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Title : In response to 'Volume loss and altered neuronal composition in the brainstem reticular zone may not cause sudden unexpected death in epilepsy'

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Dear Editor,

We are grateful to Dr Finsterer and colleagues for the important points raised on our study which also highlight more general issues in conducting neuropathological research in SUDEP. The SUDEP cases were mainly community deaths in domestic settings, with post-mortem investigations falling under the jurisdiction of the Coroner and local pathology services. In the UK, the Royal college of Pathologists has provided guidance for the methodological approaches to these post-mortem examinations since 2006, recommending referral of brain and heart for examination to specialist centres or mandatory minimum histology of organs. Brains referred to neuropathology centres as 'suspected SUDEP' are accompanied with confirmation that the general post-mortem histology (and toxicology) has been inconclusive. Pulmonary oedema is a frequent autopsy finding; in our previous audit of 145 SUDEP cases this was present in 68% [1] and in 55% of the current study (as detailed in the supplemental table). Although presumed to be neurogenic, mediated by (medullary) sympathetic drive post-ictally, we agree that a more objective evaluation of the degree and mechanisms of pulmonary oedema/congestion as well as stress-induced cardiac changes (Takotsubo cardiomyopathy; not reported in any cases in current series) is required to correlate with medullary neuropathology in SUDEP.

The available clinical information in post-mortem series is variable as many have not been recently or systematically investigated, genetically screened or as deeply phenotyped as in epilepsy surgical programmes. Regarding the comments on 'structural causes' for epilepsy, we categorised cases into 'pathology negative' (normal brains) or those with lesions (hippocampal sclerosis, dysembryoplastic neuroepithelial tumours, old scars etc., potentially relevant to epilepsy) but we reported no significant differences in 9.4T MRI or pathology medullary measures between these two groups. Regarding genetic classification, we included six cases with Dravet syndrome in the study (proven *SCN1A* mutation) in both SUDEP and epilepsy control groups; as these were included in the pathology but not the 9.4T MRI studies, we did not analyse them as a separate group and the remaining cases were not genotyped which we acknowledge as a limitation. On the question of the manual co-registration between brainstem MRI and pathology and inter-observer variability, as stated in the manuscript this was conducted independently by two observers on all cases to ensure agreement in the alignment. Regarding the seizure frequencies, drug compliance and epilepsy duration we have acknowledged, we did not have complete datasets on all cases and further, this study was not sufficiently powered to factor these

variables on brainstem measurements. However, in both the epilepsy-control and SUDEP groups, as stated, we were careful to include cases with recent onset of epilepsy (in the last year two years of life) to reduce selection bias. We classified and included cases as definite and probable SUDEP according to accepted definitions [2], but as we state in the paper there were no differences in the pathology and volume measurements between these SUDEP groups. Regarding the comments that the group sizes were small, as the first post-mortem study investigating medulla volumes changes, we agree it is essential these findings are replicated by other SUDEP research groups in larger, stratified cohorts and we encourage the authors to take up this challenge. We also acknowledged that the axial length of the brainstem under investigation was 2mm; we focussed on the medulla in the first instance as this is the region with key cardiorespiratory nuclear groups and we agree that similar studies on the upper brainstem are essential to evaluate any global brainstem alterations in epilepsy and SUDEP. Due to the relatively time-intensive nature of stereology and complexity of medullary nuclei, this necessitates a careful and stepwise series of studies.

We concur with the authors that seizure networks and underlying epilepsy causes (lesional or genetic) influence patterns of cortical and subcortical atrophy, exemplified in the ENIGMA-consortium meta-analysis of MRI data from 2149 epilepsy patients [3], which is now addressing structural alterations specific to SUDEP [4]. Of relevance, few *in vivo* MRI studies (including ENIGMA and the smaller studies referenced [5-7]) have addressed volume changes in the brainstem in epilepsies or the pathological correlates of 'volume loss', an essential element to ascertaining disease process and cellular mechanisms. Indeed, medullary pathology may represent a 'common denominator' for SUDEP regardless of epilepsy cause. Studies on diverse SUDEP genetic models show post-ictal spreading depolarisation in the ventro-lateral medulla as a final event preceding respiratory arrest [8, 9]. It is plausible that seizure-related structural alterations in the medulla, that we postulate based on our findings, determine residual auto-resuscitative capabilities following such physiological phenomena. Indeed, very recent MRI studies report postictal brainstem hypoperfusion in medullary respiratory centres following generalised seizures [10] and volume loss in the medulla correlating with peri-ictal hypoxia in cases with both focal and generalised onset of seizures [11].

The Centres for SUDEP research initiated in 2015, that supported this work, proposed integrated research and augmentation of tissue bio-banks in epilepsy deaths [12]. A recent review article in this journal addressed the challenges faced in establishing research collections in the forensic setting where priorities and human tissue legislation is primarily geared toward just establishing the cause of death [13]. Unlike brain-banking in neurodegenerative disease where donations are

largely pre-consented during life and brain-removal expedited following death, in SUDEP the consents for tissue retention/post-mortem imaging are mainly not in place and the intervals between death, finding the body and arranging the post-mortem are highly variable. We were careful to factor these inherent post-mortem variables into the current analysis based on existing tissue resources, but we agree an important aim is to minimise these in future, even if collected from different participating centres. An idealised vision for future brain-banking would enable stratification of SUDEP cases on all risk variables; genetics, circumstances of death, epilepsy history, autonomic phenomena, drug history/compliance as well as organ pathology. This could be achieved through formation of collaborative networks, data/sample sharing, adequate funding and recognition of the importance of tissue-based studies in SUDEP.

Conflict of interest statement

The authors in this article have no conflicts of interest to disclose. The Editors of Neuropathology and Applied Neurobiology are committed to peer-review integrity and upholding the highest standards of review. As such, this article was peer-reviewed by independent, anonymous expert referees and the corresponding author had no role in either the editorial decision or the handling of the paper.

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