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Decreased Numbers of Somatostatin-Expressing Neurons in the Amygdala of Subjects with Bipolar Disorder or Schizophrenia: Relationship to Circadian Rhythms

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Abstract

Background—Growing evidence points to a key role for somatostatin (SST) in schizophrenia (SZ) and bipolar disorder (BD). In the amygdala, neurons expressing SST play an important role in the regulation of anxiety, often comorbid in these disorders. We tested the hypothesis that SST-immunoreactive (IR) neurons are decreased in the amygdala of subjects with SZ and BD. Evidence for circadian SST expression in the amygdala and disrupted circadian rhythms and rhythmic peaks of anxiety in BD suggest a disruption of rhythmic expression of SST in this disorder.

Methods—Amygdala sections from 12 SZ, 15 BD, and 15 control subjects were processed for immunocytochemistry for SST and neuropeptide Y (NPY), a neuropeptide partially co-expressed in SST-IR neurons. Total numbers (N_t) of IR neurons were measured. Time of death (TOD) was used to test associations with circadian rhythms.

Results—SST-IR neurons were decreased in the lateral amygdala nucleus in BD (N_t, p= 0.003) and SZ (N_t, p=0.02). In normal controls, N_t of SST-IR neurons varied according to TOD. This pattern was altered in BD, characterized by decreases of SST-IR neurons selectively in subjects with TOD corresponding to the day (06:00–17:59). Numbers of NPY-IR neurons were not affected.

Conclusions—Decreased SST-IR neurons in the amygdala of SZ and BD, interpreted here as decreased SST expression, may disrupt responses to fear and anxiety regulation in these subjects. In BD, our findings raise the possibility that morning peaks of anxiety depend on a disruption of circadian regulation of SST expression in the amygdala.

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Keywords

anxiety; stress; mood disorder; circadian; interneuron; GABA

Introduction

Growing evidence indicates expression of somatostatin (SST) and neuropeptide-Y (NPY) in amygdala neurons plays key roles in fear and stress responses, and in modulation of anxiety (1–6). Intraventricular, and intra-amygdala infusions of SST in rodents result in anxiolytic and antidepressant effects (1, 7). Mice lacking SST display increased anxiety-like behaviors (2), and neuroendocrine and molecular abnormalities reported in subjects with anxiety and depression (8). NPY infusion counteracts the effects of corticotropin releasing factor (CRF) (4, 5, 9), a molecule essential for stress response (10, 11). In the amygdala, NPY levels decrease following restraint stress (12). Together, these observations point to marked anxiolytic effects of SST and NPY, with prominent involvement of amygdalar circuitry.

Severe anxiety is often comorbid in schizophrenia (SZ) and bipolar disorder (BD) (13–15). Approximately 38% of subjects with SZ and 50% with BD meet criteria for anxiety (16, 17). In both disorders, anxiety is associated with more severe symptoms and/or poorer treatment responses (16, 17). Findings from postmortem and genetic studies suggest abnormal SST and NPY signaling in SZ and BD (18–23). A somatostatin receptor SSTR5 genetic polymorphism has been associated with BD (24). Decreased SST and NPY mRNA was reported in the prefrontal cortex in SZ (18–20). In two of these studies, SST showed the largest decrease with respect to all other interneuron markers examined in SZ (19, 20). Furthermore, decreased SST mRNA was observed in the orbitofrontal cortex (21), and decreased SST mRNA and SST-IR neurons were observed in the hippocampus in both SZ and BD (22, 23). In a quantitative meta-analysis of gene expression studies in BD, SST was identified as one of the most consistently decreased genes (25). Surprisingly, the hypothesis that amygdala neurons expressing SST and NPY are impacted in SZ and BD has not been tested thus far.

Although anxiety represents a shared phenotype in SZ and BD, some differences are notable. For instance, SZ is commonly associated with social phobias, followed by post-traumatic stress disorder and obsessive compulsive disorder (16), while panic disorders and generalized anxiety disorder are common in BD (17). Furthermore, anxiety in BD may be linked more distinctly to disease states, such as depression and, notably, circadian rhythm dysfunction (26–33). In BD, the most severe anxiety and depression symptoms commonly occur in the morning (34–37) (“morning-worse“), with a less common peak in the evening (“evening-worse”) (34–36), suggesting a circadian component to severity. Consistent with these observations, mounting evidence supports a role for circadian rhythm abnormalities in BD (27–32). Sleep and biological rhythms are implicated in this disorder, and genetic polymorphisms for clock-associated genes are associated with BD and lithium responsiveness (32, 38–44). Notably, the most effective treatments, lithium and valproic acid, lengthen circadian period and modulate the expression of clock genes (45–49). A link between SST and circadian rhythms in BD is suggested by evidence that SST is decreased in

cerebrospinal fluid (CSF) sampled in the morning, but not in samples taken in the evening, from the same subjects (50).

Together, these considerations support the hypothesis that SST and NPY expression is decreased in the amygdala of SZ and BD subjects. In BD, altered amygdalar SST expression may be associated with circadian dysfunction (34–37). The observation that SST and NPY expression in the rodent amygdala varies in a circadian manner (2) supports this possibility. The present studies tested the hypothesis that SST- and NPY- immunoreactive (IR) neurons are reduced in the amygdala of subjects with SZ or BD, and that reductions in BD are pronounced in the morning, coinciding with reported increased severity of anxiety and depression at this time (34–37).

Methods and Materials

Human Subjects

Tissue blocks containing the whole amygdala from 12 SZ, 15 BD and 15 normal control donors were obtained from the Harvard Brain Tissue Resource Center, McLean Hospital, Belmont, MA (Tables 1 and S3). Diagnoses were made by two psychiatrists on the basis of retrospective review of medical records and extensive questionnaires concerning social and medical history provided by family members. A neuropathologist examined several regions from each brain for a neuropathology report. The cohort for this study did not include subjects with evidence for gross and/or macroscopic brain changes, or clinical history consistent with cerebrovascular accident or other neurological disorders. Subjects with Braak & Braak stages III or higher were not included. Subjects had no significant history of substance dependence, other than nicotine and alcohol, within 10 years from death.

Tissue Processing (see Supplemental Materials)

Immunocytochemistry—Free-floating tissue sections were carried through antigen retrieval in citric acid buffer (0.1 M citric acid, 0.2 M Na2HPO4) heated to 80 degrees °C for 30 minutes, and incubated in primary antibody monoclonal rat anti-SST (1:500, Millipore, MAB354, lot# NG1934075) raised against synthetic SST peptide corresponding to amino acids 1–14; rabbit anti-NPY (1:1000, Chemicon, AB1915, lot#0604027825), raised against synthetic porcine neuropeptide tyrosine) and subsequently in biotinylated secondary antibody (SST, goat anti-rat IgG; 1:500; Vector Labs, Inc. Burlingame, CA; NPY, goat anti-rabbit IgG (1:500; Vector Labs, Inc. Burlingame, CA), followed by streptavidin conjugated with horse-radish peroxidase for two hours (1:5000 µl, Zymed, San Francisco, CA), and, finally, in nickel-enhanced diaminobenzidine/ peroxidase reaction (0.02% diaminobenzidine, Sigma-Aldrich, 0.08% nickel-sulphate, 0.006% hydrogen peroxide in PB). All solutions were made in PBS with 0.5% Triton X (PBS-Tx) unless otherwise specified.

Immunostained sections were mounted on gelatin-coated glass slides, coverslipped and coded for blinded quantitative analysis. All sections included in the study were processed simultaneously within the same session to avoid procedural differences. Each six-well staining dish contained sections from SZ, BD and control subjects and was carried through each step for the same duration of time. Omission of the primary or secondary antibodies did

not result in detectable signal. The primary antibody for SST has been shown to have no cross-reactivity with enkephalins, endorphins, or substance P (Spec sheet, Millipore Corporation, Temecula, CA). Specificity of these antibodies was confirmed by our group (Supplemental Materials) and others (51).

Data Collection—SST- and NPY-IR neurons were counted in the lateral (LN), basal (BN), accessory basal (AB) and cortical (CO) nuclei of the amygdala using a Zeiss Axioskop-2 Plus interfaced with Stereo Investigator 6.0 (Microbrightfield Inc., Willinston, VT). Intra-rater (J.W.) reliability of at least 95% was established before formal data collection and reassessed regularly. The borders of amygdala nuclei (Fig. 1) were traced and confirmed in adjacent Nissl stained sections according to cytoarchitectonic criteria described by Amaral et al, 1992 and Sims and Williams, 1990 (52, 53). The nomenclature adopted was used by Sorvari et 1995 (54). The central, medial and anterior nuclei could not be quantified because their dorso-medial portion was damaged in several samples. Each traced nucleus was systematically scanned through the full x, y, and z-axes to count each SST- and NPY-IR neuron over complete sets of serial sections (6–10 sections) representing the whole extent of the amygdala from each subject (section interval 1040 μm).

Statistical Analysis—Differences between groups relative to the main outcome measures were assessed for statistical significance using stepwise linear regression (ANCOVA). Effect sizes were calculated according Hedges' g. Logarithmic transformation was uniformly applied to all values because data were not normally distributed. Statistical analyses were performed using JMP v5.0.1a (SAS Institute Inc., Cary, NC). BD and SZ were compared separately to normal controls. Potential confounds (Supplemental Materials) were tested systematically for their effects on main outcome measures, and included in the model if they significantly improved goodness-of-fit. Time of death (TOD) was obtained from the death certificate for each subject and tested for potential effects on outcome measures. TOD was also used to divide subjects into subjective day (s-Day TOD, 06:00–17:59 hours) and subjective night (s-Night, 18:00–05.59 hours) groups on the basis of previous literature indicating circadian fluctuations in SST levels (50, 55, 56). Effects of TOD on outcome measures were analyzed using three steps: 1) Effect of TOD was tested in stepwise linear regression analyses. 2) Subjects were divided into s-Day vs. s-Night groups for comparisons using stepwise linear regression analysis 3) We used quartic regression analysis on plots of $\mathbf{N_t}$ of SST-IR neurons by TOD for each group according to methods used to detect similar relationships in postmortem studies (57–59). Quartic regression models were chosen on the basis of expression patterns reported in the mouse amygdala consisting of two peaks and two troughs in SST expression levels (2). Quartic regression, commonly used to fit plots consisting of four real roots, or x-intercepts of a graph, represented by two expected peaks, has been used to fit dual-peak circadian rhythms in several studies (60–63). The t-ratios and p-values for all main outcome comparisons are reported in Supplementary Table S2. Covariates found to significantly affect outcome measures are also reported.

Numerical Densities and Total Numbers Estimates—Total number ($\mathbf{N_t}$) of IR neurons was calculated as $\mathbf{N_t = i \bullet \Sigma n}$ where $\mathbf{\Sigma n}$ = sum of the cells counted in each subject, and **i** is the section interval (i.e. number of serial sections between each section and the next

within each compartment=26) as described previously (64). Numerical densities were calculated as $\mathbf{N_d = \Sigma N / \Sigma V}$ where **V** is the volume of each amygdala nucleus, calculated as $\mathbf{V = z \bullet ssf \bullet \Sigma\ a}$ where ***z*** is the thickness of the section (40 μm), ***ssf*** is the section sampling fraction (1/26; i.e. number of serial sections between each section within a compartment) and **a** is the area of the region of interest.

Results

Amygdala Volumes

Volumetric results confirm previous findings (64). In subjects with SZ, no volume changes were detected (Supplementary Fig. 1). A significant effect of hemisphere was observed on the volume of the overall amygdala. In subjects with BD, significant volume decreases were detected in the LN ($p<0.005$, $g=-1.22$; adjusted for a significant effect of lifetime exposure to CPZ), and CO ($p<0.007$, $g=-1.09$) (Supplementary Fig. 1).

SST-IR Neurons

In the healthy human amygdala, SST-IR neurons were detected in all amygdala nuclei examined, with the highest numbers in the LN and the highest densities in the AB and CO (Figure 1, Supplementary Table S1), consistent with observations in primates (3). In subjects with SZ, $\mathbf{N_t}$ and $\mathbf{N_d}$ of SST-IR neurons were decreased selectively in the LN ($\mathbf{N_t}$, $p= 0.03$, $g= -0.98$; adjusted for hemisphere $p=0.03$; $\mathbf{N_d}$, $p = 0.02$, $g=-0.92$; Figure 2, Supplementary Table S2). Similarly, in subjects with BD, SST-IR neurons were decreased in the LN ($\mathbf{N_t}$, $p = 0.005$, $g=-1.11$; $\mathbf{N_d}$, $p = 0.005$, $g=-0.91$; Figure 2, Supplementary Table S2, with a significant effect of TOD, $p<0.005$). In both disease groups, decreases of SST-IR neurons did not correlate with years of illness, and were not affected by exposure to antipsychotics, lithium, SSRIs, alcohol or nicotine (Supplemental Materials). No changes were observed in any of the other amygdala nuclei examined (Figure 2, Supplementary Table S2).

NPY-IR Neurons

NPY-IR neurons are less numerous than SST-IR neurons, and evenly distributed across amygdala nuclei (Figure 3, Supplementary Table S1). In subjects with SZ, no changes were detected in any of the nuclei examined. In subjects with BD, a marginally significant decrease of $\mathbf{N_t}$, but not $\mathbf{N_d}$, of NPY-IR neurons was detected in the CO ($\mathbf{N_t}$, $p<0.04$, $g= -0.81$, adjusted for PMI) (Figure 4, Supplementary Table S2).

Effects of Time of Death

In control subjects, $\mathbf{N_t}$ of SST-IR neurons in the LN, as well as in the combined LN-BN-AB-CO, were significantly higher in subjects with s-Night TOD, as compared to subjects with a s-Day TOD (LN, $\mathbf{N_t}$, $p<0.03$, $g= -1.34$; with a significant effect of sex ($p<0.01$), and cause of death ($p<0.02$); $\mathbf{N_d}$, $p<0.03$, $g= -1.24$; LN-BN-AB-CO, $\mathbf{N_t}$, $p= 0.14$, $g= -0.81$; $\mathbf{N_d}$, $p<0.04$, $g= -1.15$). To further test this relationship, $\mathbf{N_t}$ of SST-IR neurons in the LN were plotted by TOD and fit to quartic regression analysis. A rhythmic-like relationship was observed, with a significant quartic regression fit ($F=6.9$, $p<0.006$; Fig. 5), displaying two peaks of SST-IR neurons in diurnal humans, identical to amygdala SST rhythms reported in

nocturnal mice (2). A first peak in $\mathbf{N_t}$ of SST-IR neurons at approximately 12 AM was followed by a trough at approximately 6 AM, and by a second, smaller, peak at approximately 12 PM, followed by a second trough at approximately 6 PM.

Subjects with BD showed an enhanced day/night difference with respect to controls. $\mathbf{N_t}$ of SST-IR neurons in the LN were significantly higher in BD subjects with a s-Night TOD ($\mathbf{N_t}$, $p<0.01$, $g= -1.49$; $\mathbf{N_d}$, $p<0.002$, $g= -2.04$; Fig. 5) and in the combined LN-BN-AB-CO ($\mathbf{N_t}$, $p<0.0009$, $g= -2.24$; Fig. 5) with respect to BD subjects with a s-Day TOD. Notably, healthy control versus BD between-group comparison showed that $\mathbf{N_t}$ of SST-IR neurons were significantly lower in BD in the s-Day groups (LN-BN-AB-CO: $p= 0.04$, $g= -1.12$; LN: $p= 0.01$, $g= -1.39$) but not in the s-Night groups (Fig. 5). Quartic regression analysis showed $\mathbf{N_t}$ of SST-IR neurons in subjects with BD display an altered rhythmic-like relationship with respect to control subjects (quartic regression fit: $F= 5.08$, $p<0.02$, Fig. 5). The appearance of a ‘reversed rhythm’ with a trough at approximately 12 AM, a peak at approximately 6 AM, a second trough at approximately 12 PM and a second peak at approximately 6 PM, is driven by the sharp reduction of SST-IR neurons between 6AM–12PM in the BD group.

TOD analyses could not be performed in subjects with SZ because of insufficient number of subjects with SZ with TOD between 4 PM and 12 AM. $\mathbf{N_t}$ of NPY-IR neurons in the LN did not vary between the s-Day and s-Night groups in controls or in subjects with BD (Fig. 5), consistent with lack of effect of TOD in stepwise linear regression models.

Discussion

The present studies resulted in three main, previously unreported, findings: i) SST-IR neurons are decreased in the amygdala in SZ and BD, ii) the expression of SST in the healthy human amygdala displays a circadian-like rhythm, and iii) this circadian-like SST expression is altered in BD. These findings add to growing evidence for involvement of SST in SZ and BD (18–20, 22, 23), and suggest that amygdalar SST decreases represent a common denominator, contributing to elevated anxiety in both disorders. In BD, we observed enhanced rhythmic-like SST expression in the LN defined by a marked decrease of SST-IR neurons in the morning, when numbers of SST-IR neurons increase in control subjects. The lowest portion of that cycle corresponds to the ‘morning worse’ time period typical of BD. Consistent with these findings, comparisons between control and BD subjects show a significant effect of TOD on SST-IR neuron numbers. These results contribute to emerging evidence for circadian rhythm disruption in BD, and suggest that dysregulation of circadian SST expression in the amygdala may contribute to morning peaks of anxiety and depression (34–37). In subjects with SZ, relationships with circadian rhythms were not investigated for technical reasons.

Technical Considerations

TOD Analysis as a Proxy for Circadian Time—In these postmortem studies, TOD for each subject was used to monitor expression changes as they may relate to circadian rhythms. TOD represents a single measure per subject at a specific time point, rather than repeated measures across time. Therefore, we refer to SST-IR neuron numbers plotted by TOD as “circadian-like” and “rhythmiclike”. This approach has been successfully used in

several postmortem brain studies (57–59, 65, 66). In the present study, the validity of this method is supported by the observation that “rhythmic-like’ changes of SST expression observed in healthy human amygdala (Fig. 5) are consistent with those reported in the rodent amygdala (2). In addition, SST rhythmic-like abnormalities detected in BD parallel similar abnormalities reported in cerebrospinal fluid of live subjects with mood disorders (50).

Treatment of Antipsychotics and Lithium—We did not detect significant effects of exposure to antipsychotics, lithium, or valproic acid on SST-IR and NPY-IR neurons. Although effects of these confounding factors cannot be ruled out with certainty, our results indicate that pharmacological treatment did not contribute to decreases of SST-IR neurons. In support, chronic treatment with antipsychotics or lithium in rodents increases SST and NPY expression (67–71). For additional considerations, see ‘Supplemental Material’.

Decreases of SST-IR neurons

Decreased numbers of SST-IR neurons in the amygdala may reflect neuronal loss or decreased expression. Several considerations point to decreased expression as the most likely interpretation. First, in both SZ and BD, numbers of NPY-IR neurons, co-expressed in a large percentage of SST neurons, were not altered. This discrepancy suggests that either changes in SST expression occur in NPY-negative neurons, or differential factors may regulate SST and NPY in the same neurons. This latter possibility is supported by studies on SST mutant mice and chronic stress reporting altered SST expression but normal levels of NPY (72). Second, previous studies in a largely overlapping cohort showed that amygdalar $\mathbf{N_t}$ and $\mathbf{N_d}$ of Nissl-stained neurons and volumes were unchanged in SZ (64), a finding inconsistent with neuronal loss. Third, in BD, significant decreases of $\mathbf{N_t}$ and $\mathbf{N_d}$ of SST-IR neurons in the LN do not parallel normal $\mathbf{N_d}$ of Nissl-stained neurons in a largely overlapping subject cohort, even in the presence of volume decreases (64). Fourth, fluctuations of SST in the amygdala of rodents (2) and SST-IR neurons in healthy humans (this study), and s-Day decreases of SST-IR neurons in BD across all amygdala nuclei (i.e. including those with normal total neuron number and volume (64)) are not consistent with neuronal loss. The likelihood that we detect SST-IR cells above a certain threshold of protein expression rather than absolute measures of protein, together with the short circulating half-life of SST (<3 minutes) (73, 74), further suggest that SST-IR neuron numbers across TOD represent fluctuating levels of SST within neurons. Together, these observations provide strong support for decreased SST expression in the amygdala of subjects with SZ and BD.

Decreased SST expression in SZ and BD: Implications for Amygdala Activity and Comorbidity with Anxiety and Stress Vulnerability

Amygdala SST-IR neurons are primarily GABAergic (75), and form inhibitory synapses onto distal dendrites of local pyramidal neurons (76). The proximity of these synapses to excitatory inputs suggests that SST synapses on pyramidal neurons affect synaptic plasticity related to emotional learning (76). SST exerts an inhibitory effect on amygdala pyramidal neurons and, in several brain regions, modulates GABAergic inhibition (77–82). These considerations suggest that decreased SST may contribute to reduced inhibitory regulation and disruption of amygdala intrinsic circuits. Consistent with this possibility, increased amygdala activity has been reported in SZ during the processing of emotional stimuli (83,

84), and in subjects with BD during mania (85). Predominant SST-IR neuron decreases in the LN, known to mediate plasticity and rapid behavioral responses to fearful stimuli (86, 87), point to abnormal processing of sensory stimuli and fear response. In addition, it is possible that small populations of LN SST-IR neurons projecting to the entorhinal cortex (88), and BN and CO SST-IR neurons to the basal forebrain (89) may be involved, potentially contributing to disrupted sensory gating in SZ (90–94), and dysregulation of sleep-wake patterns in BD (95–97) through the basal forebrain (98, 99), respectively.

Growing evidence suggest that SST in the amygdala powerfully reduces anxiety (1, 2, 4–6), indicating that SST represents a potential pharmacological target against depression and anxiety (100, 101). Animal models show anxiolytic, and possibly antidepressant, effects of SST in the amygdala, and suggest a role in fear learning and expression (102, 103). Increased SST receptors in the amygdala in response to threatening stimuli (104) suggest that SST activation in this region may counteract anxiety-related responses, perhaps contributing to maintain a balance between adaptive fear responses and maladaptive, or generalized, anxiety. Thus, it is reasonable to speculate that decreased SST expression in the amygdala of subjects with SZ and BD may result in increased anxiety and heightened vulnerability to stress. Consistent with this hypothesis, SST−/− mice display high cortisol levels and increased anxiety and depressive-like behaviors (8). Furthermore, decreased SST levels in subjects with depression correlate with increased cortisol levels (50, 105–107), and are associated with a greater plasma cortisol response to dexamethasone (50), suggesting that decreased SST contributes to disinhibited stress response. Finally, SST expression in subjects with SZ and BD was reported to correlate with levels of inflammatory cytokines (108), in turn associated with altered expression of stress-related markers (109). Alternatively, amygdala SST expression may decrease as a consequence to chronic stress experienced by patients with SZ or BD. Lack of correlation of SST-IR neuron numbers with years of illness suggests otherwise.

SST-IR Neurons in the Amygdala of Subjects with BD: Potential Link to Circadian Rhythm Dysregulation

In subjects with BD, SST-IR neuron decreases across amygdala nuclei were selective for the s-Day group, suggesting a link with circadian rhythm dysregulation (34, 35, 37, 50, 110). These findings are consistent with results from another group, showing decreased levels of SST in the CSF of subjects with affective disorders selectively in the morning (50). Notably, the significant difference in rhythmic-like SST expression in the LN of subjects with BD (Fig. 5) points to an abnormal circadian phase in the amygdala. The enhanced rhythmic-like distribution observed, with its appearance of a reversed rhythm in the regression plot driven by the sharp decrease of SST-IR neurons in BD subjects with a morning TOD, represents an enhanced day/night difference with respect to the moderate s-Day decrease of SST-IR neurons in control subjects. In BD, the sharp decrease between 6AM–12 PM may contribute to the morning-worse phase of anxiety and depression (34–37).

Circadian rhythms are controlled by clock genes in the suprachiasmatic nucleus (SCN), which coordinates rhythms throughout the brain and body (111, 112). However, clock genes exist in many neural and non-neural tissues (113–115), and can impact mood in a variety of

manners (116). Animal studies have shown that clock gene rhythms in distinct brain regions can change phase irrespective of the SCN rhythm (117, 118). Thus, altered circadian rhythm of SST expression in the amygdala of subjects with BD may result from at least two, non-mutually exclusive, mechanisms, i.e. i) core circadian dysfunction in the SCN, and ii) dysregulation of intrinsic amygdala circadian rhythms. Support for reduced synchrony of rhythms by the SCN comes from studies reporting reduced circadian amplitude of activity rhythms in subjects with BD (119–121), and from observations on the effects of bright-light therapy (122), which may resynchronize the circadian clock through established effects of light input to the SCN (123–127). Support for intrinsic amygdala dysregulation is based on evidence that molecular rhythms within this region change phase in response to fearful stimuli (118). Thus, altered amygdala SST rhythms may result from heightened responses to negative environmental factors, as observed in subjects with BD (128–130). Notably, glucocorticoids are regulated by the circadian clock and affect amygdalar circadian rhythms (131–136). It is plausible that altered SST rhythms in this region may be induced by interactions between heighted responses to stress and circadian rhythm dysregulation. In addition, the basolateral amygdala displays a circadian rhythm anti-phase to the central amygdala (137), the later of which is regulated by glucocorticoids (135). Glucocorticoid receptors (GR) are present throughout the amygdala (138, 139), with highest concentrations in the central nucleus (140, 141). Furthermore, glucocorticoids also directly regulate CLOCK genes through glucocorticoid response elements located in the promoter region of Per1 and Per2 genes (142, 143). A complex interaction of stress and CLOCK genes may contribute to enhanced SST amygdala rhythms in BD.

Evidence that neurons in the hypothalamus switch between dopamine and SST expression under altered light-dark cycles (144), suggest a complex relationship of circadian rhythms with SST and dopamine. CLOCK has been shown to regulate dopamine expression (145–147). Altered CLOCK function resulting in enhanced dopamine transmission to the amygdala, together with disrupted SST rhythms, may contribute to an imbalance of mood regulation.

In summary, reductions of amygdalar SST-IR neurons add to growing evidence of the involvement of this neuropeptide in SZ and BD. Reduced SST expression in the amygdala may contribute to anxiety and stress vulnerability frequently comorbid in these disorders. Our findings also suggest SST expression in the normal human amygdala varies according to circadian rhythms. In BD, a disruption of this rhythm, with decreased SST expression in the subjective day, may contribute to peaks of anxiety and depression in the morning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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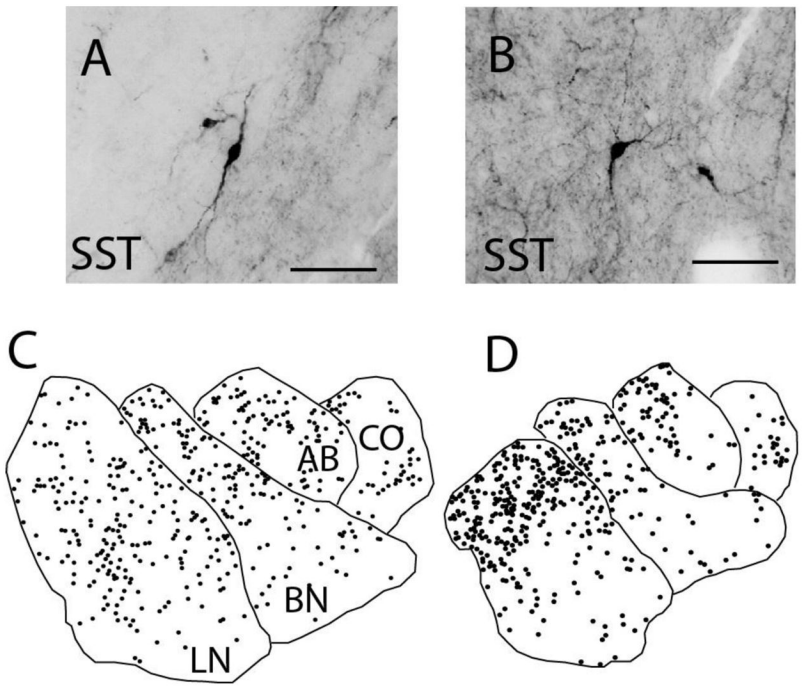

Figure 1. Somatostatin Immunoreactive Neurons in the Human Amygdala

Somatostatin immunoreactive (SST-IR) neurons display various morphological subtypes in the human amygdala, including fusiform (A) and multipolar (B) neurons. SST-IR neurons are widely distributed across all of the amygdala nuclei examined, as shown by representative plots from a control subject depicting the distribution of SST-IR neurons in the rostral (C) and caudal (D) amygdala. Scale bars = 50 μm. LN: lateral nucleus, BN: basal nucleus, AB: accessory basal nucleus, CO: cortical nucleus.

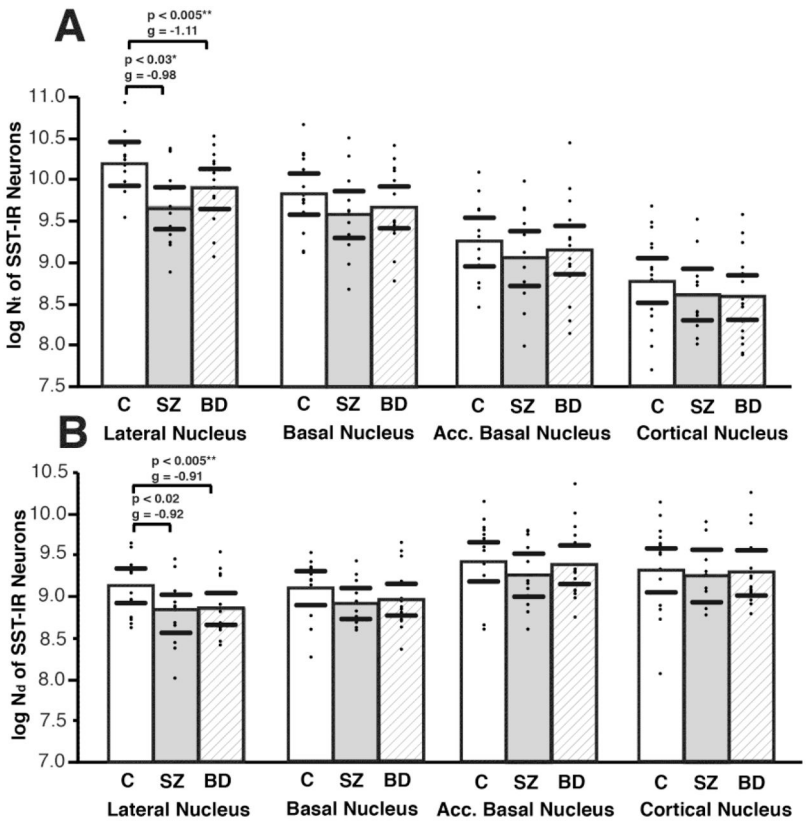

Figure 2. Decreased Total Numbers and Numerical Densities of Somatostatin Neurons in the Lateral Nucleus of SZ and BD Subjects

Scatterplots depicting total numbers (N_t) (A), and numerical densities (N_d) of SST-IR neurons in control, SZ, and BD subjects. Significant decreases of N_t (A) and N_d (B) were detected in the lateral nucleus of SZ and BD subjects. Significance values are derived from stepwise linear regression models. Scatterplots show the mean (histogram) and 95% confidence intervals (black lines). *Adjusted for significant effect of hemisphere. ** Adjusted for significant effect of time of death.

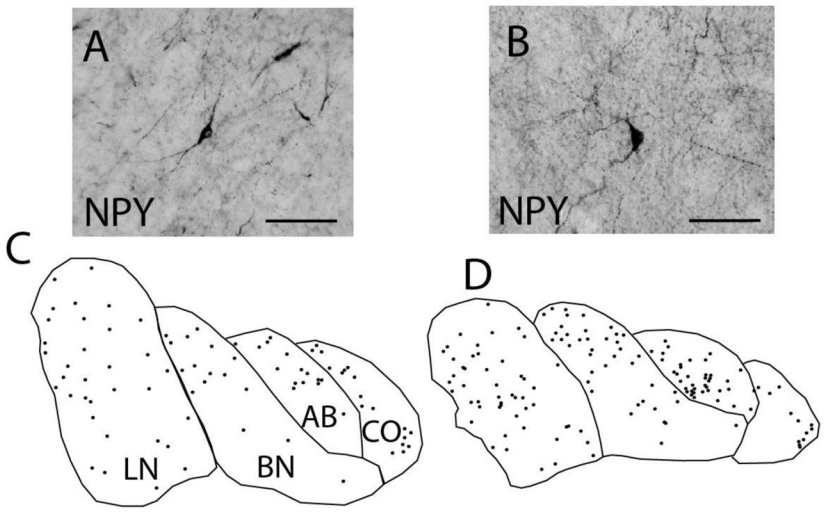

Figure 3. Neuropeptide-Y Immunoreactive Neurons in the Human Amygdala

Neuropeptide-Y immunoreactive (NPY-IR) neurons morphological subtypes in the human amygdala, including fusiform (A) and multipolar (B) neurons. NPY-IR neurons are widely distributed across all of the amygdala nuclei examined as shown by representative plots from a control subject depicting the distribution of NPY-IR neurons in the rostral (C) and caudal (D) amygdala. Scale bars = 50 μm. LN: lateral nucleus, BN: basal nucleus, AB: accessory basal nucleus, CO: cortical nucleus.

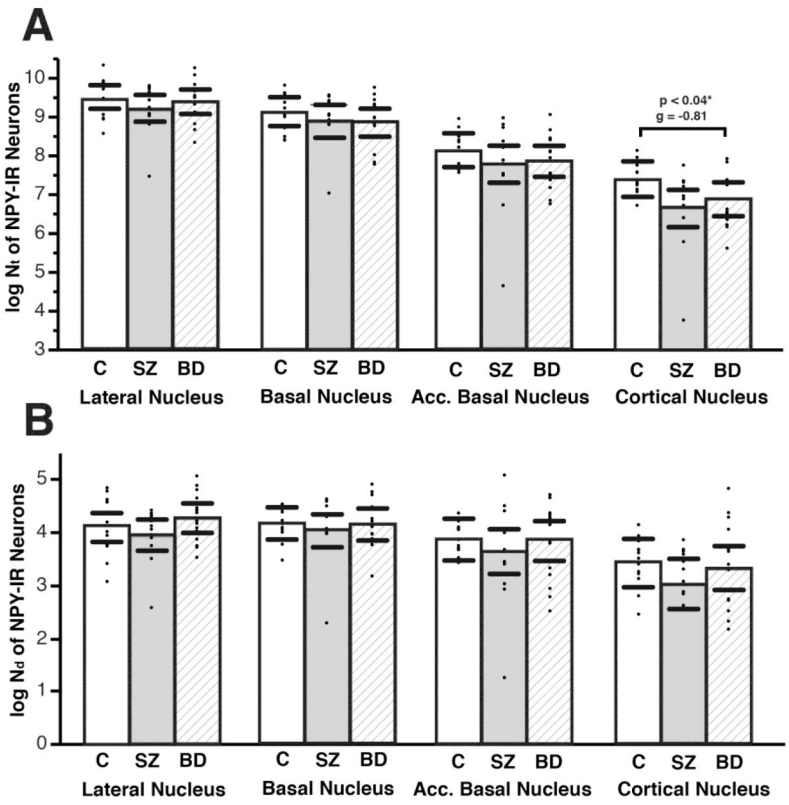

Figure 4. Total Numbers and Numerical Densities of Neuropeptide-Y Immunoreactive Neurons Are Not Altered in the Amygdala of SZ or BD Subjects

Scatterplots depicting total numbers (N_t) (A), and numerical densities (N_d) of NPY-IR neurons in control, SZ, and BD subjects. A marginally significant decrease of N_t, but not N_d, of NPY-IR neurons was detected only in the CO nucleus (N_t, $p<0.04$, $g= -0.81$, adjusted for effect of PMI). No other significant changes were observed in N_t or N_d of NPY-IR neurons when SZ or BD subjects were compared to normal control subjects. Significance values are derived from stepwise linear regression models. Scatterplots show the mean (histogram) and 95% confidence intervals (black lines).

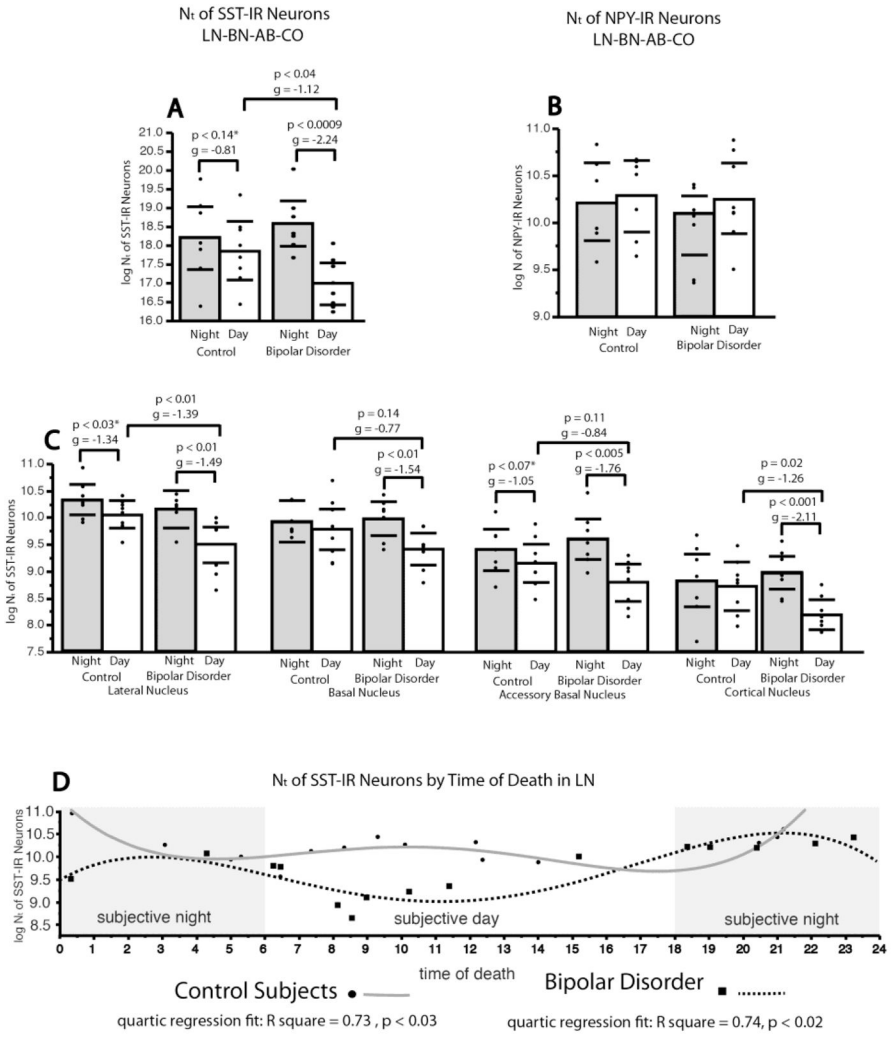

Figure 5. SST-IR Neurons are Decreased Selectively in BD Subjects with Subjective Day Time of Death

(A) Scatterplot depicts total numbers ($\mathbf{N_t}$) of SST-IR neurons in the combined LN-BNAB-CO of subjects with a time of death (TOD) in the subjective day (06:00–17:59) in comparison to subjects with a time of death in the subjective night (18:00–05:59). Within the normal control group, there is a trend toward higher numbers of SST-IR neurons in the subjective night TOD group with respect to subjective day TOD group. Within the BD group, this comparison is significant, with subjective night TOD group showing higher numbers of SST-IR neurons with respect to the subjective day TOD group ($p< 0.009$). Between group comparisons of subjective day and subjective night respectively show a significant effect of diagnosis selectively for the subjective day TOD group ($p< 0.04$).. (**B**) No relationship was observed between TOD and total number of NPY-IR neurons in the LN in either diagnosis group. (**C**) Scatterplots of Nt of SST-IR neurons in the subjective day TOD vs. subjective night TOD across each amygdala nucleus. SST-IR neuron numbers were found to be lower in the subjective day TOD in both control and BD subjects in most amygdala nuclei. In subjects with BD, decreases of total number of SST-IR neurons were present in the subjective day TOD group in the LN, and CO, with statistical trends for

decreases in the BN and AB. Furthermore, comparisons of subjective day vs. subjective night groups in subjects with BD revealed a significant decrease of Nt of SST-IR neurons in the subjective day across all amygdala nuclei examined in this disorder (C). **(D)** Plots display quartic regression analysis of total numbers of SST-IR neurons in the LN by TOD for each diagnosis group. A rhythmic relationship is evident for both normal control and BD groups. In control subjects, total numbers of SST-IR neurons display a peak at approximately 12 AM, followed by a trough at approximately 6 AM, and a second peak at approximately 12 PM followed by a second trough at approximately 6 PM (Black circles, gray solid line). Subjects with BD show a reverse rhythmic-like relationship, with a trough at approximately 12 AM, a peak at approximately 6 AM, a second trough at approximately 12 PM and a second peak at approximately 6 PM (black squares, black dashed line). Scatterplots show the mean (histogram) and 95% confidence intervals (black lines). *Adjusted for significant effect of sex and cause of death.

Table 1

Disease-Related Descriptive Characteristics

case	AGE OF ONSET	Duration of illness (years)	CPZ (g)	CPZ last 6 (g)	LI (g)	LI last 6 months (g)	nicotine	ethanol	ECT	SSRI	VPA (g)
SCHIZOPHRENIA											
4375	19	12	1654	171	0	0	0	0	0	no	0
4496	19	30	801.5	36	0	0	1	2	2	no	913.1
4544	16	16	671	30	0	0	3	2	0	no	0
4548	-	-	0	0	0	0	-	-	0	no	0
4702	35	20	793	27	0	0	1	2	2	yes	0
4707	38	22	365.2	0	0	0	1	NA	0	yes	0
4907	22	51	498	24	0	0	3	0	4	yes	0
4942	20	41	3550	36.18	0	0	2	2	3	yes	0
5100	24	48	420	31.5	0	0	1	0	0	yes	0
5656	19	54	37	0	36.8	0	2	0	0	no	0
5785	31	31	1169	162	0	0	0	3	1	yes	1370
5920	20	38	2430	81	648	108	3	-	0	no	730
case	AGE OF ONSET	Duration of illness (years)	CP Z (g)	CPZ last 6 (g)	LI (g)	LI last 6 months (g)	nicotine	ethanol	ECT	SSRI	VPA (g)
BIPOLAR DISORDER											
4403	67	9	77.9	2.7	1321.0	27.0	4	4	0	no	0
4464	52	22	102.5	29.25	3945	0	1	2	0	no	547.9
4518	-	-	0	0	0	0	-	-	0	-	-
4545	30	43	10.8	3.6	0	0	1	0	0	no	0
4661	15	10	328.7	90	3945	216	3	2	0	yes	0
4665	35	31	731.3	1.0	1096.0	54.0	4	0	0	no	0
4914	20	53	202.7	13.95	4273.0	0	1	0	0	no	0
4928	27	48	0	0	0	0	0	0	0	no	0
5044	35	38	82.2	20.3	2191.5	0	0	0	0	no	182.6
5265	43	40	0	0	2190.0	0	-	0	0	yes	803.6
5357	50	12	0	0	216.0	0	1	0	0	yes	292.2
5391	23	44	237.5	54	4131	0	4	2	3	no	1825

case	AGE OF ONSET	Duration of illness (years)	CPZ (g)	CPZ last 6 (g)	LI (g)	LI last 6 months (g)	nicotine	ethanol	ECT	SSRI	VPA (g)
5431	22	18	1116	55.8	4320	0	2	4	0	-	-
5802	33	18	1242.0	81.0	0	0		1	4	yes	912
5888	27	43	0	0	3240	0	4	4	3	no	456.25