

The anti-P2X7R antibody suppressed CSD and CSD-induced TNF- α gene expression

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Background:

- The purinergic P2X7 receptors, are ionotropic membrane channels, which can be activated by extracellular ATP.
- ATP is known to be involved cortical spreading depression (CSD), a substrate of migraine [1, 2].
- P2X7R activation promotes IL-1 β [3] and TNF- α [4] release and CSD triggers these neuroinflammatory factors release[5].
- Inhibition of the P2X7-Panx1 complex suppresses spreading depolarization in rats and the induction of IL-1 β post CSD [6]
- Whether the anti-P2X7R antibody could suppress CSD and the subsequent induction of neuroinflammatory factors is

Objectives:

- To examine the effects of anti-P2X7R antibody on cortex susceptibility to CSD.
- To address whether P2X7R contributes to the induction of IL-1 β and TNF- α induced by CSD

Methods:

Electrophysiological technique was performed to detect the effect of anti-P2X7R antibody on CSD.

Real time PCR was then performed to analyze the gene level of IL-1 β and TNF- α post CSD with or without antibody treatment

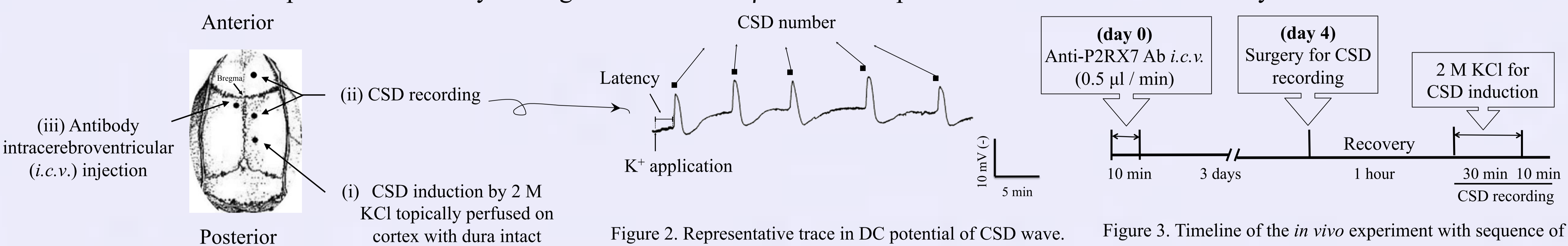


Figure 1. Schematic representation of surgical coordinates on parietal bone of isoflurane anaesthetic rats.

Figure 2. Representative trace in DC potential of CSD wave. Latency: the time required to elicit the 1st CSD wave from the start of high K⁺ application.

Figure 3. Timeline of the *in vivo* experiment with sequence of pharmacological and electrophysiological performance followed by tissue dissection for the subsequent molecular analysis.

Results:

1. Anti-P2X7R antibody suppressed CSD in rats

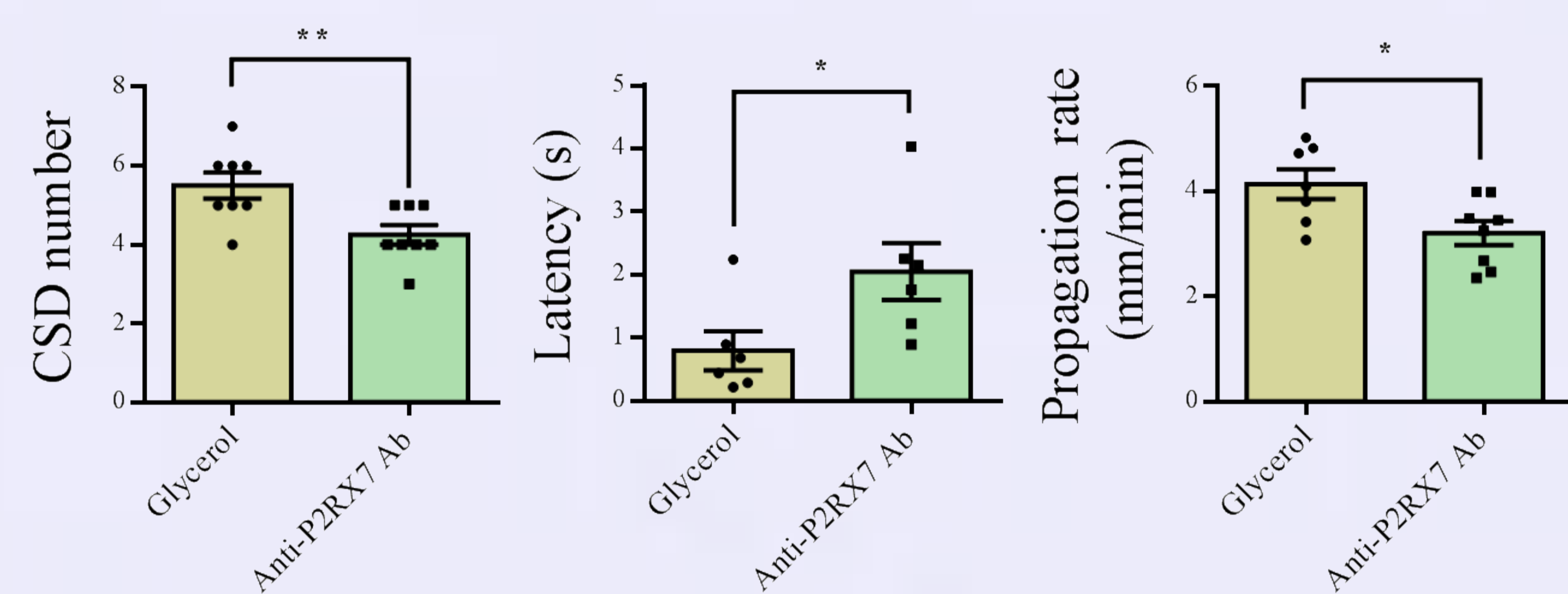


Figure 4. The anti-P2X7R antibody reduced cortex susceptibility to CSD in rats. (n=8 in each group) *p < 0.05, **p < 0.01. Two-tailed, unpaired t-test.

2. Anti-P2X7R antibody reduced the induction of mRNA of TNF- α , but not IL-1 β by CSD

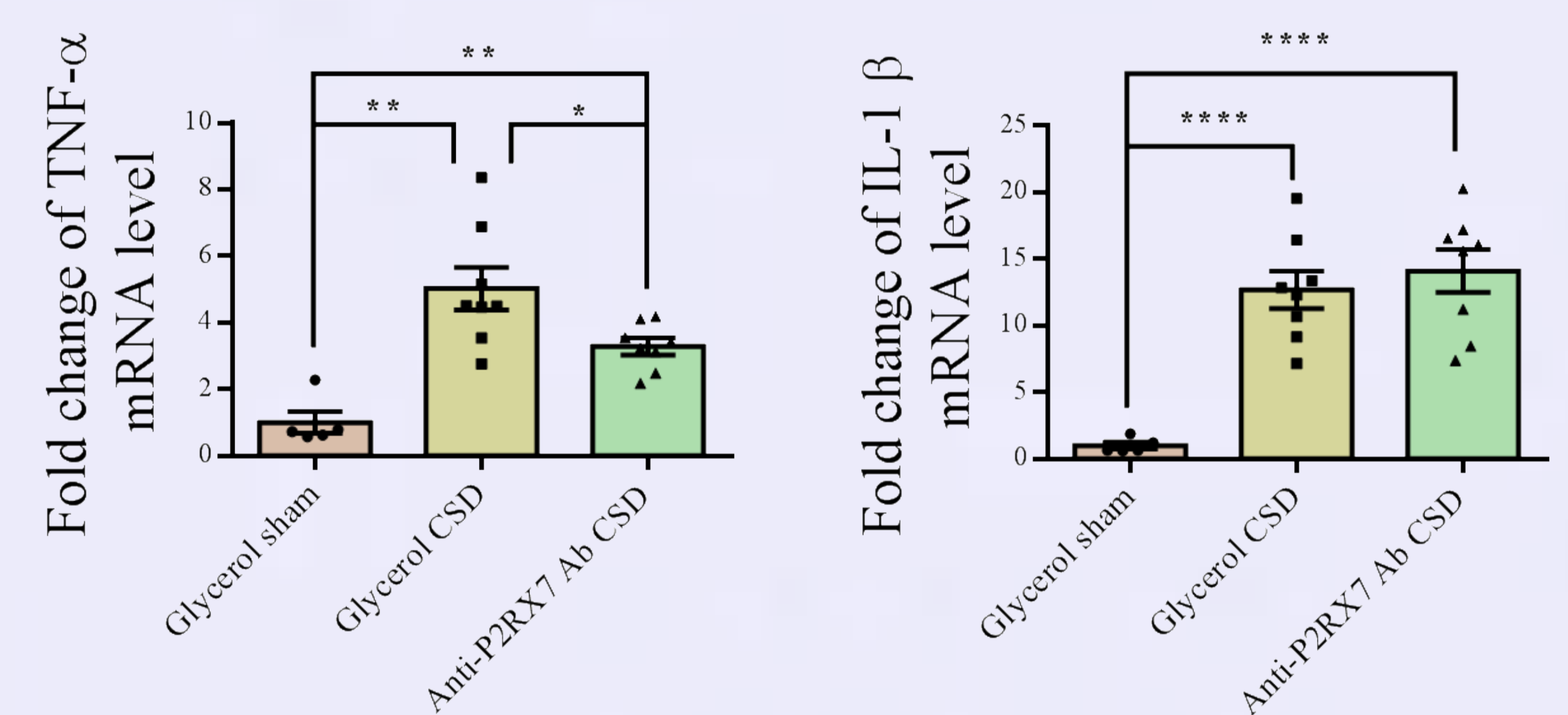


Figure 5. gene expression of TNF- α and IL-1 β , (n=5 in glycerol sham group and n=8 in another two group). *p < 0.05, **p < 0.01, ****p < 0.0001. Two-tailed unpaired t-test.

Conclusion and Further Work:

- Blockade of P2X7R by antibody reduced cortex susceptibility to CSD and rapidly reduced CSD induced gene expression of TNF- α in rats, indicating P2X7R as a potential target for migraine prophylaxis and treatment.
- The anti-P2X7R antibody did not reduce the induction of gene expression of IL-1 β immediately after CSD. This may be due to: 1) the time points, as a significant down-regulation of IL-1 β mRNA level was reported by inhibition of the P2X7/PANX1 pore complex 4 hours post CSD[6]. 2) suppression of spreading depolarization-induced IL-1 β mRNA upregulation was due to inhibition of the P2X7/PANX1 pore complex, not the P2X7R cation channel [6].

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