

# Coverage of Blood Vessels by Astrocytic Endfeet Is Reduced in Major Depressive Disorder

## *Supplemental Information*

### **Supplemental Methods**

#### **Coverage Analysis**

Images were analyzed in two different areas: 1) gray matter of the orbitofrontal cortex and, 2) white matter of the ventro-medial prefrontal cortex (Figure 1). We sampled a selective area of gray matter that was cytoarchitectonically localized since neuronal and glial cell pathology was reported specifically here in our previous work (1, 2). We deemed it crucial to link this study with our prior work by examining vessel coverage by glia in the same precise region. We needed a larger area of white matter because 1) vessel density is less in white matter than gray matter and, 2) the white matter region corresponds to the ventro-medial prefrontal bundle which connects prefrontal cortex with other cortical and subcortical areas. We examined AQP4 pathology in this region because diffusion tensor imaging studies report a decrease in fractional anisotropy in this bundle in depression (3-6). Ten to 14 images were analyzed per each area.

#### **Sampling Procedure**

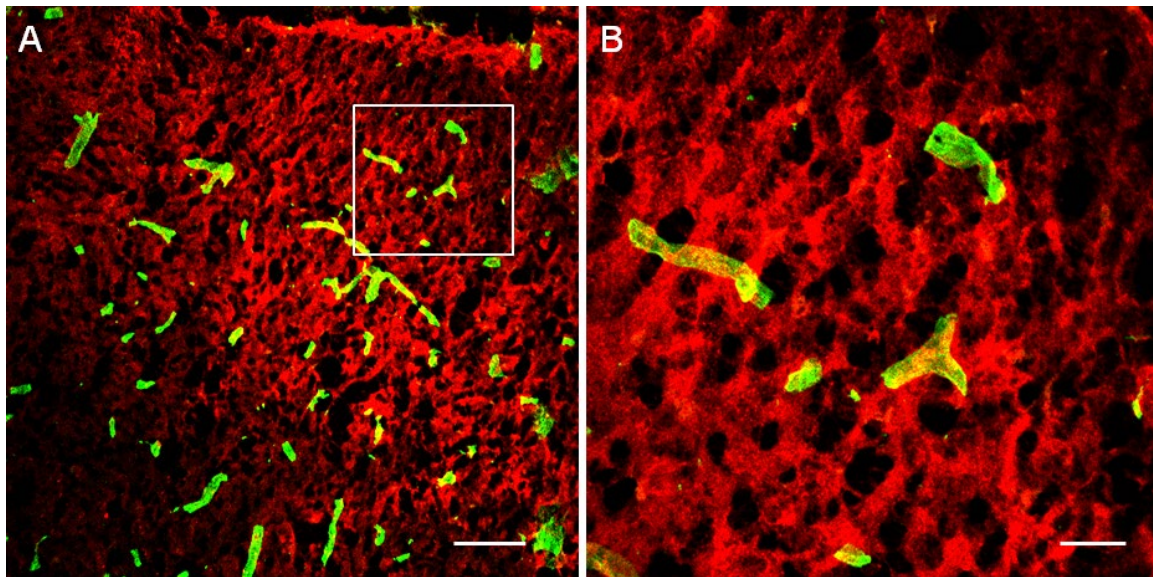
The gray matter selected in this study consisted of a contiguous stack of frames (175  $\mu\text{m}$  x 175  $\mu\text{m}$ ) spanning the entire width of the gray matter from the precise region of medial orbitofrontal in which densities of neurons and glia were assessed in previous studies (1, 2). In this specific gyrus, gray matter was selected from the flat surface

avoiding the curvatures of the bottom of the sulcus and top of the gyrus. The white matter selected in this study corresponds to the ventromedial prefrontal white matter bundle from neuroimaging studies. The first sampling image of 175  $\mu\text{m}$  x 175  $\mu\text{m}$  was randomly selected and 10-14 adjacent sampling images were selected at intervals of 525  $\mu\text{m}$  to include the entire bundle as outlined in Figure 1.

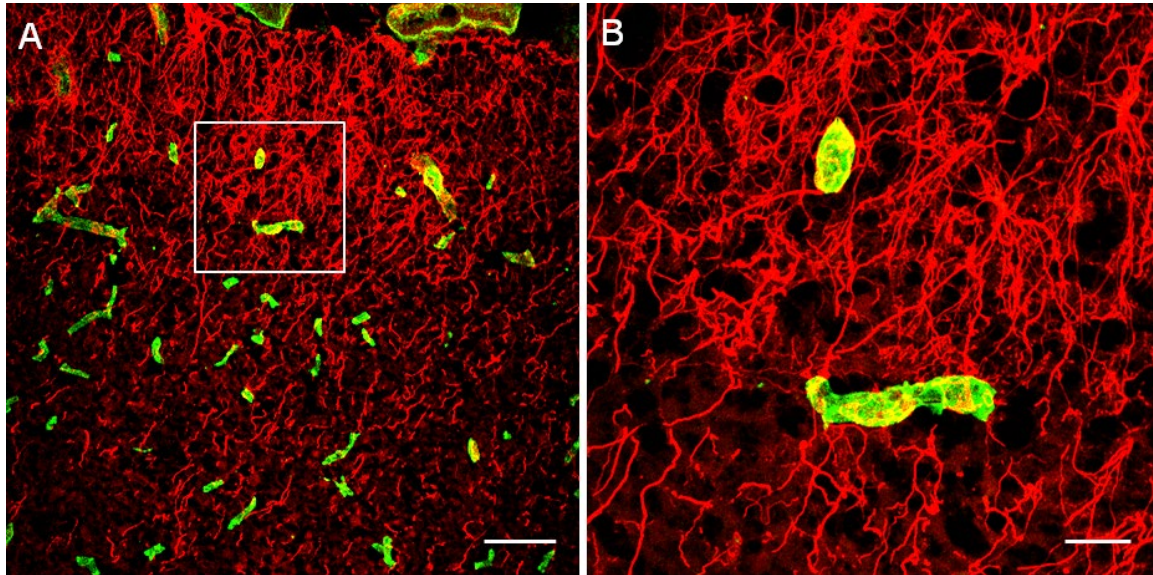
**Table S1.** Statistical analyses of AQP4 coverage in subgroups of MDD

Variable	Group	Mean $\pm$ SEM	Statistics
Antidepressants in postmortem toxicology screening	MDD with no AD in toxicology, $n = 7$	$1.12 \pm 0.27 \times 10^{-2}$	ANCOVA, $F(1,10) = 4.285$ , $p = 0.07$
	MDD with AD in toxicology, $n = 6$	$1.16 \pm 0.58 \times 10^{-2}$	
Antidepressant prescription within the last month of life	MDD with no AD prescribed, $n = 6$	$1.22 \pm 0.26 \times 10^{-2}$	ANCOVA, $F(1,10) = 0.515$ , $p = 0.49$
	MDD with AD prescribed, $n = 7$	$1.47 \pm 0.53 \times 10^{-2}$	
Gender	MDD females, $n = 6$	$0.96 \pm 0.17 \times 10^{-2}$	ANCOVA, $F(1,10) = 4.337$ , $p = 0.06$
	MDD males, $n = 7$	$1.38 \pm 0.52 \times 10^{-2}$	
Suicide	MDD suicide, $n = 8$	$1.11 \pm 0.24 \times 10^{-2}$	ANCOVA, $F(1,10) = 0.583$ , $p = 0.46$
	MDD non-suicide, $n = 5$	$1.68 \pm 0.7 \times 10^{-2}$	

AD, antidepressant; ANCOVA, analysis of covariance; AQP4, aquaporin-4; MDD, major depressive disorder.



**Figure S1.** Low power (**A**) and higher power (**B**) fluorescent micrograph of gray matter of area 47 from the orbitofrontal cortex of a non-psychiatric control subject. Pictures represent confocal merged images of immunofluorescent staining of blood vessels with an antibody to collagen IV (green) and astrocytes with an antibody to AQP4 (red). Yellow-to-orange colors represent coverage of vessels by astrocytic AQP4-immunoreactive processes. White square on picture (**A**) indicates the place from which picture (**B**) was taken. Scale bar on (**A**) = 80  $\mu$ m; on (**B**) = 20  $\mu$ m. AQP4, aquaporin-4.



**Figure S2.** Low power (**A**) and higher power (**B**) micrograph of gray matter of area 47 from the orbitofrontal cortex of a non-psychiatric control subject. Pictures represent confocal merged images of immunofluorescent staining of blood vessels with an antibody to collagen IV (green) and astrocytes with an antibody to GFAP (red). Yellow-to-orange colors represent coverage of vessels by GFAP immunoreactive processes. White square on (**A**) indicates the place from which (**B**) was taken. Scale bar on (**A**) = 80  $\mu$ m; on (**B**) = 20  $\mu$ m. GFAP, glial fibrillary acidic protein.

## Supplemental References

1. Rajkowska G, Miguel-Hidalgo JJ, Wei J, Pittman SD, Dilley G, Overholser J, *et al.* (1999): Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry* 45:1085-1098.
2. Rajkowska G, Miguel-Hidalgo JJ, Dubey P, Stockmeier CA, Krishnan RR (2005): Prominent reduction in pyramidal neurons density in the orbitofrontal cortex of elderly depressed patients. *Biol Psychiatry* 58:297-306.
3. Sexton CE, Mackay CE, Ebmeier KP (2009): A systematic review of diffusion tensor imaging studies in affective disorders. *Biol Psychiatry* 66:814-823.
4. Shimony JS, Sheline YI, D'Angelo G, Epstein AA, Benzinger TL, Mintun MA, *et al.* (2009): Diffuse microstructural abnormalities of normal-appearing white matter in late life depression: a diffusion tensor imaging study. *Biol Psychiatry* 66:245-252.
5. Maller JJ, Thomson RH, Lewis PM, Rose SE, Pannek K, Fitzgerald PB (2010): Traumatic brain injury, major depression, and diffusion tensor imaging: Making connections. *Brain Res Rev* 64:213-240.
6. Kieseppä T, Eerola M, Mäntylä R, Neuvonen T, Poutanen VP, Luoma K, *et al.* (2010): Major depressive disorder and white matter abnormalities: a diffusion tensor imaging study with tract-based spatial statistics. *J Affect Disord* 120:240-244.