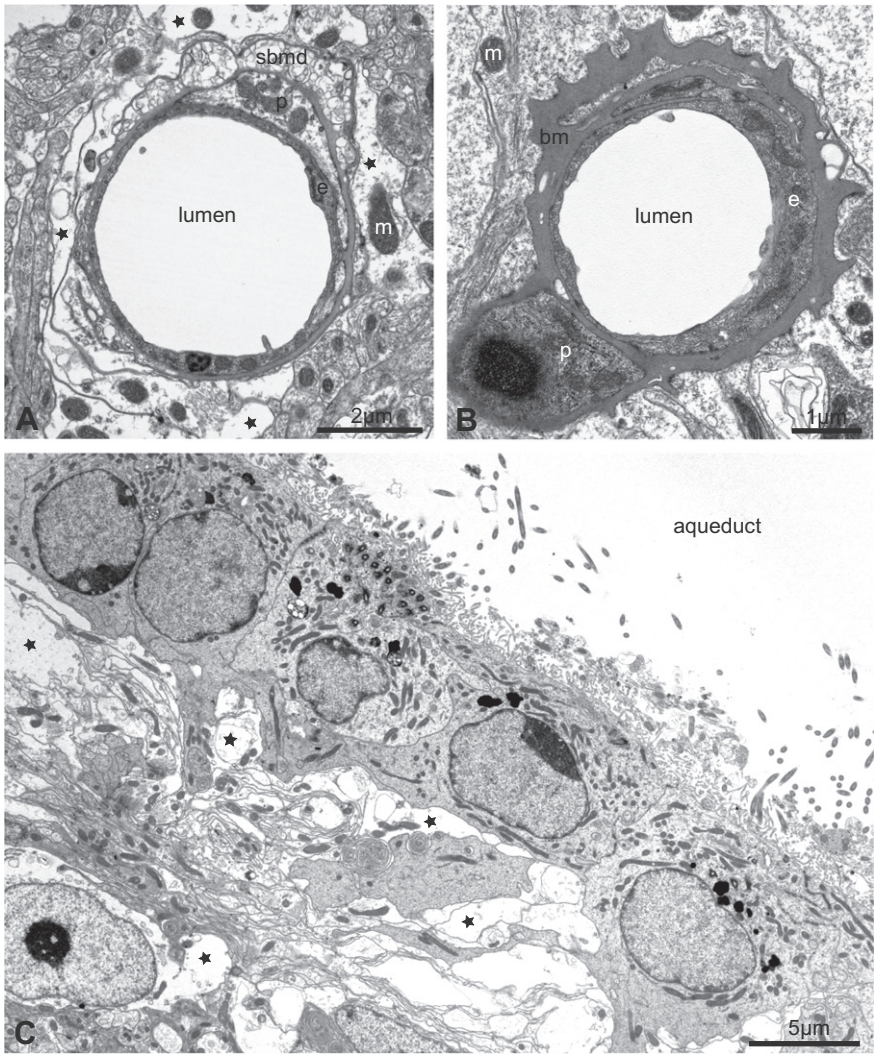

Fig. 5. Capillary with sbmd covering the complete abluminal side of a pericyte process. Note the edematous tissue (asterisks) surrounding the capillary. e, endothelial cytoplasm; m, mitochondrion; p, pericyte process. (B) Microvessel in PAG showing basement membrane thickening. Within the bm small translucent vacuoles (split basement membrane) can be observed. (C) Ependymal cells lining the aqueduct. The subependymal layer displays areas with extensive edema (asterisks).

During aging these collagen fibrils may develop in compartments that become gradually filled with high molecular translucent substances that do not pass the bm-lamina rara externa. In that case, capturing of substances in the spumiform spaces may be the result of a selective permeability change in the different bm-components.

The finding that sbmd is region specific is in agreement with reported region specificity of other aberrations (Threatt et al., 1971; Nandy et al., 1975; Goldman et al., 1992; Shah and Mooradian, 1997; Zlokovic, 2008, 2011) and may be associated with serious consequences for neural functioning.

The present study focused on the rostro-caudal extent of this new microvascular basement membrane (bm) aberrations within the mesencephalic PAG. Our quantitative studies showed that in aged hamster PAG various stages of sbmd were present in 91.6 ± 0.6% of all capillaries. No clear evidence for regional differentiation between rostral, intermediate and caudal parts of the PAG was observed. Within the old animals, the different levels of the PAG did not differ significantly in terms of mild (stages IIA–C) and severe aberrations (stages III and IV). Nonetheless, compared to young animals, there were more aberrations in old animals in all locations with the exception of the rostral PAG. Apparently, capillaries in the rostral PAG are relatively protected from the structurally damaging effects of aging. This is not in agreement with the hypothesis that pressure and pulsations induced by the formation and flow of CSF play an important role in the etiology of sbmd and other microvascular pathology in the brainstem.

In addition to sbmd, capillary bm splitting and vacuolization were common features at all five PAG locations. In agreement with our preceding studies, several other types of microvascular aberrations were observed in addition to general aspects of aging and also some ependymal structural peculiarities (Gerrits et al., 2009, 2012a,b).The finding that considerable amounts of

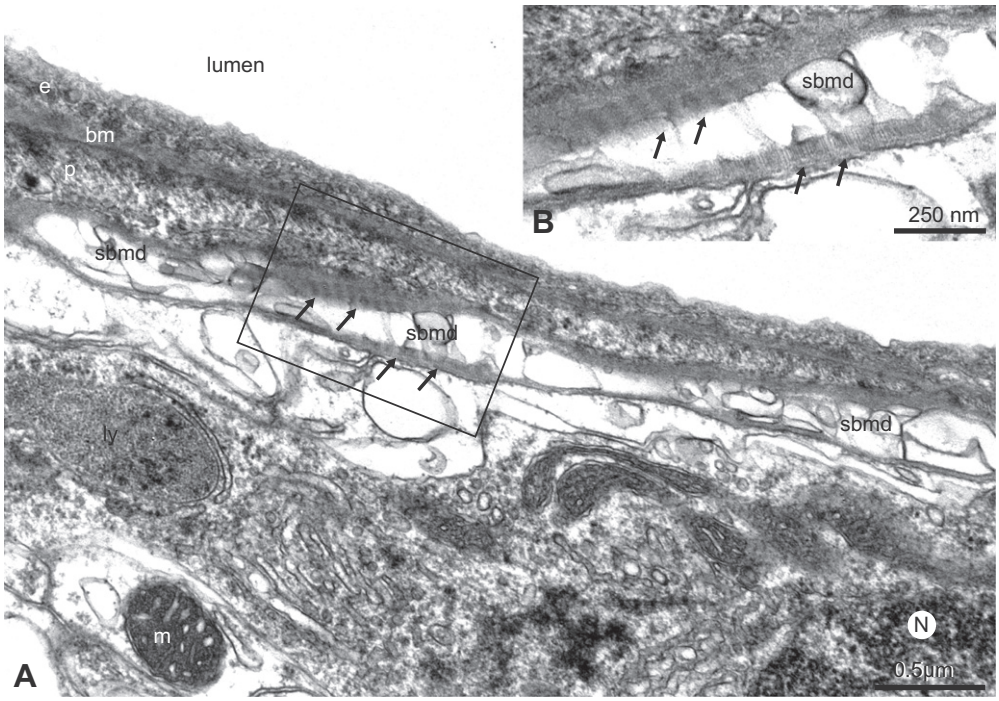

Fig. 6. Microvascular fibrosis in senescent hamster periaqueductal gray, case H575. (A) Sbmd and collagen fibrils are embedded within a split basement membrane. (B) shows a high magnification of the indicated area in panel A. Collagen fibrils (arrows) showing their characteristic periodicity are located intermingled with vacuoles of sbmd.

split basement membrane (19.5 ± 5.3%) and small amounts of the early stages of sbmd (4 ± 1.1%) were already present in young hamsters (22 weeks) suggests that the onset of bm alterations of the spumiform type starts in the hamster PAG already at the young adult age.

Possible mechanisms of the development of perivascular cuffs

The PAG is localized around the Sylvian aqueduct, and may be vulnerable to hydrodynamic processes as a result of exposure to continuous passage of pulsating CSF (Sakka et al., 2011). Recent insights into the hydrodynamics of CSF provide evidence that water, which constitutes 99% of CSF and interstitial fluid (ISF) bulk, is rapidly absorbed into microvessels adjacent to the CNS (Bulat et al., 2008; Klarica et al., 2009; Bulat and Klarica, 2011). It appears that a process of water filtration across the walls of microvessels in the central nervous system is a key step in the production of ISF and CSF. Plasma osmolytes are retained, however, for generating capillary osmotic counter-pressure, which is essential for maintenance of ISF/CSF balance of water absorption into capillaries. The concentration of other macromolecular substances in the periventricular regions including PAG, depends on the rate of their removal into microvessels (for review (Bulat and Klarica, 2011). Ultramicroscopic data provide evidence that a decreased cerebral blood flow is associated with the accumulation of fibrous collagen in the microvascular walls (Farkas et al., 2000b). It can be argued that structural capillary wall changes are adaptations to altered perfusional and physiological conditions since cerebral hypoperfusion has been found to have a deleterious impact on neural tissue (Farkas et al., 2000a,b). Human aging has been shown to lead to reduced cerebral blood – and CSF – flows (Grubb et al., 1977; Raichle, 1981; Buckner et al., 2000; Stoquart-ElSankari et al., 2007; Yang et al., 2011).

Stoquart-ElSankari et al. (2007) demonstrated that the CSF stroke volumes are significantly reduced in the elderly, i.e. at aqueduct levels. Further, their results show a decrease of total cerebral blood flow, a proportional aqueductal and cervical CSF pulsations-reduction as a result of arterial loss of pulsatility, and preserved intracerebral compliance with aging (Stoquart-ElSankari et al., 2007). Disturbances of CSF dynamics play a role in CSF-mobility decline with aging especially in cases of unknown origin (Onen et al., 2005). Considering the above mentioned studies and our recent findings, it is suggested that regional differences in the occurrence of the characteristic sbmd may be due to structural changes, related to hydrodynamics of ISF/CSF during aging. Since PAG capillaries are located close to the aqueduct, they may be significantly more vulnerable for capillary changes including sbmd than capillaries in more caudally located brainstem structures.

CONCLUSION

In the present study we describe the rostro-caudal extent of a new category of microvascular degenerative changes

in the midbrain periaqueductal gray in aging hamster which appeared to be highly region specific. From our statistical analysis it can be concluded that the occurrence of these spumiform basement membrane degenerations in the PAG of senescent hamsters is a general 'PAG-phenomenon'. Pressure fluctuations induced by the flowing CSF have been postulated as a causal factor in the induction of the spumiform aberrations (Jones et al., 1987; May et al., 1990; Kleine et al., 1993; Reiber, 1994, 2003; Redzic et al., 2005) but further evidence is needed to support this view, and presently unknown pathogenic factors cannot be excluded. Sbmds in the PAG may have serious consequences for BBB function and may eventually impair autonomic and motor control functions of the PAG region.

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REFERENCES

Alba C, Vidal L, Diaz F, Villena A, de Vargas IP (2004) Ultrastructural and quantitative age-related changes in capillaries of the dorsal lateral geniculate nucleus. Brain Res Bull 64(2):145–153.

Ball RY, Stowers EC, Burton JH, Cary NR, Skepper JN, Mitchinson MJ (1995) Evidence that the death of macrophage foam cells contributes to the lipid core of atheroma. Atherosclerosis 114:45–54.

Ballabh P, Braun A, Nedergaard M (2004) The blood-brain barrier: an overview: structure, regulation, and clinical implications. Neurobiol Dis 16:1–13.

Bandler R, Depaulis A (1991) Midbrain periaqueductal gray control of defensive behavior in the cat and the rat. In: Depaulis A, Bandler RA, editors. The midbrain periaqueductal gray matter: functional, anatomical and neurochemical organization. New York: Plenum Press. p. 175–198.

Bandler R, Keay KA (1996) Columnar organization in the midbrain periaqueductal gray and the integration of emotional expression. Prog Brain Res 107:285–300.

Bandler R, Shipley MT (1994) Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? Trends Neurosci 17:379–389.

Bandler R, Carrive P, Zhang SP (1991) Integration of somatic and autonomic reactions within the midbrain periaqueductal grey: viscerotopic, somatotopic and functional organization. Prog Brain Res 87:269–305.

Basbaum AI, Fields HL (1978) Endogenous pain control mechanisms: review and hypothesis. Ann Neurol 4:451–462.

Beitz AJ (1985) The midbrain periaqueductal gray in the rat. I. Nuclear volume, cell number, density, orientation, and regional subdivisions. J Comp Neurol 237:445–459.

Beitz AJ, Shepard RD (1985) The midbrain periaqueductal gray in the rat. II. A Golgi analysis. J Comp Neurol 237:460–475.

Besson JM, Fardin V, Olivéras JL (1991) Analgesia produced by stimulation of the periaqueductal gray matter: true antinoceptive effects versus stress effects. In: Depaulis A, Bandler RA, editors. The midbrain periaqueductal gray matter: functional, anatomical and neurochemical organization. New York: Plenum Press. p. 121–138.

Buckner RL, Snyder AZ, Sanders AL, Raichle ME, Morris JC (2000) Functional brain imaging of young, nondemented, and demented older adults. J Cogn Neurosci 12(Suppl. 2):24–34.

Bulat M, Klarica M (2011) Recent insights into a new hydrodynamics of the cerebrospinal fluid. Brain Res Rev 65:99–112.

Bulat M, Lupret V, Orehkovic D, Klarica M (2008) Transventricular and transpial absorption of cerebrospinal fluid into cerebral microvessels. Coll Antropol 32(Suppl. 1):43–50.

Carrive P (1989) Functional organization of midbrain periaqueductal grey neurons mediating somatic and autonomic changes. PhD Thesis. Sydney: University of Sydney, p. 159.

Carrive P, Bandler R, Dampney RA (1989) Somatic and autonomic integration in the midbrain of the unanesthetized decerebrate cat: a distinctive pattern evoked by excitation of neurones in the subtentorial portion of the midbrain periaqueductal grey. Brain Res 483:251–258.

De Jong GI, de Weerd H, Schuurman T, Traber J, Luiten PG (1990) Microvascular changes in aged rat forebrain. Effects of chronic nimodipine treatment. Neurobiol Aging 11:381–389.

De Jong GI, Farkas E, Stienstra CM, Plass JR, Keijser JN, de la Torre JC, Luiten PG (1999) Cerebral hypoperfusion yields capillary damage in the hippocampal CA1 area that correlates with spatial memory impairment. Neuroscience 91:203–210.

de la Torre JC (2000) Cerebral hypoperfusion, capillary degeneration, and development of Alzheimer disease. Alzheimer Dis Assoc Disord 14(Suppl. 1):S72–S81.

de la Torre JC (2005) Is Alzheimer's disease preceded by neurodegeneration or cerebral hypoperfusion? Ann Neurol 57:783–784.

de la Torre JC (2010a) The vascular hypothesis of Alzheimer's disease: bench to bedside and beyond. Neurodegener Dis 7:116–121.

de la Torre JC (2010b) Vascular risk factor detection and control may prevent Alzheimer's disease. Ageing Res Rev 9: 218–225.

Fardin V, Oliveras JL, Besson JM (1984a) A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal gray matter in the rat. I. The production of behavioral side effects together with analgesia. Brain Res 306:105–123.

Fardin V, Oliveras JL, Besson JM (1984b) A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal gray matter in the rat. II. Differential characteristics of the analgesia induced by ventral and dorsal PAG stimulation. Brain Res 306:125–139.

Farkas E, Luiten PG (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. Prog Neurobiol 64: 575–611.

Farkas E, De Jong GI, Apro E, De Vos RA, Steur EN, Luiten PG (2000a) Similar ultrastructural breakdown of cerebrocortical capillaries in Alzheimer's disease, Parkinson's disease, and experimental hypertension. What is the functional link? Ann N Y Acad Sci 903:72–82.

Farkas E, De Vos RA, Jansen Steur EN, Luiten PG (2000b) Are Alzheimer's disease, hypertension, and cerebrocapillary damage related? Neurobiol Aging 21:235–243.

Farkas E, De Jong GI, Apro E, Keuker JI, Luiten PG (2001) Calcium antagonists decrease capillary wall damage in aging hypertensive rat brain. Neurobiol Aging 22:299–309.

Gerrits PO, Kortekaas R, Veening JG, de Weerd H, van der Want JJ (2012) Reduced aging defects in estrogen receptive brainstem nuclei in the female hamster. Neurobiol Aging. http://dx.doi.org/10.1016/j.neurobiolaging.2012.02.018. [Epub ahead of print].

Gerrits PO, Croon DH, Holstege G (1993) A new subdivision of the rat midbrain periaqueductal gray based on its myeloarchitecture. Neurosci Lett 161:232–236.

Gerrits PO, Kortekaas R, Algra A, van der Want JJG, Veening JG (2009) Neurodegeneration and plasticity in the brainstem of estrous female golden hamster; ultrastructural studies on axo-dendritic relationships, remodeling and degeneration. In: Society for Neuroscience Chicago.

Gerrits PO, de Weerd H, van der Want JJL, Kortekaas R, Luiten PGM, Veening JG (2010) Microvascular changes in estrogen-alpha sensitive brainstem structures of aging female hamsters. Neurosci Res 67:267–274.

Gerrits PO, Kortekaas R, de Weerd H, Veening JG, van der Want JJ (2012a) Regional differences in age-related lipofuscin accumulation in the female hamster brainstem. Neurobiol Aging 33(625):621–629.

Gerrits PO, Kortekaas R, de Weerd H, Veenstra-Algra A, Luiten PGM, van der Want JJH, Veening JG (2012b) Spumiform capillary basement membrane degeneration; a new type of basement membrane degeneration in senescent hamsters. Neurobiol Aging, in press. http://dx.doi.org//10.1016/j.neurobiolaging.2012.09.009. [Epub ahead of print].

Giesler Jr GJ, Liebeskind JC (1976) Inhibition of visceral pain by electrical stimulation of the periaqueductal gray matter. Pain 2:43–48.

Goldman H, Berman RF, Gershon S, Murphy S, Morehead M, Altman HJ (1992) Cerebrovascular permeability and cognition in the aging rat. Neurobiol Aging 13:57–62.

Grubb Jr RL, Raichle ME, Gado MH, Eichling JO, Hughes CP (1977) Cerebral blood flow, oxygen utilization, and blood volume in dementia. Neurology 27:905–910.

Jones HC, Deane R, Bucknall RM (1987) Developmental changes in cerebrospinal fluid pressure and resistance to absorption in rats. Brain Res 430:23–30.

Jurgens U (1994) The role of the periaqueductal grey in vocal behaviour. Behav Brain Res 62:107–117.

Jurgens U, Pratt R (1979) Role of the periaqueductal grey in vocal expression of emotion. Brain Res 167:367–378.

Kalaria RN (2003) Vascular factors in Alzheimer's disease. Int Psychogeriatr 15(Suppl. 1):47–52.

Kamino K, Tillmann T, Mohr U (2001) Spectrum and age-related incidence of spontaneous tumours in a colony of Han:AURA hamsters. Exp Toxicol Pathol 52:539–544.

Kanai T, Wang SC (1962) Localization of the central vocalization mechanism in the brain stem of the cat. Exp Neurol 6:426–434.

Klarica M, Oreskovic D, Bozic B, Vukic M, Butkovic V, Bulat M (2009) New experimental model of acute aqueductal blockage in cats: effects on cerebrospinal fluid pressure and the size of brain ventricles. Neuroscience 158:1397–1405.

Kleine TO, Hackler R, Lutcke A, Dauch W, Zofel P (1993) Transport and production of cerebrospinal fluid (CSF) change in aging humans under normal and diseased conditions. Z Gerontol 26:251–255.

Kruth HS (2001) Macrophage foam cells and atherosclerosis. Front Biosci 6:D429–D455.

Larson CR (1985) The midbrain periaqueductal gray: a brainstem structure involved in vocalization. J Speech Hearing Res 28:241–249.

Liu RP, Hamilton BL (1980) Neurons of the periaqueductal gray matter as revealed by Golgi study. J Comp Neurol 189:403–418.

Lovick TA (1996) Midbrain and medullary regulation of defensive cardiovascular functions. Prog Brain Res 107:301–313.

Lovick TA (2006) Plasticity of GABAA receptor subunit expression during the oestrous cycle of the rat: implications for premenstrual syndrome in women. Exp Physiol 91:655–660.

Lovick TA (2008) GABA in the female brain – oestrous cycle-related changes in GABAergic function in the periaqueductal grey matter. Pharmacol Biochem Behav 90:43–50.

Lovick TA, Griffiths JL, Dunn SM, Martin IL (2005) Changes in GABA(A) receptor subunit expression in the midbrain during the oestrous cycle in Wistar rats. Neuroscience 131:397–405.

May C, Kaye JA, Atack JR, Schapiro MB, Friedland RP, Rapoport SI (1990) Cerebrospinal fluid production is reduced in healthy aging. Neurology 40:500–503.

Mayer DJ, Price DD (1976) Central nervous system mechanisms of analgesia. Pain 2:379–404.

Miller VM, Kalaria RN, Hall R, Oakley AE, Kenny RA (2007) Medullary microvessel degeneration in multiple system atrophy. Neurobiol Dis 26:615–622.

Mooradian AD (1988) Effect of aging on the blood-brain barrier. Neurobiol Aging 9:31–39.

Nandy K, Fritz RB, Threatt J (1975) Specificity of brain-reactive antibodies in serum of old mice. J Gerontol 30:269–274.

Nieuwenhuys R (1996) The greater limbic system, the emotional motor system and the brain. Progr Brain Res 107:551–580.

Nieuwenhuys R, Veening JG, van Domburg P (1988) Core and paracores; some new chemoarchitectural entities in the mammalian neuraxis. Acta Morphol Neerl-Scand 26:131–163.

Nieuwenhuys R, Voogd J, van Huizen C (2008) The human central nervous system. Berlin, Heidelberg, New York: Springer Verlag.

Nishizuka M, Pfaff DW (1989) Medial preoptic islands in the rat brain: electron microscopic evidence for intrinsic synapses. Exp Brain Res 77:295–301.

Oklejewicz M, Daan S (2002) Enhanced longevity in tau mutant Syrian hamsters, *Mesocricetus auratus*. J Biol Rhythms 17:210–216.

Olszewski J, Baxter D (1954) Cytoarchitecture of the human brain stem. Basel: Karger.

Onen F, Feugeas MC, De Marco G, Baron G, Ravaud P, Legrain S, Moretti JL, Claeys ES, Peretti II (2005) Cerebrospinal fluid MR dynamics and risk of falls in the elderly. J Neuroradiol 32:3–9.

Raichle ME (1981) Measurement of local cerebral blood flow and metabolism in man with positron emission tomography. Fed Proc 40:2331–2334.

Redzic ZB, Preston JE, Duncan JA, Chodobski A, Szmydynger-Chodobska J (2005) The choroid plexus-cerebrospinal fluid system: from development to aging. Curr Top Dev Biol 71:1–52.

Reiber H (1994) Flow rate of cerebrospinal fluid (CSF) – a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases. J Neurol Sci 122:189–203.

Reiber H (2003) Proteins in cerebrospinal fluid and blood: barriers, CSF flow rate and source-related dynamics. Restor Neurol Neurosci 21:79–96.

Ross R (1999) Atherosclerosis – an inflammatory disease. N Engl J Med 340:115–126.

Sakka L, Coll G, Chazal J (2011) Anatomy and physiology of cerebrospinal fluid. Eur Ann Otorhinolaryngol Head Neck Dis 128:309–316.

Sakuma Y, Pfaff DW (1979a) Facilitation of female reproductive behavior from mesensephalic central gray in the rat. Am J Physiol 237:R278–R284.

Sakuma Y, Pfaff DW (1979b) Mesencephalic mechanisms for integration of female reproductive behavior in the rat. Am J Physiol 237:R285–R290.

Sakuma Y, Pfaff DW (1980) Cells of origin of medullary projections in central gray of rat mesencephalon. J Neurophysiol 44: 1002–1011.

Sakuma Y, Pfaff DW (1983) Modulation of the lordosis reflex of female rats by LHRH, its antiserum and analogs in the mesencephalic central gray. Neuroendocrinology 36:218–224.

Shah GN, Mooradian AD (1997) Age-related changes in the blood-brain barrier. Exp Gerontol 32:501–519.

Stoquart-ElSankari S, Baledent O, Gondry-Jouet C, Makki M, Godefroy O, Meyer ME (2007) Aging effects on cerebral blood and cerebrospinal fluid flows. J Cereb Blood Flow Metab 27:1563–1572.

Threatt J, Nandy K, Fritz R (1971) Brain-reactive antibodies in serum of old mice demonstrated by immunofluorescence. J Gerontol 26:316–323.

Veening JG, Buma P, ter Horst GJ, Roeling PAG, Luiten PGM, Nieuwenhuys R (1991) Hypothalamic projections to the PAG in the rat: topographical, immuno-electronmicroscopical and functional aspects. In: Depaulis A, Bandler RA, editors. The midbrain periaqueductal gray matter: functional, anatomical and neurochemical organization. New York: Plenum Press. p. 387–415.

Veening JG, Kortekaas R, De Weerd H, Luiten PGM, van der Want JJH, Gerrits PO (2012) Spumiform capillary basement membrane aberrations in the microvasculature of the midbrain

periaqueductal gray (PAG) region: rostrocaudal pathogenesis? In: FENS Forum of Neuroscience Barcelona.

Yang YH, Roe CM, Morris JC (2011) Relationship between late-life hypertension, blood pressure, and Alzheimer's disease. Am J Alzheimers Dis Other Demen 26:457–462.

Zlokovic BV (2008) The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron 57:178–201.

Zlokovic BV (2011) Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci 12:723–738.

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