SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: 42476
STSM title: The NLRP3 inflammasome in epilepsy
STSM start and end date: 05/11/2018 to 09/11/2018
Grantee name: Tobias Engel

PURPOSE OF THE STSM:

Despite the progress made in the development of new antiepileptic drugs, pharmacoresistance remains as high as 30% in patients suffering from epilepsy. Emerging evidence demonstrates a causal role for brain inflammation in lowering seizure thresholds and driving epileptogenesis. Consistent with this, intervening in pro-inflammatory cascades has shown promise in animal models of epilepsy, with clinical trials of anti-inflammatory agents already underway. The NLRP3 inflammasome is a multiprotein complex mediating inflammatory responses during numerous diseases of the CNS including also epilepsy. Diverse triggers engaging ion channels such as the ATP-gated P2X7 receptor inducing intracellular Ca^{2+} mobilization and K^+ efflux were shown to be essential for NLRP3 oligomerization and inflammasome activation. Critically, we have previously shown that P2X7 antagonism provides potent seizure suppression during acute seizures and during epilepsy.

The main objective of the present study was to obtain genetic proof of the role of the inflammasome during seizures and seizure-induced pathology.

Specific Aims:
1. Subject mice knock-out for ASC, NLRP3 and Casp-1 to intraamygdala kainic acid-induced status epilepticus (n = 10 per group).
2. Analyse electroencephalogram (EEG) during and post-status epilepticus.
3. Analyse brains 72h following status epilepticus on cell death and inflammation (e.g. astrocytosis, microgliosis).

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

For our studies we used the well characterized intra-amygdala kainic acid-induced status epilepticus mouse model (>40 publications by the applicant: e.g. Jimenez-Pacheco, J Neurosci, 2016; Engel et al., FASEB J, 2012). In this model, mice develop status epilepticus shortly following intraamygdala kainic acid. Status epilepticus is then stopped 40 min later by the administration of the anticonvulsive lorazepam. When analysed 72 h later, mice show increased inflammation in the brain and wide-spread neurodegeneration, in particular in the

hippocampus. Mice deficient in either NLRP3, Caspase 1 and ASC and wildtype (n>10) were subjected to status epilepticus and encephalogram (EEG) recorded during time of status epilepticus and 1 h following seizure cessation. Mice were killed 72 h following status epilepticus and brains shipped to RCSI for analysis.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

EEG was analysed during the time of status epilepticus (40 min starting from time of kainic acid injection until administration of anticonvulsant lorazepam) and 1 h following status epilepticus. Surprisingly and in contrast to previous publications, this revealed that mice deficient in NLRP3 and ASC had an earlier seizure onset when compared to wildtype mice (***Fig. 1A***) Casp-1 deficient mice (Casp) showed a similar time to seizure onset when compared to wildtype mice. Analysis of the 40 min recording period during status epilepticus showed that mice deficient in NLRP3, ASC and Casp-1 experienced more severe seizures when compared to wildtype mice. Strongest increase could be observed for Casp-1 knockout mice reaching significance at 25 and 30 min post-kainic acid injection. (***Figure 1B***). EEG analysis of the 60 min recording period following the administration of lorazepam showed that Casp-1-deficient mice still showed stronger seizures. NLRP3 and ASC mice showed similar seizure strength when compared to wildtype mice (***Figure 1B***).

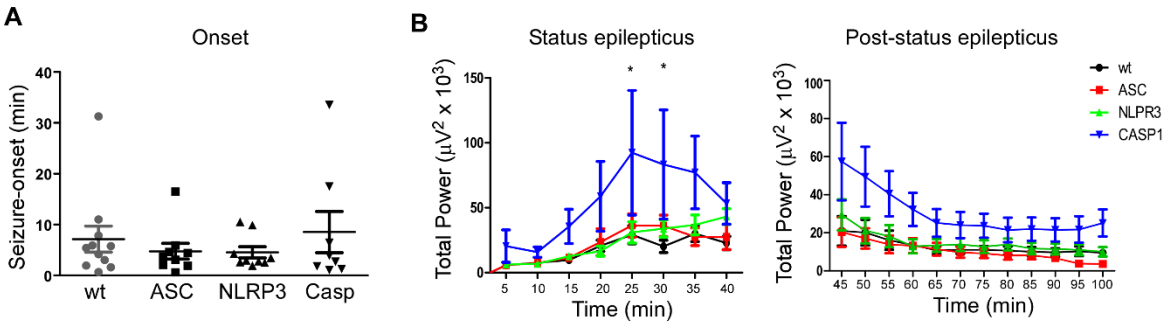

Figure 1. *EEG analysis of NLRP3, Casp-1 and ASC knockout mice during and following status epilepticus.* ***(A)*** Graph showing trend to an earlier seizure onset in mice deficient in ASC and NLRP3 (n = 11-8). **(B)** Graphs showing seizure severity measured as total power in 5 min intervals starting at time of intraamygdala kainic acid injection until 60 min post-lorazepam administration (total of 100 min)**.** Casp-1-deficient mice showed significantly more severe seizures when compared to control.

FUTURE COLLABORATIONS (if applicable)

Analysis of brain pathology including cell death and inflammation (carried out at RCSI).
If necessary, increase n numbers to reach significance.
Publish results.