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# Endogenous TRPV1 expression in the human cingulate- and medial frontal gyrus

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ABSTRACT

*Background:* The transient receptor potential vanilloid subtype-1 (TRPV1) channel is a calcium selective ion channel that responds to various stimuli such as heat, low pH, and capsaicin. Recently this channel was studied as an actuator for wireless neuromodulation in rodents, e.g., heat-induced activation of TRPV1 resulted in neuronal excitation. From a translational perspective, we addressed if TRPV1 is endogenously expressed in the human medial frontal gyrus (MFG) and cingulate gyrus (CG) in depressed and control subjects and if it can be used as a means for neuromodulation in mood and other neuropsychiatric disorders.
*Methods:* We assessed TRPV1 expression levels by Western blotting and evaluated its tissue and cellular distribution by means of immunohistochemistry.
*Results:* TRPV1 was observed in all tissue samples, i.e., depressed and control, MFG and CG, yet the expression level as assessed by Western blotting varied between individuals. No intra-individual differences were seen between the MFG and CG. Immunohistochemistry showed that TRPV1 was expressed by glial-like cells but also in neurites, endothelial cells, and to a lesser extent in neuronal cell bodies. Fluorescent co-labeling of TRPV1 and glial fibrillary acidic protein (GFAP) identified most glial cells expressing TRPV1 to be astrocytes.
*Conclusion:* These findings indicate that TRPV1 is endogenously expressed in the human CG and MFG. As TRPV1 is predominantly expressed by glial cells, this may suggest an opportunity for non-neuronal network modulation.

## 1. Introduction

TRPV1 is a subfamily of the transient receptor potential cation channels and functions as a molecular integrator for multiple types of sensory input. It is selective for calcium ions and responds to capsaicin, noxious heat, low extracellular pH, divalent cations, and particular toxins (Yang and Zheng, 2017). TRPV1 has been described in the peripheral pain pathway where the receptor can initiate nociceptive signaling by generating a receptor potential (Premkumar and Sikand, 2008). In addition to peripheral expression, various reports state that TRPV1 can be found in the brain (Marrone et al., 2017). Recently, TRPV1 was used as an actuator for neuromodulation in mouse brain (Chen et al., 2015). However, due to low endogenous expression of TRPV1 in the central nervous system of rodents, the TRPV1 channel was introduced with lentiviral delivery. This method of neuromodulation seems to be a promising approach for future clinical application, although lentiviral delivery of TRPV1 might be undesirable.

To explore the possible clinical use of TRPV1 for neuromodulation we investigated if TRPV1 is endogenously expressed in the human brain. A sufficient expression of TRPV1 in neurons is necessary for this technique to work properly. We focused on subjects with depression since TRPV1 channels have been implicated in depression and anxiety (Madasu et al., 2015; Marsch et al., 2007; Wang et al., 2017; Sartim et al., 2019; Reyes-Mendez et al., 2018).

We investigated the MFG and CG of subjects whom experienced depression in their medical histories and compared them to non-demented control subjects. The MFG, which is part of the dorsolateral prefrontal cortex, is hypoactive in MDD while the CG is hyperactive in MDD (Mayberg et al., 2005; Koenigs and Grafman, 2009). Therefore, these brain regions are potential targets for neuromodulation. In this article, we aim to examine whether TRPV1 is sufficiently expressed in these brain regions to be considered as a target for neuromodulation.

*Abbreviations:* DBS, deep brain stimulation; CG, cingulate gyrus; GFAP, glial fibrillary acidic protein; MDD, major depressive disorder; MFG, medial frontal gyrus; PD, Parkinson's disease; SN, substantia nigra; TRPV1, transient receptor potential vanilloid subtype 1

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**Table 1**
Clinical characteristics of the subjects in the NBB samples.

| Subject No. | Diagnosis | Gender | Age (years) | Resected tissue | Amyloid | Braak | Post-mortem delay |
|---|---|---|---|---|---|---|---|
| P1 | Depression | F | 84 | MFG | O | 2 | 08:45 |
| P2 | Depression | M | 89 | CG<br>MFG | O | 1 | 04:35 |
| P3 | Depression | F | 66 | CG<br>MFG | O | 1 | 07:55 |
| P4 | Non-demented control | F | 84 | MFG | A | 2 | 05:36 |
| P5 | Non-demented control | M | 89 | CG<br>MFG | O | 2 | 06:50 |
| P6 | Non-demented control | F | 82 | CG | A | 1 | 07:45 |

F: female, M: male, MFG: medial frontal gyrus, CG: cingulate gyrus, O: amyloid stage 0, A: amyloid stage I.

## 2. Materials and methods

### 2.1. Subjects

Fresh-frozen and paraffin embedded brain tissue of depressed and control subjects containing the CG and MFG were provided by the Netherlands Brain Bank (NBB) (Table 1). As a positive control, we used temporal neocortical tissue of an epileptic patient provided by Maastricht UMC+ (MUMC+) for an abundant expression of TRPV1 in patients with mesial temporal lobe epilepsy has been reported earlier (Sun et al., 2013). All experiments have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

### 2.2. Western blot

Western blot analysis was performed to investigate and compare TRPV1 expression in the CG and MFG of depressed and control subjects. Fresh frozen brain tissue was cut on a cryostat and lysed in homogenization buffer (1 g/ 9 mL) containing 10% protease inhibitor (cat. no. 11697498001; Roche). Total tissue lysates were centrifuged at 12,000 rpm for 10 min at 4 °C. Protein concentrations in the supernatants were estimated using the Lowry protein assay (Bio-Rad). Odyssey protein molecular weight marker and samples (100 µg/lane) were loaded onto a 4% stacking gel (acrylamide/Bis 29:1, 1 M Tris-HCl pH 6.8, 20% SDS, 10% APS, TEMED) / 8% running gel (acrylamide/Bis 29:1, 3 M Tris-HCl pH 8.8, 20% SDS, 10% APS, TEMED).The resolved proteins were transferred onto a PVDF membrane using a mini-protein transfer system (Bio-Rad) at 100 V for 2 h in transfer buffer (1.4% glycine, 0.3% trizma base, 20% methanol). Next, membranes were blocked for 1 h at room temperature (RT) with Odyssey blocking buffer (cat. no. 927-40003; LI-COR) and then incubated overnight at 4 °C with primary antibodies (mouse anti-glycerylaldehyde-3-phosphate-dehydrogenase (GAPDH) (1:1,000,000, cat. no. 10R-G109a; Fitzgerald) and rabbit anti-TRPV1 (1:1,000; cat. no. PA1-748; Thermo Fisher Scientific) diluted in Odyssey blocking buffer. The following day, the membranes were washed and subsequently incubated with secondary antibody (goat anti-rabbit IR Dye 800 CW, Alexa 488 (1:10,000, 925-32211; LI-COR) and donkey anti-mouse IR Dye 680, Alexa 700 (1:10,000, 925-68072; LI-COR) for 1 h at RT in the dark. After washing, the membranes were dried between filter paper and analyzed using an Odyssey scanner.

To validate TRPV1 specificity and to visualize TRPV1 degradation products, one membrane was stained with anti-TRPV1 (1:1000; cat. no. PEP-202; Thermo Fisher Scientific) that was preabsorbed with TRPV1 synthetic peptide (1:1).

Optical densities (OD) of TRPV1 for each sample were calculated relative to the GAPDH protein band in the same sample using ImageJ software. To plot TRPV1 degradation against post-mortem delay, we calculated the ratio of the relative OD of TRPV1 degradation products and TRPV1 degradation products together with intact TRPV1.

### 2.3. Immunohistochemistry

Paraffin-embedded tissue was cut into 5 µm thick sections using a microtome.

All sections were deparaffinized followed by antigen retrieval in boiled citrate buffer (10 mmol) for 10 min and allowed to cool down a consecutive 20 min. Sections were blocked for endogenous peroxidase activity using a 0.3% $H_2O_2$ in TBS solution for 30 min. After washing, primary antibody was added overnight at 4 °C (TRPV1; 1:100 or 1:200; cat. no. PA1-748; Thermo Fisher Scientific). The following day, all sections were incubated with secondary antibody for 1 h at RT (biotinylated donkey anti-rabbit; 1:200; cat. no. 711-065-152; Jackson ImmunoResearch). All sections were then washed and stained with an ABC kit (1:400; cat. no: PK-6100; Vector Labs) followed by an incubation in DAB-NiCl (1 DAB : 1 Tris-HCl, 0.5% NiCL); cat. no: d5637-10 g; Sigma-Aldrich) for 10 min. After washing, the sections were dehydrated in ethanol and coverslipped with Pertex. Photomicrographs were taking using an AX-70 microscope (Olympus Provis) and Cellˆ P software.

### 2.4. Immunofluorescence double-labeling

For immunofluorescence all sections underwent deparaffinization and antigen retrieval as described above. The primary antibodies were added overnight at 4 °C (TRPV1; 1:50; cat. no. PA1-748; Thermo Fisher Scientific and anti-GFAP, 1:50, G3893; Sigma-Aldrich). The next day secondary antibodies were added for 1 h at RT (for TRPV1; donkey anti-rabbit Alexa 647 (1:100, cat. no. A-31573, Invitrogen), for GFAP; donkey anti-mouse Alexa 488 (1:100, cat. no. A-21202, Invitrogen)). All sections were washed and stained with Hoechst (1:500). A final step of 10 min incubation with Sudan black (0.5% in 100% ethanol) at RT in the dark was done to prevent autofluorescence from lipofuscin. All sections were coverslipped with glycerol. Microscopy was performed using an upright fluorescence microscope (Olympus BX51WI) and Stereo Investigator software.

## 3. Results

### 3.1. Western blotting

Results showed an immunoreactive band of 95 kDa in control tissue and the MFG and CG in both depressed and control subjects (Fig. 1 and supplementary Fig. 1).

Pre-absorption of the anti-TRPV1 antibody with synthetic peptide resulted in a loss of the 95 kDa immunoreactive band. In addition, this condition also resulted in the loss of several lower immunoreactive bands (supplementary Figs. 2 and 3). Analysis of the OD showed a high inter-individual variability of the 95 kDa immunoreactive band and thus TRPV1 expression (Fig. 2).

Relative OD values were compared between the CG and MFG regions of all subjects (Fig. 3). For the CG, both samples from the

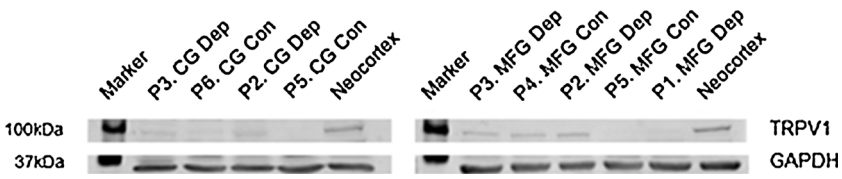


**Fig. 1.** Western blot TRPV1 expression. Western blot of TRPV1 expression per subject and brain region. CG: cingulate gyrus, MFG: medial frontal gyrus, Con: Control, Dep: depressed, P#: subject number.

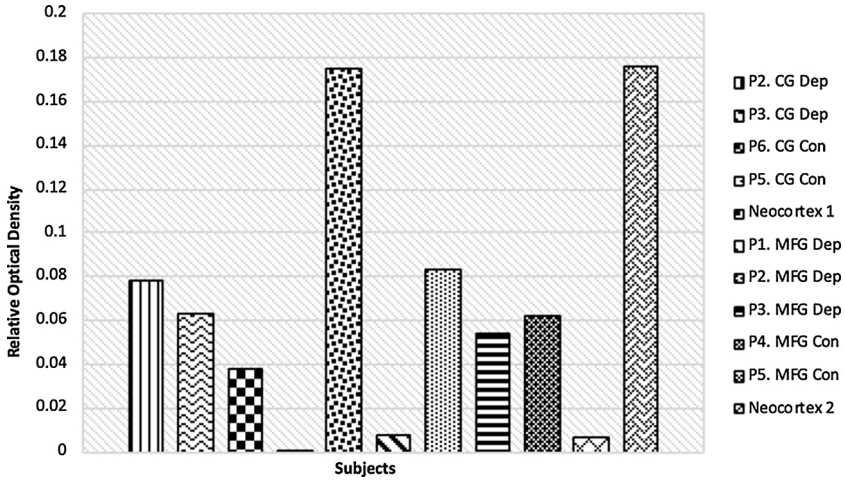


**Fig. 2.** Individual TRPV1 expression levels. The individual TRPV1 relative OD values per subject and brain region represented by bar charts. CG: cingulate gyrus, MFG: medial frontal gyrus, Con: Control, Dep: depressed, P#: subject number.

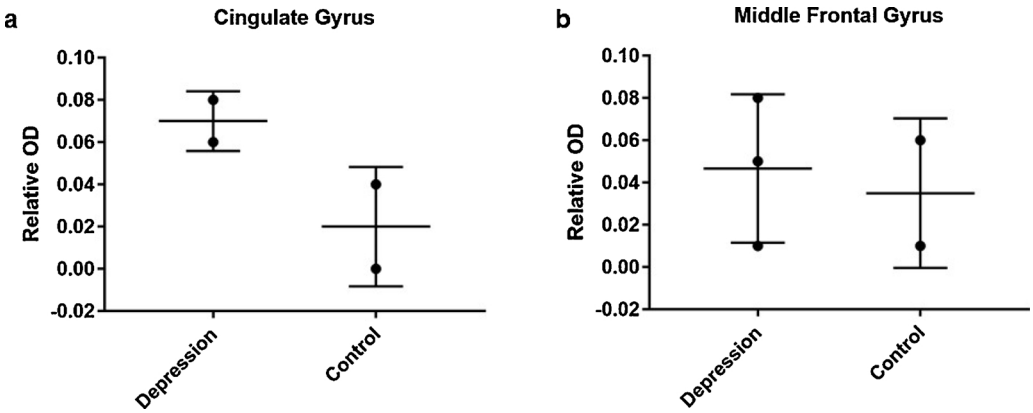


**Fig. 3.** Differences in TRPV1 expression levels between depression and control within one brain region. Relative OD values measured in **a)** the depression (n = 2) and control group (n = 2) in the CG. **b)** the depression (n = 3) and control group (n = 2) in the MFG. Each dot represents one individual value and the average is represented with a horizontal line. CG: cingulate gyrus, MFG: medial frontal gyrus.

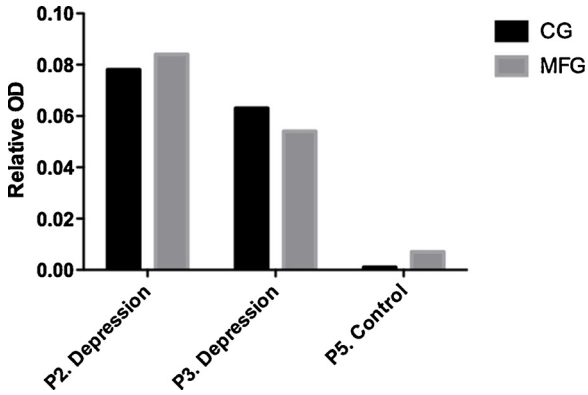


**Fig. 4.** Relative OD values of the CG and MFG in the same subjects. The relative OD values of TRPV1 in the CG and MFG of the same subject (n = 3) represented by bar charts. CG: cingulate gyrus, MFG: medial frontal gyrus.

depression group showed a higher relative OD as compared to the controls. However, a univariate ANOVA showed no significant difference in the interaction effect between regions and the subjects state (depression vs control; p = 0.442). No differences were found for the MFG region.

In subjects of whom we were able to investigate both the CG and MFG, no significant difference in relative OD values of TRPV1 expression was found (n = 3; Student's paired *t*-test p = 0.083; Fig. 4). For the other subjects either the CG or MFG was not available for research.

We found a moderate negative correlation between the post-mortem time and the expression level of TRPV1 (Spearman correlation coefficient $r_s = -0.456$). A short post-mortem time of 4.5 h resulted in 91.48% degradation of TRPV1 95 kDa into 50–75 kDa bands compared to 99.34% with a post-mortem time of 7 h (Fig. 5). No strong correlation was detected between age and the expression level of TRPV1.

### 3.2. Immunohistochemistry and double immunofluorescence labelling

To investigate in which cell types TRPV1 is expressed we executed immunohistochemistry and double immunofluorescence labelling on paraffin-embedded tissue of the CG and MFG of the subject that showed the highest TRPV1 expression in the western blots. We observed a high TRPV1 expression in glial-like cells in both the CG and MFG (Figs. 6 and

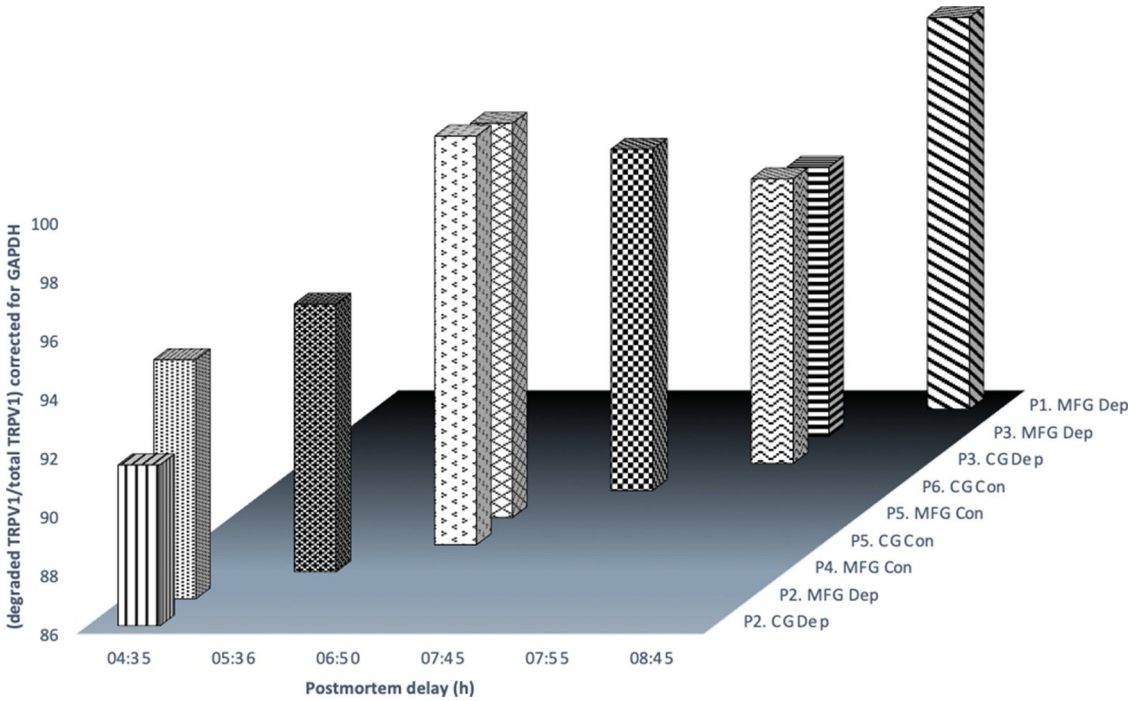


**Fig. 5.** TRPV1 degradation and post-mortem delay. The amount of degraded TRPV1 and post-mortem delay per subject and brain region represented by bar charts. Degraded TRPV1 is represented as a percentage by calculating the ratio of the relative OD of 50–75 kDa : (the relative ODs of 50–75 kDa + 95 kDa).

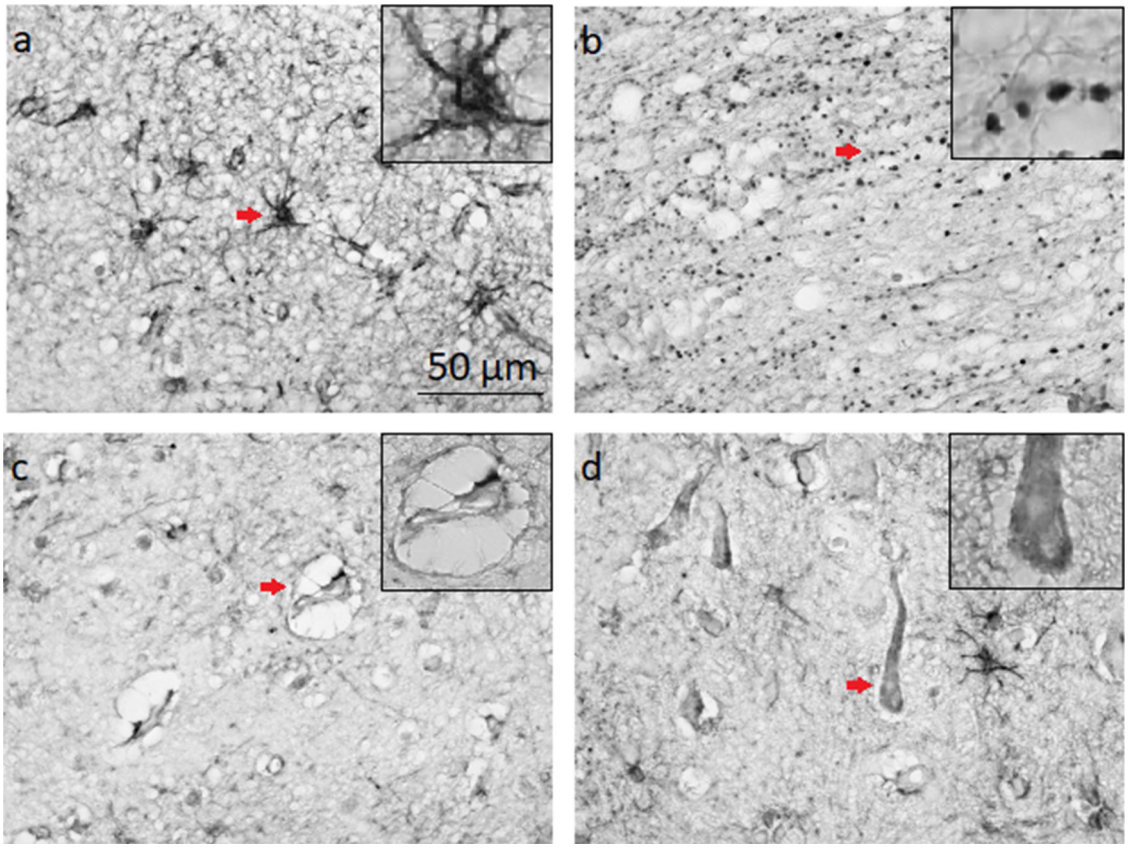


**Fig. 6.** Cellular distribution of TRPV1 in the CG. Representative photomicrographs of anti-TRPV1 stained sections containing the CG from subject #2. Note the expression of TRPV1 (1:200) in cells with a morphological like appearance of a) glial cells, b) dendritic structures in white matter, c) endothelial cells, and d) neuronal cells. The images were acquired using a 40x objective. The scale bar of 50 µm is applicable to a, b, c, and d. Insets in the upper right corner: photomicrograph of cells indicated by the arrow at 100x magnification.

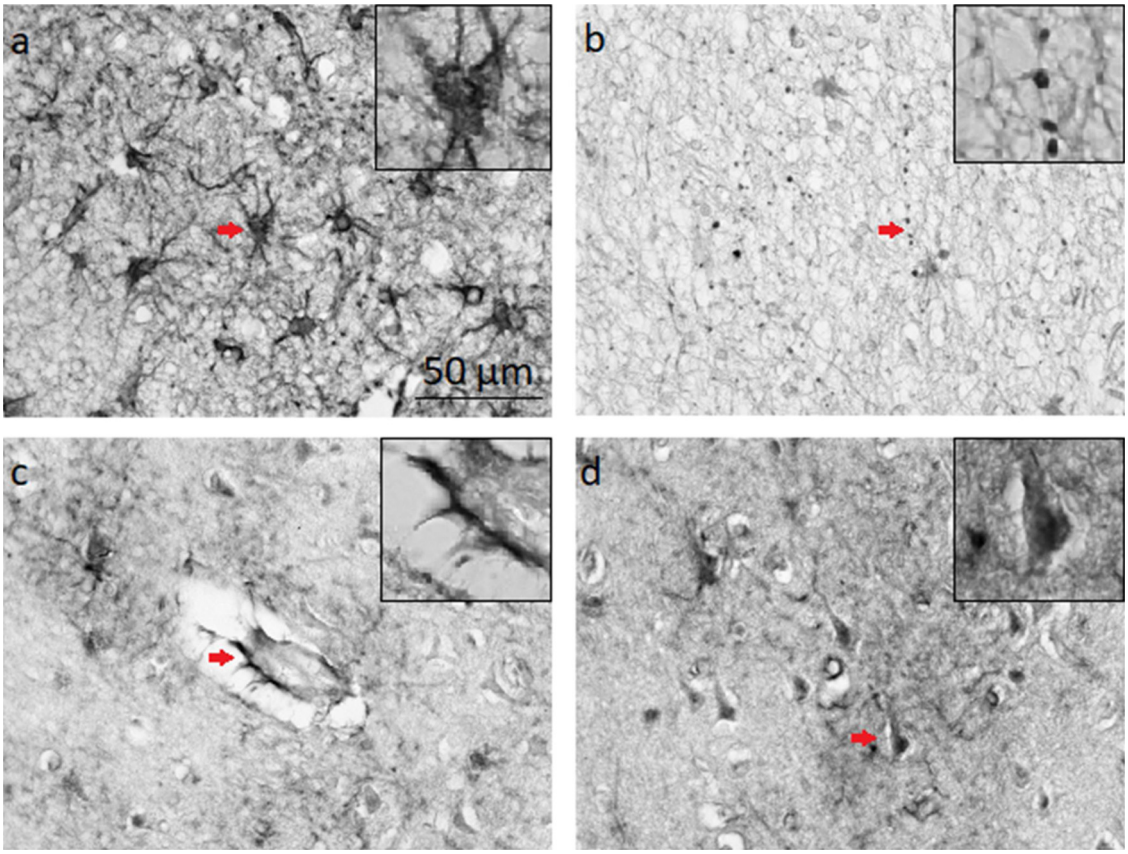


**Fig. 7.** Cellular distribution of TRPV1 in the MFG. Representative photomicrographs of anti-TRPV1 stained sections containing the MFG from subject #2. Note the expression of TRPV1 (1:200) in cells with a morphological like appearance of a) glial cells, b) dendritic structures in white matter, c) endothelial cells, and d) neuronal cells. The images were acquired using a 40x objective. The scale bar of 50 µm is applicable to a, b, c, and d. Insets in the upper right corner: photomicrograph of cells indicated by the red arrow at 100x magnification.

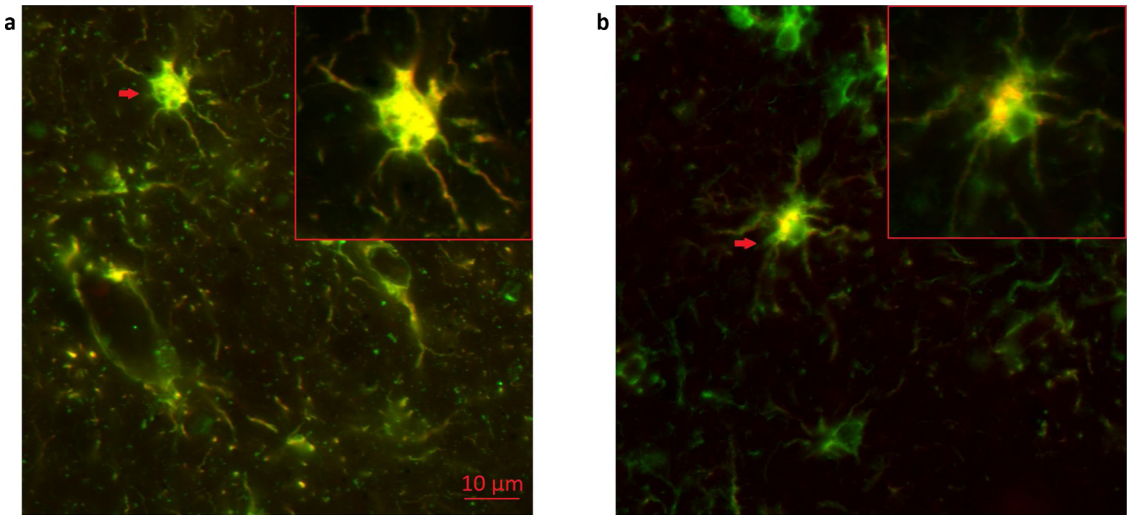


**Fig. 8.** TRPV1 expression in astrocytes in the CG and MFG of subject no. 2. Representative photomicrographs of anti-TRPV1 (1:50, red), anti-GFAP (1:50, green) stained sections containing the a) CG and b) MFG from subject #2 showing double-labeling of TRPV1 and GFAP. The images were acquired using a 40x objective. The scale bar of 10 µm is applicable to a. and b. Insets in the upper right corner: photomicrograph of a co-labeling cell indicated by the red arrow at 100x magnification.

7), endothelial cells in the CG and MFG, neurite structures in the CG, and to a lesser extend in the MFG and neuronal cell bodies in the CG and MFG. Neuronal cell bodies were found scarcely and stained less intensely than the others mentioned structures (Figs. 6 and 7).

Fluorescent double labelling revealed TRPV1 co-expression with GFAP containing cells both in the CG and MFG (Fig. 8).

## 4. Discussion

In the present study we showed that the TRPV1 channel was expressed in both the CG and MFG of subjects with a history of depression as well as controls. No differences in TRPV1 expression levels were found between the depression and control groups. However, strong inter-individual differences in TRPV1 expression level were detected.

Immunohistochemistry revealed TRPV1 expression in morphologically glial-like cells, endothelial cells, neurites, and neurons. In addition, fluorescent double labeling showed abundant co-expression of TRPV1 with GFAP indicating its expression in astrocytes.

The TRPV1 channel has been implicated in depression and anxiety in rodents before (Sartim et al., 2019). However, TRPV1 expression in the human brain has only been reported scarcely (Sun et al., 2013). In rodents, the function of TRPV1 in depression has shown some contradictory results. Reports have stated both antidepressant-like and depressant-like effects when activating TRPV1 channels (Wang et al., 2017; Sartim et al., 2019; Reyes-Mendez et al., 2018). In this study, TRPV1 did not seem to significantly differ between the depressed and control groups, indicating that TRPV1 expression is neither up- nor downregulated in depression. Nevertheless, the expression level of TRPV1 might be related to the severity of depression. This study did not examine this interrelation since extensive diagnostic information regarding depression pathology of the subjects was not available in the clinical information reports from the NBB. In addition, it should be noted that as we studied post-mortem tissue a potential *in vivo* difference may have been missed. Furthermore, within subjects no differences were found in TRPV1 expression between the CG and MFG, although only three subjects were examined due to scarcity of available tissue.

In this study, in addition to observing the presence of TRPV1 in our western blots, pre-absorption with anti-TRPV1 resulted in the absence of not only the 95 kDa but also several lower immunoreactive bands. This illustrates that the 95 kDa band is TRPV1 specific and that the few bands below the 95 kDa are most likely degradation products of TRPV1. Additionally, we found a moderate negative correlation between the post-mortem time and TRPV1 expression indicating that TRPV1 is an unstable protein sensitive to degradation. We therefore conclude that the interpretation of post-mortem TRPV1 expression levels needs to be done with precaution.

Immunohistochemistry showed that TRPV1 is expressed in presumably glial cells, neurites, endothelial cells, and neuronal cell bodies. Neuronal expression of TRPV1, however, was rather scares. More abundant TRPV1 expression was seen in astrocytes, shown by the co-labeling of TRPV1 and GFAP. Expression of TRPV1 in microglia was also considered, but given the fact that microglia cells are much more mobile than astrocytes, we did not consider them stable enough to fulfill the purpose of neuromodulation. For this reason TRPV1 expression in microglia cells was not studied. TRPV1 expression in astrocytes in rats and humans has been shown before. Studies state that TRPV1 in astrocytes can mediate the production of ciliary neurotrophic factor inhibiting the degeneration of nigral dopaminergic neurons in rodent models of Parkinson's disease (PD). Furthermore, PD individuals express more TRPV1 and GFAP in their substantia nigra compared to healthy subjects (Nam et al., 2015). Given these results, astrocytic TRPV1 might play a more crucial role than previously expected.

DBS outcomes have thus far mainly been attributed to a direct effect on neural elements. However, there is a growing insight into the role of astrocytes in neuronal communication and DBS (Perea et al., 2009; Vedam-Mai et al., 2012). Astrocytes communicate with neurons via so called 'tripartite synapses' and potentially participate in synaptic transmission through $Ca^{2+}$ and gliotransmitter signaling such as ATP (Perea et al., 2009; Halassa and Haydon, 2010). One human astrocyte interacts with approximately two million synapses making it a feasible candidate for the modulation of a neural network (Oberheim et al., 2006). Astrocytes can be triggered directly by electrical stimulation, releasing the neuromodulators ATP and glutamate (Bekar et al., 2008; Tawfik et al., 2010). This makes it plausible that DBS-induced modulation of network activity is partially due to astrocytic gliotransmission. Modulation of astrocytes, and thereby network activity, could potentially be done using the TRPV1 channel.

Given our results, using endogenous TRPV1 as a mean for neuromodulation can be a less effective approach. TRPV1 expression shows a great inter-individual variability, so using TRPV1 for this purpose requires the assessment of TRPV1 expression in each individual, which does not seem to be feasible at this time. Furthermore, TRPV1 appears to be scarcely expressed in neurons, thereby restricting direct neuronal neuromodulation. Nevertheless, abundant expression of TRPV1 was shown in astrocytes making neuronal modulation through these cells a potential approach in future research.

## 5. Conclusion

This study showed that TRPV1 is present in the human CG and MFG in both depressed and control subjects. TRPV1 expression levels differ between subjects and do not seem to be dependent on depression in one's medical history. TRPV1 expression was extensively co-localized with GFAP indicating its abundant expression in astrocytes. Since neuronal TRPV1 expression is scarce, the potential use of TRPV1 for neuromodulation seems restricted. Interestingly, glial expression of TRPV1 may indicate an alternative approach for neuromodulation.

## Declaration of Competing Interest

All authors declare to have no conflict of interest.

## Acknowledgements

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.brainresbull.2019.07.018.

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