Archival Report

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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, which is 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, a neuropeptide partially coexpressed in SST-IR neurons. Total numbers (N_t) of IR neurons were measured. Time of death was used to test associations with circadian rhythms.

RESULTS: SST-IR neurons were decreased in the lateral amygdala nucleus in BD (N_t, $p = .003$) and SZ (N_t, $p = .02$). In normal control subjects, N_t of SST-IR neurons varied according to time of death. This pattern was altered in BD subjects, characterized by decreases of SST-IR neurons selectively in subjects with time of death corresponding to the day (6:00 AM to 5:59 PM). Numbers of neuropeptide Y-IR neurons were not affected.

CONCLUSIONS: Decreased SST-IR neurons in the amygdala of patients with SZ and BD, interpreted here as decreased SST expression, may disrupt responses to fear and anxiety regulation in these individuals. 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.

Keywords: Anxiety, Circadian, Gamma-aminobutyric acid, Interneuron, Mood disorder, Stress

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Growing evidence indicates that expressions of somatostatin (SST) and neuropeptide Y (NPY) in amygdala neurons play key roles in fear and stress responses and in modulation of anxiety (1–6). Intraventricular and intra-amygdalar 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 similar to those reported in subjects with anxiety and depression (8). Infusion of NPY counteracts the effects of corticotropin-releasing factor (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% of subjects 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 messenger RNA (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). The hypothesis that amygdala neurons expressing SST and NPY are impacted in SZ and BD has not been tested thus far.

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Although anxiety represents a shared phenotype in SZ and BD, some differences are notable. For example, SZ is commonly associated with social phobias, posttraumatic stress disorder, and obsessive-compulsive disorder (16), whereas 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 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). The most effective treatments, lithium and valproic acid, lengthen circadian periods 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 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 subjects with SZ and BD. 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 study tested the hypothesis that SST-immunoreactive (IR) and NPY-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 (Table 1 and Supplemental Table S3). Diagnoses

Table 1. Disease-Related Descriptive Characteristics

Case	Age of Onset (Years)	Duration of Illness (Years)	CPZ (g)	CPZ Last 6 Months (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
Bipolar Disorder											
4403	67	9	77.9	2.7	1321	27	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	1096	54	4	0	0	No	0
4914	20	53	202.7	13.95	4273	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	Yes	803.6
5357	50	12	0	0	216	0	1	0	0	Yes	292.2
5391	23	44	237.5	54	4131	0	4	2	3	No	1825
5431	22	18	1116	55.8	4320	0	2	4	0	—	—
5802	33	18	1242	81.0	0	0		1	4	Yes	912
5888	27	43	0	0	3240	0	4	4	3	No	456.25

CPZ, chlorpromazine; ECT, electroconvulsive therapy; Li, lithium; NA, not available; SSRI, selective serotonin reuptake inhibitor; VPA, valproic acid.