The spatial and cell-type distribution of SARS-CoV-2 receptor ACE2 in human and mouse brain

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Abstract

By engaging angiotensin-converting enzyme 2 (ACE2 or Ace2), the novel pathogenic SARS-coronavirus 2 (SARS-CoV-2) may invade host cells in many organs, including the brain. However, the distribution of ACE2 in the brain is still obscure. Here we investigated the ACE2 expression in the brain by analyzing data from publicly available brain transcriptome databases. According to our spatial distribution analysis, ACE2 was relatively highly expressed in some important brain areas, such as the substantia nigra and brain ventricles. According to our cell-type distribution analysis, the expression of ACE2 were found in many neurons (both excitatory and inhibitory neurons) and some non-neuron cells (mainly astrocytes and oligodendrocytes) in human middle temporal gyrus and posterior cingulate cortex, but the ACE2-expressing cells was none in the prefrontal cortex and very few in the hippocampus. Except for the additional high expression of Ace2 in the olfactory bulb areas for spatial distribution as well as in the pericytes and endothelial cells for cell-type distribution, the distribution of Ace2 in mouse brain was similar to that in the human brain. Thus, our results reveal an outline of ACE2/Ace2 distribution in the human and mouse brain, which indicates the brain infection of SARS-CoV-2 may be capable to result in serious central nervous system symptoms in coronavirus disease 2019 (COVID-19) patients.

Keywords: Angiotensin-converting enzyme 2; ACE2; Brain; SARS-coronavirus 2;

Introduction

Since December 2019, much attention has focused on the novel SARS-coronavirus 2 (SARS-CoV-2) and related coronavirus disease 2019 (COVID-19), which is rapidly spreading around the world and resulted in a global health emergency (Liu et al., 2020). In addition to atypical pneumonia, the central nervous system (CNS) symptoms of COVID-19 patients have been observed in the clinic (Li et al., 2020). According to a recent retrospective case series study, 53 out of 214 (24.8%) COVID-19 patients had CNS symptoms, including dizziness, headache, impaired consciousness, acute cerebrovascular disease, ataxia, and epilepsy (Mao et al., 2020). More importantly, it has been found that SARS-coronavirus (SARS-CoV), a previous similar coronavirus to SARS-CoV-2, spread into the brain after it was cleared from the lung in mice, which could be more concealment than that in the lung (Glass et al., 2004). Thus, it is necessary and urgent to pay attention to the CNS infection of SARS-CoV-2.

Angiotensin-converting enzyme 2 (ACE2 or Ace2) has been identified as a key entry receptor for novel pathogenic SARS-CoV-2 as well as previous SARS-coronavirus (SARS-CoV) (Hoffmann et al., 2020). By binding of the spike protein of the virus to ACE2, SARS-CoV-2 and SARS-CoV could invade host cells in human organs (Walls et al., 2020; Yan et al., 2020). However, the distribution of ACE2 in the brain is still obscure and even inconsistent. Hamming et al. found ACE2 may be express only in endothelium and vascular smooth muscle cells in the human brain tissue in 2004 (Hamming et al., 2004). Since then, few studies focused on the distribution of ACE2 in the human brain. On the other hand, previous study has reported that Ace2 could express in mouse neuron cells, which may contribute to the development of hypertension (Xia et al., 2013); however, in another neurocytometry study, Ace2 is a potential marker for non-neurons in zinc-fixed mouse brain cortical section (Martin et al., 2017). Thus, further clarifying brain tissue distribution of ACE2 may help to bring light to the CNS infection of the novel SARS-CoV-2 and previous SARS-CoV.

Here, we investigated the distribution of ACE2 in the brain by analyzing publicly available brain transcriptome databases. We revealed an uneven spatial and cell-type distribution of ACE2 in the human and mouse brain.

Methods

Brain transcriptome databases

Seven publicly available brain transcriptome databases that can be accessed without specialized computational expertise were used. All databases were listed in Table 1. Except for the Single Cell Portal database (https://singlecell.broadinstitute.org), these databases have been also introduced in a recent review study (Keil et al., 2018). All databases and datasets were appropriately used and cited according to their citation policy, license or terms of use.

Table 1. Database used for current study

Analysis	Web Interface	Reference	Species	Brain area	Method
Spatio-temporal	http://hbatlas.org	Johnson et al., 2009	Human	Multi	Microarray
Spatial	http://human.brain-map.org	Hawrylycz et al., 2012	Human	Multi	Microarray
Spatial	https://www.gtexportal.org	Consortium, 2015	Human	Multi	RNA-seq
Spatial	https://mouse.brain-map.org/	Lein et al., 2007	Mouse	Multi	Microarray
Spatial and cell-type	https://hipposeq.janelia.org	Cembrowski et al., 2016	Mouse	Hippocampus	RNA-seq
Single-cell	https://singlecell.broadinstitute.org	Not available	Many	Multi	RNA-seq
Single-cell	https://celltypes.brain-map.org/rnaseq/	Tasic et al., 2016	Human	Cortex	RNA-seq

Analysis of the spatial distribution of ACE2 in the human brain

Three databases were used, including Allen human brain atlas database (http://human.brain-map.org), Human Brain Transcriptome database (https://hbatlas.org), and GTExportal database (https://www.gtexportal.org), to analyze the spatial distribution of ACE2 in the human brain.

Cell-type distribution of ACE2 in human brain

Two single-cell sequencing databases, including Single Cell Portal database (https://singlecell.broadinstitute.org, Single-cell sequencing) and Allen Cell Types Database (http://celltypes.brain-map.org, Single-cell sequencing), were used. The summary of the

included three datasets for human brain were shown in Table 2.

Table 2. Summary of cell-type or single-cell sequencing databases for human and mouse brain

Brain area	Reference	Method	Nuclei	Species n (age)	Source
Middle temporal gyrus	Tasic et al., 2016	SMART- Seq	15928	Human n=8 (24–66)	https://celltypes.brain-map.org/rnaseq/human/mtg
Posterior cingulate cortex	Gaublomme et al., 2019	Multiplexing snRNA-seq	9923	Human n=20 (>□65)	https://singlecell.broadinstitute.org/single_cell/study/SCP371
Prefrontal cortex and Hippocampus	(Habib et al., 2017)	DroNc-Seq	19550	Human n=5 (40–65)	https://singlecell.broadinstitute.org/single_cell/study/SCP90/
Whole cortex	Ding et al., 2019	10x Chromium, SMART-seq2, DroNc-seq and sci-RNA-seq	13783	Mouse (one month old)	https://singlecell.broadinstitute.org/single_cell/study/SCP425/
SNr, SNc, VTA	U19 - Huang BICCN data (1U19MH114821-01)[a]	Unclear	13861	Mouse (unclear)	https://singlecell.broadinstitute.org/single_cell/study/SCP478
Cerebellum	Kozareva et al., 2020	High-throughput single-nucleus RNA-seq	611034 (10000 used)	Mouse 2 female, 4 male, (60 days)	https://singlecell.broadinstitute.org/single_cell/study/SCP795
Prefrontal cortex and hippocampus	Habib et al., 2017	DroNc-Seq	29543	Mouse 4, (adult)	https://singlecell.broadinstitute.org/single_cell/study/SCP60
Hippocampus	Cembrowski et al., 2016	Div-Seq	1367	Mouse unclear	https://singlecell.broadinstitute.org/single_cell/study/SCP1

[a] This dataset is provided by Huang et al (https://biccn.org/teams/u19-huang) and from BRAIN Initiative Cell Census Network (BICCN, https://biccn.org/). Multiplexing snRNA-seq: Nuclei multiplexing with barcoded antibodies for single-nucleus genomics Smart-Seq: Switching mechanism at 5' end of the RNA transcript; DroNc-Seq: Deciphering cell types in human archived brain tissues by massively-parallel single nucleus RNA-seq; sci-RNA-seq: single-cell combinatorial-indexing RNA-sequencing analysi; SNr: substantia nigra pars reticulate; SNc: substantia nigra pars compacta; VTA: ventral tegmental area;

Spatial and cell-type distribution of Ace2 in mouse brain

Allen mouse brain atlas database (http://mouse.brain-map.org) was used to analyze the general spatial distribution of Ace2 in the mouse brain. Four cell-type sequencing datasets from hippocampus RNA-seq atlas, (https://hipposeq.janelia.org/) and five single-cell sequencing datasets form Single Cell Portal database were used for cell-type distribution of Ace2 in the mouse brain. The summary of the used five single-cell sequencing datasets was also shown in Table 2.

Data processing and statistical analysis

Datasets were independently searched and analyzed by two authors (R.C. and J.Y.) and any disagreements were discussed and resolved by consensus with the corresponding author (Z.X.). The data from Allen human brain atlas database and Allen Cell Types Database was exported to Microsoft Excel 2017 and GraphPad Prism 6.0 for further analysis. ACE2 expression data from the Allen human and mouse brain atlas database were also imported to Brain Explorer 2.0 software to get visualization. The data from the other databases were analyzed online.

To minimize false positive of expression as previous studies (Gonzalez-Castellano et al., 2019; Mortazavi et al., 2008), a gene with calculate counts per million (CPM), transcripts per million (TPM), unique molecular identifier (UMI) count or Fragments Per Kilobase Million (FPKM) > 1 were considered to be positive, which also is the same as log10 (CPM), log10 (TPM) or log10 (UMI) ≥ 0 as well as log10 (CPM+1), log10 (TPM+1), and log10 (UMI+1) ≥0.3. Beside, Z score of ACE2 expression > 1 were considered as high ACE2 expression in Allen human brain atlas data.

Where applicable, data are expressed as median or mean. Interquartile range (IQR), 95%CI, range and/or all sample points, were also provided if possible.

Results

The general expression of ACE2 in the human brain.

According to the GETx portal database (Consortium, 2015), compared with the lung, the general expression of ACE2 was lowe but not none in the brain (**Figure 1A**). The expression

intensity of ACE2 was similar among different brain regions. Excepted for the lung (38 out of 578, 6.57%), the samples with log10(TMP+1) of ACE2 expression >0.3 were also found in amygdala (1 out of 152, 0.65%), anterior cingulate cortex (2 out of 176, 1.14%), caudate (3 out of 246, 1.22%), cortex (1 out of 255, 0.39%), frontal cortex (2 out of 209, 0.96%), hippocampus (4 out of 197, 2.03%), hypothalamus (3 out of 202, 1.49%), nucleus accumbens (1 out of 246, 0.41%), putamen (1 out of 205, 0.48%), spinal cord (cervical c-1, 4 out of 159, 2.52%), substantial nigra (5 out of 139, 3.60%), but none in cerebellum (0 out of 241) and cerebellar hemisphere (0 out of 215).

Besides, according to the Human Brain Transcriptome database (Johnson et al., 2009), the expression of ACE2 was also similar among cortex and other brain regions, which may not change a lot with the age (**Figure 1B and C**).

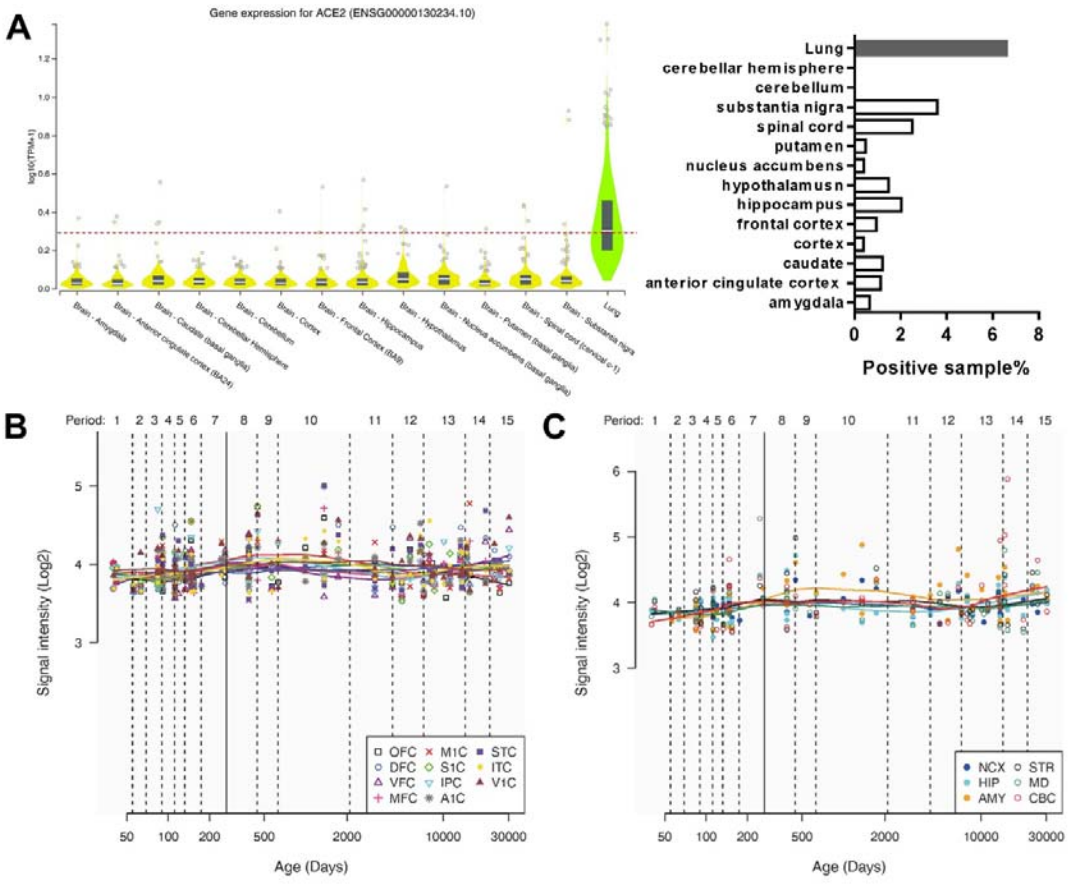

Figure 1. The general expression of ACE2 in different human brain areas. (**A**)The expression of ACE2 in different human brain areas and the lung according to GETx portal database (Consortium, 2015). The dotted line means log10 (CPM+1) = 0.3, which means a

threshold of the positive sample (>0.3 could be positive). (**B** and **C**) Change of intensity of ACE2 expression with age in different human brain areas according to Human Brain Transcriptome database (Johnson et al., 2009). Data are expressed as the median, interquartile range (IQR) and all sample points in **A**, while data are expressed as mean and all sample points in **B** and **C**.

Spatial distribution of ACE2 in the human brain.

By providing slice images (**Figure 2A and B**), Allen Human Brain Atlas may prove a more detail Spatial distribution of ACE2 in the human brain than the GETx portal database and Human Brain Transcriptome database (Hawrylycz et al., 2012). Two microarray datasets using the different probes of ACE2 (A_23_P252981, CUST_16267_PI416261804) were found and used in Allen Human Brain Atlas (Http://human.brain-map.org/microarray/search). By analyzing the intensity of ACE2 expression, we found 20 brain areas with z score of ACE2 expression >1.0 in both datasets, which represents ACE2 expression in these brain areas is 1 standard deviation greater than the mean (**Figure 1C**).

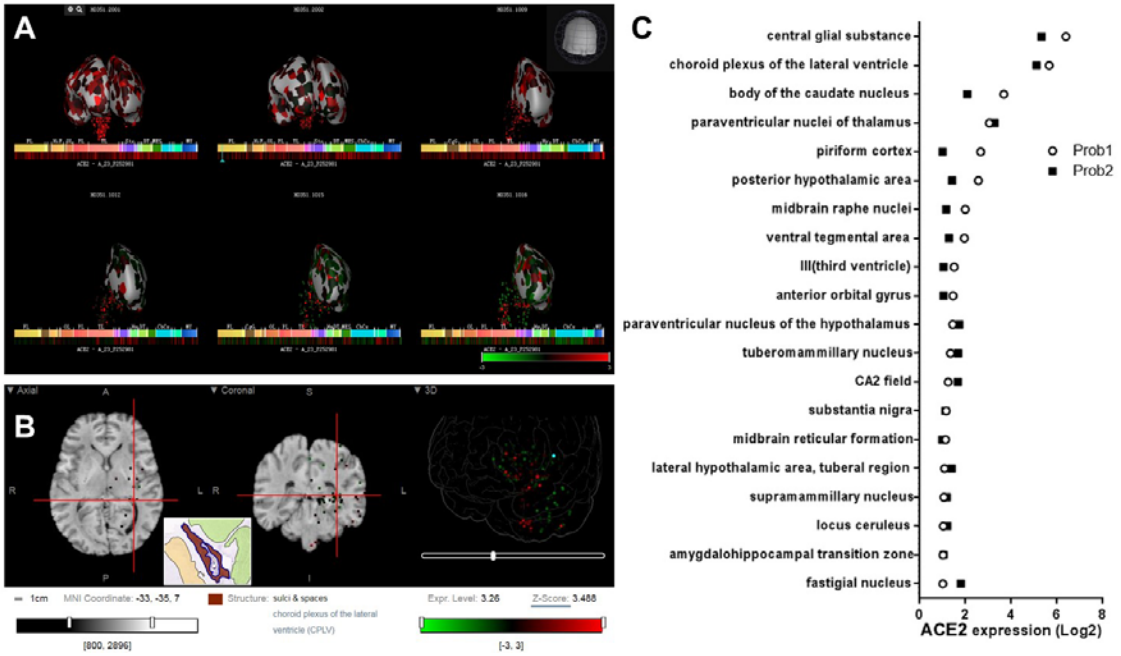

Figure 2. Spatial distribution of ACE2 in the human brain according to Allen Brain Atlas. (**A**) 3D view of the expression of ACE2 in different human brain areas based on the data of probe A_23_P252981 (probe 1). (**B**) Planar view of the expression of ACE2 in the

choroid plexus of the lateral ventricle. The inset shows a sampled area of the choroid plexus of the lateral ventricle (the dark area in the inset). (**C**) the 20 brain areas with log2 intensity of ACE2 expression >1.0 in both datasets (probe 1: A_23_P252981; probe 2: CUST_16267_PI416261804). All data were generated from the Allen Human Brain Atlas (Http://human.brain-map.org/microarray/search). Images in A and B are directly form the Allen Human Brain Atlas (© 2010 Allen Institute for Brain Science. Allen Human Brain Atlas. Available from: human.brain-map.org).

Spatial distribution of Ace2 in mouse brain.

As shown in **Figure 3,** We additionally analyzed the spatial distribution of Ace2 in the mouse brain based on the Allen Mouse Brain Atlas (http://mouse.brain-map.org/gene/show/45849; Lein et al., 2007). Similar to the human brain, we found the Ace2 expression is relatively high in the choroid plexus of lateral ventricle, substantia nigra pars reticulata (SNR) and some cortical areas (including piriform cortex). Maybe different from the human brain, we additionally found the ACE2 expression is also relatively high in the olfactory bulb, while it was very low in most hippocampal areas.

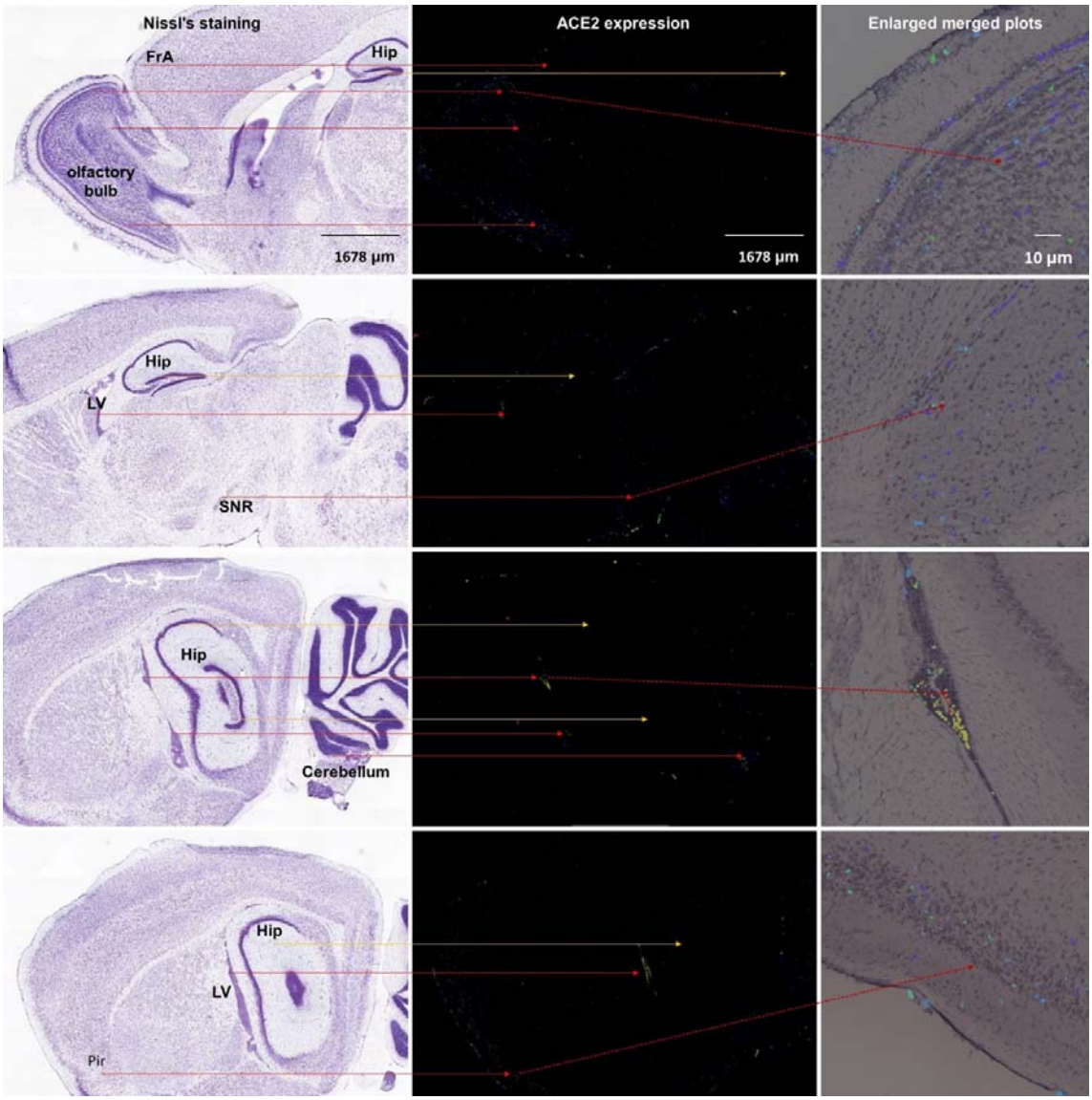

Figure 3. Spatial distribution of ACE2 in mouse the brain. The left column means Nissl's staining of mouse brain slice, the middle column means the ACE2 expression by antisense, the right column means the enlarged merged plots form Brain Explorer 2.0 software. Hip: hippocampus; LV: lateral ventricle; SNR: substantia nigra pars reticulate. Pir: piriform cortex. All images are from the Allen Mouse Brain Atlas (© 2004 Allen Institute for Brain Science. Allen Mouse Brain Atlas. Available from: https://mouse.brain-map.org/).

Cell-type distribution of ACE2 in the human brain

We further collected and analyzed single-cell sequencing data, which may provide all mRNAs present in every single cell of tested brain tissue.

By analyzing the single-cell sequencing data of human middle temporal gyrus (https://celltypes.brain-map.org/rnaseq/human/mtg; Tasic et al., 2016; **Figure 4A and B**), we found the expression of ACE2 is relatively high in excitatory neurons (164 out of 10525, 1.56%) and inhibitory neurons (41 out of 4164, 0.98%), but relatively low in non-neural cells (5 out of 914, 0.55%). The highest ACE2-expression cell type of excitatory neuron was FEZ Family Zinc Finger 2 (FEZF2) and Sodium Voltage-Gated Channel Beta Subunit 4 (SCN4B) expressing excitatory neurons of lay 4-5 (2 out of 25, 8%), while the highest ACE2-expression cell type of inhibitory neuron was somatostatin (SST) and Neuronal acetylcholine receptor subunit alpha-4 (CHRNA4) expressing inhibitory neurons in Lay1 (3 out of 52, 5.77%). Of note, two types of non-neural cells, glial fibrillary acidic protein (GFAP) positive astrocytes (2 out of 61, 3.27%) and oligodendrocytes (ODCs, 8 out of 313, 2.56%), also have a relatively high proportion of cells with the expression of ACE2. The details of ACE2 expression in each sub-cluster of brain cells in human middle temporal gyrus were shown in **Figure 4C and D**.

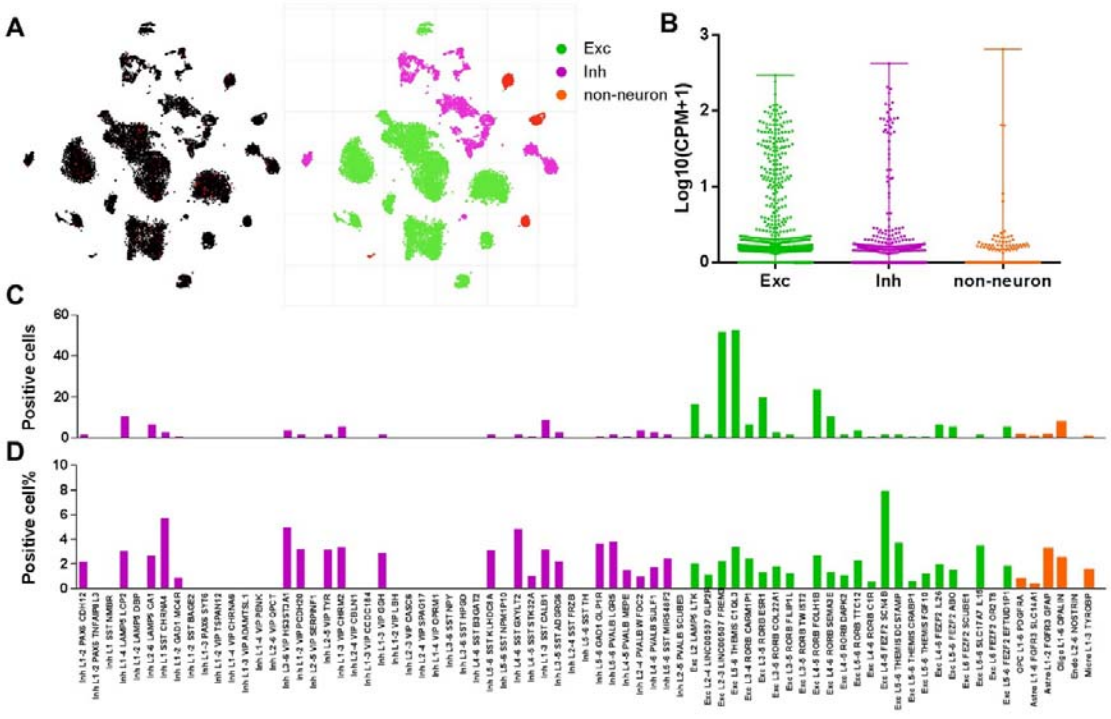

Figure 4. ACE2 expression in the human middle temporal gyrus. (**A**) The expression of ACE2 in the cells of the human middle temporal gyrus. Left: Red means potential ACE2-positive cells, while black means ACE2-negative cells; Right: the annotation map of

cell clusters. (**B**) The expression of ACE2 in brain cell subtypes. (**C**) The number of ACE2-positive cells in each sub-cluster of brain cells. (**D**) The percentage of ACE2-positive cells in each sub-cluster of brain cells. Cells with log10 (CPM+1) >0.3 were considered as positive cells. CPM: calculate counts per million; Inn: Inhibitory neuron; Exc: Excitatory neuron; Oligo: oligodendrocyte; Astro: astrocytes; Micro: microglia. Original data are from https://celltypes.brain-map.org/rnaseq/human/mtg. Images in A were directly generated by the RNA-Seq Data Navigator from Allen Cell Types Database (© 2015 Allen Institute for Brain Science. Allen Cell Types Database. Available from: https://celltypes.brain-map.org/). Data are expressed as median, range and all sample points in B.

By analyzing the single-cell sequencing data of human posterior cingulate cortex (https://singlecell.broadinstitute.org/single_cell/study/SCP371; Gaublomme et al., 2019 **Figure 5A and B**), we also found the expression of ACE2 was found in glutamatergic neurons (excitatory neurons, 88 out of 4308, 2.18%), GABAergic neurons (Inhibitory neurons, 14 out of 1327,1.06%) and astrocytes (14 out of 1274,1.10%), Oligodendrocyte progenitor cells (OPCs, 5 out of 342, 1.46%), ODCs (9 out of 2138, 0.42%), microglia (2 out of 355, 0.56%), and endothelial cells (2 out of 179, 1.12%). The details of ACE2 expression in each subtype of brain cell in human posterior cingulate cortex were shown in **Figure 5C**

By analyzing the single-cell sequencing data of archived human prefrontal cortex and hippocampus samples (https://singlecell.broadinstitute.org/single_cell/study/SCP90/; Habib et al., 2017; **Figure 5D and E**), we found the expression of ACE2 may be low in the hippocampus (2 out of 9530, **Figure 5F**) and none in the prefrontal cortex (0 out of 5433, **Figure 5F**). Besides, the expression of ACE2 was not found in prefrontal excitatory neurons (0 out of 2501), prefrontal GABAergic neurons (0 out of 1048), hippocampal GABAergic neurons (0 out of 452), and hippocampal Cornu Ammonis (CA) excitatory neurons (0 out of 1166).

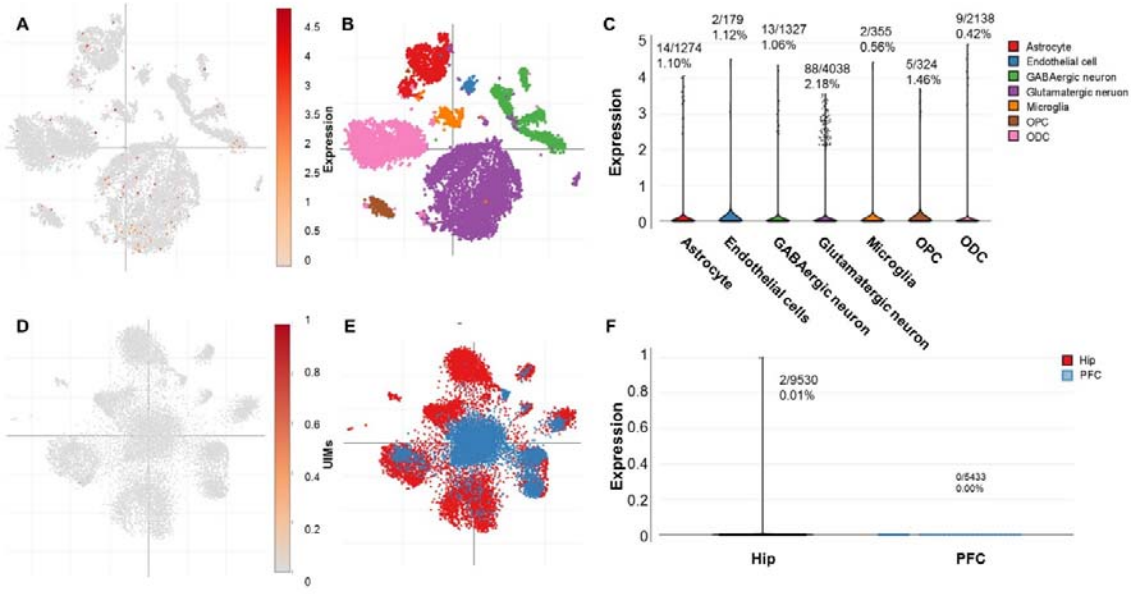

Figure 5. Expression of ACE2 in the human posterior cingulate cortex, prefrontal cortex and hippocampus. (**A**) Expression of ACE2 in the human posterior cingulate cortex. (**B**) The annotation map of cell clusters. (**C**) Expression of ACE2 in brain cell subtypes. (**D**) Expression of ACE2 in human prefrontal cortex and hippocampus. (**E**) The annotation map of brain regains, red points mean cells from the prefrontal cortex, and blue points mean cells from the hippocampus. (**F**) Expression of ACE2 in human prefrontal cortex and hippocampus. Original data are available from: https://singlecell.broadinstitute.org. Data are expressed as median, range and all sample points in C and F.

Cell-type distribution of Ace2 in the mouse brain.

In view of the difficulty in obtaining human brain samples, mousse brain was frequently used alternately. Thus, we also analyzed the expression of Ace2 receptors in the mouse brain.

According to the single-cell sequencing data of multiple mouse cortex (https://singlecell.broadinstitute.org/single_cell/study/SCP425/; Ding et al., 2019; **Figure. 6A-C**), the expression of Ace2 was found in endothelial cells (31 out of 403, 7.7%), pericytes (6 out of 29, 20.69%), OPC (3 out of 276, 1.1%), excitatory neurons (26 out of 4040, 0.64%), inhibitory neurons (15 out of 2472, 0.61%), microglia (1 out of 313, 0.32%), ODC (1 out of 798, 0.12%) and astrocytes (1 out of 1412, 0.07%), but none in other unassigned cells (0 out of 257).

Hippocampus and prefrontal cortex were important brain areas for learning and memory (Eichenbaum, 2017). According to the single-cell sequencing dataset of archived mouse brain samples (https://singlecell.broadinstitute.org/single_cell/study/SCP60; Habib et al., 2017; **Figure. 6D-F**), we found the expression of Ace2 is found in endothelial cells (61 out of 416, 14.66%), ODC (1 out of 502, 0.20%), prefrontal cortex excitatory neurons (12 out of 4452, 0.27%) and astrocytes (1 out of 927, 0.10%), but it was none in GABAergic neurons (0 out of 816), hippocampal excitatory neurons (0 out of 1602), smooth muscle cell (0 out of 183), microglia (0 out of 209), OPC (0 out of 186) and Choroid plexus cell (0 out of 28). Besides, in another single-cell sequencing data of mouse hippocampus (https://singlecell.broadinstitute.org/single_cell/study/SCP1; Cembrowski et al., 2016; **Figure. 6G-I**.), the Ace2 expression was found in one CA1 excitatory neuron (1 out of 155, 0.65%) and one glial cell (1 out of 88, 1.1%) in the mouse hippocampus. Besides, the Ace2 expression was not found in the CA2 neurons (0 out of 43), CA3 neurons (0 out of 72), DG neurons (0 out of 672), GABAergic neurons (0 out of 133) or ependymal cells (0 out of 25).

Substantia nigra was a high ACE2 expression area as suggest according to our spatial distribution analysis in both human and mouse brains. According to single-cell sequencing data of mouse substantia nigra pars reticulata (SNr), substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) areas (https://singlecell.broadinstitute.org/single_cell/study/SCP478; BICCN dataset proved by Huang et al. Available from: biccn.org/teams/u19-huang; **Figure. 6J-L**), the expression of Ace2 is found in many endothelial cells (81 out of 647, 12.5%), and some dopamine neurons (3 out of 811, 0.37%), inhibitory neurons (3 out of 2166, 0.21%), extra neurons (4 out of 1875, 0.14%), ODCs (3 out of 2492, 0.12%), and microglia/macrophages (1 out of 179, 0.56%), but none in astrocyte (0 out of 1547) and polydendrocyte cells (0 out of 283).

the expression of Ace2 in the cerebellum was inconsistent according to our spatial distribution analysis in human and mouse brains. According to single-cell sequencing data of mouse cerebellum (https://singlecell.broadinstitute.org/single_cell/study/SCP795; Kozareva et al., 2020; **Figure. 6M-O**), the expression of Ace2 was found in endothelial mural cells (33 out of 592, 5.5%), endothelial stalk cells (6 out of 592, 1.01%) and ODCs (3 out of 593, 0.51%), choroid cells (2 out of 591, 0.34), Golgi cells (2 out of 592, 0.34%), OPCs (2 out of

592, 0.34%), molecular layer type 2 interneurons (1 out of 592, 0.17%), Purkinje layer interneurons (2 out of 592, 0.17%), and Purkinje cells (1 out of 593, 0.17%). Ace2 expression was not found did in any astrocytes, Bergmann cells, ependymal cells, granule cells, molecular layer type 1 interneurons, macrophages, microglia, and unipolar brush cells.

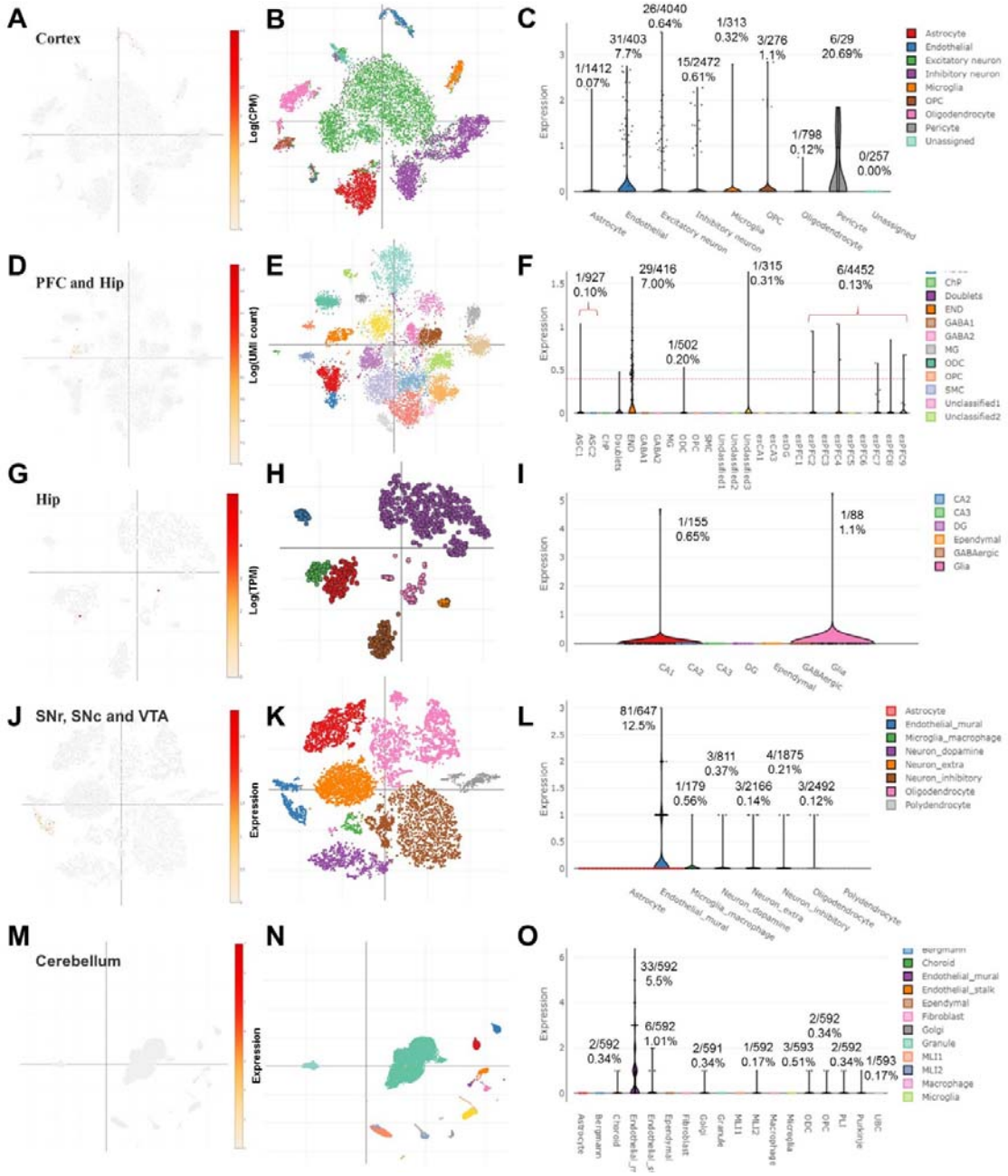

Figure 6. Cell-type distribution of Ace2 in the mouse brain. (**A-C**) Cell-type distribution of Ace2 in the mouse cortex. (**A**): Ace2 expression map; (**B**): the annotation map; (**C**) distribution plot. (**D-F**) Cell-type distribution of Ace2 in the mouse prefrontal cortex and

hippocampus. (**D**): Ace2 expression map; (**E**): the annotation map; (**F**) distribution plot. (**G-I**) Cell-type distribution of Ace2 in mouse hippocampus. (**G**): Ace2 expression map; (**H**): the annotation map; (**I**) distribution plot. (**J-L**) Cell-type distribution of Ace2 in mouse SNr, SNc, and VTA. (**J**): expression map; (**K**): the annotation map; (**L**) distribution plot. (**M-O**) Cell-type distribution of Ace2 in mouse cerebellum. (**M**): Ace2 expression map; (**N**): the annotation map; (**O**) distribution plot. ACS: astrocyte; ChP: choroid plexus cell; END: endothelial cells; Hip: hippocampus; MG: microglia; MLI: molecular layer interneuron; ODC: oligodendrocyte; OPC: Oligodendrocyte progenitor cell. PFC: prefrontal cortex; SMC: smooth muscle cell; SNr: substantia nigra pars reticulate; SNc: substantia nigra pars compacta; VTA: ventral tegmental area; UBC: unipolar brush cells. Data are expressed as median, range and all sample points in **C, F, I, L, and O**. Original data are available from: https://singlecell.broadinstitute.org.

As the inconsistent results were found in the hippocampus based on the single-cell sequencing, we additionally analyzed the expression of Ace2 in a cell-type sequencing database of mouse hippocampus, the Hippocampus RNA-seq atlas database (https://hipposeq.janelia.org; Cembrowski et al., 2016). As shown in **Figure 7**, we found: (1) Ace2 expression were only found in intermediate and ventral areas of the hippocampus with the mean FPKM<1 (**Figure 7A**); (2) Ace2 expression were found in ventral pyramidal cells but not in dorsal CA1 pyramidal cells, dorsal CA3 pyramidal cells, dorsal CA2 pyramidal cells, dorsal DG granule cells, dorsal DG mossy cells, ventral CA3 pyramidal cells and ventral DG granule cells (**Figure 7B**); (3) Ace2 expression were not found in PV-positive or SST-positive interneurons in the hippocampus (**Figure 7C**); (4) Ace2 expression were not found in any neurons that project to post-subiculum, NAc or amygdala (**Figure 7D**).

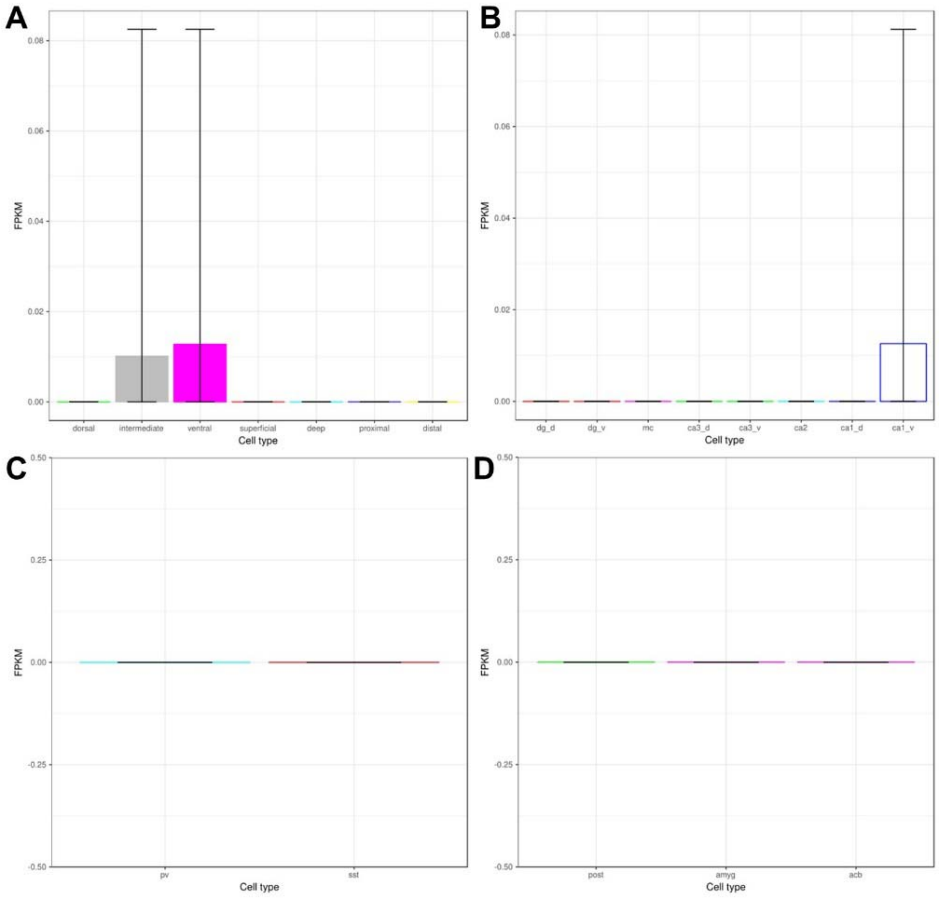

Figure 7. Spatial and cell-type distribution of ACE2 in the mouse hippocampus. (**A**) Spatial distribution of ACE2 in mouse hippocampus. (**B**) Spatial distribution of ACE2 in mouse hippocampal pyramidal cells and granule cells. dg_d: Dorsal DG granule cell; dg_v Ventral DG granule cell; mc: Dorsal DG mossy cell; ca3_d: Dorsal CA3 pyramidal cell; ca3_v: Ventral CA3 pyramidal cell; ca2: Dorsal CA2 pyramidal cell; ca1_d: Dorsal CA1 pyramidal cell; ca1_v: Ventral CA1 pyramidal cell (**C**) Cell-type distribution of ACE2 in mouse hippocampal interneurons. Pv: parvalbumin-expressing neurons; sst: somatostatin-expressing neurons. (**D**) Distribution of ACE2 in mouse hippocampal neurons that project to post-subiculum, nucleus accumbens or amygdala. post: post-subiculum projecting neuron; amyg: amygdala projecting neuron. NAc; nucleus accumbens projecting neuron. FPKM: Fragments Per Kilobase Million. Data are expressed as mean and 95%CI. Original data are available from: https://hipposeq.janelia.org (Cembrowski et al., 2016)

Discussion

ACE2 is an important entry receptor for SARS-CoV-2 and SARS-CoV infecting host organs (Hoffmann et al., 2020). Though the infection of SARS-COV in the brain was reported in the past (Gu et al., 2005; Xu et al., 2005), the distribution of ACE2 in the brain is still unclear. Here we mainly found: (1) The expression of ACE2 was relatively high in several specific brain areas in human, such as such as the substantia nigra and brain ventricles; (2) the expression of ACE2 located in many neurons (both excitatory and inhibitory neurons) and some non-neuron cells (mainly astrocytes and ODCs) in human middle temporal gyrus and posterior cingulate cortex, but the ACE2-expressing cells was none in PFC and very few in hippocampus; (3) Except for the additional expression of ACE2 in the olfactory bulb areas for spatial distribution and the pericytes and endothelial cells for cell-type distribution analysis, the main distribution figure of ACE2 in mouse brain was similar to that in human. Thus, our results reveal an outline of ACE2 distribution in the human and mouse brain, which support the hypotheses that the SARS-CoV-2 is capable to infect the brain and may result in serious CNS symptoms in COVID-19 patients (Kabbani and Olds, 2020).

SARS-CoV-2 shares a 77.2% amino acid identity, 72.8% sequence identity and high structural similarity to previous SARS-CoV (Brielle et al., 2020; Wu et al., 2020). Similar to SARS-CoV, experimental affinity measurements show a high affinity of the receptor-binding domain of SARS-CoV-2 and ACE2 (Brielle et al., 2020; Lu et al., 2020). Here we found the expression ACE2 was relatively highly expressed in some special brain regions in human (**Figure 2**), though the total expression of ACE2 is low and similar in most brain areas. Even more, the percent of ACE2 positive samples of substantial nigra was almost comparable to that of lung (3.60% vs 6.57%), though the total expression in substantial nigra seems much lower than that in the lung (**Figure 1A and B**). Besides, the total expression of ACE2 seems not change with age (**Figure 1C**). Thus, our results highlight the importance of spatial distribution rather than general total expression of ACE2 in the brain.

For the spatial distribution of ACE2 in human brain, we found ACE2 may be relatively high (Z score>1) expressed in many important brain nuclei as follows: (1) brain areas where located the neural cell bodies of different neuromodulators, including dopaminergic nuclei (midbrain reticular formation, VTA and substantia nigra), serotoninergic nuclei (midbrain raphe nuclei) (Pollak Dorocic et al., 2014), histaminergic nuclei (tuberomammillary nucleus,

TM) (Hu and Chen, 2017), and norepinephrinergic nuclei (locus ceruleus) (Wood and Valentino, 2017); (2) Brain areas participating in important physiologic functions, including posterior hypothalamic area (involved in the control of the sleep-wake cycle, cardiovascular regulation and the expression of defensive-aggressive behavior)(Katagiri et al., 2013), paraventricular nuclei of thalamus (involved in the control of wakefulness, feeding, appetitive motivation, drug addiction, regulation of stress and negative emotional behavior, and epilepsy)(Chen et al., 2020; Ren et al., 2018), paraventricular nucleus of the hypothalamus (neuroendocrine neurons regarding oxytocin, vasopressin, corticotropin-releasing hormone, thyrotropin-releasing hormone)(Qin et al., 2018), and lateral hypothalamic area (the central regulation of hunger, thirst, rewarding, and autonomic nervous system) (Stuber and Wise, 2016); (3) Other brain areas, including amygdalo-hippocampal transition area (related to fear expression) (Fujisaki et al., 2004), hippocampal CA2 field (related to learning and memory) (Dudek et al., 2016), fastigial nucleus (related to body and eye movements) (Zhang et al., 2016), and piriform cortex (related to the sense of smell and epilepsy) (Cheng et al., 2020). Thus, our results may provide some clues to further study on the brain infection of SARS-CoV-2 in the COVID-19 patients, and suggesting SARS-CoV-2 might be able to result in serious CNS symptoms in COVID-19 patients (if it could infect these important brain areas by binding ACE2). Brain imaging and long-term follow up may be needed in COVID-19 patients for the possibility of SARS-CoV-2 brain infection and the following brain disorders.

The routes or pathways for SARS-CoV and novel SARS-CoV-2 entering the brain are still unclear. According to experiments in mice transgenic for human ACE2, intranasally given SARS-CoV may enter the brain by olfactory nerves (Netland et al., 2008). Consistent with this, we found the expression of ACE2 in the olfactory bulb is higher than that in most other cortexes (Figure 3). In human brain, we found piriform cortex, a brain area directly connected with olfactory bulb, were ACE2 high-expression. Though no ACE2 expression data of olfactory bulb in human was available, our results indirectly support the hypothesis that SARS-CoV-2 might enter the human brain by olfactory nerves. On the other hand, we additionally found the central glial substance and choroid plexus of lateral ventricle were with very high ACE2 expression (Z score > 5) in the human brain. Relatively high expression of ACE2 in choroid plexus of lateral ventricle was also found in the mouse brain in current

study. The central glial substance refers to an area of grey matter surrounding the central canal, which carries CSF and helps to transport nutrients to the spinal cord (Thouvenin et al., 2020); Besides, the choroid plexus of ventricles is an important brain area for the generation of CSF (Lun et al., 2015), the main location of the blood-cerebrospinal fluid barrier (Ghersi-Egea et al., 2018), and a crucial gateway for immune cells entering the brain (Valente et al., 2019). Recently, the SARS-CoV-2 has also been found in cerebrospinal fluid (CSF) sample from a 56-year-old COVID-19 patient by genetic sequencing in China (http://www.ecns.cn/news/society/2020-03-05/detail-ifzuhesu4119860.shtml). SARS-CoV-2 may also infect the brain in a Japanese male patient (https://www3.nhk.or.jp/nhkworld/en/news/20200308_07). Thus, our results suggest the high expression of ACE2 in the central glial substance and ventricles may provide another potential pathway for the SARS-CoV-2 or SARS-CoV entering CSF and/or spreading around the brain.

Single-nucleus RNA-seq provides a high resolution of cellular gene-expression of each individual cell (Grun and van Oudenaarden, 2015). According to single-nucleus RNA-seq data, we further found that ACE2 located in many neurons (especially excitatory neurons) and some non-neuron cells (especially astrocytes and ODCs) in both posterior cingulate cortex and middle temporal gyrus, where the expression level of ACE2 seems low under normal conditions. Here, the highest number of ACE2 positive cells were excitatory neurons, which may be projection neurons that make up many important brain networks. For example, excitatory neurons in the posterior cingulate cortex may project dense connections to the hippocampal formation and parahippocampal cortex, which are related to emotion and memory (Leech and Sharp, 2014). On the other hand, though the positive cell number of inhibitory neurons is lower than excitatory neurons (**Figure 4C**), the percentage of positive cells in some sub-types inhibitory neuron were comparable or slightly low compared with excitatory neurons (**Figure 4D**). Inhibitory neurons are also crucial for normal brain function (Cardin, 2018). Especially, the neurons in SNr are mainly inhibitory GABAergic neurons, which is one important note in the neural circuits that contribute to epilepsy (Chen et al., 2020). In addition, we also found some dopaminergic neurons and cerebellar cells in the mouse brain are also ACE2-positive. Thus, our results may help to explain the previous

finding that SARS-CoV particles are mainly located in the neurons in the brain samples from SARS patients (Gu et al., 2005), and suggest SARS-CoV-2 may also invade many neurons in the human brain and hence contribute to the CNS symptoms in COVID-19 patients.

In addition, different from previous microarray finding that ACE2 expressed in the endothelium (Hamming et al., 2004), single-nucleus RNA-seq data showed that the ACE2 was none or low expression in endothelial cells in the human brain. Endothelial cells are the main component of blood vessel endothelium. Thus, one possible reason for these differences could be that the blood vessels were probably removed from the tested human brain tissues. This reason may also explain why endothelial cells and pericytes were the two highest ACE2-expression cell types in the mouse brain, where the blood vessels may be hard to be removed. Both endothelial cells and pericytes are closely related to the blood-brain barrier integrity (Daneman et al., 2010). Endothelial cells and pericytes could also build blocks of the neurovascular unit together with astrocytes and neurons (Sweeney et al., 2016). Thus, it is possible endothelial cells and pericytes may help the SARS-CoV-2 to enter the brain by crossing the BBB and also infect the neurovascular unit, which may contribute to the cerebrovascular events in COVID-19 patients (Khosravani et al., 2020). The CSF biomarkers of pericytes injury and blood-brain barrier integrity, such as sPDGFRβ and albumin respectively (Miners et al., 2019), may be needed to detect whether SARS-CoV-2 damaged the BBB in COVID-19 patients in future studies.

Conclusion

Our results reveal an outline of ACE2 or Ace2 distribution in the human and mouse brain, which indicates the brain infection of SARS-CoV-2 may be capable to infection the brain and result in serious CNS symptoms in COVID-19 patients. The finding of high ACE2 expression of in central glial substance and brain ventricles suggest two potential novel routes for the SARS-CoV-2 entering the CSF and/or spreading around the brain. In addition, the differences of ACE2/Ace2 distribution between human and mouse may be also useful to further “bench to bedside” translational studies regarding SARS-CoV-2. Our results may help to bring light to the brain infection of the present novel SARS-CoV-2 and previous SARS-CoV. Further studies are warranted to confirm our results and related predictions.

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Competing interests

None declared.

Authors' contributions

Z.X. and C.W. designed the study. R.C. and J.Y. performed the search and analysis. Z.X. and K.W. checked the analyzed data. Z.X. wrote the manuscript in consultation with J.Y., Z.C. and C.W.

References

Brielle, E. S., et al., 2020. The SARS-CoV-2 exerts a distinctive strategy for interacting with the ACE2 human receptor. bioRxiv. 2020.03.10.986398.

Cardin, J. A., 2018. Inhibitory Interneurons Regulate Temporal Precision and Correlations in Cortical Circuits. Trends Neurosci. 41, 689-700.

Cembrowski, M. S., et al., 2016. Hipposeq: a comprehensive RNA-seq database of gene expression in hippocampal principal neurons. Elife. 5, e14997.

Chen, B., et al., 2020. A disinhibitory nigra-parafascicular pathway amplifies seizure in temporal lobe epilepsy. Nat Commun. 11, 923.

Cheng, H., et al., 2020. The piriform cortex in epilepsy: What we learn from the kindling model. Exp Neurol. 324, 113137.

Consortium, G. T., 2015. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. Science. 348, 648-60.

Daneman, R., et al., 2010. Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature. 468, 562-6.

Ding, J., et al., 2019. Systematic comparative analysis of single cell RNA-sequencing methods. bioRxiv. 632216.

Dudek, S. M., et al., 2016. Rediscovering area CA2: unique properties and functions. Nat Rev Neurosci. 17, 89-102.

Eichenbaum, H., 2017. Prefrontal-hippocampal interactions in episodic memory. Nat Rev Neurosci. 18, 547-558.

Fujisaki, M., et al., 2004. Role of the amygdalo-hippocampal transition area in the fear expression: evaluation by behavior and immediate early gene expression. Neuroscience. 124, 247-60.

Gaublomme, J. T., et al., 2019. Nuclei multiplexing with barcoded antibodies for single-nucleus genomics. Nat Commun. 10, 2907.

Ghersi-Egea, J. F., et al., 2018. Molecular anatomy and functions of the choroidal blood-cerebrospinal fluid barrier in health and disease. Acta Neuropathol. 135, 337-361.

Glass, W. G., et al., 2004. Mechanisms of host defense following severe acute respiratory syndrome-coronavirus (SARS-CoV) pulmonary infection of mice. J Immunol. 173, 4030-9.

Gonzalez-Castellano, I., et al., 2019. De novo gonad transcriptome analysis of the common littoral shrimp Palaemon serratus: novel insights into sex-related genes. BMC Genomics. 20, 757.

Grun, D., van Oudenaarden, A., 2015. Design and Analysis of Single-Cell Sequencing Experiments. Cell. 163, 799-810.

Gu, J., et al., 2005. Multiple organ infection and the pathogenesis of SARS. J Exp Med. 202, 415-24.

Habib, N., et al., 2017. Massively parallel single-nucleus RNA-seq with DroNc-seq. Nat Methods. 14, 955-958.

Hamming, I., et al., 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 203, 631-7.

Hawrylycz, M. J., et al., 2012. An anatomically comprehensive atlas of the adult human brain transcriptome. Nature. 489, 391-399.

Hoffmann, M., et al., 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell.

Hu, W., Chen, Z., 2017. The roles of histamine and its receptor ligands in central nervous system disorders: An update. Pharmacol Ther. 175, 116-132.

Johnson, M. B., et al., 2009. Functional and evolutionary insights into human brain development through global transcriptome analysis. Neuron. 62, 494-509.

Kabbani, N., Olds, J. L., 2020. Does COVID19 infect the brain? If so, smokers might be at a higher risk. Mol Pharmacol.

Katagiri, A., et al., 2013. Posterior hypothalamic modulation of light-evoked trigeminal neural activity and lacrimation. Neuroscience. 246, 133-41.

Keil, J. M., et al., 2018. Brain Transcriptome Databases: A User's Guide. J Neurosci. 38, 2399-2412.

Khosravani, H., et al., 2020. Protected Code Stroke: Hyperacute Stroke Management During the Coronavirus Disease 2019 (COVID-19) Pandemic. Stroke. STROKEAHA120029838.

Kozareva, V., et al., 2020. A transcriptomic atlas of the mouse cerebellum reveals regional specializations and novel cell types. bioRxiv. 2020.03.04.976407.

Leech, R., Sharp, D. J., 2014. The role of the posterior cingulate cortex in cognition and disease. Brain. 137, 12-32.

Lein, E. S., et al., 2007. Genome-wide atlas of gene expression in the adult mouse brain. Nature. 445, 168-76.

Li, Y. C., et al., 2020. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. J Med Virol.

Liu, Y., et al., 2020. Secondary attack rate and superspreading events for SARS-CoV-2. Lancet. 395, e47.

Lu, R., et al., 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 395, 565-574.

Lun, M. P., et al., 2015. Development and functions of the choroid plexus-cerebrospinal fluid system. Nat Rev Neurosci. 16, 445-57.

Mao, L., et al., 2020. Neurological Manifestations of Hospitalized Patients with COVID-19 in Wuhan, China: a retrospective case series study. medRxiv. 2020.02.22.20026500.

Martin, D., et al., 2017. Neurocytometry: Flow Cytometric Sorting of Specific Neuronal Populations from Human and Rodent Brain. ACS Chem Neurosci. 8, 356-367.

Miners, J. S., et al., 2019. CSF evidence of pericyte damage in Alzheimer's disease is associated with markers of blood-brain barrier dysfunction and disease pathology. Alzheimers Res Ther. 11, 81.

Mortazavi, A., et al., 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nat Methods. 5, 621-8.

Netland, J., et al., 2008. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. J Virol. 82, 7264-75.

Pollak Dorocic, I., et al., 2014. A whole-brain atlas of inputs to serotonergic neurons of the dorsal and median raphe nuclei. Neuron. 83, 663-78.

Qin, C., et al., 2018. The Paraventricular Nucleus of the Hypothalamus: Development, Function, and Human Diseases. Endocrinology. 159, 3458-3472.

Ren, S., et al., 2018. The paraventricular thalamus is a critical thalamic area for wakefulness. Science. 362, 429-434.

Stuber, G. D., Wise, R. A., 2016. Lateral hypothalamic circuits for feeding and reward. Nat Neurosci. 19, 198-205.

Sweeney, M. D., et al., 2016. Pericytes of the neurovascular unit: key functions and signaling pathways. Nat Neurosci. 19, 771-83.

Tasic, B., et al., 2016. Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. Nat Neurosci. 19, 335-46.

Thouvenin, O., et al., 2020. Origin and role of the cerebrospinal fluid bidirectional flow in the central canal. Elife. 9.

Valente, L. A., et al., 2019. Updating Neuroimmune Targets in Central Nervous System Dysfunction. Trends Pharmacol Sci. 40, 482-494.

Walls, A. C., et al., 2020. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell.

Wood, S. K., Valentino, R. J., 2017. The brain norepinephrine system, stress and cardiovascular vulnerability. Neurosci Biobehav Rev. 74, 393-400.

Wu, F., et al., 2020. A new coronavirus associated with human respiratory disease in China. Nature. 579, 265-269.

Xia, H., et al., 2013. Brain angiotensin-converting enzyme type 2 shedding contributes to the development of neurogenic hypertension. Circ Res. 113, 1087-1096.

Xu, J., et al., 2005. Detection of severe acute respiratory syndrome coronavirus in the brain: potential role of

the chemokine mig in pathogenesis. Clin Infect Dis. 41, 1089-96.

Yan, R., et al., 2020. Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2. Science.

Zhang, X. Y., et al., 2016. Cerebellar fastigial nucleus: from anatomic construction to physiological functions. Cerebellum Ataxias. 3, 9.