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ORIGINAL ARTICLE

Encephalopathy and death in infants with abusive head trauma is due to hypoxic-ischemic injury following local brain trauma to vital brainstem centers

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Abstract
Background Infants with abusive head trauma (AHT) have diffuse brain damage with potentially fatal brain swelling. The pathogenesis of the brain damage remains unclear. We hypothesize that brain damage in AHT is due to hypoxic-ischemic injury with hypoxic-ischemic encephalopathy (HIE) rather than primary traumatic brain injury (TBI) with traumatic diffuse axonal injury (tDAI).
Methods We studied brain tissue of AHT victims. Primary outcome measure was the presence of primary traumatic versus hypoxic-ischemic brain injury. The diagnosis of tDAI followed a standardized semiquantitative diagnostic approach yielding a 4-tiered grading scheme (definite, possible, improbable, and none). In addition, results of quantitative immunohistochemical analysis in a subgroup of AHT victims with instant death were compared with matched SIDS controls.
Results In our cohort of 50 AHT victims, none had definite tDAI (no tDAI in 30, tDAI possible in 2, and tDAI improbable in 18). Instead, all AHT victims showed morphological findings indicative of HIE. Furthermore, the subgroup with instant death showed significantly higher counts of damaged axons with accumulation of amyloid precursor protein (APP) in the brainstem adjacent to the central pattern generator of respiratory activity (CPG) (odds ratio adjusted for age, sex, brain weight, and APP-count in other regions=3.1; 95 % confidence interval=1.2 to 7.7; p=0.015).
Conclusions AHT victims in our cohort do not have diffuse TBI or tDAI. Instead, our findings indicate that the encephalopathy in AHT is the due to hypoxic-ischemic injury probably as the result of respiratory arrest due to local damage to parts of the CPG in the brainstem.

Keywords Abusive head trauma · Non-accidental head injury · Shaken baby syndrome · Child abuse · Diffuse axonal injury · Neuropathology

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Introduction

Abusive head trauma (AHT) is the most frequent cause of traumatic brain injury (TBI) in infants, occurring annually in 14.9–38.5/100,000 infants [1]. Clinically, AHT features subdural hemorrhage (SDH), retinal hemorrhage (RH), and signs of a diffuse encephalopathy, typically occurring in a context of an inappropriate history [2, 3]. The severity of the encephalopathy varies from only slight irritability to deep coma; often, the infants are lifeless at the time when medical help is sought [4]. The cause of the encephalopathy is usually attributed to primary traumatic mechanisms resulting in "classic" traumatic

brain injury (TBI) with traumatic diffuse axonal injury (tDAI); more recently, hypoxic-ischemic brain injury has been discussed as a further or main contributing factor [5–8]. Specifically, hyperextension injury to the neck during a violent shaking event has been suggested to cause damage to the central pattern generator (CPG) of respiration in the brainstem [9]. As a consequence, AHT victims would experience sudden respiratory collapse leading to hypoxic-ischemic brain injury. We hypothesize (i) that if hypoxic-ischemic injury was indeed a significant cause of brain damage in AHT, the brain tissue of AHT victims should feature findings typical of hypoxic-ischemic encephalopathy (HIE) rather than tDAI. We further hypothesize (ii) that if AHT victims had serious damage to the brainstem, morphological analysis should reveal findings of local TBI. To test these hypotheses, we investigated a large group of AHT victims and specifically studied the immunohistochemical profile of brain tissue samples obtained at autopsy using markers for both tDAI/TBI and HIE. In addition, results of immunohistochemistry in a subgroup of AHT victims with rapid or instant death were compared with victims of the sudden infant death syndrome (SIDS).

Methods

Selection of AHT and SIDS victims Autopsy files from four institutions in Northern Germany (two in Hamburg, one each in Rostock and Bremen) were scanned, overall spanning a time period of 50 years from 1960 through 2010. AHT victims were identified (i) on the basis of a perpetrator's confession, (ii) conviction for child abuse in a criminal court, or (iii) by case conference, as defined previously [2]. Briefly, the presence of at least three out of the following criteria was mandatory for a positive decision: serious external injury, unexplained fractures, SDH, traumatic intracerebral pathology, RH, and/or an alleged history overtly inadequate to the clinical picture. We sought to minimize the problem of circularity in diagnosing AHT by meticulously considering all available data in each case. Age limit was 24 months. Victims of sudden infant death syndrome (SIDS) were identified in the autopsy files of the Institute of Neuropathology, Hamburg, using the most rigorous operational criteria as suggested by an international expert panel of pediatric and forensic pathologists and pediatricians (category IA SIDS from Krous et al. [10]).

Ethical considerations Autopsies had been performed as legal autopsies on behalf of investigating authorities or as clinical autopsies with first-line relatives, next of kin, or their legally authorized representatives giving oral informed consent. Studies were in accordance with ethical standards and regulations at the University Medical Center Hamburg-Eppendorf; furthermore, this study was approved by the official ethical institutional review board of the Hamburg medical association (WF 042/12).

Clinical and macromorphological analysis Available records from investigative authorities, court proceedings, and expert witnesses were analyzed regarding history and clinical data. Autopsy reports were screened for peripheral hematomas, fractures, SDH, RH, and epidural, subarachnoid, or intracerebral hemorrhage.

Neuropathologic studies Brain tissue samples included paraffin blocks of cerebral cortex, white matter including corpus callosum, central grey matter, hippocampus, brainstem, cerebellum, spinal cord, eye balls, and optic nerve. Processing of samples was performed according to standard laboratory procedures and included hematoxylin and eosin (H&E), van Gieson's stain, Kluver's stain, Nissl's stain, modified Bielschowsky, Bodian, and Turnbull's stain for siderin. Interpretation of results was performed according to guidelines from established protocols [11–15].

Immunohistochemical studies Four micrometer sections were submitted to immunohistochemical staining on an automated Ventana HX system (Ventana-Roche Medical systems, Tucson, AZ, USA) following the manufacturer's instructions. Antibodies used for staining and their rationale are detailed in Table 1. All immunohistochemical studies were conducted and analyzed in the Institute of Neuropathology, Hamburg.

Diagnosis of tDAI The literature concerning a valid morphological diagnosis of tDAI in the forensic setting is only sparse [24, 35–38]. To guarantee the most reliable data and results from this study, we have extracted and refined the literature for the development of a customized step-wise standardized approach. These steps included (i) axons show typical stigmata of traumatic damage, (ii) there is a substantial amount of traumatically damaged axons in each histological section (according to grade 3 in a 4-tiered semiquantitative grading scheme; see below), and (iii) the amount of traumatically damaged axons surpasses a threshold value in anatomically defined brain regions. Specifically, concerning step (i), traumatically injured axons were defined as axonal swellings, spheroids, bulbs, or varicosities, seen in standard histology (H&E, Bodian) and/or by immunopositivity for amyloid-precursor protein (APP; see Fig. 1a–d). In addition, APP-positive axons where only then considered traumatically injured if scattered within the white matter with the orientation along white matter bundles, while a geographical, zigzag, or perivascular staining pattern was considered as vascular, ischemic, or brain swelling-associated axonal injury which was discarded from analysis ([38]; see Fig. 1h). To overcome the subjectivity associated with the term "widespread" in step (ii), we developed a 4-tiered quantitative approach. The count of

Table 1 Antibodies and their rational for use in this study

Antibody	Clone	Manufacturer	Dilution[1]	Rationale as marker
Microtubulus-associated protein 2c (MAP2c)	HM-2	Sigma M4403	1:3000	Loss of neuronal MAP2 immunoreactivity marker of HIE [16, 17]
Amyloid-Precursor-Protein (APP)	40.10	Novocastra NCL-APP	1:40	Accumulation of APP early marker of TBI and tDAI [18, 19]
CD68	PG-M1	DAKO M0876	1:100	Activation of microglia part of inflammatory response after TBI [20–23]; microglia/macrophagic clusters indicative of remote TBI [24]
Caspase-3	269518	R&D Systems MAB835	1:1000	Crucial role for apoptosis in neuronal death following TBI [25, 26]
α-synuclein	KM51	Novocastra NCL-ASYNC	1:30	α-synuclein accumulates in axonal swellings following TBI [27, 28]
p53	DO-7	DAKO M7001	1:800	Increase of p53 mRNA/protein following TBI [29, 30]
β-amyloid	6 F/3D	DAKO M0872	1:50	β-amyloid accumulating in axons following TBI [31–33]
Tau	AT8	Thermo MN1020	1:1,500	Accumulation of tau in axons, oligodendrocytes, and neurons following TBI [28, 34]
GFAP	6 F2	DAKO M0761	1:200	Reactive gliosis part of reparatory processes after TBI [24]

[1] Ventana Benchmark HX, Tucson/AZ (USA)

traumatically injured APP-positive axons per square millimeter of white matter was determined and graded as follows: A count of more than 50 mm^2 was assigned to grade 3, 49–10 mm^2 to grade 2, 9–1 mm^2 to grade 1, and less than 1 mm^2 to grade 0 (see Fig. 1e–g). Only grade 3 was considered as "widespread". Finally, concerning step (iii), we chose a set of ten defined anatomical brain regions including corpus callosum (anterior (i) and posterior (ii)), white matter of both cerebral hemispheres (iii, iv), internal capsule including the posterior limb bilaterally (v, vi), upper brainstem (midbrain (vii), pons (viii)), and cerebellar peduncles bilaterally (ix, x; precise neuroanatomical locations are shown in the online resource 1) [35–37]. After assessment of all three steps, the morphological diagnosis of tDAI was graded as follows: A diagnosis of "definite" tDAI required traumatically damaged axons corresponding to grade 3 in at least six out of the ten regions. tDAI could not be excluded ("possible") with grade 3 axonal injury in 3–5 regions, while with grade 3 axonal injury in only 1–2 regions, tDAI was considered "improbable". No tDAI was assigned when no region showed grade 3 axonal injury. For a diagnosis of remote tDAI (older than 7 days), evidence of organizational and/or reparative processes (focal collections of microglia/macrophages; reactive astrocytosis) in anatomical brain regions characteristic of tDAI was required [24].

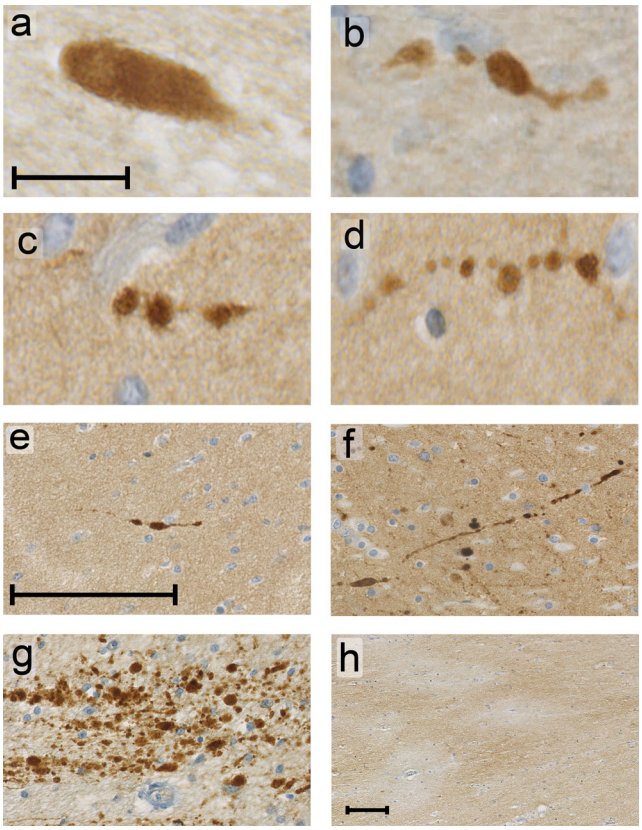

Fig. 1 Identification of traumatically damaged axons with immunopositivity for APP (**a**–**d**; scale bar 25 μm); illustration of grading protocol of tDAI with different counts of APP-positive traumatically damaged axons per millimeter squared (according to grade 1 in **e**, grade 2 in **f**, and grade 3 in **g**; scale bar 125 μm); typical faint zig-zag statining pattern for APP indicating VAI (**h**; scale bar 125 μm)

Comparative quantitative immunohistochemical analysis: As an additional study, a panel of immunohistochemical markers (Caspase 3, CD68, MAP2c, and APP; see Table 1 for details) was used in a standardized approach allowing for statistical comparison with non-AHT controls. We chose SIDS victims as controls, since this forms a homogenous population without TBI or any other intracranial pathology by definition [10]. Furthermore, since SIDS is believed to result from defective regulatory mechanisms in cardiorespiratory activity with resulting hypoxic-ischemic injury leading to instant or rapid death [39–41], we took the immunohistochemical profile in

SIDS victims as a model for very early HIE. Therefore, for this comparative approach, only AHT victims in whom instant or rapid death could be assumed with certainty were studied. As a standardized approach, paraffin blocks covering anatomic brain regions essential for the diagnosis of tDAI, any other TBI-associated damage, and HIE-related changes were analyzed in both AHT victims and SIDS controls: white matter of cerebral hemispheres (frontal lobe/temporal lobe), hippocampus including stratum pyramidale with all sectors CA1–CA4, frontal isocortex, brainstem, and cerebellum/cerebellar peduncles (precise neuroanatomic locations are depicted in the online resource 1; since sampling in SIDS victims generally had been not as comprehensive as in AHT victims, we had to omit central grey matter/internal capsule and corpus callosum from the standardized panel in this part of the study. Because all AHT victims in this part of the study had been included in the prior part including analysis of corpus callosum and central grey matter/internal capsule with no evidence of tDAI, this approach was considered legitimate by all authors). Quantitative evaluation was performed by two neuropathologists (J.M. and M.G.) blinded to the AHT/SIDS status of the case. To assure uniformity of staining, two slides of each region were used for immunohistochemistry with each antibody. After scanning all sections and identifying "hot spot" regions, three adjacent high-power fields measuring 0.19 mm^2 each were analyzed and the mean of each ratio (Caspase-3, MAP2c) or score per mm^2(CD68, APP) was calculated. Specifically, quantification for each marker was performed as follows:

Caspase-3: Nuclei with accumulation of caspase-3 indicating apoptotic injury were counted in each layer of the frontal isocortex and in each sector of the pyramidal layer of the hippocampus. Since apoptotic nuclei appear similar shrunken, condensed, and pyknotic irrespective of their origin [42], no attempt was made to differentiate astrocytic, oligodendrocytic, and neuronal nuclei.

Microtubule-associated-protein 2c (MAP 2c): Following a recently published protocol [16], positively stained neurons were counted separately in each of the cortical layers II–VI of the frontal isocortex and in each sector of the pyramidal layer of the hippocampus (CA1–CA4) and the ratio to all neurons in the respective region was calculated.

CD68: For the quantification of activated microglia, CD68-positive cells with clearly rod-shaped nuclei and delicately ramified processes were counted in the white matter of the cerebral hemispheres, cerebellar peduncles, and in the brainstem. Only regions with evenly distributed CD68-positive cells were analyzed; accumulations of cells adjacent to focal processes or rounded perivascular cells considered to be macrophages were discarded.

APP: Traumatically injured APP-positive axons were counted in the white matter of the cerebral hemispheres, cerebellar peduncles, and in the brainstem including the upper dorsolateral quadrant as described above.

Statistics Data were analyzed using IBM SPSS Statistics version 21. General demographic features were analyzed using standard methods of descriptive statistics. Comparison of frequency distributions was performed with binomial or χ^2 tests including Fisher's exact test. Gaussian distribution of data was affirmed by Kolmogorov–Smirnov test. Gaussian distribution and equal variance provided means of two samples were compared using unpaired two-tailed *t* test. Welch's *t* test modification was used for parameters with unequal variance and the Mann–Whitney test for parameters of non-normal distribution. To further analyze the capacity of each parameter as a possible predictor for AHT, binary logistic regression was first performed separately for each parameter with the AHT/SIDS status as the binary outcome variable. Significant findings at the 0.10 α level were then included in a full multivariate logistic regression model to assess the impact of each variable separately while adjusting for possible confounders (age, sex, and brain weight). Actual *p* values were presented wherever possible and the 95 % confidence interval (95 % CI) was calculated. Statistical significance was assumed with $p<0.05$.

Results

AHT occurs mainly in young male infants and is fatal within the first 24 h in nearly 50 %

From 50 AHT victims from all four institutions (Bremen: 7, Rostock: 11, Hamburg: 32), 31 were male and 19 female (see Table 2 for details). Mean age at death was 5.5 months.

Table 2 General demographic features in 50 AHT victims

	Total	Male	Female	*p* value
N (%)	50/(100)	31/(62)	19/(38)	0.119 (binomial-test)
Age at death [months]				
Mean	5.5	5.8	4.8	0.408 (*t* test)
Median	4.0	4.5	4.0	
Range	1–24	1–24	1–14	
Survival [days]				
Mean	7.3	7.9	6.4	0.707 (*t* test)
Median	2	1	4	
Range	1–90	1–90	1–35	

Survival time was known in 49/50, with 24 (49 %) dying instantly or within the first 24 h. Further demographic details can be found in the supplementary data (Online resource 2).

Nearly all AHT victims show subdural and intraocular bleedings

In 48 from all 50 AHT-victims data allowing for a diagnosis of SDH were available. In 47 from these, SDH was present, mostly in typical diffuse-bilateral distribution. Twenty-four from 27 AHT victims had RH (89 %) and 19/25 (76 %) had hemorrhage inside the optic nerve sheath (ONSH). One AHT victim without RH had ONSH; thus, 93 % had intraocular bleeding (RB and/or ONSB). For further morphological details see the supplementary data (Online resource 2).

AHT victims do not have severe tDAI

In n=42 AHT victims brain tissue sampling was included all ten defined anatomical brain regions [35, 36]. No single case fulfilled the requirements of a diagnosis of definite tDAI. In 30/42 cases, there was no accumulation of traumatically injured axons according to grade 3 in any of the ten regions (no tDAI; 71 %). In 10/42 cases, tDAI was categorized as improbable. In the remaining 2/42 cases, tDAI could neither be proven nor excluded (possible). Finally, in eight AHT victims, sampling was considered not adequate to allow for a assessment for tDAI; since in these cases all available regions had negative immunohistochemistry for APP, we considered it safe to classify these cases also as tDAI improbable.

All 25 AHT victims with a survival of >24 h show signs of HIE

All AHT victims with a survival of >24 h (n=25) showed the classical histopathological profile of HIE with hypoxic-ischemic necrosis in various stages of resorption. Pronounced eosinophilia of neuronal cytoplasm accompanied by nuclear pyknosis followed by swelling of endothelial cells of cerebral vessels and extravasation of polymorphous granulocytes were interpreted as reaction of between 1–3 days. Invasion of macrophages and proliferation of reactive astrocytes was considered as later events beginning from day 3 onwards [11–15].

Case-control analysis of 20 AHT-victims with instant death with 20 SIDS victims

The immunohistochemical findings in 20 AHT victims with instant or rapid death were compared with the findings in a control group of 20 SIDS victims (composition of both groups see Table 3). The classical histopathological profile of severe diffuse TBI is characterized by tDAI with diffuse and widespread accumulation of traumatically injured axons accompanied by activation of CD68-positive microglia throughout the supratentorial white matter, pathological accumulation of α-synuclein, tau, ubiquitin, p53, β-amyloid, and signs of pathological apoptosis with an increased percentage of caspase 3-positive nuclei. In our analysis, there was no significant difference between AHT victims and SIDS controls for activation of microglia as mirrored by the counts of CD68-positive microglia (see Fig. 2e) and for pathological apoptosis as indicated by the percentage of Caspase-3-positive nuclei (see Fig. 2c, d). Concerning the presence of axonal injury, there were only very few and single APP-positive axons in the white matter of the frontal and temporal lobe following preexisting white matter bundles in both AHT victims and SIDS controls, clearly below the threshold for a diagnosis of tDAI. Accordingly, statistical analysis revealed no significant difference of the mean count of APP-positive axons in the white matter of the frontal and temporal lobe between AHT victims and SIDS controls (frontal 4.8 vs. 3.9; p=0.07 and temporal 6.3 vs. 5.3; p=0.27; see Fig. 2f, e, Table 1 with complete original data in the online resource 3). Interestingly though, in AHT victims, there were significantly more APP-positive axons in the brainstem (15.8 vs. 4.6; p<0.001) and the cerebellar peduncles (14.5 vs. 5.4; p=0.001), with the typical configuration of groups of positive axons alongside white matter fiber tracts (see Fig. 3). Logistic regression run separately for these two parameters adjusted for age, sex, and brain weight as possible confounding factors showed a significant effect for APP-positive axons in the brainstem (odds ratio [OR]=3.5; 95 % confidence interval [CI]=1.3 to 9.2; p=0.010) in the cerebellar peduncles (OR=1.3; 95 % CI=1.1 to 1.7; p=0.012; see Table 2 with complete original data in the online resource 3). Most importantly, in selected cases, accumulation of APP-positive axons indicative of local traumatic axonal injury was seen in the immediate vicinity of parts of the automatic respiratory centers in the brainstem (central pattern generator or CPG; see discussion and Fig. 3). Further analysis showed that the ratio of MAP2c-positive neurons to all neurons in the frontal isocortex and the hippocampus indicating

Table 3 Demographic features of 20 AHT victims with instant death and SIDS-control cases used for morphometry and statistical analysis

	AHT	SIDS
N	20	20
M:F	13:7	14:6
Age at death [months]		
Mean	3.8	3.4 (p=0.62)
Median	3.8	3.5
Standard deviation	2.5	1.5
Range	1–9	2–8

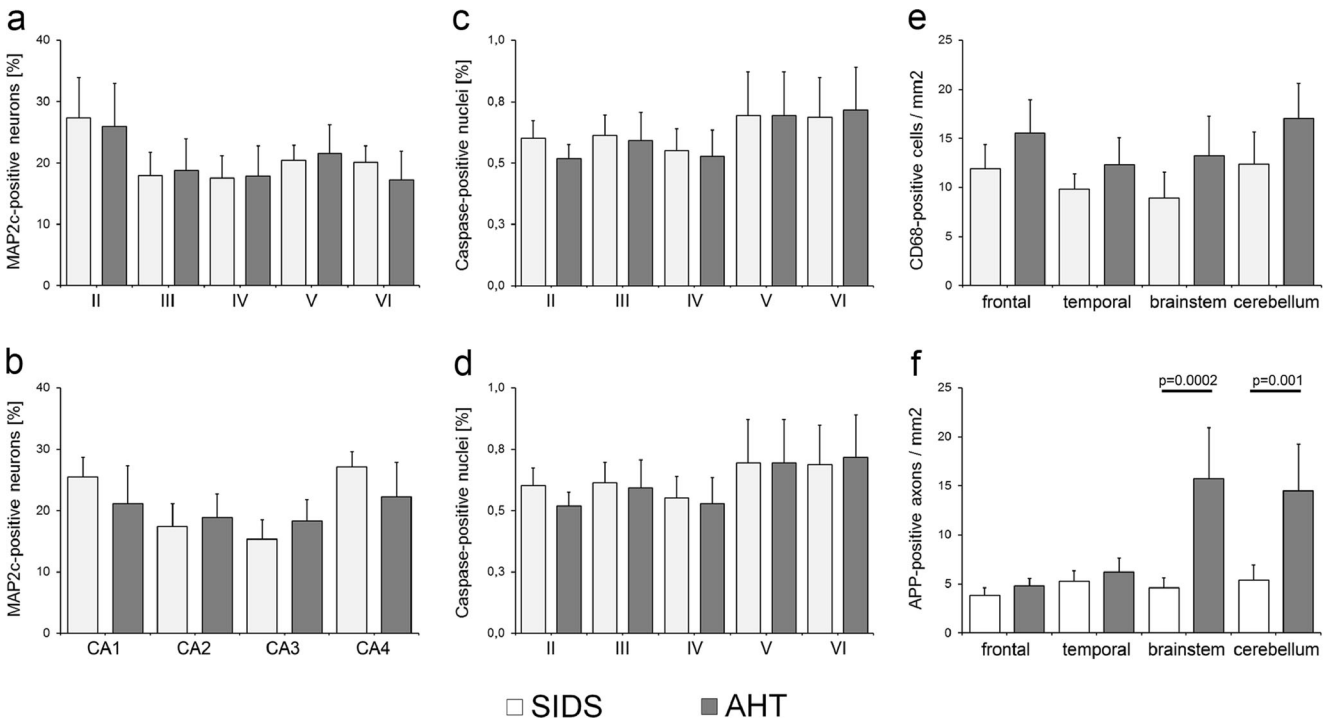

Fig. 2 Quantitative morphometric analysis in AHT victims and SIDS controls: Mean percentage of MAP2c-positive neurons and of Caspase-3-positive nuclei in layers II–VI in frontal isocortex (**a**, **c**) and hippocampal sectors CA1–CA4 (**b**, **d**). Mean count of CD68-positive microglia and of APP-positive axons in the white matter of the frontal lobe, temporal lobe, brainstem, and cerebellar peduncles (**e**, **f**) [error bars=95 % confidence intervals]

hypoxic-ischemic injury [16] in AHT victims did not differ significantly from those seen in SIDS controls (Fig. 2a, b; online resource 3). Finally, there was no relevant immunopositivity for p53, β-amyloid, α-synuclein, ubiquitin, and tau in neither AHT victims nor SIDS controls.

Discussion

Since the concept of AHT in infants has evolved some 50 years ago, further research has helped to elucidate its complex pathophysiology (for review cf. [43–48]). Although it has been known for decades that AHT victims suffer brain damage that may be severe enough as to cause death in these infants, the intrinsic nature of this brain damage has remained enigmatic. The classical theory holds that shaking an infant would lead to shearing stress causing widespread tDAI as seen in adults with severe acceleration-deceleration TBI [49]. Considering the importance of understanding the precise mechanisms of the brain damage in AHT, surprisingly few detailed neuropathological studies have been performed (Table 4). Furthermore, these have in part led to contradictory results: For example, after Calder et al. did not find tDAI using silver stains (Palmgren method, Glees' and Marsland's stain) in nine AHT victims [51], Vowles et al. in their reevaluation of the very same material using a different set of silver stains (Naoumenko and Feigin) 3 years later described "retraction balls and reactive axonal swellings" indicating tDAI in seven of the original nine patients [52]. In 1998, Shannon et al. reported APP-positive axons in the white matter of AHT victims but also in infants with non-traumatic HIE [18]. On the other hand, Gleckman et al. in 1999 identified APP-positive axons in the brains of AHT victims but not in controls (which were significantly older than the infants with AHT) [53]. In 2001, Geddes et al. found signs of tDAI only in 2/37 AHT victims while signs of widespread axonal damage interpreted as "vascular" were present in 13 and HIE was the most common finding [6, 7]. Reichard et al. described vascular axonal injury (VAI) in 11/14 AHT victims and tDAI in two of them [54]. In a study from 2008, Oehmichen et al. analyzed 18 AHT victims using immunohistochemistry for APP and hypothesized that since tDAI had not been detected, "global cerebral ischemia secondary to SDH, focal vasospasm, trauma-induced transitory respiratory and/or circulatory failure" led to the death of the infants [55]. Finally, Johnson and coworkers found accumulation of APP-positive axons indicating tDAI not only in AHT victims, but also in two SIDS controls, leading these authors to conclude that the "utility of APP is quite powerful if not confounded by global hypoxic-ischemic injury" [56].

These conflicting results are in part due to the evolving difficulties surrounding the concept of DAI, especially concerning the morphological and immunohistochemical

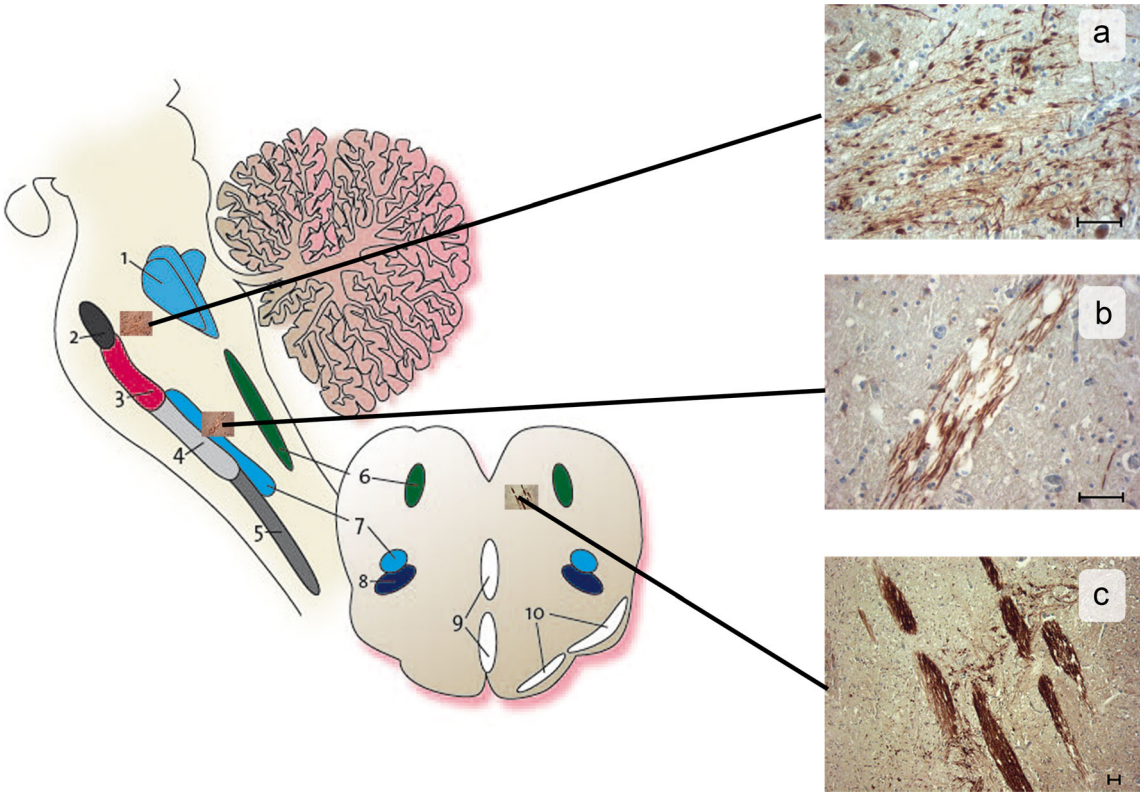

Fig. 3 Schematic drawing of the central pattern generator in the brainstem with case examples of adjacent local traumatic axonal injury in a 4-month-old female (**a**), a 2-month-old male (**b**), and a 4-month-old male (**c**). Immunohistochemistry for APP; scale bar=50 μm. Anatomical parts of the CPG are Parabrachial–Kölliker-Fuse complex (*1*), Bötzinger complex (*2*), Pre-Bötzinger complex (*3*), rostral and caudal ventral respiratory group (*4*, *5*), nucleus of the solitary tract (*6*), nucleus ambiguous (*7*), ventral respiratory column (*8*), medullary raphe, and arcuate nucleus (*9*). Artwork by Ms. Monika Thiel

diagnostic methods. DAI has been initially considered a consequence of severe acceleration-deceleration injury to the skull with resulting shear stress and damage to axons in the white matter [49]. The resulting pathological axonal accumulation of proteins due to impairment of axoplasmic transport was considered a characteristic morphological change that can be demonstrated by silver impregnation techniques and immunohistochemistry of APP [57, 58]. DAI was initially described in adults with severe TBI, but in the following years it became increasingly clear, that axonal damage with immunopositivity for APP was seen not only in TBI but in various other circumstances as, for example, in inflammation or ischemia [59–62]. Thus, a reliable differentiation between tDAI and DAI from other causes requires widespread sampling of cerebral tissue and profound expertise in diagnostic neuropathology; yet, there are no standardized or widely accepted diagnostic criteria [35–37, 56, 63, 64].

Table 4 (modif. from Geddes 2001 [6, 7]): Neuropathological studies of AHT (Abbreviations: *DAI* diffuse axonal injury; *APP* amyloid-precursor protein; *NF* neurofilament; *HIE* hypoxic-ischemic encephalopathy; *VAI* vascular axonal injury)

Study	N (<2 years)	Controls	Methods	Main findings
Lindenberg 1969 [50]	16	No	Silver stains	Contusional tears
Calder 1984 [51]	11	Yes	Silver stains	Contusional tears, no DAI
Vowles 1987 [52]	10	No	Silver stains	Contusional tears, DAI
Shannon 1998 [18]	14	Yes	APP, NF	HIE/DAI; axonal damage in cervical cord
Gleckman 1999 [53]	7	Yes	APP	DAI
Geddes 2001 [6, 7]	37	Yes	APP, CD68	HIE, no DAI, axonal damage in brainstem
Reichard 2003 [54]	14	No	APP	DAI/VAI
Oehmichen 2008 [55]	18	Yes	APP, CD68	No DAI
Johnson 2011 [56]	5	Yes	APP	DAI (seen also in SIDS controls)

Therefore, we developed a standardized stepwise approach incorporating quantitative measurement of traumatically damaged APP-positive axons in a set of ten anatomically defined regions covering the sites affected by traumatic axonal injury thus minimizing the possibility of missing the diagnosis of tDAI [35, 36]. According to our data, definite tDAI was not present in any of the 50 AHT victims of our study group. However, AHT victims do have classical morphological findings of widespread HIE (if survival >1 day) and the immunohistochemical profile in sections of supratentorial brain tissue does not differ significantly from that seen in SIDS (if survival <1 day). In conclusion, AHT victims do not have severe TBI or relevant tDAI but widespread HIE. Lack of signs of TBI and presence of HIE must therefore not be used as evidence against AHT [65].

Finally, our results indicate (i) the lack of relevant "classic" tDAI and on the other hand (ii) the presence of strictly focal, yet significant traumatic axonal damage in the brainstem of AHT victims. Since central control of respiration is governed by various interconnected nuclear groups in the brainstem [66–68], local lesions here can lead to various abnormal respiratory patterns including potentially life-threatening apnea, as exemplified by numerous reports of fatal central hypoventilation syndromes in adults [69–71]. The network of neurons generating the respiratory rhythm (central pattern generator; CPG) is mostly located in the medulla oblongata with modulating influences from the pons and other higher centers [66]. The CPG is further divided in a dorsal respiratory group (DRG) located bilaterally around the nucleus tractus solitarii containing mainly inspiratory neurons and in a ventral respiratory group (VRG) with both inspiratory and expiratory neurons arranged in a continuous column of various nuclei extending from the ventral pons through the medulla to the spinomedullary junction [66, 71]. More specifically, the VRG contains the rostral VRG, also termed the Bötzinger complex, an intermediate VRG with the pre-Bötzinger-complex, and the caudal VRG [72, 73]. In our study, we were able to demonstrate focal traumatic axonal injury in the immediate vicinity of parts of the CPG in selected AHT victims (Fig. 3). We interpret this finding as a morphological correlate indicative of morphological and / or functional damage to the CPG with resulting catastrophic disturbance of respiratory pattern causing the often reported sudden fatal collapse in AHT victims during or immediately after a shaking event [74–77]. Furthermore, we ascribe the HIE in AHT to a respiratory arrest due to traumatic damage to the CPG most probably following hyperextension injury to the neck in a shaking event. This assumption has been suggested as early as 1995 [8] and has been further corroborated since by the findings of morphological damage to the neck and/or cervical spinal cord and the brainstem [6, 7, 9, 78–82].

Our study has a number of limitations. A comparison of the findings in AHT and SIDS victims with infants that died of accidental head trauma would have strengthened the power of our study; but as severe accidental head trauma is exceedingly rare in infancy, we were not able to assemble a control group of reasonable size. At this point, it is not clear whether, and if, how, our findings in fatal AHT translate to non-fatal cases. Further in vivo studies using sophisticated neuroimaging techniques for visualizing local TBI in the brainstem are needed. Owing to the retrospective design of our study, sampling of brain tissue varied somewhat between the institutions and/or with changing protocols throughout the study time span of 50 years; consequently, eight AHT victims lacked the complete set of brain regions and the sampling protocol in SIDS was not absolutely congruent with those in AHT-victims.

In summary, our investigations on the nature of encephalopathy in AHT identify hypoxic-ischemic injury as the cause of brain damage and consequently as the cause of death in infants with AHT. Our results further corroborate the hypothesis that AHT victims experience a sudden respiratory collapse due to focal traumatic axonal damage in the brainstem. The fact that the cause of the encephalopathy in AHT is due to secondary hypoxic-ischemic injury following only very focal traumatic damage, must not be regarded as an argument in favor of an alleged benign nature of the causative event. Shaking an infant is always an extremely harmful act leading to irreversible brain damage and even death.

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Ethical standards As indicated earlier, all studies were in accordance with ethical standards and regulations at the University Medical Center Hamburg-Eppendorf; furthermore, this study was approved by the official ethical institutional review board of the Hamburg medical association (WF 042/12).

Conflict of interest The authors declare no conflicts of interest

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