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Clinical paper

# Hippocampus and basal ganglia as potential sentinel sites for ischemic pathology after resuscitated cardiac arrest

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## Abstract

**Aims of the study:** Neurological impairment after resuscitated cardiac arrest (CA) remains a significant unmet medical need. Brain ischemia associated with CA and subsequent reperfusion is evident as two fundamentally different types of damage on neuropathological examination: frank necrosis (involving all cell types) and selective eosinophilic neuronal death (SEND). These types of damage are not only dissimilar in micromorphology, but also differently detectable with clinical brain imaging methods. In a previous study, SEND was reported in most patients surviving the initial CA. This study was undertaken to further characterize and map SEND in an expanded dataset.

**Methods:** A cohort of 46 cases was included from an observational study on targeted temperature management (TTM) of resuscitated CA. Six brain and brain stem regions and 21 subregions were examined, and SEND severity was tested for correlation with time to ROSC. Representativity of all regions vis-à-vis global SEND was assessed, to investigate whether any particular region could be used as a "sentinel site" for overall damage.

**Results:** The thalamus, the CA4 subregion of the hippocampus and the Purkinje cell layer of the cerebellum were the most severely affected subregions. Involvement of the hippocampus, cerebellum, cortex or basal ganglia indicated presence of SEND in other regions. There was a significant correlation between time to ROSC and SEND.

**Conclusion:** There are regional differences in SEND distribution. Cases free of SEND in the hippocampus or basal ganglia are unlikely to have significant SEND in other regions, suggesting that these regions could be used as "sentinel sites" for global SEND in future studies.

**Keywords:** neuropathology, selective eosinophilic neuronal death;, targeted temperature management

## Introduction

Neurological impairment is common after cardiac arrest. Clinical trials indicate that this impairment can be reduced by targeted temperature management (TTM),[1,2] though controversy remains.[3,4] Nevertheless, the unmet medical need is significant, as prognosis after CA is dire.[5,6] The neuropathological substrate of clinical impairment is either frank necrosis or, in cases with less extensive immediate injury, selective eosinophilic neuronal death (SEND).[7] SEND constitutes a state of neuronal death with sparing of other cell types, which sets it apart from frank necrosis, which involves all cell types. The process leading to post-ischemic and post-resuscitational neuronal death may be a target for therapeutic intervention, and since SEND develops over hours or days after CA, this interval may constitute a time window for such therapy.

It can be surmised, analogous to the concept of selective ischemic vulnerability,[8] that different brain regions would be differently susceptible to SEND. Prior studies in animals indicate that the dynamics of ischemic damage differ significantly between brain regions.[9–11] More refined knowledge on the differential rescue potential of neurons in the different brain regions may help with

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targeted endpoint development in future clinical trials, in order to focus on symptoms likely associated with the respective region, rather than crude global functional measures. A recent study showed long-term verbal memory impairment and structural hippocampal damage in CA survivors considered neurologically intact at discharge.[12] In the longer term, future imaging techniques may be able to identify patients with greater potential for successful therapeutic intervention, and could potentially complement the present use of electroencephalogram (EEG) and/or somatosensory evoked potential (SSEP) in prognostication.

Prior research from our group has indicated that SEND can be reliably assessed and graded using routine neuropathological sections.[7] In that study, SEND was mapped in 6 regions and 14 subregions in 23 CA survivors treated with TTM. The hippocampus exhibited the highest median SEND score, and the brainstem was comparatively spared. These neuropathological findings support the results of clinical studies on CA survivors, where memory impairment is generally a prominent long-term finding.[12–14] Furthermore, in the Bjorklund study, neuropathological damage burden was found to correlate significantly with time to ROSC, which appears to validate the proposed method of SEND grading.

In the present study, 6 brain regions and 21 subregions were mapped for SEND in 46 patients having undergone TTM after CA. The main purposes were to (1) expand the available data on the regional differences in SEND development by including a greater number of subjects and brain regions and (2) to identify potential regions particularly representative of overall SEND burden to facilitate future neuropathological studies.

## Materials and methods

Histopathological brain sections from 46 CA non-survivors treated at the Skane University Hospital in Lund were included. These patients were all part of a prospective observational study including patients receiving hypothermia treatment[15] -they were hence monitored comprehensively and in most cases all pertinent data was accessible. Consecutive patients with clinical data and sufficient available neuropathology at the time of the study were included. Clinical data were collected from the available hospital and ambulance records. Of the 46 cases, 23 were included in a prior publication, but were re-investigated as per the extended methods described below for this report. The study was approved by the Regional Ethical Review Board in Lund (223/2008).

### *Histopathological evaluation*

Tissue preparation was conducted as described previously.[7] Briefly, tissues were fixed in formalin, embedded in paraffin and sectioned at 4 μm. For microscopical analysis, 6 regions and 21 subregions were selected for microscopic evaluation and stained with hematoxylin-eosin. The regions were: the limbic system (hippocampus (CA1-4 and dentate gyrus) and amygdala), cerebellum (dentate nucleus, Purkinje cell layer), neocortex (frontal, temporal, parietal, occipital, insular), the basal ganglia/nigrostriatal (globus pallidus, putamen, caudate nucleus, substantia nigra), the brainstem (mesencephalon, pons, the medulla with inferior olive). The thalamus was also assessed but not assigned to a region.

The microscopical evaluation was conducted similar to the method described previously[7] for all regions included in this study (Table 1).

**Table 1 – Scale used for assessing SEND.**

| SEND score | Definition |
|---|---|
| 0 | No SEND in the subregion |
| 1 | SEND involving <30% of neurons in the most severely affected field of view |
| 2 | SEND involving 30–60% of neurons in the most severely affected field of view |
| 3 | SEND involving 60–90% of neurons in the most severely affected field of view |
| 4 | SEND involving >90% of neurons in the most severely affected field of view |

The mean damage score (MDS) for each individual patient was also calculated. The MDS was defined as the average of the damage score in all examined sections. An X4 objective was used to screen the entire section from a subregion, and the field of view harbouring the most extensive degree of pathology (determined subjectively) was analysed with an X10 objective for neuronal counting. Briefly, eosinophilic neurons were counted as % of total neurons per microscopic field and SEND scores 1–4, signified red neurons accounting for <30%, 30–60%, 60–90% and >90%, respectively. Regional scores were calculated by averaging subregional scores for each region (including the thalamus). All ratings were conducted masked to clinical data.

### *Statistical analysis*

Data analysis was performed using R 3.5.0. Differences between brain regions were assessed using Kruskal–Wallis test, and the relationship between time to ROSC and damage score for individual regions as well as MDS were analysed using Spearman rank correlation. Bonferroni correction was applied for the assessment of correlation between ROSC and individual brain regions.

## Results

### *Demographics*

In total, 14 women and 32 men were included. Median age at death was 66.5 years (IQR 60.0–73.0), with median time to ROSC 27.0 min (IQR 18.5–38.8). The decision to withdraw intensive care was made was made on day 4 (median, IQR 2–5.25), and the median time to death was 4 days (IQR 2–6). The most frequent initial rhythm was ventricular fibrillation (VF). Demographics are summarized in Table 2.

### *SEND distribution*

There was a significant difference in SEND severity across subregions ($p < 0.001$, Kruskal–Wallis test). The highest regional SEND score was observed in the thalamus, where the median score was 3 out of 4 (mean 2.79), whereas the most severely involved subregion was the

**Table 2 – Demographics of the study cohort.**

| Demographics | |
|---|---|
| Age | Median age 66.5 years (IQR 60–73) |
| Gender | Male 32; Female 14 |
| Initial rhythm | 16 ventricular fibrillation; 13 asystole; 3 pulseless electrical activity; 1 ventricular tachycardia; 10 cases unclear or not described |
| ROSC | Median 27 min (IQR 18.5–38.75) |
| Time to decision to withdraw | Median 4 days (IQR 3–6) |
| Time to death | Median 4 days (IQR 2–6) |

*IQR = Interquartile range

Purkinje cell layer of the cerebellum (median 3 out of 4, mean 2.83). The brainstem was the least affected region, with a median score of 0 (mean 0.63). Scores for each region, and subregional outliers, are shown in Table 3.

### *SEND analyses*

There was a significant correlation between ROSC and mean SEND damage score (MDS) ($p = 0.019$; Spearman's rho = 0.36; coefficient of determination (COD)/Spearman rho2 = 0.13). The SEND scores of the neocortex and basal ganglia correlated significantly with time to ROSC ($p = 0.048$ and 0.036, respectively, after Bonferroni correction), whereas the correlation between limbic system SEND and ROSC was nominally significant at $p = 0.049$ but not after Bonferroni correction. Scores in the cerebellum ($p = 0.09$) trended towards a correlation with time to ROSC, but no trend was observed for brainstem or thalamus, nor for age and MDS (data not shown).

Time to death did not correlate with MDS or any regional score ($p > 0.1$ for the MDS and all regions). Cases with ROSC < 30 min had significantly lower MDS scores than cases with ROSC ≥ 30 min ($p = 0.003$, Mann–Whitney U test). A post hoc analysis of degree of SEND based on initial rhythm showed that the MDS was greater in patients presenting with asystole than in patients presenting with VF. The low numbers of patients presenting with pulseless electrical activity (n = 3) and ventricular tachycardia (n = 1) precluded statistical analysis.

### *Representativity*

No subject with an MDS greater than 1 was free of cortical, limbic, cerebellar or basal ganglia involvement, whereas the brainstem and thalamus were completely spared in some cases with severe SEND in other regions. Post-hoc exclusion of the amygdala from the limbic region and the substantia nigra from the basal ganglia (for simplicity) yielded similar results.

## Discussion

These results are largely consistent with those reported in the previous study,[7] and confirm those findings in a larger (albeit partly overlapping) material, with the analyses including additional brain subregions. Through this assessment, we conclude that the method of grading SEND proposed by Bjorklund et al. appears to be a valid scale for assessing CNS damage induced by cardiac arrest with subsequent resuscitation. This is supported by the significant statistical correlation between time to ROSC and overall SEND extent as evaluated with MDS as well as extent of SEND development in key brain regions. It should be emphasized that the overall hypoxic/ischemic burden is influenced by a number of factors beyond ROSC. Such factors may include flow conditions at time of CA, pre-arrest risk and protective factors, post-resuscitational conditions and more. Importantly, we found a greater degree of SEND in patients presenting with asystole than in patients presenting with VF, which is in accord with studies showing that patients presenting with asystole have a worse clinical outcome.[16,17]

In a recent study by van Putten et al.,[18] there was a numerically slightly higher degree of hypoxic/ischemic damage/SEND in the hippocampus and the cerebellum than in the cortex and thalamus. Based on the limited number of cases and the different scales employed in our respective studies, we would consider these results largely compatible with the present study. In calculating regional scores based on several subregions, less extensively damaged subregions (such as the inferior olive of the cerebellum and CA1-3 of the hippocampus) will reduce the regional total, which may complicate cross-study comparisons. Prior studies by Horn and Schlote[19] encompassed the frontal cortex, CA1 and the Purkinje cells only, whereas Hinduja et al.[20] noted hypoxic/ischemic damage as present or absent only, making comparisons of the regional pattern of injury difficult.

It can be argued that SEND is unlikely to be an immediate result of ischemia related to the CA alone, since it does not appear in non-

**Table 3 – SEND scores in each region and subregional outliers.**

| Region | Median SEND score (IQR) | Subreqional outlier(s) |
|---|---|---|
| Neocortex | 1.75 (0.75–2.75) | Sparing of the insular cortex |
| Limbic | 2.17 (0.88–2.90) | Sparing of the amygdala and dentate gyrus |
| Basal ganglia | 1.13 (0.50–1.73) | Sparing of the substantia nigra |
| Cerebellum | 2.50 (1.00–3.50) | Purkinje cell layer more affected than dentate nucleus |
| Brainstem | 0.00 (0.00–1.00) | Inferior olive more affected than pons and mesencephalon |
| Thalamus | 3.00 (2.00–4.00) | N/A |

resuscitated CA patients. This raises the intriguing possibility that SEND is the neuropathological substrate of reperfusion injury. Another potential explanation is that SEND represents delayed cell death initiated by the ischemic insult, developing gradually over hours and days thereafter. In case the resuscitation is unsuccessful and ROSC is not achieved, the lack of SEND would then be explained by the interceding death rather than absence of a pathogenetic insult related to the reperfusion. As to the lack of a correlation between time to death and SEND severity, it should be noted that no subject in this series had a time to death shorter than 12 h, which may be long enough for post-resuscitational or ischemia-induced damage to reach its maximum intensity.

For future neuropathological studies, we conclude that SEND can be reliably assessed in either the cortex, basal ganglia, cerebellum or the limbic region/hippocampus. If either of these regions are free of SEND, the likelihood of significant SEND in other brain regions is low. Mapping SEND in several different brain regions and subregions, as done in this report, does not appear to be necessary to achieve a reliable assessment of overall neuropathological SEND burden. Since examination of cortical sections (and to a lesser extent the basal ganglia and cerebellum) is more time-consuming, assessing sections from the hippocampus would be appropriate as a first step. However, the correlation between time to ROSC and hippocampal/limbic SEND did not achieve formal statistical significance (as opposed to the cortex and basal ganglia), suggesting that examination of the basal ganglia is a useful alternative (or complement). The need for a comprehensive assessment must be balanced vis-à-vis feasibility (based on available sections) and time expenditure.

## Conflict of interest

The authors declare that they have no conflicts of interest.

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