Silencing of amygdala circuits during sepsis prevents the development of anxiety-related behaviours

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Abstract

Sepsis is a life-threatening condition induced by a deregulated host response to severe infection. Post-sepsis syndrome includes long-term psychiatric disorders, such as persistent anxiety and post-traumatic stress disorder (PTSD), which neurobiological mechanisms remain unknown. Using a reference mouse model of sepsis, we showed that mice that recovered from sepsis further developed anxiety-related behaviours associated with an exaggerated fear memory. In the brain, sepsis induced an acute pathological activation of a specific neuronal population of the central nucleus of the amygdala (CeA) which projects to the ventral bed nucleus of the stria terminalis (vBNST). Using viral-genetic circuit tracing and in vivo calcium imaging, we observed that sepsis induced persistent changes in the connectivity matrix and in the responsiveness of vBNST-projecting CeA neurons. The transient and targeted silencing of this subpopulation only during the acute phase of sepsis with a viral pharmacogenetic approach, or with the antiepileptic and neuroprotective drug Levetiracetam, prevented the subsequent development of anxiety-related behaviours. Specific inhibition of brain anxiety- and fear circuits during the sepsis acute phase constitutes a preventive approach to preclude the post-infection psychiatric outcomes.

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Abbreviations: AAV = adeno-associated virus; AI = agranular insular cortex; AP = area postrema; BLA = basolateral amygdala; CeA = central nucleus of the amygdala; CeL = lateral subdivision of the CeA; CLP = cecal ligation and puncture; CNO = clozapine-N-oxide; CS = conditional stimulus; CTB = cholera toxin B; DREADD = designer receptor exclusively activated by designer drugs; EEG = electroencephalogram; EMG = electromyogram; FC = fear conditioning; IL = interleukin; IP = intra-peritoneal; LDB = light/dark box test; LEV = levetiracetam; LFP = local field potential; LPS = liposaccharide; NOL = novel object localization; NOR = novel object recognition; NTS = solitary tract nucleus; OF = openfield; PBN = parabrachial nucleus; PBS = phosphate buffered saline; PKCδ = protein kinase Cδ; PTSD = post-traumatic stress disorder; PVT = paraventricular thalamus; SAE = sepsis-associated encephalopathy; SC = subcutaneous; SI = substantia innominata; SO = supraoptic nucleus of the hypothalamus; SOM = somatostatin; TNF = tumor necrosis factor; US = unconditional stimulus; vBNST = ventral bed nucleus of the stria terminalis; vHIP = ventral hippocampus
Supplementary material

Supplementary Figure 1 (related to Figure 1): Molecular, electrophysiological and behavioural characterization of the acute and long-term effects of CLP.

Supplementary Figure 2 (related to Figure 2): CLP-induced transient brain activation at H6 and H24 post-CLP.

Supplementary Figure 3 (related to Figure 3): Projection-pattern of genetically labeled CeA neurons activated by sepsis and occurrence of epileptiform-like events in the amygdala following sepsis.

Supplementary Figure 4 (related to Figure 5 and Figure 6). Control experiments related to the effects of LEV administration during sepsis on neuronal activation and anxiety-related behaviours.

Supplementary Figure 5 (related to Figure 7). Control experiments related the pharmacogenetic silencing of vBNST-projecting CeA neurons.
Supplementary Figure 1. Molecular, electrophysiological and behavioural characterization of the acute and long-term effects of CLP. A. Plasmatic cytokines levels were significantly increased during...
sepsis acute phase (Two-way ANOVA, \( n_{\text{control}}=8 \), \( n_{H6}=4 \), \( n_{H24}=2 \), time: \( F(2,74)=26.11 \), ****\( P<0.0001 \), multiple comparison, IL6: control-H6 ****\( P<0.0001 \), G-CSF: control-H6 ****\( P<0.0001 \); Control condition for IL-1a, IL-2, IL-6, IL-10, IL-12 and TNF: below detection threshold. B. Survival after 400 hours post-CLP was approximately 50% (log-rank test, *\( P=0.0208 \). C. CLP is followed by EEG abnormalities (Paired t-test: \( n=5 \), Total power: **\( P=0.0011 \); Mean Frequency: ***\( P=0.0008 \); Delta/Theta ratio: **\( P=0.0060 \). D. CLP mice showed an altered sleeping behaviour compared to control animal (\( n=5 \), Unpaired t-tests, % of prolonged rest (time at rest\( >120s \)) ****\( P<0.0001 \); Mean resting bout duration: *\( P=0.0194 \); % of time at rest (\( >20s \)): \( P=0.4662 \). E. In the OF (\( n_{\text{Sham}}=13 \), \( n_{\text{CLP}}=15 \), CLP showed no significant effect on the Total distance moved (Unpaired t-test: \( P=0.0518 \)) or the Time spent moving (Unpaired t-test, \( P=0.0938 \). F-H. CLP caused no significant effect on pain threshold during FC (F) (Mann-Whitney test, \( n_{\text{control}}=11 \), \( n_{\text{CLP}}=10 \), \( P=0.8444 \), or on fear retention after several extinction sessions (G) (Unpaired t-test: Auditory: \( n=9 \), \( P=0.6101 \); Contextual: \( n_{\text{Sham}}=13 \), \( n_{\text{CLP}}=15 \), \( P=0.0680 \), or on freezing behaviour during FC recall at 45 days post-CLP (H) (\( n_{\text{control}}=7 \), \( n_{\text{CLP}}=12 \), Contextual: Unpaired t-test, \( P=0.5436 \); Auditory: Mann-Whitney test, \( P=0.9399 \). I-K. CLP does not impact long-term behaviour in the Morris water maze (I) (Learning curve AUC: Unpaired t-test, \( n_{\text{control}}=7 \), \( n_{\text{CLP}}=6 \), \( P=0.4778 \); Latency to reach platform: Two-way ANOVA, \( n=28 \), group: \( F(1, 54)=2.438 \), \( P=0.1243 \), in the NOL/NOR test (J) (Unpaired t-test: NOL: \( n_{\text{control}}=9 \), \( n_{\text{CLP}}=24 \), \( P=0.1797 \); NOR: \( n_{\text{control}}=17 \), \( n_{\text{CLP}}=20 \), \( P=0.3583 \), or in the Olfactory test (K) (Two-way ANOVA, \( n_{\text{Sham}}=5 \), \( n_{\text{CLP}}=10 \), *\( P=0.0148 \), group: \( F(1, 13)=1.393 \), \( P=0.2591 \). L-M. Higher sepsis score between H24 and H48 post-surgery correlates with later increased freezing behaviour in both contextual and auditory FC recall (L) (\( n_{\text{Contextual}}=33 \), \( n_{\text{Auditory}}=40 \), Pearson test) and higher cytokines levels in the brain during the acute phase of sepsis (M) (\( n=7 \), Pearson test). Data shown as mean ± s.d. (except A, I, K ± s.e.m. and L ± 95% confidence interval)
Supplementary Figure 2. CLP-induced transient brain activation at H6 and H24 post-CLP. A.
Images of c-fos staining in different brain areas at H6 and H24 post-CLP. B-E. H6 transient c-fos expression variations were returned to baseline at H24 (n_{shamH24}=4, n_{CLPH24}=6 (except NTS and AP: n_{shamH24}=2 and vHIP CA3: n_{shamH24}=3, n_{CLPH24}=5), CLP_{H6-CLPH24}; Unpaired t-test: NTS: ***P=0.0003; AP: **P=0.0023; PBN: P=0.1376; SO: **P=0.0036; vHIP CA3: P=0.5294; BLA: P=0.3391; vBNST: **P=0.0019; Mann-Whitney test: CeA: **P=0.0072) Scale=200 μm. Data shown as mean ± s.d.
Supplementary Figure 3. Projection pattern of genetically labeled CeA neurons activated by sepsis and occurrence of epileptiform-like events in the amygdala following sepsis. A. Representative pictures showing the soma (top) and the axonal projections (bottom) of neurons tagged using c-fos-CreER transgenic mice and two different Cre-dependant viruses injected in the CeA. In the literature, CeA PKCδ+ neurons are known to project to the vBNST, the SI, the BLA, the Retrorubral field (RRf) and the PBN. Sepsis-activated CeA neurons only projected to the vBNST and the SI. B-C. LFP recording in the CeA during sepsis. (B) top left, sagittal brain section showing electrodes positioning in the CeA (dapi counterstaining). Bottom, example LFP trace one hour after CLP showing the presence of some spontaneous large recurrent stereotyped epileptiform-like spikes. Higher magnification of the waveform of sorted epileptiform-like spikes (C) Quantification of the presence of epileptiform-like spikes before and after CLP. Two mice over 10 presented epileptiform-like spikes in the first hours following CLP.
Supplementary Figure 4. Control experiments related to the effects of LEV administration during sepsis on neuronal activation and anxiety-related behaviours. A. LEV administration in sham
animals caused no significant effect on c-fos quantification at H6, except in the PBN (Mann-Whitney test: vBNST: nControl=3, nControl+LEV=4, P=0.0571, NTS: nControl=3, nControl+LEV=3, P=0.2000; Unpaired t-test: CeA: nControl=9, nControl+LEV=3, P=0.7127, PBN: nControl=7, nControl+LEV=3, **P=0.0061). B. Multiplex dosage of cytokines in serum from CLP mice treated or not with Lev. Data are presented as fold increase in LEV treated compared to placebo treated CLP mice and show no significant effect of LEV on cytokines levels post-CLP (nCLP=12, nCLP+LEV=11, Repeated measures ANOVA summary, LEV effect F(1,21) =0.01092, P=0.9178). C. In control (nControl=13, nControl+LEV=12) or CLP (nCLP=20, nCLP+LEV=24) mice, early LEV administration did not impact the OF Total distance moved (Unpaired t-test, Control: P=0.8339, CLP: P=0.5968), or the Time spent moving (Mann-Whitney test, Control: P=0.8225, CLP: P=0.4021), or the Mean distance to arena centre in control mice (Mann-Whitney test, Control: P=0.5034). D. Nociception during FC was similar in CLP mice with or without previous LEV treatment (Mann-Whitney test: nCLP=10, nCLP+LEV=14, P=0.8210). E. Previous LEV administration did not affect the Fear recall response in control mice (nControl=17, nControl+LEV=14, Contextual: Mann-Whitney test, P=0.8739; Auditory: Unpaired t-test, P=0.2670). F-H. 48-hour LEV treatment induced no effect on the NOL/NOR task (F) (Unpaired t-test: Control: nControl=8, nControl+LEV=7, NOL: P=0.3034, NOR: P=0.2382; CLP: nCLP=13, nCLP+LEV=21, NOL: P=0.2418, NOR: P=0.1783), the Olfactory test (G) (Two-way ANOVA, nCLP=10, nCLP+LEV=8, group: F(1,16)=0.0050, P=0.9443) and the Morris Water Maze (H) (Latency to reach platform: Two-way ANOVA: n=28, group: F(1,54)=0.1561, P=0.6943; AUC: Unpaired t-test: nCLP=7, nCLP+LEV=6, P=0.8991). I-J. Transient intracerebro-ventricular (ICV) administration of LEV does not improve long-term anxiety and fear behaviour in CLP mice. (nSaline= 9, nLEV= 7) (I) Timeline and setup of the experiment. (J) Early ICV infusion of LEV shows no effect on anxiety behaviour in the OF (Unpaired t-test: Total distance moved: P=0.6516, Time spent moving: P=0.8546, Mean distance to arena centre: P=0.3398) or on the freezing behaviour during FC recall in CLP surviving mice (Unpaired t-test, Contextual: P=0.8526, Auditory: P=0.7210). Control ip LEV- or CLP ip LEV- data are the same that Control or CLP data in SFig.1 and 2. Data shown as mean ± s.d. (except G, H ± s.e.m.).
Supplementary Figure 5. Control experiments related the pharmacogenetic silencing of vBNST-projecting CeA neurons. A. In the OF, DREADD inhibition of the vBNST-projecting CeA neurons during 24 hours did not impact the Total distance moved (Unpaired t-test: Sham: n_mch=6, n_Gi=7, P=0.4481, CLP: n_mch=5, n_Gi=9, P=0.2128), or the Time spent moving in CLP or Sham mice (Unpaired t-test: Sham: n_mch=6, n_Gi=7: P=0.5750, CLP: n=10, P=0.1758), or the Mean distance to arena centre in Sham mice (Unpaired t-test, n_mch=6, n_Gi=7, P=0.8774). B. DREADD inhibition in Sham mice showed no effect on the FC recall response (Unpaired t-test: n_mch=5, n_Gi=6, Contextual: P=0.1964; Auditory: P=0.3503). Data shown as mean ± s.d.