# The distinct characteristics of somatostatin neurons in the human brain

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# Abstract

Somatostatin cells are frequently described as a major population of GABAergic neurons in the cerebral cortex, however, a comprehensive analysis of their molecular expression, morphological features and laminar distribution has not yet been performed. In this study, we provided a detailed description of somatostatin neurons in the human prefrontal cortex, including their proportion in the total neuron population, laminar distribution, neurotransmitter phenotype as well as their molecular and morphological characteristics using immunofluorescence and RNAscope *in situ* hybridization. We found that somatostatin neurons comprise around 7% of neocortical neurons in the human Brodmann areas 9 and 14r, without significant difference between the two regions. Somatostatin cells were NeuN positive and synthesized vesicular GABA transporter and glutamate decarboxylase 1 and 2, confirming their neuronal nature and GABAergic phenotype. Somatostatin cells in the upper cortical layers were small, had a high expression of somatostatin mRNA, a relatively low expression of somatostatin peptide and co-expressed calbindin. In the lower cortical layers, somatostatin cells were larger with complex somato-dendritic morphology, typically showed a lower expression of somatostatin mRNA and a high expression of somatostatin peptide, and co-expressed neuronal nitric oxide synthase (nNOS), but not calbindin. Somatostatin neurons in the white matter co-expressed MAP2. Based on their somato-dendritic morphology, cortical somatostatin neurons could be classified into at least five subtypes. The somatostatin neurons of the human prefrontal cortex show remarkable morphological and molecular complexity with substantial differences compared to rodents, both of which imply a unique function in the human brain.


# Introduction

Somatostatin (SOM) is a peptide encoded by the *SST* gene. Its isoform somatostatin-14 is expressed in a major subset of GABAergic neurons [1, 2] and is involved in the modulation of cortical microcircuits, which is essential for regulating higher cognitive functions [3–9]. $SOM^+$ neurons play an important role in spatial working memory by selectively modulating pyramidal neurons [10]. In addition, due to their involvement in synaptic plasticity, they represent an important component in the processes of learning [10].

Reduced levels of SOM expression have been reported in several neuropsychiatric and neurodegenerative disorders, such as major depressive disorder, schizophrenia, bipolar disorder and Alzheimer's disease[10, 11] and $SOM^+$ neurons appear to play an important role in the pathogenesis these disorders. Nevertheless, the exact causes of reduced SOM expression in these disorders remain largely unknown. Most of these disorders affect the prefrontal cortex (PFC), which plays a key role in the delicate integration of relevant information from other regions of the brain. It is possible that even smaller alterations to a particular subpopulation of $SOM^+$ neurons could potentially lead to serious dysfunction of the PFC. Therefore, providing a detailed understanding the molecular organization of $SOM^+$ neurons in the human PFC is of great importance and would enable determining the intricate physiological roles of

SOM$^+$ neurons. Furthermore, SOM and its analogs may have potential as future pharmacological treatments for brain disorders characterized by reduced SOM expression, which gives even more incentive to focus research on SOM$^+$ neurons [10, 11]. Nevertheless, data on the molecular characterization of SOM$^+$ neurons in the human PFC is severely lacking.

In general, the calcium-binding proteins parvalbumin (PV), calbindin (CB) and calretinin (CR) are the most common markers used to define the major populations of GABAergic neurons [12], though other molecular, morphological and electrophysiological classification criteria are also described [5, 13–15]. In most classifications, SOM immunolabelling is considered to visualize the vast majority of CB$^+$ neurons as well as additional GABAergic neurons that are not visualized by CB immunolabelling. This is why SOM is typically considered a more eligible marker that encompasses a larger subset of cortical GABAergic neurons than CB [9] – in essence CB$^+$ neurons are increasingly being regarded as merely a subpopulation of SOM$^+$ neurons and are rarely included in newer interneuron classifications, especially in rodents [15]. However, it is important to accentuate that this classification has never been explicitly confirmed in the primate brain and especially not in humans. This means that inferences on the GABAergic cortical network are often done by attempting to extrapolate data from rodents, i.e. descriptions of cortical network connectivity (and conclusions on its role in the pathogenesis of human diseases) are heavily reliant on whether the rodent model is correct for the human brain.

Based on their morphology, SOM$^+$ neurons are typically classified into two major subpopulations – Martinotti and non-Martinotti cells [16–19]. Martinotti cells have an ascending axon with synaptic targets located in layer I of the cerebral cortex, where they form significant axonal plexuses [20, 21]. There, the axons of Martinotti cells establish synapses with the terminal branches of the apical dendrites of principal cells. Unlike Martinotti cells that have a distinct axonal morphology with clearly defined synaptic targets, non-Martinotti cells are simply defined as all SOM$^+$ cells that lack significant axonal plexuses in layer I [20]. Besides their intriguing morphology, studies in rodents revealed that a portion of SOM + neurons co-express (to varying degrees) different molecular markers [22], including CR [15], CB [23, 24], neuropeptide Y (NPY) [24], cholecystokinin (CCK) [25], nNOS [16, 17, 24, 25], and substance P receptor (SPR) [18, 26].

In most species, SOM$^+$ cell bodies are present in all cortical layers, except layer I. In primates, SOM$^+$ cells have been described as less densely packed than in the rat cortex and as being located in superficial and deep bands in the cerebral cortex [27]. In the PFC of the cynomolgus monkey (*Macaca fascicularis*), SOM$^+$ cell bodies were found in layers II – III and V – VI [28]. In the human brain, their laminar distribution has been described as uneven, with some studies describing them only in layers IV – VI [29], others describing them as predominantly present in layers II and III [30], and yet others describing them in all cortical layers, including in layer I [31, 32]. In general, previous studies on SOM$^+$ neurons in humans used different methodology (either only immunohistochemistry or only RNA *in situ* hybridization), analyzed different cortical regions (though none of them focused on the PFC) and assessed varying pathologies, while coming to somewhat conflicting findings. In order to resolve these discrepancies in literature, it is

necessary to conduct systematic studies that first assess $SOM^+$ neurons in non-pathological tissue utilizing multiple complementary histological methods.

The role of $SOM^+$ neurons in the human brain is still understudied and their laminar distribution as well as morphological and molecular features in the human brain are not resolved. In particular, no studies so far addressed the molecular expression, morphological features and laminar distribution of $SOM^+$ neurons in the human PFC. Therefore, the aim of this paper is to give a comprehensive molecular and morphological characterization of $SOM^+$ neurons in phylogenetically and functionally entirely different cortical areas (Brodmann areas 9 and 14r) of the human PFC using novel histological methodology combining immunofluorescence and RNAscope *in situ* hybridization.

# Materials And Methods

### Brain tissue samples

The brain samples of five male human subjects aged from 29 to 51 years with a postmortem delay of 6 to 11 hours were analyzed in this study (Table S1).

The subjects had no medical history of neurological or psychiatric disorders and no neuropathological deviations in the brain on autopsy. Relevant medical history was obtained from both autopsy reports and medical records. All analyzed subjects died without preagonal state, and the postmortem delay represents the actual interval of neuron death. The brain tissue is a part of the Zagreb Neuroembryological Collection. It was obtained with the approval of the Ethics Committee of Zagreb University School of Medicine (380-59-10106-14-55/152). The information on the subject's identity and history is stored in secure records, and the brain tissue is given a code indicating only the subject's age.

The brain tissue was cut into blocks following Talairach's coordinates [33]. Tissue blocks of the dorsolateral and ventral prefrontal cortex (dlPFC and vPFC) containing the superior frontal gyrus (Brodmann area 9, BA9) and straight gyrus (Brodmann area 14r, BA14r) respectively were selected for double labelling immunofluorescence [34]. BA9 and BA14r were delineated on NeuN immunolabelled sections according to relevant literature [35–37]. Cortical layers were also distinguished using NeuN immunolabelled sections.

The tissue was first fixed by immersion in 4% paraformaldehyde for 24 h, then dehydrated in an ethanol cascade (70%, 96%, 100%), embedded in paraffin, kept in toluene [38] and finally cut on a microtome into 20 µm thick coronal slices [39] and mounted on VitroGnost Plus Ultra adhesive microscope slides (BioGnost, Zagreb, Croatia).

### Double labelling immunofluorescence

Double labelling immunofluorescence was performed according to standard protocols for paraffin-embedded tissue [40]. Histological sections were first photobleached for 48 h using a LED light source[41, 42] in order to reduce autofluorescence. The sections were deparaffinized and heat antigen retrieval was

performed in citrate-based (pH 6.0) unmasking solution [43]. Next, protein blocking was done by incubating the sections for 1 h at room temperature (RT) in normal donkey serum (NDS; Chemicon, USA) diluted 1:30 in permeabilization solution (0.3% Triton X-100 in 1X PBS; Sigma-Aldrich, USA). Afterwards the sections were incubated overnight in primary antibodies (Table S2) at 4°C and in secondary antibodies (Table S2) for 2 h at RT. The sections were treated with TrueBlack® Lipofuscin Autofluorescence Quencher (Biotium, USA) to further reduce autofluorescence[44] and coverslipped with VECTASHIELD® Antifade Mounting Medium (Vector Laboratories, USA).

Somatostatin antibodies were used in combination with all other primary antibodies listed in Table S2. Furthermore, the calbindin antibody was used in combination with the mouse monoclonal NeuN antibody.

### **RNAscope** in situ **hybridization**

The expression of mRNA in the tissue samples was evaluated using RNAscope *in situ* hybridization (Advanced Cell Diagnostics, USA). The RNAscope® Fluorescent Multiplex Assay (Advanced Cell Diagnostics) was used to detect multiple target mRNAs. A standard protocol, according to the manufacturer's instructions (Advanced Cell Diagnostics) was used [45–47]. Pretreatment included deparaffinization, target retrieval with Target Retrieval Reagent, and incubation in Protease Plus reagent (Advanced Cell Diagnostics). The slides were then incubated in the appropriate Probe Mix reagent (Table S3) followed by successive amplifier (AMP) reagents (Advanced Cell Diagnostics). Depending on the probes used, visualization was done using channel appropriate dyes (TSA™ Plus Fluorescein, TSA™ Plus Cyanine 3 System and/or TSA™ Plus Cyanine 5 System; Akoya Biosciences, Mariborough, MA, USA).

After RNAscope *in situ* hybridization, single labelling immunofluorescence was performed on the same slides, according to the previously described protocol.

Histological sections were imaged using a laser confocal microscope (Olympus FLUOVIEW FV3000RS, Japan) on high power magnification and using Z-stack in order to visualize the entire section thickness.

### **Quantification of immunoreactive cells**

Quantification of immunoreactive cells was done using Neurolucida 2020 (MBF Bioscience, Williston, Vermont, USA) and Neurolucida Explorer (MBF Bioscience) on confocal images of double labelled immunofluorescent histological slides (NeuN/SOM and NeuN/CB immunolabeling). NeuN immunolabeling was used to delineate the cortical layers in Neurolucida. During analysis, layer I was further divided into layer Ia (superficial part of layer I) and layer Ib (deep part of layers I). From each brain specimen (n = 5) at least three slides were analyzed using the *Detect cells* function in Neurolucida software.

The results of the quantification are presented as: a) the number of immunolabelled neurons per countour surface (further: surface density) and b) the proportion of $SOM^+$ and $CB^+$ neurons within the total cortical neuron population (defined as the total number of NeuN immunolabelled cells).

### Morphometric analysis

Morphometric analysis of $SOM^+$ cells was done using Neurolucida 2020 and Neurolucida Explorer. The cell bodies of $SOM^+$ cells were reconstructed on confocal images using the following criteria: the cell body was visible in its entirety and the most proximal parts of the cell's processes could be identified. A total of 552 $SOM^+$ cell bodies were chosen for reconstruction (278 from BA9 and 274 from BA14r). The following morphometric parameters were analyzed: *Area*, *Aspect ratio* and *Roundness*.

### Statistical analysis

The statistical analysis was done using GraphPad Prism, version 9.2.0 (GraphPad Software, La Jolla, USA).

Data referring to different brains (proportions and surface density) as well as morphometric parameters for which a normal distribution was determined to be more likely (*Aspect ratio* and *Roundness*) are shown as arithmetic mean ± standard deviation (SD).

Morphometric parameters for which a lognormal distribution was determined to be more likely (Area) are shown as geometric mean ×÷ geometric standard deviation factor (GSDF).

In subsequent statistical analysis, data from the same brain were paired. A *P*-value of less than 0.05 was considered to be statistically significant for all statistical tests.

The paired t-test was used to test the differences between the proportions and surface densities of somatostatin neurons between BA9 and BA14r.

Welch's t-test was used to test the differences in *Roundness* and *Aspect ratio* between supragranular and infragranular layer somatostatin neurons. Mann Whitney's test was used to test the differences in cell body *Area*.

# Results

There were no observable qualitative differences between Brodmann area 9 (BA9) and Brodmann area 14r (BA14r), and quantitative analysis confirmed no significant differences between these two regions. Therefore, the results represent pooled data from both regions of the PFC, unless stated otherwise.

### $SOM^+$ cells are GABAergic neurons present in cortical layers II – VI as well as in the white matter (WM)

$SOM^+$ cells in all cortical layers co-expressed NeuN (Figure S1A). Both immunofluorescence and RNA *in situ* hybridization confirmed that somatostatin neurons are present in cortical layers II – VI as well as in the superficial white matter (WM) (Fig. 1A). On average, $SOM^+$ neurons represented 6.83 ± 1.46% of the total neuron population. Somatostatin fibers were generally highly immunoreactive and present in all cortical layers and the superficial white matter (Fig. 1B), but were most abundant in layers I, IV and VI.

The proportion of $SOM^+$ neurons within the total neuron population was mostly uniformly distributed across cortical layers II – VI (Fig. 1C), while their surface density was almost twice as high in layers II and IV compared to other cortical layers (Fig. 1D).

RNA *in situ* hybridization confirmed the GABAergic neurotransmitter phenotype of $SOM^+$ neurons as they expressed *SLC32A1* (VGAT) as well as *GAD1* (GAD67) and *GAD2* (GAD65) mRNA, but did not express *SLC17A7* (VGLUT1) mRNA (Fig. 1E and Figure S1B).

$SOM^+$ neurons were also present in the superficial white matter, where they were more abundant than any other analyzed interneuron population (CR, PV or CB).

### $CB^+$ cells are GABAergic neurons present predominantly in supragranular cortical layers

In contrast to $SOM^+$ cells, $CB^+$ cells were significantly more abundant in supragranular than infragranular layers (Figure S1C). As with $SOM^+$ cells, all $CB^+$ interneurons co-expressed NeuN as well as the GABAergic markers *SLC32A1*, *GAD1* and *GAD2*. $CB^+$ neurons were only sporadically present in the deep parts of layer I.

### $SOM^+$ neurons co-express CB predominantly in supragranular cortical layers

Double labelling immunofluorescence revealed that in layers V and VI and the WM, $SOM^+$ and $CB^+$ neurons represented two almost separate neuronal populations, while in layers II and III almost all cells that expressed SOM co-expressed CB (Fig. 2A and Figure S1D). Overall, throughout all cortical layers, 67.73% of $CB^+$ interneurons co-expressed SOM, and 49.94% of $SOM^+$ neurons co-expressed CB.

SOM/CB double labelling revealed three neuron subpopulations with characteristic laminar distributions. $SOM^+/CB^+$ cells were the most abundant in layers II and III, $SOM^+/CB^-$ cells being most abundant in layers IV – VI, and $SOM^-/CB^+$ cells being most abundant in layers II and VI (Fig. 2B).

Co-expression of SOM and CB in the supragranular layers was also confirmed using RNA *in situ* hybridization (Fig. 2C).

### The expression of SST mRNA and SOM peptide differs between supragranular and infragranular layer neurons

Evaluation of somatostatin mRNA and peptide (using RNA *in situ* hybridization and immunofluorescence) revealed that layer II and III $SOM^+$ neurons typically had a very high expression of *SST* mRNA, but a comparatively lower expression of SOM peptide (Fig. 2D). The reverse was true for layer V and VI as well as the WM, where $SOM^+$ neurons had a low to medium expression of *SST* mRNA, but a comparatively higher expression of SOM peptide (Fig. 2D). These findings were consistent in all analyzed subjects and when using different combination of markers. Occasionally, individual cells with both high *SST* mRNA and high peptide expression were observed (Fig. 2E).

### There are morphological differences between supragranular and infragranular $SOM^+$ neurons

Morphological analysis revealed that most $SOM^+$ neurons could be classified into one of five different types based on their size and cell body shape and their laminar position (for detailed overview see Table 1 and Figure S2A).

Table 1

Morphological types of $SOM^+$ neurons in the human PFC and laminar diversity of $SOM^+$ neurons.

| **Morphological types of SOM + neurons** | | | | |
|---|---|---|---|---|
| **Morphological type** | **Immunoreactivity** | **Cell body size** | **Cell body shape** | **Laminar distribution** |
| 1 | medium | medium | slightly elongated, oval | layers II – VI |
| 2 | low | small | circular | layer II (predominantly) and layer III |
| 3 | low | very small | circular | layer IV (almost exclusively), layer V (occasionally only in the most superficial parts) |
| 4 | high | very large | extremely elongated | layers V – VI and superficial WM (predominantly), layer III (sporadically) |
| 5 | high | large | pleomorphic | layers V – VI and superficial WM |
| **Laminar diversity of $SOM^+$ neurons** | | | | |
| **Cortical layer** | **Morphological cell type** | | **Co-expression of other molecular markers** | **Immunoreactivity and** SST **mRNA expression** |
| I | predominantly nerve fibers, cell bodies only sporadically found | | CB (sporadically) | - |
| II | predominantly type 2, type 1 also present | | CB (high) | low – medium immunoreactivity; high mRNA expression |
| III | predominantly types 1 and 2, type 4 sporadically found | | CB (high) | |
| IV | predominantly type 3, type 1 also present | | CB (low – medium) | low immunoreactivity: medium mRNA expression |
| V | predominantly types 1 and 4, types 3 (at the border with layer IV) and 5 also present | | nNOS (high) | high immunoreactivity; low – medium mRNA expression |
| VI | predominantly types 1, 4 and 5 | | nNOS (high) | |
| WM | exclusively types 4 and 5 | | nNOS (high), MAP2 (medium – high) | |

The axons of a substantial portion of $SOM^+$ neurons likely arborized in layer I where somatostatin fibers were particularly abundant.

The differences in morphometric parameters between supragranular and infragranular layers $SOM^+$ neurons were particularly pronounced and statistically significant ($P < 0.0001$ for all analyzed morphometric parameters) (Figure S2B, S2C and S2D) confirming that supragranular and infragranular $SOM^+$ neurons differ not only in their molecular expression, but also in their morphological properties.

**Some $SOM^+$ neurons co-express specific molecular markers**

Double labelling immunofluorescence further revealed that almost all infragranular layer and WM $SOM^+$ neurons co-expressed nNOS (Fig. 3A), unlike in supragranular layers where the level of co-expression was low. Furthermore, $SOM^+$ neurons in the WM also frequently co-expressed MAP2 (Fig. 3B), however, practically no MAP2 co-expression was observed in supragranular and infragranular cortical layers. No co-expression was observed between SOM and CR (Fig. 3C), PV or SMI-32 (Figure S3).

# Discussion

In this study, we provided a detailed description of $SOM^+$ GABAergic neurons in the human PFC, including their proportion in the total neuron population, laminar distribution, neurotransmitter phenotype as well as their molecular and morphological characteristics.

**The laminar distribution of $SOM^+$ neurons is uniform across ventral and dorsal areas of the human PFC**

One of the most relevant findings of our study is the uniform laminar distribution of $SOM^+$ neurons in the human PFC. The major issue when addressing the laminar distribution of any cell population seems to be the lack of consensus on how to report laminar distributions [27, 29–32]. The surface density of $SOM^+$ neurons is almost twice as high in layer II and IV than in other layers, while the proportions of $SOM^+$ neurons in these layers are only slightly higher. This indicates that the high density/number of $SOM^+$ neurons in layers II and IV is mostly due to the high neuronal densities of these layers in general, and not due to a disproportionate increase of $SOM^+$ neurons in these layers. Indeed, the proportions of $SOM^+$ neurons vary rather little between cortical layers, with the notable exception of layer I, where $SOM^+$ neurons are present only sporadically.

Quantification of $SOM^+$ neurons with low immunoreactivity is methodologically challenging and is most reliably done using double labelling with NeuN, CB or *SST* mRNA.

Previous studies on $SOM^+$ neurons in the human brain differed vastly in methodology, pathology and cortical regions analyzed [29–32] and none analyzed the PFC in normotypical postmortem brain tissue. Some of these studies came to conflicting findings using immunohistochemistry, with González-Albo et al. [30] concluding that $SOM^+$ neurons were more prevalent in layers II – III and Zhu et al. [29] stating that they were more prevalent in layers IV – VI. In addition, the study by Zhu et al. [29] found that $SOM^+$

neurons constituted only half the proportion we determined for the PFC. Regional differences as well as the fact that this study used intraoperative tissue of epileptic patients might explain the different findings.

### Specific co-expression of SOM and other molecular markers redefines the human GABAergic network

Another major finding in this study is a detailed molecular redefinition of the somatostatin subpopulation in the human brain. We noted a significant difference in the level of colocalization between SOM and CB in the human PFC. This is particularly evident when comparing supragranular with infragranular layers, with almost all of the double labeled cells being located in layers II and III, and it was confirmed both by immunofluorescence and RNAscope *in situ* hybridization. Such a difference in co-expression between upper and lower cortical layers was previously not described in humans, and contrasts with findings in rodents, especially rats where the level of co-expression seems to be the highest in layers V and VI [16, 23, 24]. The study by González-Albo et al. [30] also indicated that $CB^+$ interneurons were about 50% more numerous than $SOM^+$ neurons, though detailed quantification seems to have only been done in layers II and III. The differences in our findings might also arise from the fact that González-Albo et al. [30] identified relatively few $SOM^+$ cells in layers V and VI (see Fig. 4 from González-Albo et al., 2001) whereas in our study $SOM^+$ cells were numerous in these layers. As previously mentioned, this could be due to regional differences between different parts of the cortex or methodological differences between the studies.

Furthermore, we clearly demonstrated that, in the human PFC, interneurons that express either SOM or CB represent a neuron population that does not overlap with either CR or PV, which is distinct from the findings in rodents [15, 16, 18, 23, 24]. In addition, this neuron population based on SOM and CB expression can be divided into three subpopulations: $SOM^+/CB^+$ cells, $SOM^+/CB^-$ cells and $SOM^-/CB^+$ cells. All of the aforementioned provides important evidence that the SOM network of the human PFC differs fundamentally from what has previously been described in rodents. In particular, the finding that $SOM^+$ and $CB^+$ neurons are only partially overlapping GABAergic neuron populations challenges the notion that CB could be replaced as an important interneuron marker in the human brain and calls for a reevaluation of the applicability of rodent interneuron classifications in the human PFC.

### Somatostatin neurons of different layers have different molecular and morphological profiles with important functional implications within cortical neural circuits

In this study we demonstrated that there are at least five morphological types of somatostatin neurons in the human PFC, each of which has characteristic immunoreactivity, cell body shape and size, and even laminar distribution (Table 1). The fact that certain morphological cell types are largely constrained to certain cortical layers suggests that they may also have specific functional properties [13, 48, 49]. In particular, the type 3 cells found predominantly in layer IV and the most superficial part of layer V might correspond to the non-Martinotti cells targeting layer IV PV + neurons described in rodents, whereas the type 1 cells found in layers II – VI most likely correspond to the Martinotti cells whose synaptic targets are the apical dendrites in layer I. The morphological types 4 and 5 are particularly interesting since they are

very large cells present predominantly in layers V and VI as well as the white matter. So far, these cell types have received little attention in most research on somatostatin neurons, even though their morphology, size and molecular profile (most co-express nNOS, but do not co-express CB) are vastly different from the other types of somatostatin neurons. The $SOM^+$ neurons found in the white matter expressed exclusively GABAergic molecular markers, and also co-expressed MAP2 – a marker of projection neurons (but not SMI-32, another marker of projection neurons). These neurons are, therefore, very intriguing since they could represent long-projecting GABAergic cells.

Overall, $SOM^+$ neurons in the human PFC have different morphological and molecular characteristics in different cortical layers, which is particularly pronounced between supragranular and infragranular layers (Table 1).

# Conclusion

In conclusion, $SOM^+$ neurons represent a relatively large population of GABAergic neurons in the human PFC, constituting on average 6.83% of the total neuron population. They are found in cortical layers II – VI as well as in the white matter, and their molecular and morphological characteristics differ between cortical layers, particularly between supragranular and infragranular layers. Our findings challenge the notion that data on $SOM^+$ neurons can be reliably extrapolated from rodents to humans and give reason to shift focus from models reliant solely on rodents to models incorporating studies of the human brain. In particular, understanding the characteristics and function of $SOM^+$ neurons in the human PFC is of great importance for determining the possible effects of their alterations in diseases affecting the central nervous system, especially psychiatric disorders such as major depressive disorder, schizophrenia, and bipolar disorder.

# Declarations


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## Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

## Author Contributions

Ivan Banovac and Dora Sedmak designed the study. Ivan Banovac acquired the data. All authors analyzed the data, drafted the manuscript and performed a critical revision of the manuscript.

### Data Availability

All data presented in this study are available in the article and accompanying supplementary information. Raw data are available on request from the corresponding author.

### Ethics approval

This study was approved by the Ethics Committee of Zagreb University School of Medicine (380-59-10106-14-55/152).

### Consent to participate

Not applicable.

### Consent to publish

Not applicable.

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# Figures

### Figure 1

Laminar distribution and GABAergic phenotype of $SOM^+$ cells in the human PFC. (**A**) Dot plot showing laminar distribution of $SOM^+$ neurons. (**B**) Heat map showing relative quantities of SOM+ fibers in different cortical layers. (**C**) Binary confocal image of SOM immunolabeling in human BA14r. Scale bar:

200 µm. (**D**) Surface density of $SOM^+$ neurons in different cortical layers. Data presented as mean ± SD. (**E**) Proportion of $SOM^+$ neurons in different cortical layers. Data presented as mean ± SD. (**F**) SOM/*SLC32A1* (VGAT)/*SLC17A7* (VGLUT1) triple labeling showing a $SOM^+$ neuron co-expressing only *SLC32A1* (VGAT) mRNA. Scale bar: 20 µm.

**Figure 2**

Differences in molecular expression between supragranular and infragranular layer $SOM^+$ cells in the human PFC. (**A**) Confocal acquisition of CB/SOM double labeling in the human BA9. Scale bar: 200 µm. (**B**) Dot plots showing laminar distributions of $CB^+/SOM^-$, $CB^-/SOM^+$ and $CB^+/SOM^+$ cells. (**C**) CB/*SST* double labeling showing $CB^+$ neurons co-expressing *SST* mRNA in human BA14r. Scale bar: 20 µm. (**D**) SOM/*SST* double labeling showing differences between SOM peptide and *SST* mRNA expression in supragranular (SG) and infragranular (IG) layers of human BA14r. Scale bar: 10 µm. (**E**) SOM/*SST* double labeling showing a sporadically present $SOM^+$ neuron in human BA9 with both high SOM peptide and *SST* mRNA expression. Scale bar: 20 µm.

**Figure 3**

Molecular co-expression of SOM and other molecular markers in the human PFC.

(**A**) SOM/nNOS double labeling showing high level of SOM/nNOS co-expression (*) in the infragranular layers of human BA9. Scale bar: 40 µm. (**B**) SOM/MAP2 double labeling showing SOM/MAP2 co-expression (*) in the white matter of human BA14r. Scale bar: 40 µm. (**C**) SOM/CR double labelling showing lack of SOM/CR co-expression in the human BA9. Scale bar: 200 µm.

# Supplementary Files

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