Published in final edited form as:

Neurobiol Dis. 2015 January ; 73: 213–219. doi:10.1016/j.nbd.2014.10.005.

Laminar and Cellular Analyses of Reduced Somatostatin Gene Expression in the Subgenual Anterior Cingulate Cortex in Major Depression

Marianne L. Seney^{1,2,#}, Adam Tripp^{1,#}, Samuel McCune¹, David Lewis^{1,2}, and Etienne Sibille^{1,2,3,§}

¹Department of Psychiatry, University of Pittsburgh Medical School, Pittsburgh, PA, USA

²Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA, USA

³Campbell Family Research Institute, Centre for Addiction and Mental Health, Departments of Psychiatry, Pharmacology and Toxicology, University of Toronto, Toronto, CA

Abstract

Somatostatin (SST), a neuropeptide expressed in dendritic-targeting gamma-aminobutyric acid (GABA) neurons, is decreased across corticolimbic areas in major depressive disorder (MDD). SST-positive GABA neurons form heterogeneous subgroups with different laminar distributions and electrophysiological properties, so knowing the anatomical and cellular localization of reduced SST may provide insight into the nature of the pathology in MDD. In cohorts of MDD subjects with known reduction of SST in postmortem sgACC gray matter, we used in situ hybridization to quantify the laminar and cellular patterns of altered SST mRNA expression. SST mRNA levels were lower across all cortical layers in the MDD subjects. Expression levels per cell were also lower, but the density of labeled neurons did not differ between subject groups. Consistent with the previous tissue level analysis, differences were more robust in females. In summary, we report MDD-related reduction in SST expression per cell across cortical layers in sgACC, suggesting a general vulnerability of SST neurons independent of specific cell type.

Keywords

somatostatin; depression; postmortem; anterior cingulate cortex

§Corresponding author: Centre for Addiction and Mental Health (CAMH), 250 College Street, Toronto, ON M5T1R8, Canada Tel: 416-535-8501 ext 36751; etienne.sibille@camh.ca.

#These authors contributed equally to this work

Conflict of Interest

Authors declare no conflict of interest

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Somatostatin (SST), a neuromodulatory peptide, is expressed in a subtype of GABA neurons that inhibits the dendritic compartment of principal excitatory glutamatergic neurons (Viollet et al., 2008). Lower SST expression has been reported in psychiatric and neurodegenerative disorders, including schizophrenia (Morris et al., 2008), bipolar disorder (Konradi et al., 2011) and Alzheimer's disease (Gahete et al., 2010). In major depressive disorder (MDD), we reported a downregulation of SST mRNA expression in the dorsolateral prefrontal cortex (Sibille et al., 2011), subgenual anterior cingulate cortex (sgACC) (Tripp et al., 2011) and in the lateral and basomedial nuclei of the amygdala (Guilloux et al., 2012) compared to control subjects. Differences were more robust in female MDD subjects in sgACC and were restricted to women with MDD in the amygdala (Guilloux et al., 2012; Sibille et al., 2009). These findings were consistent with postmortem studies showing reduced calbindin-positive GABA neuron density in MDD (Maciag et al., 2010; Rajkowska et al., 2007), as SST is largely co-localized with calbindin [reviewed in (Viollet et al., 2008)].

SST cells represent ~18–20% of interneurons across cortical regions, but are not evenly distributed across cortical layers (Lewis et al., 1986; McDonald and Mascagni, 2002; Weckbecker et al., 2003). At least three different subtypes of SST neurons have been identified through the generation of transgenic mice expressing green fluorescent protein (GFP) in SST cells (Ma et al., 2006; Oliva et al., 2000). So-called GIN and X98 mice express GFP in layers 2/3 and 5 SST neurons, and send abundant projections to layer 1. These neurons include the traditional low threshold spiking Martinotti cells that provide inhibitory inputs to the distal dendrites of pyramidal neurons. In contrast, GFP-expressing cells in X94 mice represent a population of SST neurons in layer 4 (or deep layer 3 in agranular sgACC), which do not project to layer 1, display “stuttering” electrophysiological properties, and target layer 4 parvalbumin-positive GABA neurons (Xu et al., 2013). Consistent with these differences in innervation patterns, optogenetic-induced activity of layer 2/3/5 SST neurons resulted in pyramidal cell inhibition, while activity of layer 4 (or deep layer 3 in agranular sgACC) resulted in pyramidal cell disinhibition (Xu et al., 2013). Accordingly, in MDD, a reduction in SST function in agranular layer 3 of the sgACC might result in decreased excitation of pyramidal cells. On the other hand, reduction in SST function in layers 2/3/5 might result in increased excitation of pyramidal cells.

Accordingly, understanding whether all or a specific subset of SST cells are affected in MDD has important implications for understanding the nature of the circuit dysfunction. Here, we tested two alternative hypotheses regarding the role of SST reduction in MDD. First, SST reduction seen in MDD represents shared biological vulnerability of SST cells in general. Based on this hypothesis, we would predict similar changes in SST cells across all layers. Second, SST reduction represents secondary, adaptive changes in the brain due to a putative primary deficit in pyramidal cell function. Based on this alternative hypothesis, we would predict different results in sgACC deep layer 3 versus layers 2/3 and 5, based on their opposite effects on pyramidal cells. To test these hypotheses, we used in situ hybridization to quantify the laminar and cellular patterns of altered SST mRNA expression in the sgACC of MDD subjects. Note that these studies were performed in the same subjects for which we

had a priori information on reduced SST expression, as measured by gene array and quantitative PCR in combined gray matter samples (Tripp et al., 2011), so it is not a replication of those initial findings, but rather a follow-up on reduced SST expression at the cortical layer and cell level.

MATERIALS AND METHODS

Human Postmortem Subjects

Brain specimens were obtained during routine autopsies conducted at the Allegheny County, PA, Medical Examiner's Office after consent was obtained from next of kin. An independent committee of experienced research clinicians made consensus DSM-IV diagnoses for each subject using structured interviews with family members and review of medical records. The absence of psychiatric diagnoses was confirmed in comparison subjects through the same approach. To control for biological variance, subjects with MDD were matched to comparison subjects for sex and as closely as possible for age (Table 1). There were no statistical differences between MDD and control groups on any demographic or technical parameters. Subjects were selected from a previous study (Tripp et al., 2011) so as to enrich our cohort in MDD subjects with robust SST expression reduction, with the goal of characterizing the layer and cellular specificity of the previously reported SST reduction. Twenty MDD and nineteen control subjects (~50% female) were investigated. All procedures were approved by the University of Pittsburgh Committee for the Oversight of Research Involving the Dead and Institutional Review Board for Biomedical Research.

In situ hybridization

In situ hybridization probes, methods and analytical protocols were as previously described in (Morris et al., 2008). The templates for the synthesis of the antisense and sense riboprobes were generated by PCR. The specific primers amplified a 337 base pair fragment, corresponding to bases 112–448 of the human SST gene (GenBank NM_001048). This SST riboprobe was used and described previously (Guilloux et al., 2012; Morris et al., 2008).³⁵ S-labeled riboprobes were generated by in vitro transcription, and hybridized to three sections from each subject; the three sections were separated by 200µm. MDD and control subjects were processed in parallel to reduce experimental variance. Each section was randomly coded, so that subject number and diagnosis were unknown to experimenter. Glass slide sections were trans-illuminated and brightfield images were captured on video camera, digitized, and recorded. Areas were defined on brightfield digitized images of the dried in situ hybridization slides. After digitizing each autoradiographic film section, the slides were coated with liquid emulsion at 43°C. (KODAK Autoradiography Emulsion, Type NTB for light Microscope Autoradiography Rochester, NY). Slides were dipped for one minute using an automated dipping machine. Slides were wrapped in aluminum foil in a plastic box and incubated at 4°C. After 12 days, the coated slides were dipped for 4 minutes in developer, 1 minute in water and 8 minutes in fixer. After the emulsion coated slides were developed, they were immediately stained for 10 seconds with 0.5% Cresyl Violet solution and washed two times in Milli-Q water. We used published measures (Gittins and Harrison, 2004) to delineate each cortical layer on these cresyl violet stained sections.

Optical density of SST mRNA expression by layer

Optical density was measured using film autoradiographs. Using the Microcomputer Imaging Device (MCID; Imaging Research Inc., London, Ontario, Canada) system coupled to a microscope equipped with a motor-driven stage, laminar expression was calculated using a series of cortical traverses (1 mm in width, 1–2mm in depth) extending from the pial surface to the white matter, as previously described (Morris et al., 2008). Two to five cortical traverses (based on best available space and orientation of slides) were sampled for each section, 3 sections per subject, for a total of approximately 9 quantified traverses per subject. Each traverse was divided into 100 equal bins parallel to the pial surface and the optical density was determined for each bin. These bins were then combined into five zones that approximated the laminar boundaries of the sgACC based on previous studies (Gittins and Harrison, 2004). These zones (i.e., bins 1–12, 13–22, 23–53, 54–70 and 71–100) corresponded to cortical layers 1, 2, 3, 5 and 6, respectively. The sgACC is agranular and does not contain a layer 4.

Number of SST positive cell clusters and expression of SST mRNA per cluster

To evaluate SST mRNA expression at the cellular level, the numbers of silver grains generated in emulsion-dipped sections were counted over neurons in sampling frames using a fixed diameter circle placed over cresyl violet-stained nuclei in layers 1, 2/3, 3, 5 and 6 in sgACC. SST-positive clusters were similar in size to those found in the DLPFC, and the sampling circle diameter from a previous study (Morris et al., 2008) fixed at 22 μ m (380 μ m²) was used. See Figure 1 for a representation of the sampling strategy for grain analysis. Five “layer points” were selected based on mean SST expression from laminar analysis, and representing layer 1 (at 6.2% depth from pia to white matter boundary), the first peak of SST expression on the border of layer 2 and layer 3 (at 22.5% depth), the trough of SST expression in layer 3 (35% depth), the second peak of SST expression in layer 5 (62%), and the last layer point in layer 6 (85% depth). Five traverses per section (1mm wide by 2–4mm depth) and one section per subject were used to study grain counts. Four sampling boxes (170 μ m $\times$ 120 μ m) were used per “layer point” for analysis; the sampling boxes were separated by 64 μ m and 64 μ m separated the first and fourth sampling boxes from the edge of the traverse (see Figure 1). Each traverse contained 20 sampling boxes. We measured grain density and cluster density (we use cluster density as a proxy measure of the number of SST cells) in each sampling box using the sampling circle (22 μ m diameter) to count grains in all pyramidal, interneuron, and glial cell bodies (endothelial cells were excluded). For each darkfield slide section, background noise was first minimized (similarly across all analyzed sections). After background minimization, grain clusters were counted in each sampling box. We then used MCID imaging software to count the number of grains within each sampling circle placed over grain clusters (Morris et al., 2008). The total number of grain clusters sampled for the sgACC was 10,769 and 11,021 for control and MDD subjects, respectively. The basis for cutoff of specific SST labeling was determined as in (Morris et al., 2008): a histogram of all clusters and SST grain counts was generated, and clusters with grain counts greater than seven times the statistical mode of grain count of all cell clusters were analyzed as SST+ clusters. 25% of the sampling boxes for each “layer point” were used to generate background measurement of SST grains.

Statistical analysis

To determine relevant scale covariates to include in the analysis of covariance (ANCOVA) model, Pearson correlation was used to assess the effect of age, postmortem interval, brain pH, and RNA integrity (RIN) on in situ hybridization measures. To determine relevant categorical covariates (sex, antidepressant use, benzodiazepine use, death by suicide) to include in the ANCOVA model, in situ hybridization measures were tested by analysis of variance (ANOVA); note that while all subjects were used to assess potential effects of sex, only MDD subjects were used to assess potential effects of antidepressant use, benzodiazepine use, and death by suicide. We did not examine race as a contributing factor, as all but two subjects examined were Caucasian. The modified Bonferroni-Holm method was used to correct for testing of multiple covariates. Although this method of multiple testing correction of potential covariates decreases the power to detect significant correlations, statistical significance of the main outcome measures by ANCOVA did not change when we used an alternative method of applying a cutoff of $p < 0.01$ for inclusion of a cofactor in the ANCOVA. The only covariates that survived multiple testing were an effect of sex in layer 1 laminar OD analysis and an effect of sex on layer 3 SST cell counts; thus these covariates were included in the ANCOVA model when testing that specific dependent measure (see Supplemental Table 1 for p-values associated with covariate testing). Uncorrected ANCOVA p values are reported since we have a priori information of reduced tissue level SST expression in these subjects.

RESULTS

Reduced SST expression across sgACC cortical layers in MDD

Given our previous findings of reduced SST mRNA and protein in the sgACC (Tripp et al., 2011), we first evaluated whether there was an overall decrease in optical density of SST mRNA with all layers combined. Replicating our previous findings on tissue homogenate by qPCR, we found a statistically significant 25% decrease in SST optical density in MDD subjects compared to controls ($p < 0.01$; mean controls: 838.44; mean MDD: 627.84). We next examined the layer specificity of the reduction in SST in MDD by examining the optical density of SST mRNA levels in each cortical layer. Since there was an effect of sex on optical density in layer 1, we included sex as a cofactor in the ANCOVA model for layer 1 optical density analysis (see Materials and Methods). We found a general, uniform reduction in SST expression across cortical layers in MDD subjects (Layer 1: -21% , $p < 0.1$; Layer 2: -24% , $p < 0.015$; Layer 3: -30% , $p < 0.005$; Layer 5: -27% , $p < 0.005$; Layer 6: -21% , $p < 0.05$) (Figure 2 **on left**; Table 2 **upper panel**).

As an additional level of analysis and based on the observation of a significant cofactor effect of sex in SST expression in layer 1, we examined SST expression separately in males and females. Results indicate that SST expression was significantly reduced in all cortical layers in female MDD subjects (Layer 1: -27% , $p < 0.05$; Layer 2: -25% , $p < 0.015$; Layer 3: -33% , $p < 0.005$; Layer 5: -28% , $p < 0.015$; Layer 6: -26% , $p < 0.005$; all layers combined: -28% , $p < 0.005$) (Figure 2 **in center**; Table 2 **upper panel**), and was not significantly reduced in male MDD subjects (Layer 1: -5% , $p > 0.75$; Layer 2: -20% , $p > 0.15$; Layer 3: -24% , $p > 0.1$; Layer 5: -26% , $p > 0.1$; Layer 6: -30% , $p > 0.4$; all layers

combined: -20% , $p > 0.15$) (Figure 2 **on right**; Table 2 **upper panel**). When we alternatively analyzed the optical density of SST with a 2-way ANOVA (sex by diagnosis), there was no significant interaction for any layer ($p > 0.1$ for each measure).

MDD subjects have no loss of detectable SST positive cells, or grain clusters, in the sgACC

We next examined the density of SST grain clusters (number of clusters within a defined area), as a presumptive measurement of the number of SST expressing cells. Since there was an effect of sex on the density of SST grain clusters in layer 3, we included sex as a cofactor in the ANCOVA model for layer 3 SST grain cluster density analysis (see Materials and Methods). When males and females were analyzed together, we found no difference in the density of SST positive cells in any layer between controls and MDD subjects ($p > 0.4$ for layers; Table 2 **middle panel**). Males and females were also analyzed separately. In the female cohort, we found a significant reduction in the density of SST positive cells in layer 6 of MDD subjects, with no differences in other layers (Layer 1: $p > 0.6$; Layer 2: $p > 0.95$; Layer 3: $p > 0.9$; Layer 5: $p > 0.9$; Layer 6: -20% , $p < 0.05$). When males were analyzed independently, there were no differences in the density of SST positive cells across layers (Layer 1: $p > 0.7$; Layer 2: $p > 0.35$; Layer 3: $p > 0.95$; Layer 5: $p > 0.25$; Layer 6: $p > 0.15$). A survey of the total number of cresyl violet stained cell bodies (as a measure of both SST positive and SST negative cells) showed no significant changes in any layer point (Supplementary Figure 1).

MDD subjects have reduced SST mRNA per cell across cortical layers in the sgACC

As a measure of the expression of SST per SST-positive cell, we counted the number of silver grains per cluster. Interestingly, the numbers of silver grains per cluster were significantly reduced across all cortical layers (layer 1: -47% , $p < 0.002$; layer 2/3: -42% , $p < 0.002$; layer 3: -39% , $p < 0.002$; layer 5: -41% , $p < 0.001$; layer 6: -31% , $p < 0.01$) (Figure 3; Table 2 **bottom panel**). As performed for optical density and cluster number, we also analyzed SST expression per cell in males and females separately. In females, there was a reduction in number of silver grains per SST-positive cell in layers 1, 2/3, 3, and 5 (layer 1: -40% , $p < 0.07$; layer 2/3: -40% , $p < 0.01$; layer 3: -34% , $p < 0.07$; layer 5: -42% , $p < 0.01$; layer 6: $p > 0.15$). In the male cohort, the reduction in number of silver grains per SST cell was significant in all layers (layer 1: -53% , $p < 0.01$; layer 2/3: -43% , $p < 0.05$; layer 3: -33% , $p < 0.002$; layer 5: -40% , $p < 0.01$; layer 6: -45% , $p < 0.05$). To address the possibility of different SST subtypes across layers, we also analyzed the number of silver grains per cluster across layers in control subjects only. The number of silver grains per cluster did not differ between layers 2/3, 5, and 6 ($p > 0.2$ for all comparisons). SST cells in layer 3 had a slightly decreased number of silver grains compared to layer 6 SST cells ($p < 0.05$). SST positive cell clusters in layer 1 had the lowest number of silver grains ($p < 0.02$ compared to all other layers).

DISCUSSION

Results from this study show that the previously-reported reductions in sgACC tissue levels of SST expression in MDD reflect changes across all cortical layers. Cell- and sex-based

specificities were also noted. Reduced SST expression was observed across all layers in the MDD subjects, when males and females were combined (albeit at trend level in layer 1); when males and females were analyzed separately, this reduction was significant only in females with MDD. At the cellular level, we observed a robust decrease in grain count per cell across all layers, with no change in density of SST-positive grain clusters (i.e. putative SST cells). Interestingly, we saw a reduction in grain count per SST cell in both males and females, potentially reflecting the increased sensitivity of grain counting compared to optical density analysis, although other unknown factors may have contributed. Together, these findings suggest reduced SST expression per cell affecting all SST-positive neuron subtypes in the sgACC in subjects with MDD.

In this study, we tested two alternative hypotheses regarding SST reduction in MDD. If the observed MDD-related changes in SST were to represent a compensatory mechanism to maintain excitation/inhibition balance due to putative altered function of specific excitatory principal neurons, one would predict a specific reduction in SST in layer 3 of the sgACC of MDD subjects in order to decrease excitation of pyramidal cells (due to parvalbumin cell-mediated indirect targeting; see Introduction); reductions in SST in layers 2/3/5 (Martinotti cells) would not be expected in this scenario, as this would lead to increased pyramidal cell excitation. On the other hand, if the reduction in SST in MDD represents a cellular vulnerability that is intrinsic to all classes of SST neurons, one would predict similar SST reduction across cortical layers. Indeed, our data support this second hypothesis. Also since SST neurons located in layer 1 and 6 originate at different development time points compared to layers 2–5 SST neurons (Bayer and Altman, 1990; Chun and Shatz, 1989; Kostovic and Rakic, 1980), the observed pan-layer findings suggest that events specific to a particular developmental window are unlikely to have caused the cortical changes.

We also examined whether the number of silver grains per cluster varied by cortical layer (control subjects only), as this information might give insight into potential different subtypes of SST positive cells across layers. The number of silver grains per cluster did not differ between layers 2/3, 5, and 6, suggesting that these SST cells are of a similar subtype; this conclusion is consistent with SST cells in these layers representing the typical low threshold spiking Martinotti cell that innervates pyramidal neurons in layer 1. Interestingly, SST cells in layer 3 had a slightly decreased number of silver grains compared to layer 6 SST cells; SST cells in layer 3 are thought to represent the SST cell population that exhibits “stuttering” electrophysiological properties and that innervates parvalbumin interneurons. As expected due to the low expression of SST in layer 1, clusters in layer 1 had the lowest number of silver grains.

What potential mechanisms could lead to the observed MDD-related changes in SST mRNA levels? The cumulative evidence supports a model of intrinsic vulnerability of adult SST-positive neurons that results in reduced SST expression per cell. Pathways underlying this high vulnerability may include oxidative stress, inflammation, and dependence on neurotrophic environment (See (Lin and Sibille, 2013) for review): Specifically, (1) SST-positive neurons display high expression of neuronal nitric oxide synthase (nNOS) and NADPH diaphorase (NADPHd), two enzymes that produce reactive oxidative species (Di Martino et al., 2007; Dun et al., 1994; Figueredo-Cardenas et al., 1996; Jaglin et al., 2012);

(2) SST neurons contribute to the modulation of, and are conversely affected by, peripheral and central inflammation (Markovics et al., 2012; Pinter et al., 2006); and (3) importantly, SST gene expression robustly depends on brain derived neurotrophic factor (BDNF) and TrkB signaling (Glorioso et al., 2006; Martinowich et al., 2011). Ample evidence exists for the presence of inflammation, oxidative stress, and low neurotrophic support in MDD, and these events are often considered putative causal mechanisms for onset of the biological and behavioral phenotypes of MDD (Belmaker and Agam, 2008; Dantzer et al., 2008). Taken together, the reduced SST expression per cell may represent a common downstream event that integrates several upstream pathological mechanisms.

As noted in the Introduction, cortical SST neurons comprise heterogeneous subgroups of neurons, potentially reflecting different tasks across related but distinct local cell circuits. The molecular basis supporting the SST cellular diversity is not known, nor is its relevance to human brain function, but it suggests tissue- and cell type-specific transcriptional programs, likely reflecting the differential distribution of SST neurons with specific functional roles across related but distinct local cell circuits. These cellular and functional diversities also offer different substrates for maladaptive changes involved in pathological mechanisms of psychiatric disorders. This suggests that etiological factors leading to low SST may be shared across disorders, although the functional outcome of low SST may be dictated by the layer in which SST cells are affected.

Sex is emerging as an important moderating factor of the SST phenotype, as demonstrated here in sgACC. Putative sex-specific factors that may influence our findings include the roles of X/Y chromosome genes (i.e., genetic sex), of gonadal sex during development, and of circulating hormones in adulthood. We recently reported an unexpected role of XY genetic sex on SST and other GABA-related genes (Seney et al., 2013), showing that factors encoded on the X-chromosomes indirectly regulate frontal cortex expression of those genes both in humans and in mouse models, and that genetic sex modulates aspects of mood-related behaviors in rodents. Thus, we speculate that sex- and cell-specific transcriptional programming may provide different substrates for cellular vulnerability or resiliency when faced with adverse biological events. This model is consistent with findings of reduced SST expression in other brain disorders, since the potential mechanistic events listed above occur in multiple contexts and are not specific to MDD. Indeed, reduced SST expression has been reported in schizophrenia (Morris et al., 2008), bipolar disorder (Konradi et al., 2004; Konradi et al., 2011), Alzheimer's disease (Epelbaum et al., 2009; Gahete et al., 2010), as well as during normal aging of the human brain (Glorioso et al., 2011). See (Martel et al., 2012) for a comprehensive review of SST changes across brain disorders.

How could “non-specific” changes in SST expression be conceptualized as contributing factors in disorders, and to what extent do these changes contribute to symptom dimensions? At the local cell circuit level, the consequence of low SST will depend on (1) whether all SST neurons are affected, (2) whether the inhibitory GABA-related function of SST-positive neuron is also affected or (3) whether the deficits are restricted to its peptide neuromodulatory function. Here, we only examined expression of SST in the sgACC of MDD subjects, so it is unclear whether the reduction in SST we observe influences the GABA-related inhibitory function of the SST cells and/or the peptide neuromodulatory

function of the SST cells. GABA neurons can be subdivided according to their expression of neuropeptides and targeted pyramidal cell compartment: parvalbumin- and cholecystokinin-positive GABA neurons target the perisomatic area, while SST, neuropeptide Y (NPY) and cortistatin (CORT)-expressing GABA neurons specifically target pyramidal neuron dendrites (reviewed in (Kubota, 2014)). Notably, expression of NPY and CORT expression were reduced in concert with low SST in MDD, while other markers of GABA-neuron subtypes are either rarely or not affected in MDD (Guilloux et al., 2012; Tripp et al., 2011), suggesting a functional phenotype that selectively affects dendritic inhibition in MDD. Notably the outcome of low SST and associated dendritic inhibition is expected to be different in the context of additional pathology (including other GABA-related deficits), such as reduced perisomatic inhibition (i.e. low parvalbumin-mediated inhibition) in schizophrenia and bipolar disorder (e.g. (Hashimoto et al., 2008; Hashimoto et al., 2003; Sibille et al., 2011)), or altered pyramidal cell function due to amyloid plaque and neurofibrillary tangles in Alzheimer's disease. In addition to this modularity of GABA-related phenotypes at the local circuit, the extent to which the low SST phenotype is present in other corticolimbic areas and related neural networks will further contribute to determining the putative functional outcome of low SST and associated symptom dimensions.

One potential limitation of this study is that it was performed in selected cohorts of subjects with already-established MDD-related reduction in SST; thus it does not represent an independent validation of prior findings, but rather a characterization of the cortical layer of the reduced SST in MDD, with cellular level specificity. Systematic cofactor analyses in the current and prior studies in these subjects (Guilloux et al., 2012; Tripp et al., 2011) showed that these effects were not due to demographic, technical parameters, or other clinical parameters. Hence, based on the small cohort size and pre-selection approach, we did not investigate those factors in further details beyond correction of putative effects in analyses of covariance (See Materials and Methods).

In summary, the current study provides information about the anatomical and cellular localization of reduced SST expression in sgACC of subjects with MDD, and suggests a SST-cell intrinsic vulnerability that is moderated by sex. The investigation of biological factors underlying this selective vulnerability is thus critical for the development of treatments targeted at the primary pathology of MDD and has implications for other brain-related disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institute of Mental Health (NIMH) MH084060 (ES) and MH085111 (ES). The funding agency had no role in the study design, data collection and analysis, decision to publish and preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIMH or the National Institutes of Health. David A. Lewis currently receives investigator-initiated research support from Bristol-Myers Squibb and Pfizer and in 2012-2014 served as a consultant in the areas of target identification and validation and new compound development to Autifony, Bristol-

Myers Squibb, Concert Pharmaceuticals, and Sunovion. Marianne Seney in 2014 served as a consultant for NeuroPhase Pharmaceuticals, providing technique-based support.

References

- Bayer SA, Altman J. Development of layer I and the subplate in the rat neocortex. *Exp Neurol*. 1990; 107 (1):48–62. [PubMed: 2295319]
- Belmaker RH, Agam G. Major depressive disorder. *N Engl J Med*. 2008; 358 (1):55–68. [PubMed: 18172175]
- Chun JJ, Shatz CJ. Interstitial cells of the adult neocortical white matter are the remnant of the early generated subplate neuron population. *J Comp Neurol*. 1989; 282 (4):555–569. [PubMed: 2566630]
- Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008; 9 (1):46–56. [PubMed: 18073775]
- Di Martino M, Degli Esposti L, Filigheddu F, Veronesi C, Salerno G, Saragoni S, Glorioso N, Didoni G, Degli Esposti E. Use of antihypertensive and lipid-lowering drugs: the management of cardiovascular risk in clinical practice. *J Hum Hypertens*. 2007; 21 (1):53–59. [PubMed: 17036042]
- Dun NJ, Dun SL, Wong RK, Forstermann U. Colocalization of nitric oxide synthase and somatostatin immunoreactivity in rat dentate hilar neurons. *Proc Natl Acad Sci U S A*. 1994; 91 (8):2955–2959. [PubMed: 7512719]
- Epelbaum J, Guillou JL, Gastambide F, Hoyer D, Duron E, Viollet C. Somatostatin, Alzheimer's disease and cognition: an old story coming of age? *Prog Neurobiol*. 2009; 89 (2):153–161. [PubMed: 19595735]
- Figueredo-Cardenas G, Morello M, Sancesario G, Bernardi G, Reiner A. Colocalization of somatostatin, neuropeptide Y, neuronal nitric oxide synthase and NADPH-diaphorase in striatal interneurons in rats. *Brain Res*. 1996; 735 (2):317–324. [PubMed: 8911672]
- Gahete MD, Rubio A, Duran-Prado M, Avila J, Luque RM, Castano JP. Expression of Somatostatin, cortistatin, and their receptors, as well as dopamine receptors, but not of neprilysin, are reduced in the temporal lobe of Alzheimer's disease patients. *J Alzheimers Dis*. 2010; 20 (2):465–475. [PubMed: 20164562]
- Gittins R, Harrison PJ. A quantitative morphometric study of the human anterior cingulate cortex. *Brain Res*. 2004; 1013 (2):212–222. [PubMed: 15193531]
- Glorioso C, Oh S, Douillard GG, Sibille E. Brain molecular aging, promotion of neurological disease and modulation by sirtuin 5 longevity gene polymorphism. *Neurobiol Dis*. 2011; 41 (2):279–290. [PubMed: 20887790]
- Glorioso C, Sabatini M, Unger T, Hashimoto T, Monteggia LM, Lewis DA, Mirnics K. Specificity and timing of neocortical transcriptome changes in response to BDNF gene ablation during embryogenesis or adulthood. *Mol Psychiatry*. 2006; 11 (7):633–648. [PubMed: 16702976]
- Guilloux JP, Douillard-Guilloux G, Kota R, Wang X, Gardier AM, Martinowich K, Tseng GC, Lewis DA, Sibille E. Molecular evidence for BDNF- and GABA-related dysfunctions in the amygdala of female subjects with major depression. *Mol Psychiatry*. 2012; 17 (11):1130–1142. [PubMed: 21912391]
- Hashimoto T, Arion D, Unger T, Maldonado-Aviles JG, Morris HM, Volk DW, Mirnics K, Lewis DA. Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Mol Psychiatry*. 2008; 13 (2):147–161. [PubMed: 17471287]
- Hashimoto T, Volk DW, Eggen SM, Mirnics K, Pierri JN, Sun Z, Sampson AR, Lewis DA. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J Neurosci*. 2003; 23 (15):6315–6326. [PubMed: 12867516]
- Jaglin XH, Hjerling-Leffler J, Fishell G, Batista-Brito R. The origin of neocortical nitric oxide synthase-expressing inhibitory neurons. *Front Neural Circuits*. 2012; 6:44. [PubMed: 22787442]
- Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Arch Gen Psychiatry*. 2004; 61 (3):300–308. [PubMed: 14993118]

- Konradi C, Zimmerman EI, Yang CK, Lohmann KM, Gresch P, Pantazopoulos H, Berretta S, Heckers S. Hippocampal interneurons in bipolar disorder. *Arch Gen Psychiatry*. 2011; 68 (4):340–350. [PubMed: 21135314]
- Kostovic I, Rakic P. Cytology and time of origin of interstitial neurons in the white matter in infant and adult human and monkey telencephalon. *J Neurocytol*. 1980; 9 (2):219–242. [PubMed: 7441294]
- Kubota Y. Untangling GABAergic wiring in the cortical microcircuit. *Curr Opin Neurobiol*. 2014; 26:7–14. [PubMed: 24650498]
- Lewis DA, Campbell MJ, Morrison JH. An immunohistochemical characterization of somatostatin-28 and somatostatin-281-12 in monkey prefrontal cortex. *J Comp Neurol*. 1986; 248 (1):1–18. [PubMed: 2873154]
- Lin LC, Sibille E. Reduced brain somatostatin in mood disorders: a common pathophysiological substrate and drug target? *Front Pharmacol*. 2013; 4:110. [PubMed: 24058344]
- Ma Y, Hu H, Berrebi AS, Mathers PH, Agmon A. Distinct subtypes of somatostatin-containing neocortical interneurons revealed in transgenic mice. *J Neurosci*. 2006; 26 (19):5069–5082. [PubMed: 16687498]
- Maciag D, Hughes J, O'Dwyer G, Pride Y, Stockmeier CA, Sanacora G, Rajkowska G. Reduced density of calbindin immunoreactive GABAergic neurons in the occipital cortex in major depression: relevance to neuroimaging studies. *Biol Psychiatry*. 2010; 67 (5):465–470. [PubMed: 20004363]
- Markovics A, Szoke E, Sandor K, Borzsei R, Bagoly T, Kemeny A, Elekes K, Pinter E, Szolcsanyi J, Helyes Z. Comparison of the anti-inflammatory and anti-nociceptive effects of cortistatin-14 and somatostatin-14 in distinct in vitro and in vivo model systems. *J Mol Neurosci*. 2012; 46 (1):40–50. [PubMed: 21695504]
- Martel G, Dutar P, Epelbaum J, Viollet C. Somatostatinergic systems: an update on brain functions in normal and pathological aging. *Front Endocrinol (Lausanne)*. 2012; 3:154. [PubMed: 23230430]
- Martinowich K, Schloesser RJ, Jimenez DV, Weinberger DR, Lu B. Activity-dependent brain-derived neurotrophic factor expression regulates cortistatin-interneurons and sleep behavior. *Mol Brain*. 2011; 4:11. [PubMed: 21388527]
- McDonald AJ, Mascagni F. Immunohistochemical characterization of somatostatin containing interneurons in the rat basolateral amygdala. *Brain Res*. 2002; 943 (2):237–244. [PubMed: 12101046]
- Morris HM, Hashimoto T, Lewis DA. Alterations in somatostatin mRNA expression in the dorsolateral prefrontal cortex of subjects with schizophrenia or schizoaffective disorder. *Cereb Cortex*. 2008; 18 (7):1575–1587. [PubMed: 18203698]
- Oliva AA Jr, Jiang M, Lam T, Smith KL, Swann JW. Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. *J Neurosci*. 2000; 20 (9):3354–3368. [PubMed: 10777798]
- Pinter E, Helyes Z, Szolcsanyi J. Inhibitory effect of somatostatin on inflammation and nociception. *Pharmacol Ther*. 2006; 112 (2):440–456. [PubMed: 16764934]
- Rajkowska G, O'Dwyer G, Teleki Z, Stockmeier CA, Miguel-Hidalgo JJ. GABAergic neurons immunoreactive for calcium binding proteins are reduced in the prefrontal cortex in major depression. *Neuropsychopharmacology*. 2007; 32 (2):471–482. [PubMed: 17063153]
- Seney ML, Chang LC, Oh H, Wang X, Tseng GC, Lewis DA, Sibille E. The Role of Genetic Sex in Affect Regulation and Expression of GABA-Related Genes Across Species. *Front Psychiatry*. 2013; 4:104. [PubMed: 24062698]
- Sibille E, Morris HM, Kota RS, Lewis DA. GABA-related transcripts in the dorsolateral prefrontal cortex in mood disorders. *Int J Neuropsychopharmacol*. 2011; 14 (6):721–734. [PubMed: 21226980]
- Sibille E, Wang Y, Joeyen-Waldorf J, Gaiteri C, Surget A, Oh S, Belzung C, Tseng GC, Lewis DA. A molecular signature of depression in the amygdala. *Am J Psychiatry*. 2009; 166 (9):1011–1024. [PubMed: 19605536]
- Tripp A, Kota RS, Lewis DA, Sibille E. Reduced somatostatin in subgenual anterior cingulate cortex in major depression. *Neurobiol Dis*. 2011; 42 (1):116–124. [PubMed: 21232602]

- Tripp A, Oh H, Guilloux JP, Martinowich K, Lewis DA, Sibille E. BDNF signaling and subgenual anterior cingulate cortex dysfunction in major depression. *American Journal Psychiatry*. 2012; 169 (11):1194–1202.
- Viollet C, Lepousez G, Loudes C, Videau C, Simon A, Epelbaum J. Somatostatinergic systems in brain: networks and functions. *Mol Cell Endocrinol*. 2008; 286 (1–2):75–87. [PubMed: 17997029]
- Weckbecker G, Lewis I, Albert R, Schmid HA, Hoyer D, Bruns C. Opportunities in somatostatin research: biological, chemical and therapeutic aspects. *Nat Rev Drug Discov*. 2003; 2(12):999–1017. [PubMed: 14654798]
- Xu H, Jeong HY, Tremblay R, Rudy B. Neocortical somatostatin-expressing GABAergic interneurons disinhibit the thalamorecipient layer 4. *Neuron*. 2013; 77 (1):155–167. [PubMed: 23312523]

Highlights

- Somatostatin (SST) mRNA was reduced across cortical layers in major depression
- The number of SST positive cells was not changed in depressed subjects
- The expression of SST per cell was decreased in depressed subjects
- Findings were more robust in females with major depression

Figure 1. Sampling strategy in sgACC

In situ hybridization signal using antisense ^{35}S -labeled SST riboprobe in sgACC (5mm scale bar). The dorsal cut is at the level of the genu of the corpus callosum. A sample traverse measuring SST expression across cortical layers is indicated by the black box (1mm $\times$ 2–4mm) on the tissue section, with a blown-up version of the sampling strategy on the right. The traverse was placed perpendicular to the pial surface and away from obvious curvatures in cortex. Five “layer points” were selected based on mean SST expression from laminar analysis: layer 1 is at 6.2% depth from pia to white matter boundary, the border of layers 2 and 3 represent the first peak of SST expression (at 22.5% depth), the trough of SST expression in layer 3 (35% depth), the second peak of SST expression in layer 5 (62%), and the last layer point in layer 6 (85% depth). Five traverses per section and one section per subject were used to study grain counts. Four sampling boxes (170 μm $\times$ 120 μm) were used per “layer point” for analysis. The sampling boxes were separated by 64 μm and 64 μm separated the first and fourth sampling boxes from the edge of the traverse. The sampling strategy in sgACC was as described in Morris et al. (2008).

Figure 2. SST transcript levels across layers in the sgACC of controls and subjects with MDD
 When males and females are analyzed together (on left), MDD subjects show a reduction in SST mRNA expression across all layers. When females are analyzed independently (in center), MDD subjects again show a reduction in SST mRNA expression across all layers. However, when males are analyzed independently (on right), SST mRNA expression does not differ between MDD subjects and controls. *, $p < 0.05$, **, $p < 0.01$, #, $p < 0.1$.

Figure 3. SST-positive grain cluster analysis in control and MDD subjects

Across all cortical layers, MDD subjects have reduced SST expression per neuron compared to controls. A similar pattern was observed when males and females were examined independently. Female subjects are indicated by red circles (controls) or triangles (MDD), while male subjects are indicated by blue circles (controls) or triangles (MDD).

Table 1
Demographic and postmortem characteristics of human subjects for in situ hybridization study.

Comparison subjects										MDD subjects							
Case	Sex	Race	Age	PMI	pH	RIN	Cause of death	Case	Sex	Race	Age	PMI	pH	RIN	Suicide	Cause of death	Medications ATOD
546	F	W	37	23.5	6.7	8.6	ASCVD	666	F	W	16	10	7.3	9.2	N	Trauma	D
818	F	W	67	24	7.1	8.4	Anaphylactic reaction	934	F	W	54	17.9	6.2	8.2	N	ASCVD	D O
1081	F	W	57	14.9	6.8	9	COPD	1041	F	W	52	10.3	6.5	8.4	N	Drug overdose	B D O P
1092	F	B	40	16.6	6.8	8	Mitral valve prolapse	1221	F	B	28	24.8	6.6	7.2	N	Pulmonary thrombosis	N
1099	F	W	24	9.1	6.5	8.6	Cardiomyopathy	1249	F	W	40	11.2	6.5	9	N	Drug overdose	B C D O
1247	F	W	58	22.7	6.4	8.4	ASCVD	1254	F	W	39	12.8	6.4	9	Y	Incised wounds	D
1280	F	W	50	23.5	6.7	7.7	Pulmonary thromboemboli	1289	F	W	46	25	6.3	7.3	N	ASCVD	U
1391	F	W	51	7.8	6.6	7.1	ASCVD	1315	F	W	28	12.4	7	7.9	Y	Asphyxiation by hanging	N
1403	F	W	45	12.3	6.7	8.2	ASCVD	1356	F	W	60	20.6	6.1	8.5	N	Intraperitoneal hemorrhage	D O
10013	F	W	16	9.3	6.7	9	Trauma	1360	F	W	59	18.1	6.4	7.6	Y	Drowning	D
615	M	W	62	7.2	6.4	7.8	Ruptured aortic aneurysm	513	M	W	24	13.1	6.9	7.0	Y	Asphyxiation by hanging	N
634	M	W	52	16	7	8.1	ASCVD	600	M	W	63	9.9	6.7	7.1	Y	Asphyxiation by hanging	O
789	M	W	22	20	7	7.4	Asphyxiation	619	M	W	55	18.8	6.9	7.9	Y	GSW of head	B D
852	M	W	54	8	6.8	9.1	Cardiac tamponade	809	M	W	50	20	6.9	8.5	N	ASCVD	D O
1031	M	W	53	23.1	6.8	8.2	Arteriosclerotic/hypertensive CVD	863	M	W	51	28.3	7.2	8.4	N	ASCVD	N
1047	M	W	43	12	6.9	9	ASCVD	943	M	W	56	15.4	6.6	8.2	Y	GSW to mouth	O
1086	M	W	51	24.2	6.8	8.1	ASCVD	1001	M	W	53	7.3	6.6	7.6	N	Arteriosclerotic/hypertensive heart disease	O
1317	M	W	56	22.9	6.4	6.9	ASCVD	1312	M	W	51	24.6	6.5	8.1	N	Drug overdose	O
1372	M	W	37	20.5	6.6	9	Accidental asphyxiation	1320	M	W	55	24.4	6.5	7.2	N	ASCVD	N
								10031	M	W	36	20	6.8	8.9	N	Drug overdose	C D P
Mean			46.1	16.7	6.7	8.2					45.8	17.2	6.6	8.1			
SD			13.8	6.4	0.2	0.7					13.3	6.2	0.3	0.7			

Abbreviations: ASCVD, arteriosclerotic cardiovascular disease; ATOD, at time of death; B, benzodiazepines; B, black subject; C, anticonvulsants; COPD, chronic obstructive pulmonary disease; D, antidepressants; F, female; GSW, gunshot wound; M, male; MDD, major depressive disorder; N, no medications; O, other medication(s); P, antipsychotics; PMI, postmortem interval in hours; RIN, RNA integrity number W, white subject.

Table 2

MDD SST Reduction in Cortical Layer and Expression per Cluster in sgACC.

	Combined				Females				Males						
	Control ¹	MDD ¹	%	F	p-value	Control ¹	MDD ¹	%	F	p-value	Control ¹	MDD ¹	%	F	p-value
Laminar analysis ²															
Layer 1	86.61 (40.8)	68.6 (24.5)	-21	4.01	0.053	112.6 (30.9)	82.3 (23.4)	-27	6.12	0.024	57.8 (29.7)	54.9 (17.4)	-5	0.07	0.797
Layer 2	170.9 (56.6)	130.6 (35.4)	-24	7.21	0.011	195.6 (49.5)	145.9 (28.5)	-25	7.58	0.013	143.5 (53.3)	115.2 (36.2)	-20	1.86	0.190
Layer 3	193.2 (66.3)	136.1 (8.0)	-30	11.4	0.002	212.2 (56.7)	142.0 (27.1)	-33	12.5	0.002	172.1 (72.9)	130.1 (43.4)	-24	2.39	0.141
Layer 5	226.6 (72.6)	165.5 (46.2)	-27	9.92	0.003	243.4 (63.4)	131.2 (27.8)	-28	8.27	0.010	208.0 (81.3)	154.9 (53.1)	-26	2.90	0.107
Layer 6	161.4 (51.5)	127.2 (34.6)	-21	5.96	0.020	177.3 (33.9)	131.2 (27.8)	-26	11.1	0.004	143.6 (63.3)	123.3 (41.5)	-30	0.70	0.414
Total	172.2 (54.5)	128.4 (32.6)	-25	9.39	0.004	941.0 (64.8)	677.5 (41.3)	-28	11.8	0.003	724.5 (95.8)	578.2 (56.1)	-20	1.83	0.194
Cluster Number ³															
Layer 1	6.3 (2.2)	6.9 (2.6)	10	0.43	0.515	5.4 (2.0)	6.0 (2.8)	11	0.28	0.602	7.2 (2.1)	7.6 (2.2)	6	0.12	0.737
Layer 2/3	7.7 (2.2)	8.2 (2.3)	6	0.46	0.503	7.6 (2.1)	7.6 (2.3)	4	0.00	1.000	7.8 (2.4)	8.8 (2.2)	13	0.82	0.378
Layer 3	6.9 (2.1)	7.0 (2.1)	1	0.01	0.917	5.9 (1.4)	5.9 (1.6)	0	0.01	0.906	8.0 (2.3)	8.0 (2.1)	0	0.002	0.966
Layer 5	8.2 (1.8)	8.7 (2.4)	6	0.57	0.454	7.9 (1.5)	7.8 (2.5)	-1	0.00	0.983	8.6 (2.1)	9.6 (2.1)	12	1.15	0.299
Layer 6	5.0 (1.4)	5.0 (1.5)	0	0.001	0.980	5.4 (1.1)	4.3 (0.9)	-20	5.54	0.030	4.5 (1.6)	5.6 (1.8)	24	2.11	0.165
Grain Count ⁴															
Layer 1	62.2 (33.2)	33.1 (13.9)	-47	12.99	0.001	56.1 (32.6)	33.4 (15.9)	-40	3.92	0.063	69.0 (34.5)	32.8 (12.5)	-53	9.68	0.006
Layer 2/3	122.5 (50.6)	71.8 (31.9)	-42	14.17	0.001	125.1 (38.1)	75.2 (37.8)	-40	8.64	0.009	119.6 (64.0)	68.5 (26.4)	-43	5.40	0.033
Layer 3	110.9 (36.6)	67.4 (35.3)	-39	14.34	0.001	111.5 (41.2)	74.0 (44.7)	-34	3.80	0.067	110.3 (33.1)	60.7 (23.1)	-33	14.61	0.001
Layer 5	138.3 (52.0)	80.9 (28.7)	-41	18.41	0.000	144.8 (53.2)	83.7 (37.2)	-42	8.86	0.008	131.0 (52.9)	78.1 (18.5)	-40	8.85	0.008
Layer 6	153.6 (58.0)	106.7 (46.8)	-31	7.77	0.008	156.1 (40.0)	130.4 (41.4)	-16	1.99	0.176	150.9 (75.8)	83.0 (40.8)	-45	6.09	0.025

¹ Results are expressed as mean values (standard deviation).

² Optical density of SST expression (μCi/g).

³ Cluster Density per mm².

⁴ Grain count per cell body. Grey shades indicate significant effects (p < 0.05).