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ORIGINAL ARTICLE

Hypoxic changes in Purkinje cells of the human cerebellum

R. Hausmann · S. Seidl · P. Betz

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Abstract The significance of both Purkinje cell numbers and various neuronal changes for the diagnosis and timing of hypoxic-induced brain lesions was investigated in tissue samples from the cerebellar cortex of 52 individuals with a history of acute or prolonged cerebral hypoxia/ischemia before death. Furthermore, the area of the Purkinje cell somata (PC size) was measured using an automatic image processing and analysis system (LEICA QWin®). Significantly reduced numbers of Purkinje cells (<6 cells/unit length of 1 mm) and a decreased portion (<50%) of intact Purkinje cells could be detected in individuals with a period of resuscitation of at least 2 h after acute circulatory arrest. Average cell numbers of less than 4 cells/unit were found in individuals who suffered from diffuse brain swelling and were ventilated for at least 3 days, as well as in individuals who died of brain death. Moreover, the Purkinje cells in these cases exhibited shrunken somata compared to the controls. Specimens that were stored at room temperature up to 30 h after removal at autopsy showed no significant autolytic changes of the Purkinje cells. After 46 h, however, reduced Purkinje cell numbers and shrunken cell bodies were found.

Keywords Brain hypoxia · Purkinje cells · Histomorphology · Forensic medicine

R. Hausmann (✉) · S. Seidl · P. Betz
Institute of Legal Medicine,
University of Erlangen-Nuremberg, Universitätsstrasse 22,
91054 Erlangen, Germany
e-mail: roland.hausmann@recht.imed.uni-erlangen.de

Introduction

The morphological demonstration of hypoxic brain injury is of considerable interest in forensic pathology for determining the cause of death [20]. In this context, the occurrence of plasmolysis, cellular degeneration with shrinkage, acidophilic behavior, and loss of Nissl substance are the most important general microscopic criteria. But morphological studies revealed considerable polymorphism of ganglion cell lesions, predominantly determined by the severity, duration and form of hypoxia, as well as by the survival interval after the onset of oxygen deficiency [3]. Furthermore, a selective vulnerability to hypoxic and ischemic damage could be demonstrated for definite regions of the central nervous system such as layers 3, 5, and 6 in the cerebral cortex, the CA1 area (Sommer sector), the end plate in the hippocampus, and the Purkinje neurons of the cerebellum. [1, 4, 14, 17, 22].

With respect to the Purkinje cells, the primarily reported criteria for hypoxic damage are cell swelling, autolytic necrosis characterized by the lack of distinct nuclear staining, shrunken cells, and dark cell degeneration [21]. However, the accurate determination of such histological findings is possibly more difficult in autopsy material than in experimental animals [21], as similar patterns of neuronal changes may possibly be induced by postmortem influences [15]. Furthermore, there is an inconsistency of data reported in the literature concerning the earliest manifestation of Purkinje cell necrosis after hypoxia. With respect to a limited number of systematic investigations in the literature, this study was performed to investigate the significance of histological changes in cerebellar Purkinje neurons for the diagnosis and timing of hypoxic-induced lesions in forensic autopsy cases.

Materials and methods

Specimens

Tissue samples from the cerebellar cortex of 52 individuals aged between 6 and 82 years (average age 43 years) with a history of acute or prolonged hypoxia/ischemia before death were obtained at autopsy. According to the cause of death and the duration of hypoxia/ischemia, the cases were divided into five groups (Table 1). In group A, cerebellar tissue from individuals who died on sudden death served as controls. Individuals who suffered from acute circulatory arrest due to coronary thrombosis (n=2), myocarditis (n=1), injury-induced bleeding (n=4), or electric shock (n=1) belonged to group B. According to the duration of resuscitation, these cases were divided into subgroups B1 (<2 h) and B2 (≥2 h). Individuals who died of suffocation from complete airway obstruction due to aspiration of blood or vomit (n=7), drowning (n=8), homicide by smothering using a pillow (n=2), or mechanical asphyxia due to thoracic compression (n=3) belonged to group C. In one of the cases of aspiration and in a further case of fatal mechanical asphyxia, resuscitation was performed for 1 and 4 h, respectively. Individuals who had sustained hypoxic/ischemic brain damage due to blunt head injury (n=2), suicidal hanging attempt (n=1), spontaneous intracerebral hemorrhage (n=2), or misplaced intubation (n=1) belonged to group D. According to the duration of artificial respiration, these cases were divided into subgroups D1 (<3 days) and D2 (≥3 days). Group E had cases in which autopsy revealed morphological hallmarks of brain death ("respirator brain") such as global softening, dusky discoloration of the gray matter, and transtentorial herniation [14]. Brain death was caused by blunt head injury (n=2), a shotgun wound to the head (n=1), trauma-induced respiratory distress (n=1), cerebral infarction due to carotid thrombosis (n=1), and global ischemia after cardiac arrest (n=1). The period of respiration ranged between 1 day and 5.5 months.

The postmortem interval before autopsy did not exceed 2 days. Tissue samples, 3 cm^3 in size, were collected from a region of the cerebellar cortex showing no focal lesions macroscopically. After fixation in 4% phosphate-buffered saline/formaldehyde solution for a maximum of 24 h, the tissue samples were embedded in paraffin and sections (3–5 μm) were stained with hematoxylin and eosin (HE).

To investigate the course of autolytic changes of Purkinje cells, cerebellar tissue samples from a 40-year-old individual who died of hanging were removed at autopsy, which took place 6 h after death occurred. The specimens were then placed for 2, 4, 8, 22, 26, 30, 46, 50, 54, and 70 h at room temperature before fixation in formaldehyde solution.

Histological evaluation

For histological evaluation, the damaged Purkinje cells (PC) were categorized according to their cytological characteristics (Fig. 1) as described in the literature [21]:

PC type I: intact Purkinje cells with distinct stained nucleus
PC type II: necrotic Purkinje cells lacking both nuclear staining and distinct cellular morphology
PC type III: shrunken cells characterized by an intensive (dark) staining of the cytoplasm (so-called dark neurons)

The different cell types (PC I–III) were counted in microscopic preparations along eight randomly selected segments of the Purkinje cell line assisted by an image processing and analysis system (LEICA QWin®) and using a unit length of 1 mm. For this purpose, an image scanning was performed with a CCD color camera. Afterwards, the Purkinje cells were selected at the screen and marked by mouse click. The number of marked cells was counted automatically by the analysis system.

To measure the Purkinje cell size (PC size), the area of Purkinje cell somata was marked by the examiner using the interactive modus of the system. The area was then evaluated by the QWin® software program.

Statistical methods

The statistical data analysis was performed using SPSS for Windows release 13.0. Descriptive analysis included means, medians, and SD. The significance of differences in quantitative data obtained in the different subgroups was tested using the Mann–Whitney U test.

Results

Purkinje cell numbers

The average number of Purkinje cells evaluated in the eight randomly selected fields in cases of sudden death (group A, n=12) ranged between 7.38 and 11.38 PC/unit (mean value, 9.21 PC/unit). The majority of these cells (76%) were characterized by intact cell bodies and distinct nuclear staining, defined as normal PC type I (Fig. 2a). The remaining cells exhibited cytological features corresponding to PC type II that lacked both nuclear staining and distinct cellular morphology.

The data obtained in individuals who suffered from acute circulatory arrest were comparable to that in the control group, if duration of resuscitation did not exceed 1 h (group

Table 1 Data of 52 individuals with survival intervals ranging from a few minutes up to 5.5 months after resuscitation

No.	Sex	Age	Subgroup	Cause of death	Survival interval
1	M	33	A	Electrical death	Sudden death
2	M	78	A	Myocarditis/pericardial tamponade	Sudden death
3	F	82	A	Coronary thrombosis	Sudden death
4	F	54	A	Stab wounds/bleeding	Sudden death
5	M	21	A	Traffic injury/aortic rupture	Sudden death
6	M	57	A	Traffic injury/run over	Sudden death
7	M	54	A	Aneurysma dissecans/pericardial tamponade	Sudden death
8	F	35	A	Endocarditis/acute cardiac death	Sudden death
9	F	40	A	Traffic injury/brain stem rupture	Sudden death
10	M	45	A	Coronary insufficiency	Sudden death
11	M	45	A	Coronary thrombosis	Sudden death
12	M	35	A	Cardiac hypertrophy/acute cardiac death	Sudden death
13	M	53	B1	Stab wounds/bleeding	20-min resuscitation
14	M	59	B1	Coronary thrombosis	30-min resuscitation
15	M	41	B1	Coronary thrombosis	1-h resuscitation
16	M	47	B2	Traffic injury/aortic rupture	2-h resuscitation
17	M	42	B2	Electrical death	2-h resuscitation
18	M	26	B2	Myocarditis	2-h resuscitation
19	M	47	B2	Traffic injury/hemorrhagic shock	8-h resuscitation
20	F	5	B2	Hemorrhagic shock/rupture of spleen	5-h resuscitation
21	M	31	C1	Chest injury/aspiration (vomit)	<10 min
22	M	40	C1	Chest injury/aspiration (vomit)	<10 min
23	F	24	C1	Chest injury/aspiration (vomit)	<10 min
24	F	79	C1	Polytrauma/aspiration (blood)	<10 min
25	M	12	C1	Polytrauma/aspiration (blood)	<10 min
26	F	7	C1	Polytrauma/aspiration (blood)	<10 min
27	F	72	C1	Drowning	<10 min
28	F	34	C1	Drowning	<10 min
29	F	24	C1	Drowning	<10 min
30	M	28	C1	Drowning	<10 min
31	M	28	C1	Drowning	<10 min
32	M	77	C1	Drowning	<10 min
33	M	45	C1	Drowning	<10 min
34	M	6	C1	Drowning	<10 min
35	F	82	C1	Suffocation/smothering	<10 min
36	F	26	C1	Suffocation/smothering	<10 min
37	M	44	C1	Mechanical asphyxia (thoracic compression)	<10 min
38	M	43	C1	Mechanical asphyxia (thoracic compression)	<10 min
39	F	24	C2	Aspiration (blood)	1-h resuscitation
40	M	8	C2	Mechanical asphyxia (thoracic compression)	4-h resuscitation
41	M	57	D1	Traumatic brain injury/brain swelling	1.5 days artificial respiration
42	M	58	D1	Hanging/carotid thrombosis	2 days artificial respiration
43	M	58	D1	Spontaneous intracerebral hemorrhage	2.5 days artificial respiration
44	M	32	D2	Misplaced intubation/suffocation	5 days artificial respiration
45	M	54	D2	Traumatic brain injury/respiratory failure	7 days artificial respiration
46	M	46	D2	Spontaneous intracerebral hemorrhage	17 days artificial respiration
47	M	25	E	Shotgun wound/brain death	1 day artificial respiration
48	M	58	E	Traumatic brain injury/brain death	3.5 days artificial respiration
49	M	49	E	Carotid thrombosis/brain death	5 days artificial respiration
50	F	31	E	Traumatic brain injury/brain death	7 days artificial respiration
51	F	52	E	Cardiac failure/hypoxic brain damage/brain death	7 days artificial respiration
52	M	60	E	Polytrauma/respiratory distress/apallic syndrome	5.5 months artificial respiration

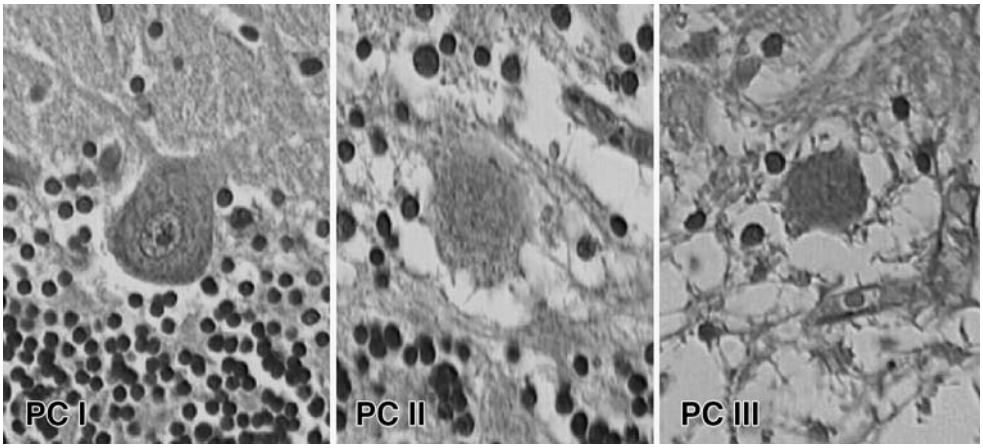

Fig. 1 Cytological characteristics of Purkinje cells (PC) in tissue samples from human cerebellum. Intact PC with distinctly stained nucleus (*PC I*), Purkinje cells lacking both nuclear staining and distinct cellular morphology (*PC II*), and shrunken cells characterized by an intensive (dark) staining of the cytoplasm (*PC III*; HE×410)

B1, n=3), and 79% of Purkinje cells were intact. In contrast, significantly reduced cell numbers (mean value, 5.53 PC/unit) were detected in individuals who were resuscitated for at least 2 h (group B2, n=5), and the majority of these cells (about 61%) were characterized by lacking nuclear staining and fading out of cytoplasm, defined as PC type II (Fig. 2b). The percentage of intact Purkinje cells was 39%. Compared to the data obtained in the controls, the differences concerning the cell numbers were statistically significant (p<0.001, Fig. 3).

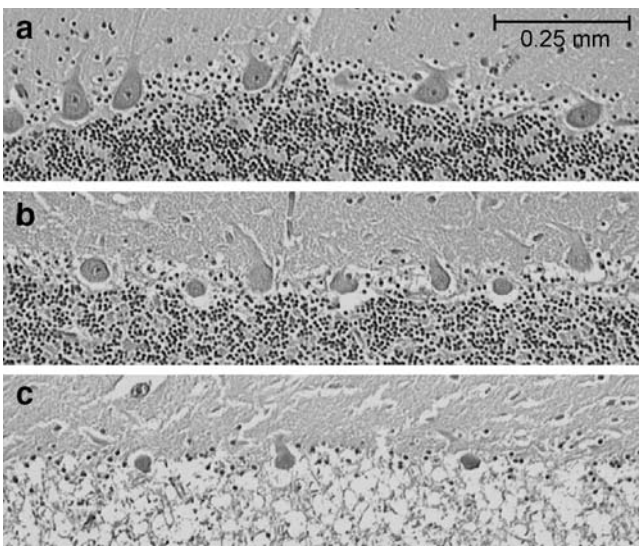

Fig. 2 Morphometrical evaluation of the Purkinje cell numbers in microscopic preparations along a segment of the PC line using a unit length of 1 mm. **a** Purkinje cells showing a distinct nuclear staining and regular cell morphology (PC I) in the cerebellum of an individual who died immediately of acute cardiac arrest due to coronary thrombosis. **b** Individual who suffered from acute hemorrhagic shock and died after a resuscitation period of 5 h. The majority of Purkinje cells are characterized by lacking nuclear staining and fading out of cytoplasm (PC II). **c** Case of brain death ("respirator brain") 7 days after traumatic head injury showing complete damage of the granular layer without any cellular or glial reaction, as well as so-called dark neurons

In cases of suffocation due to airway obstruction (aspiration, drowning, smothering) or mechanical asphyxia without resuscitation (group C1, n=18), the average cell numbers ranged between 6.12 and 9.38 PC/unit (mean value, 7.60 PC/unit) and were significantly different from the data obtained in the controls (p<0.001, Fig. 3). The percentage of intact Purkinje cells (PC I) was about 52%. More reduced cell numbers could be obtained if resuscitation was performed during 1 or 4 h after hypoxic-induced circulatory arrest (group C2, n=2).

Individuals with hypoxic brain damage who were ventilated for at least 1.5 days (group D1, n=3) and up to 17 days (group D2, n=3) showed Purkinje cell numbers ranging between 1.25 and 5.63 PC/unit, as well as a reduced percentage of intact Purkinje cells (about 30%).

In brain tissue with morphological signs of brain death at autopsy (group E, n=6), comparably low cell numbers (mean value, 4.06 PC/unit) with a significantly reduced portion of intact Purkinje cells (24%) could be detected. Furthermore, numerous dark neurons were obvious in three cases after 1 h, 3.5, and 7 days on a respirator, respectively. These findings were associated with complete damage of the granular layer of the cerebellar cortex without any cellular or glial reaction (Fig. 2c), which could not be detected in the remaining cases. The numbers of the different Purkinje cell types are shown in Table 2 and Fig. 4.

Purkinje cell size

As shown in Table 2 and Fig. 4, the somata of Purkinje cell bodies measured between 534 and 867 μm^2 in the controls (mean value, 711 μm^2). Slightly elevated average values could be obtained in individuals who suffered from acute circulatory arrest and were resuscitated for 20 min up to 1 h (mean value, 744 μm^2), whereas the data obtained in the other subgroups showed a comparably high variance. But as shown in Fig. 6, significantly reduced values of less than

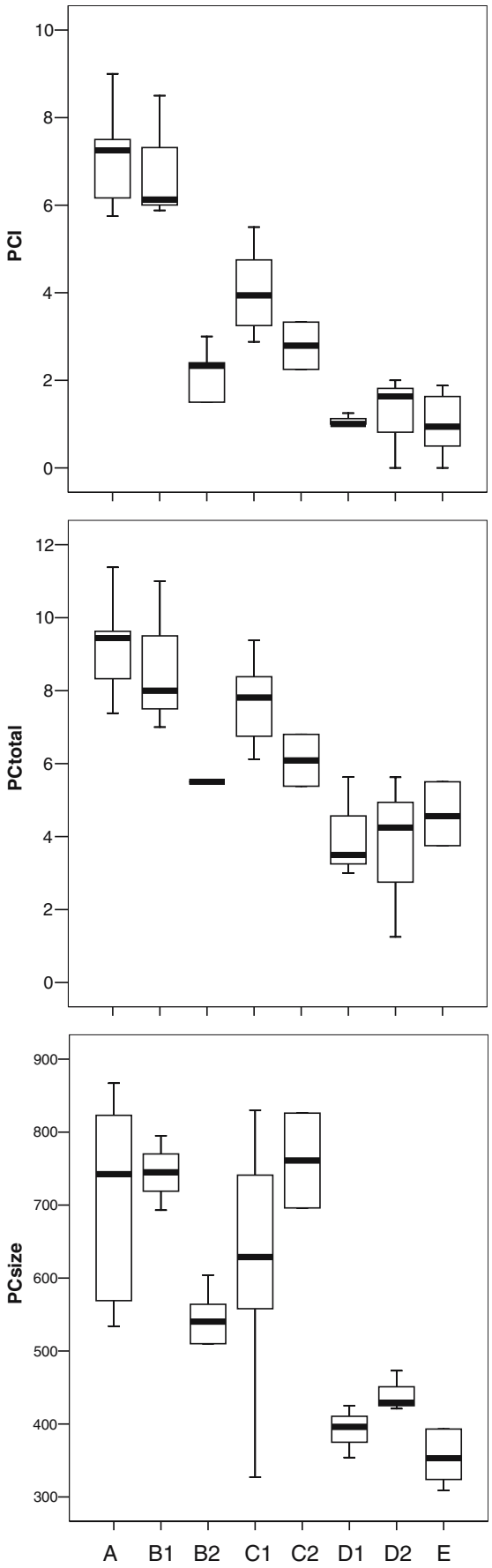

Fig. 3 Average numbers of intact Purkinje cells (PC I) and total cell numbers (PC total), as well as mean Purkinje cell size (PC size) in the different subgroups. *Boxplot* The boxes contain 50% of the measurement values, *line* in the *box* represents the median value

450 μm^2 could be obtained in individuals with hypoxic brain injury dying of elevated intracranial pressure and in generalized damaged brains of those patients who had been ventilated after intravital arrest of intracerebral circulation.

Autolytic changes

Tissue samples that had been stored at room temperature up to 30 h after removal at autopsy showed average numbers of Purkinje cells (PC total) ranging between 8.25 and 9.00 PC/unit (Table 3), with a comparable high proportion of intact Purkinje cells (63–75%). The cell numbers and the average sizes of Purkinje cell bodies (535–603 μm^2) were comparable to that in the control group. Significantly reduced cell numbers and shrunken cell bodies could be detected in specimens stored for at least 46 h at room temperature before fixation in formaldehyde solution (Fig. 5). The majority of Purkinje cells were characterized by the loss of nuclear staining and "pale" cytoplasm (Fig. 6), whereas so-called "dark" neurons were not obvious during the putrefaction process.

Discussion

As Purkinje cells of the cerebellum have been found to be most vulnerable to oxygen demand [5], the histological detection of structural changes could be of particular interest for both demonstrating and timing of hypoxic injury to human brain tissue. However, there is a limited number of systematic studies on ischemic damage in human Purkinje cells reported in the literature.

Sato et al. [24] investigated histological changes of neuronal damage in vegetative dogs induced by 18 min of complete global brain ischemia. The authors reported a two-phase damage of Purkinje cells and hippocampal CA1 pyramidal cells during cerebral ischemia and in the following recirculation period, whereby 50% of the damaged neurons had virtually recovered morphologically 1 h after recirculation but disintegrated 2–3 days later.

To obtain more detailed information on the degree of hypoxic changes in Purkinje cells, Lee et al. [18] investigated the cerebellum of hypoxic chick embryos by morphometrical analysis. The hypoxic Purkinje cells had smaller somata than those of normal cells by 15% and the density was decreased by 10% compared to normal. In contrast to healthy Purkinje cells, hypoxic-challenged PCs exhibited a more rounded, shrunken appearance with average diameter of 15±2 μm compared to 21±2 μm in controls.

Table 2 Numbers of the different Purkinje cell types (PC I–III) and the size of Purkinje cell somata (PC size) in various types of hypoxic/ischemic brain injury (groups B–E) compared to the values obtained in the control group (A)

Cell type	Ischemic brain injury group							
	A	B1	B2	C1	C2	D1	D2	E
PC I								
Min	5.75	5.88	1.50	2.88	2.25	1.00	0.00	0.00
Max	9.00	8.50	3.00	5.50	3.33	1.25	2.00	1.88
Mean	7.00	6.84	2.15	4.01	2.79	1.08	1.21	0.98
Median	7.25	6.13	2.33	3.94	2.79	1.00	1.63	0.94
PC II								
Min	1.00	0.88	2.50	1.75	3.13	1.88	1.25	0.25
Max	3.38	2.50	5.25	5.50	3.50	4.63	3.63	3.88
Mean	2.21	1.84	3.46	3.59	3.32	2.92	2.50	2.11
Median	2.10	2.13	3.17	3.38	3.32	2.25	2.63	2.26
PC III								
Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max	0.00	0.00	0.00	0.00	0.00	0.13	0.00	2.88
Mean	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.98
Median	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.13
PC total								
Min	7.38	7.00	4.38	6.12	5.38	3.00	1.25	0.50
Max	11.38	11.00	6.75	9.38	6.80	5.63	5.63	5.50
Mean	9.21	8.67	5.53	7.60	6.09	4.04	3.71	4.06
Median	9.44	8.00	5.50	7.81	6.09	3.50	4.25	4.57
Percentage PC I	76	79	39	52	46	27	33	24
PC size								
Min	534	693	381	327	696	354	421	309
Max	867	795	604	830	826	425	473	556
Mean	711	744	519	646	761	391	441	381
Median	742	741	540	629	761	396	429	353

Horn and Schlote [14] investigated delayed neuronal death and delayed neuronal recovery in the human brain after global ischemia. The earliest manifestation of ischemic neuronal necrosis was observed 5 h after cardiac arrest in cortical layers 3, 5, and 6, whereas hippocampal CA1 and Purkinje cell layer still showed no clear ischemic cell damage. In these selective vulnerable areas, the first necrotic neurons were not detected before 12 h after resuscitation from cardiac arrest. The proportion of neuronal necrosis steadily increased with advancing post-arrest survival in the neocortex and the cerebellar cortex. Complete manifestation of post-ischemic Purkinje cell changes seemed to have a delay of about 4 days. Additionally, the delayed increase of neuronal necrosis was less pronounced in the cerebellar cortex than in hippocampal CA1 neurons.

On the other hand, Purkinje cell damage could be demonstrated even after only 5 h of exposure to oxygen deficiency. A very low proportion of shrunken, dark Purkinje cells was found after 10 h steady-state exposure, and after 30 h more than 90% of neurons were damaged [23].

In the present study the damaged PCs were categorized according to simple standards, including autolysis, darkened, and shrunken characteristics, which have been employed recently by Pae et al. [21]. Autolytic cells are cells with no nucleus and no distinct cellular morphology, defined as PC type II in this study. Darkened cells are characterized by an intensive (dark) staining of the perikaryal cytoplasm (so-called dark neurons, PC type III). These features are considered typical for neuronal necrosis and were described in Purkinje cells after intermittent hypoxia in rats [21] and after complete global brain ischemia in vegetative dogs [24]. To investigate the significance of the above-mentioned cytological criteria of cell necrosis for the diagnosis and timing of hypoxic-induced brain lesions, the various cell-types (PC I–III) were counted in samples from the cerebellar cortex of individuals with a history of acute or prolonged cerebral hypoxia/ischemia before death. As Purkinje cells failed to display spherical/ovoid cytoplasmic/nuclear fragments, referred to as apoptotic bodies, or TUNEL-positive staining [25], apoptotic cell death was not investigated in this study.

Compared to control tissue, a significantly reduced number of intact Purkinje cells (PC I) could be detected as early as 2 h after recirculation after acute circulatory arrest. The majority of Purkinje cells (about 60%) were

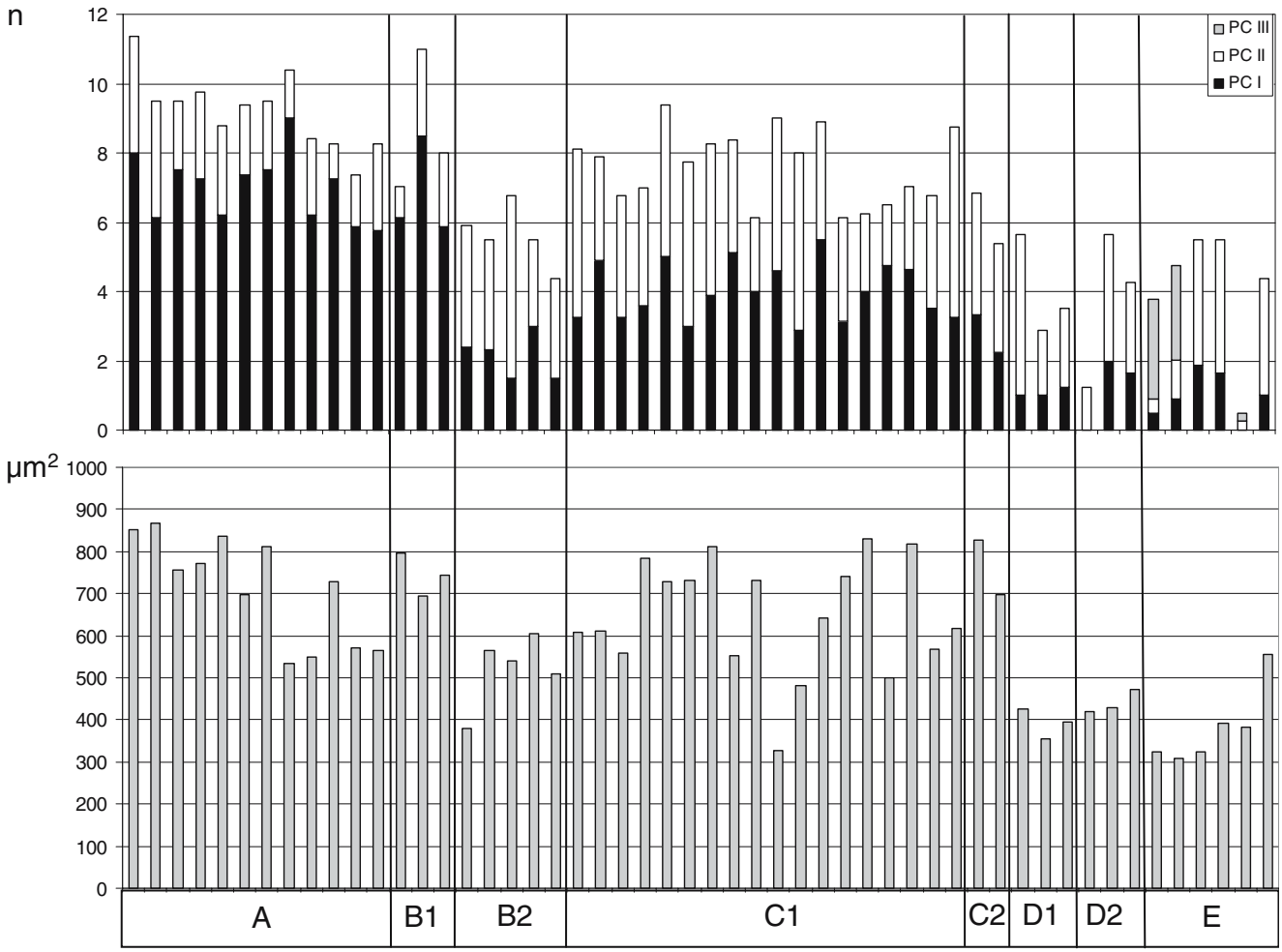

Fig. 4 Numbers (*n*) of the various Purkinje cell types (PC I–III) and the average Purkinje cell size (micrometers^2) evaluated in cerebellar samples from the control group (*A*) and from individuals who sustained different types of hypoxic/ischemic brain injury: acute circulatory arrest, resuscitation <2 h (group *B1*) or ≥2 h (group *B2*); suffocation, no resuscitation (group *C1*); suffocation, resuscitation 1–4 h (group *C2*); Hypoxic/ischemic brain damage, artificial respiration for<3 days (group *D1*) or ≥3 days (group *D2*); respirator brains (group *E*)

Table 3 Average numbers of Purkinje cells and sizes of Purkinje cell bodies related to the time since autopsy (*t*) before tissue fixation in a case of hanging

t (*h*)	PC I (*n*)	PC II (*n*)	PC III (*n*)	PC total (*n*)	PC size (µm^2)
0	6.40	2.17	0	8.57	603
4	5.75	2.50	0	8.25	599
8	5.90	2.40	0	8.30	558
22	6.20	2.80	0	9.00	562
26	5.80	2.80	0	8.60	535
30	5.70	3.30	0	9.00	542
46	1.80	5.00	0	6.80	262
50	2.40	4.60	0	7.00	279
54	2.40	4.90	0	7.30	302
70	1.00	6.10	0	7.10	305

characterized by the lack of nuclear staining and cell lysis (PC type II) and by shrunken cell bodies when compared to the data obtained in the control group. If oxygen deficiency was primarily induced by complete airway obstruction or mechanical asphyxia, about 50% of Purkinje cells showed neuronal changes, although death occurred within a comparably short period after the onset of mechanical asphyxia. Based on the traditional concept of pathophysiological mechanisms [13], it can be assumed that circulatory arrest occurred within 5–10 min in cases of complete airway obstruction or mechanical asphyxia. In addition to the diminished proportion of intact neurons in the hypoxic cerebellum, the total number of Purkinje cells decreased with advancing survival time after circulatory arrest. The minimal total of cell numbers could be obtained in cerebellar tissue from individuals who had sustained hypoxic brain damage and were ventilated for at least 3 days, as well as in individuals who died of brain death.

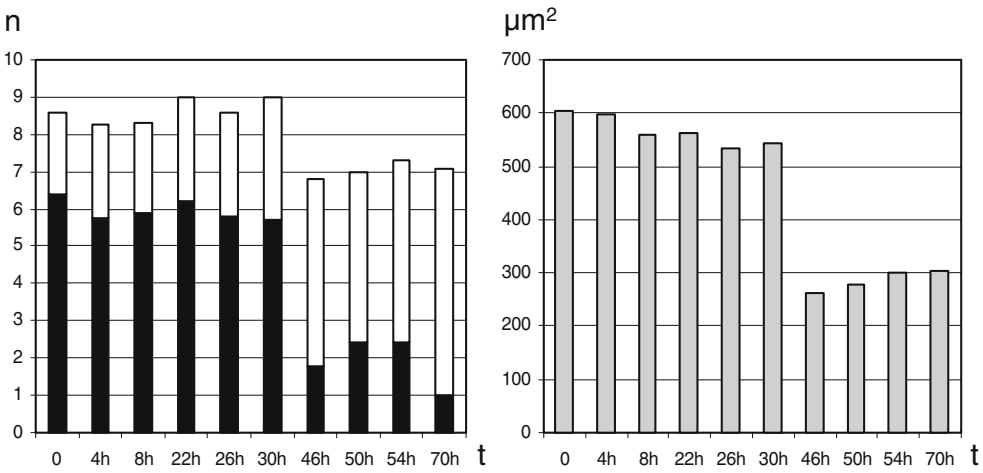

Fig. 5 Autolytic changes in cerebellar samples, which were stored at room temperature up to 70 h after removal at autopsy. *Left* Numbers of intact Purkinje cells (*black columns*) and Purkinje cells showing autolytic changes (*white columns*), *right* average Purkinje cell size (in micrometers^2)

Furthermore, several so-called dark neurons, characterized by an intensive (dark) staining of the cytoplasm in HE-stained preparations, were found in autopsy cases showing morphological hallmarks of respirator brain such as generalized swelling, global softening, and dusky discoloration of the gray matter [19]. As dark neurons are often regarded as an artifact due to autolysis or inappropriate tissue handling such as pressure [16] or inadequate fixation [6], it could be assumed that some of the type III cells demonstrated in the softened tissue from respirator brains might be artifacts. On the other hand, this cell type could be detected neither in the other subgroups nor in specimens that were exposed to putrefaction. Thus, it can be concluded that the appearance of dark neurons obviously depends on preceding longer periods of hypoxic brain damage. Similar findings were reported as a form of delayed cell death known as dark cell degeneration [2].

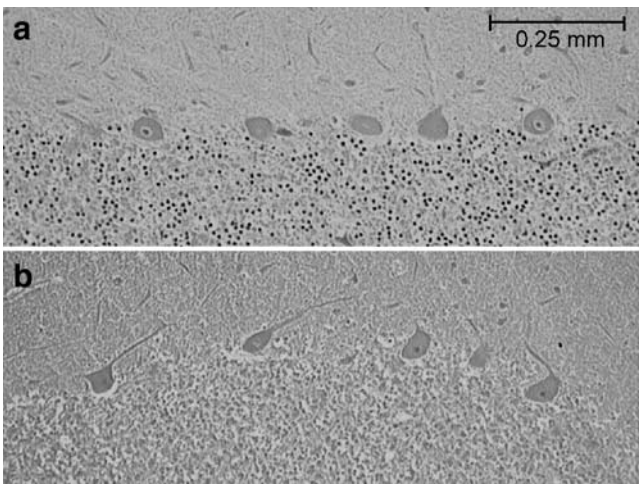

Fig. 6 Tissue samples that had been stored for 46 h (**a**) or 70 h (**b**) at room temperature before fixation in formaldehyde solution. The majority of Purkinje cells are characterized by loss of nuclear staining and "pale" cytoplasm (×260)

With respect to the different morphometrical data obtained in subgroups B–E compared to the controls, it could be of forensic interest to establish threshold values for the diagnosis of hypoxic injury in human brain tissue, as performed in quantitative morphological studies concerning traumatically induced reactions in the human brain [8–12] or postmortem features of the neuronal stem cells [7]. In this context, the most reliable indicator might be a significantly reduced proportion of intact Purkinje cells (PC I) related to the total cell numbers (PC total). Cerebellar tissue of the control group exhibited a high percentage (76%) of intact Purkinje cells, and the remaining cells corresponded to the (abnormal) type II cells. These findings are in accordance to the experimental study by Pae et al. [21], who demonstrated several damaged ("swollen/autolyzed") Purkinje cells in the control tissue from cerebellar cortex in rats. It can be discussed whether these morphological changes may possibly induced by a short stage of global brain ischemia during agonia or by postmortem influences. Significantly reduced numbers of intact Purkinje cells could be detected after hypoxic/ischemic events. Values below 50% could be observed if resuscitation was performed during a period of at least 2 h after acute circulatory arrest or mechanical asphyxia. Furthermore, the total number of Purkinje cells was significantly reduced (<6 cells/unit) in these cases. Average cell numbers of <4 cells/unit can be considered as an indicator for prolonged hypoxia, as such findings could exclusively be detected in individuals who suffered from diffuse brain swelling and were ventilated for at least 3 days, as well as in individuals who died of brain death. Moreover, cerebellar tissue from individuals out of these subgroups exhibited shrunken Purkinje cell somata, when compared to the findings obtained in the remaining cases.

Summarizing the data obtained in this study, we conclude that the morphometrical evaluation of both Purkinje cell numbers and various neuronal changes contributes to diagnosis and timing of hypoxic injury in human brain. It

must be emphasized, however, that the diagnosis of cerebral hypoxia cannot be exclusively based on microscopic findings. Furthermore, the morphometrical analysis of Purkinje cell damage does not facilitate a differentiation between the various types and causes of oxygen deficiency.

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