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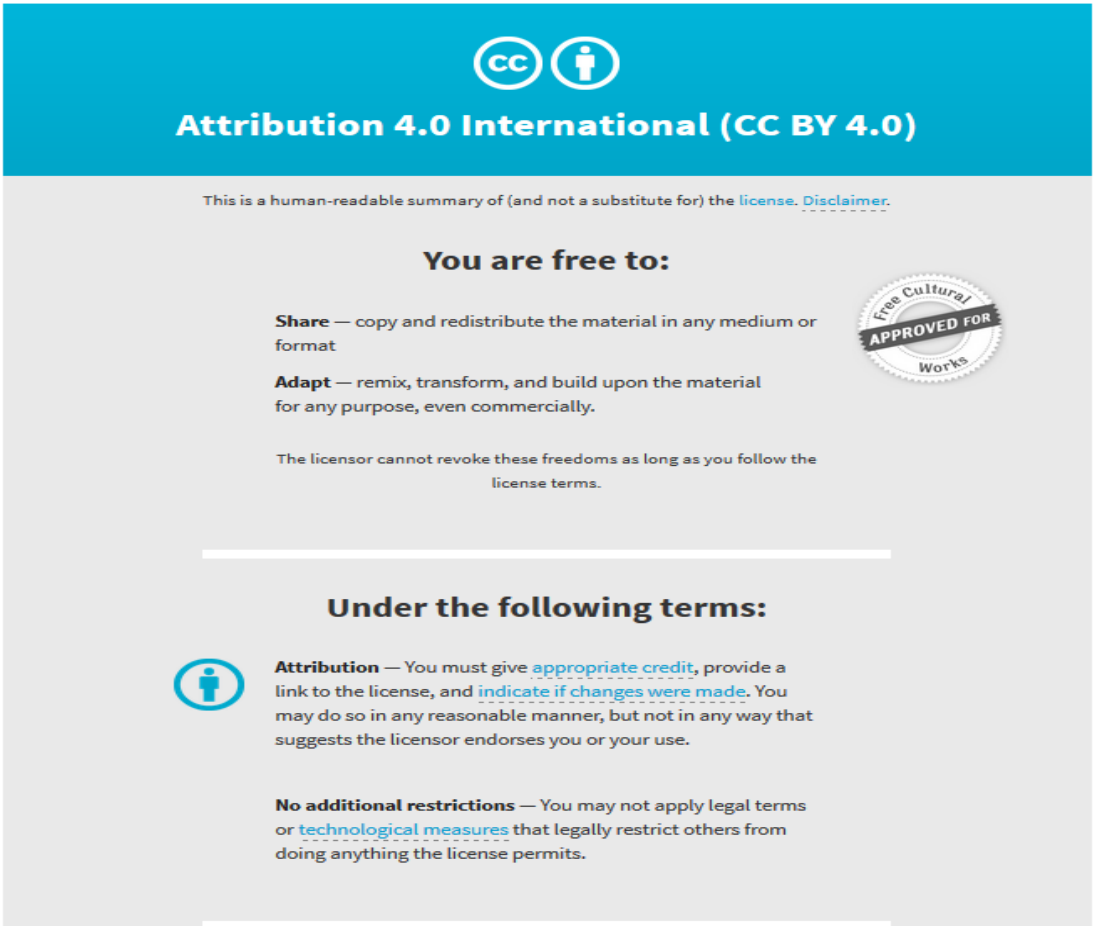

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Calretinin and parvalbumin in schizophrenia and affective disorders: a mini-review, a perspective on the evolutionary role of calretinin in schizophrenia, and a preliminary post-mortem study of calretinin in the septal nuclei

Ralf Brisch[1]†, Hendrik Bielau[2]†, Arthur Saniotis[3,4], Rainer Wolf[5], Bernhard Bogerts[2,6], Dieter Krell[2], Johann Steiner[2,6], Katharina Braun[6,7], Marta Krzyżanowska[1], Maciej Krzyżanowski[1], Zbigniew Jankowski[1], Michał Kaliszan[1], Hans-Gert Bernstein[2] and Tomasz Gos[1]*

[1] Department of Forensic Medicine, Medical University of Gdańsk, Gdańsk, Poland, [2] Department of Psychiatry and Psychotherapy, Otto-von-Guericke-University of Magdeburg, Magdeburg, Germany, [3] School of Medicine, The University of Adelaide, Adelaide, SA, Australia, [4] Institute of Evolutionary Medicine, University of Zurich, Zurich, Switzerland, [5] Department of Psychiatry and Psychotherapy, Ruhr University Bochum, Bochum, Germany, [6] Center for Behavioral Brain Sciences, Magdeburg, Germany, [7] Department of Zoology/Developmental Neurobiology, Institute of Biology, Otto-von-Guericke-University of Magdeburg, Magdeburg, Germany

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***Correspondence:**
Ralf Brisch,
Department of Forensic Medicine, Medical University of Gdańsk, ul. Dębowa 23, PL-80-204 Gdańsk, Poland
ralfbrisch@hotmail.com

† These authors have contributed equally to this work.

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Objective: The septal nuclei are important limbic regions that are involved in emotional behavior and connect to various brain regions such as the habenular complex. Both the septal nuclei and the habenular complex are involved in the pathology of schizophrenia and affective disorders.

Methods: We characterized the number and density of calretinin-immunoreactive neurons in the lateral, medial, and dorsal subregions of the septal nuclei in three groups of subjects: healthy control subjects ($N = 6$), patients with schizophrenia ($N = 10$), and patients with affective disorders ($N = 6$).

Results: Our mini-review of the combined role of calretinin and parvalbumin in schizophrenia and affective disorders summarizes 23 studies. We did not observe significant differences in the numbers of calretinin-immunoreactive neurons or neuronal densities in the lateral, medial, and dorsal septal nuclei of patients with schizophrenia or patients with affective disorders compared to healthy control subjects.

Conclusions: Most post-mortem investigations of patients with schizophrenia have indicated significant abnormalities of parvalbumin-immunoreactive neurons in various brain regions including the hippocampus, the anterior cingulate cortex, and the prefrontal cortex in schizophrenia. This study also provides an explanation from an evolutionary perspective for why calretinin is affected in schizophrenia.

Keywords: calretinin, parvalbumin, septal nuclei, post-mortem studies, schizophrenia, affective disorders, evolution of the human brain

Introduction

Ca^{2+}-binding proteins (CBPs) are classified as Ca^{2+}-puffer proteins (CPPs; parvalbumin, calretinin, calbindin, calcineurin, and the S100 family) or Ca^{2+}-sensor proteins (CSSs; calmodulin, and VILIP-1,3). The processes by which CPPs and CSSs interact are not well-understood. In schizophrenia, CBPs are used to identify altered GABAergic (γ-aminobutyric acid-producing) interneurons in various brain regions, including the prefrontal cortex, hippocampus, and amygdala (Inan et al., 2013). Calretinin belongs to a subset of inhibitory interneurons that uses the neurotransmitter GABA (Barinka and Druga, 2010; Cauli et al., 2014). GABA is converted by the action of glutamic acid decarboxylase (GAD), which exists as two isoforms, GAD65 and GAD67. GAD65 is localized in axon terminals, and GAD67 is localized in neuronal cell bodies (Blum and Mann, 2002). Calretinin has been detected in the cingulate and entorhinal cortices as well as the hippocampus, brainstem, and cerebellum in the human brain (Nitsch and Ohm, 1995; Mikkonen et al., 1997; Baizer, 2014). White matter interneurons express GABA, calbindin, and calretinin (Suárez-Solá et al., 2009), which are relevant in schizophrenia (review by Kostovic et al., 2011; Yang et al., 2011; Joshi et al., 2012).

In the present study, we assessed the presence of calretinin in the anterior, middle, and posterior portions of the human septal nuclei. Recent findings regarding the involvement of calretinin in neurogenesis and animal models of psychiatric diseases imply that it is important to study the role of calretinin in the septal nuclei in schizophrenia and affective disorders. A loss of calretinin causes a deficit in adult hippocampal neurogenesis (Todkar et al., 2012). In adult mice, calretinin-positive neurons are present in the dentate gyrus, an important neurogenic zone (Spampanato et al., 2012). The transcription factor Gsx2 (genetic screened homebox 2) proliferates in the human cortical subventricular zone and commits cortical stem cells into calretinin-expressing cells (Radonjic et al., 2014). Calretinin cells originate from the subventricular zone of the lateral and caudal ganglion eminences (González-Gómez and Meyer, 2014). The density of calretinin-positive neuronal progenitors along the septo-temporal axis of the hippocampus was decreased by unpredictable chronic mild stress (UCMS), and this effect was inhibited by the treatment with the antidepressant fluoxetine (Tanti et al., 2013). Alterations in calretinin expression have been observed in mouse models of epilepsy and psychiatric diseases (Shin et al., 2013); however, no significant differences in the number of calretinin-immunoreactive interneurons in the cerebral cortices of wild-type and DBZ (DISC1-binding zinc finger protein) knockout (KO) mice have been reported (Koyama et al., 2013). Electroconvulsive therapy (ECT) results in the neurogenesis of calretinin-positive interneurons (Inta et al., 2013). The septum, via cholinergic and GABAergic pathways, is involved in the regulation of mesolimbic dopamine transmission (Lecourtier et al., 2010). The suppression of the septohippocampal pathway and its GABAergic activity might represent a novel treatment for the symptoms of schizophrenia (Ma et al., 2012; Deidda et al., 2014). The medial habenular complex is connected to the septal nuclei through the stria medullaris (Sutherland, 1982; Hikosaka, 2013). Habenular dysfunction is involved in schizophrenia (Heldt and Ressler, 2006), and habenular calcification has been reported in schizophrenia (Sandyk, 1992). A reduction in the volume of the medial and lateral habenular complex and reductions in the cell number and area of the medial habenula have been observed in affective disorders (Ranft et al., 2010), mainly a habenular volume reduction in unmedicated bipolar patients (Savitz et al., 2011a), but not in patients with post-traumatic stress disorder (Savitz et al., 2011b). Further, diminished neuronal density has been reported in the lateral septal nucleus of brain sections from bipolar patients compared with control subjects using both Nissl and Heidenhain-Woelke methods (Brisch et al., 2011).

The aim of this sudy is to investigate whether alterations of calretinin-immunoreactive neurons exist in the lateral, medial and dorsal septal nuclei in patients with schizophrenia and patients with affective disorders in comparison with healthy control subjects, based on the pathway between the septal nuclei and the habenular complex and the importance of calretinin in neurogenesis and animal models of psychiatric diseases.

Experimental Procedures

Subjects

All brains used in this study were from the Brain Collection of the University of Magdeburg. Brains were obtained from pathologists or medical examiner offices in the years 1987–2002 according to the Declaration of Helsinki (1975) and German and EU laws and after approval by the university's ethic commission. The mean demographic data for all individual cases (all were Caucasian) including brain weight, post-mortem delay, onset of disease, and duration of disease are present in **Table 1**. The three groups were carefully matched for gender, age, post-mortem delay, the age at onset of illness, and brain weight. The post-mortem brains of six subjects lacking any signs of neurological or psychiatric symptoms were used as a control group. Brains from 10 patients with schizophrenia diagnosed according to the DSM-IV (Diagnostic and statistical manual of mental disorders) and ICD-10 (International statistical classification of diseases and related health problems) criteria were included; most of these patients had received antipsychotic treatment for at least several years. In addition, the brains of six patients with affective disorders according to DSM-IV and ICD-10 were studied. Of these, three patients were diagnosed with bipolar disorder (DSM IV-TR: 296.5; F 31.3) with manic and depressive episodes, and three patients suffered from major depressive disorder (DSM IV-TR: 296.5; F 31.5). All patients with affective disorders had received mood stabilizers consistently or periodically and/or antidepressive medication for several years before death. Only patients with detailed clinical records and well-documented psychopathology were included. The criteria for exclusion from the three groups were as follows: (i) organic brain disease; (ii) brain injury; (iii) alcoholism or chronic substance abuse; (iv) chronic somatic diseases affecting the central nervous system (i.e., cachexia, cancer, chronic liver or kidney diseases, or long

TABLE 1 | Demographic data and group parameters for healthy control subjects, patients with affective disorders, and patients with schizophrenia.

	Control subjects	Affective disorders	Schizophrenia
N	6	6	10
Males/Females	2/4	2/4	6/4
Age (years)	52.7 ± 9.7	48.7 ± 11.6	54.8 ± 8.9
Brain weight (g)	1298.3 ± 169.6	1373.3 ± 155.9	1305.7 ± 155.2
Brain volume (cm^3)	1252.0 ± 163.6	1324.3 ± 150.3	1259.1 ± 149.7
Post-mortem delay (h)	36.0 ± 20.1	29.2 ± 14.7	31.7 ± 15.2
Duration of illness (years)		9.7 ± 6.8	23.7 ± 12.9
Onset of illness (years)		39.0 ± 10.1	31.1 ± 11.1
Thickness of section (μm)	16.8 ± 1.2	16.9 ± 1.8	14.9 ± 1.9

Mean ± standard deviation.

term corticosteroid treatment); and (v) age greater than 65 years, to exclude changes related to normal aging of the brain.

Tissue Processing

Brains were removed within 4–72 h after death (see **Table 1** for the demographic data of control subjects and patients) and fixed in toto in 8% phosphate-buffered formaldehyde for at least 2 months (pH = 7.0, $T = 15–20^{\circ}C$). The frontal and occipital poles were separated by coronal cuts anterior to the genu and posterior to the splenium of the corpus callosum. After embedding all parts of the brain in paraffin, serial whole brain sections without midline cut of the middle block were cut (20 μm) with a calibrated microtome and mounted. The shrinkage factor caused by fixation and embedding and the thickness of the slices were calculated by methods described previously by Baumann et al. (1999). The mean volume shrinkage factor for brains in the schizophrenia, affective disorder, and control groups was 2.2 ± 0.3 (mean ± SD). No significant differences in the shrinkage factors were observed among the three groups. Every 50th section was stained for calretinin. The distance between the sections was 1 mm.

Stereological-based Analysis and Morphometric Delineation Criteria

For the present study, one coronal sections was randomly selected from each brain. Each section was located at the same clearly defined anatomical landmarks in either the anterior, middle, or posterior portion of the human septum. The cross-sectionals areas of the septal nuclei within each section were determined using a computerized image system (Digitrace Imaging System). The borders of the septal tissue were delineated under a microscope at low magnification with a 2.5 × objective according to the boundaries described by Horváth and Palkovits (1987). The anterior border of the septal tissue is the genu of the corpus callosum; the upper border is the body of the corpus callosum and the anterior commissure; and the lateral borders are the lateral ventricles. The septal tissue is surrounded basally by the nucleus accumbens and the stria terminalis. To determine interrater reliability, stereological measurements of eight different, randomly selected brains were performed by two investigators (R.B., R.S). The interrater reliability for the densities of calretinin-immuno-positive neurons in the septal nuclei was 0.97 (intraclass correlation coefficient). All measurements were performed blind to the diagnosis: the investigators were unaware of the patient's diagnosis, age, and gender. The cross-sectional area of the section was scanned with a 2.5 × objective using a video camera module attached to a Leica light microscope, and Digitrace software was used to project a picture on a monitor (22.0 × 15.9 mm). A magnification of 400 × was used for cell counting. Using this apparatus, the counting frame was superimposed onto one section at clearly defined anatomical landmarks, with up to 200 systemically, uniformly randomly sampled counting boxes (i.e., up to 100 counting boxes for the left and the right portions of the septal nuclei) for each septal nucleus along the entire extent of the septal nucleus. The actual section thickness of each section in the septal nuclei was determined with a 100 × oil immersion objective by focusing on the upper and lower surfaces of the section and then subtracting the z-axis distance measured by the a microcator attached to the Leica DM RB microscope (Leica, Gießen, Germany). To determine the number of neurons at a higher magnification (400X) neurons were counted by using the optical disector method as described earlier (Bernstein et al., 2001; Brisch et al., 2009; Walløe et al., 2014). The average thickness of the sections (z-axis) was 16.0 ± 1.9 μm (mean ± SD). The mean thickness of the sections was 14.9 ± 1.9 μm (mean ± SD) in the schizophrenia group, 16.9 ± 1.8 μm (mean ± SD) in the affective disorders group, and 16.8 ± 1.2 μm (mean ± SD) among healthy control subjects. The neuronal density was estimated based on the square of the counting area, which was determined by the square of the septal nuclei at the adjacent nuclei, and the number of calretinin-immunoreactive neurons within the counting boxes (Brisch et al., 2009). Neurons touching the left and lower borders of the counting boxes were excluded, and neurons touching the opposite borders were included (see **Figure 1**; Pennington et al., 2008).

Immunohistochemistry

The brain sections were dewaxed with xylol and washed with distilled water (two 10-min washes). The sections were washed in a 1% H_2O_2–10% methanol/phosphate-buffered saline solution. After repeated washings in phosphate-buffered saline solution, the sections were incubated in bovine serum albumin in a humidified chamber for 1 h. A rabbit polyclonal antiserum (Swant, Bellizona, Switzerland, Code No: 7699/4) was then applied to the brain sections as the primary antibody in a dilution of 1:500. The solution consisted of 20 μl of calretinin antibody, 10 ml of PBS, 40 μl of Triton X-100, and 200 ml of goat normal serum. The brain sections were incubated in a humidified chamber at 4°C for 48 h and washed twice for 10 min each in phosphate-buffered saline. The secondary antibody (goat-anti-rabbit-immunoglobulin E 0432, Dako, Denmark) was then applied to the sections at a dilution of 1:100, and the sections were incubated in a humidified chamber for 48 h, followed by two washes for 5 min each in phosphate-buffered saline. Streptavidin was then applied to the sections at a dilution of 1:100 (Streptavidin-biotin-peroxidase-complex, RPN 1051, Batch

177351, Amersham Biosciences, Germany). Streptavidin was used as an antibody marker. The sections were then incubated in a humidified chamber at a 4°C for 1 h, followed by two washes for 10 min each in phosphate-buffered saline. To visualize the reaction products, 3,3′-diamino nickel sulfate hexahydrate was used. Finally, repeated washings with distilled water (twice for 5 min), 60% alcohol (5 min), 70% alcohol (5 min), 96% alcohol (5 min), and absolute alcohol (5 min) were performed. To control the specificity of the immunostaining for calretinin, we either omitted the primary antiserum or replaced it with buffer or normal rabbit serum. Control reactions showed a complete disappearance of specific immunostaining.

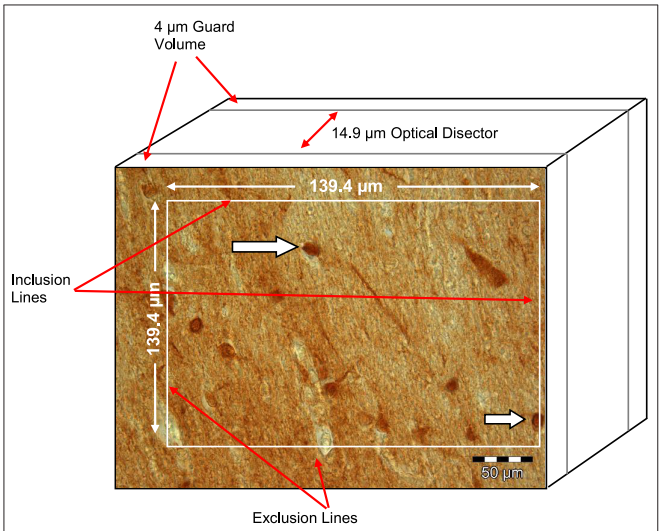

FIGURE 1 | Calretinin-immunopostive neurons (arrows) in the Ncl. lateralis of a patient with schizophrenia (modified from Pennington et al., 2008).

Statistical Analysis

The independence of frequency for the variables of gender and diagnosis was analyzed using Pearson's chi-square test. The other demographic variables are presented as their mean ± standard deviation and were compared among the three groups using a one-way ANOVA (analysis of variance; see **Table 1**). Levene's-test was used to evaluate the equality of variances for a given variable, such as the cell numbers calculated for the three groups (i.e., healthy control subjects, patients with affective disorders, and patients with schizophrenia). Some of the morphometric values had distinct asymmetric distributions (see **Table 2**) and were therefore using non-parametric tests. The Kruskal–Wallis test was performed to determine the significance of the differences in terms of mean cross-sectional area, the number of neurons, and the neuronal densities among the three groups (see **Table 2**). Results were considered significant at the 0.05 level. In cases of significance, the pairwise U-test (Wilcoxon–Mann–Whitney with the Shaffer-correction) was used to detect significant differences between pairwise groups (see **Table 2**). The statistical power of each test was also calculated.

Methods of the Mini-review

We searched PubMed in July 2015. Using the following keywords "calretinin and neuronal density and schizophrenia," we obtained 12 hits, of which three were relevant studies. We had 18 hits in PubMed for the keywords "calretinin and neuronal number and schizophrenia," two of which were relevant studies. We had two hits in PubMed for the keywords "calretinin and neuronal

TABLE 2 | Cell numbers and neuronal densities (neurons/mm^3) in the septal nuclei of patients with schizophrenia, patients with affective disorders, and the healthy control subjects.

Cell number (mean, SD, CE)	Ncl. lateralis	Ncl. medialis (pars fimbrialis and pars intermedia)	Ncl. dorsalis
Volume cell density (mean, SD, CE)			
Control subjects ($N = 6$)	123.0 (81.4; 0.27)	171.6 (85.7; 0.22) ($N = 5$)	131.0 (87.8; 0.27)
	2032 (687; 0.14)	1717 (684; 0.18)	1711 (1373; 0.33)
Patients with schizophrenia ($N = 10$)	257.4 (243.3; 0.32) ($N = 9$)	329.7 (374.1; 0.43) ($N = 7$)	224.2 (299.6; 0.45) ($N = 9$)
	2891 (1939; 0.22)	2863 (3452; 0.46)	2110 (2389; 0.38)
Patients with affective disorders ($N = 6$)	168.2 (91.3; 0.22)	165.4 (98.3; 0.27) ($N = 5$)	238.0 (150.5; 0.26)
	2378 (1193; 0.20)	1744 (730; 0.19)	2856 (1281; 0.18)
Two-group-comparisons			
Aff. vs. Ctr. (U-test)	0.48; 0.59	1.00; 1.00	0.24; 0.24
SZ vs. Ctr. (U-test)	0.18; 0.61	0.76; 0.88	0.69; 0.86
SZ vs. Aff. (U-test)	0.78; 0.78	0.88; 1.00	0.78; 0.22
Three-group-comparisons			
ANOVA Ctr./Aff./SZ (p-value)	0.34; 0.54	0.46; 0.62	0.65; 0.57
Levene's-test	**0.048**; **0.071**	**0.036**; 0.10	0.50; 0.44
K–W-test Ctr./Aff./SZ	0.38; 0.76	0.94; 0.97	0.56; 0.33

The data are presented as the mean, standard deviation (SD), and coefficient of error (CE). Please note that the number of patients with schizophrenia included in the data for the nuclei lateralis, medialis (pars fimbrialis and pars intermedia), and dorsalis data is less than the original number of patients with schizophrenia (in bold). In addition, the number of patients with affective disorders included in the nuclei medialis data is less than the original number of patients with affective disorders (in bold). K–W-test, Kruskal–Wallis-test; Ctr, Control subjects; SZ, Patients with schizophrenia; Aff, Patients with affective disorders; Ncl., nucleus. Shrinkage-corrected data are presented.

density and bipolar disorder," one of which was a relevant study. We had two hits in PubMed for the keywords "calretinin and neuronal number and bipolar disorder," one of which was a relevant study. We had two hits in PubMed for the keywords "calretinin and neuronal density and major depressive disorder," one of which was a relevant study. We had two hits in PubMed for the keywords "calretinin and neuronal number and major depressive disorder," one of which was a relevant study. We had 22 hits for the keywords "parvalbumin and neuronal density and schizophrenia," of which six were relevant studies. We had 48 hits for the keywords "parvalbumin and neuronal number and schizophrenia, three of which were relevant studies." We had two hits for the keywords "parvalbumin and neuronal density and bipolar disorder," of which one was a relevant study. We had four hits for the keywords "parvalbumin and neuronal number and bipolar disorder," two of which were relevant studies. We also searched the reference lists of published studies.

Results

Numbers of Calretinin-immunoreactive Neuron and Neuronal Densities in the septal Nuclei of Patients with Schizophrenia, Patients with Affective Disorders, and Healthy Control Subjects

No significant differences were observed in the number of calretinin-immunoreactive neurons in the lateral (AFF vs. CTR, $P = 0.48$; SZ vs. CTR, $P = 0.18$; SZ vs. AFF, $P = 0.78$), medial (pars fimbrialis and pars intermedia; AFF vs. CTR, $P = 1.00$; SZ vs. CTR, $P = 0.76$; SZ vs. AFF, $P = 0.88$), and dorsal septal nuclei (AFF vs. CTR, $P = 0.24$; SZ vs. CTR, $P = 0.69$; SZ vs. AFF, $P = 0.78$) among patients with schizophrenia, patients with affective disorders and healthy control subjects (see **Figures 2A**, **3A–E**; **Table 2**). There were no significant differences in the densities of calretinin-immunoreactive neurons in the lateral (AFF vs. CTR, $P = 0.59$; SZ vs. CTR, $P = 0.61$; SZ vs. AFF, $P = 0.78$), medial (pars fimbrialis and pars intermedia; AFF vs. CTR, $P = 1.00$; SZ vs. CTR, $P = 0.88$; SZ vs. AFF, $P = 1.00$), and dorsal septal nuclei (AFF vs. CTR, $P = 0.24$; SZ vs. CTR, $P = 0.86$; SZ vs. AFF, $P = 0.22$) among patients with schizophrenia, patients with affective disorders, and healthy control subjects (see **Figure 2B**; **Table 2**). The mean cross-sectional areas of the septal nuclei did not differ siginificantly among the patients with schizophrenia, patients with affective disorders, and healthy control subjects. The statistical power (1-β probability of error) was estimated for the *F*-tests used in the statistical analysis of number of cells and cell density, respectively. For the the dorsal septal nuclei, it was estimated as 0.612 and 0.736, for the medial septal nuclei (pars fimbrialis and pars intermedia) as 0.933 and 0.763, and for the lateral septal nuclei as 0.952 and 0.771. Therefore, it was concluded that our sample size would be large enough to detect statistically significant differences.

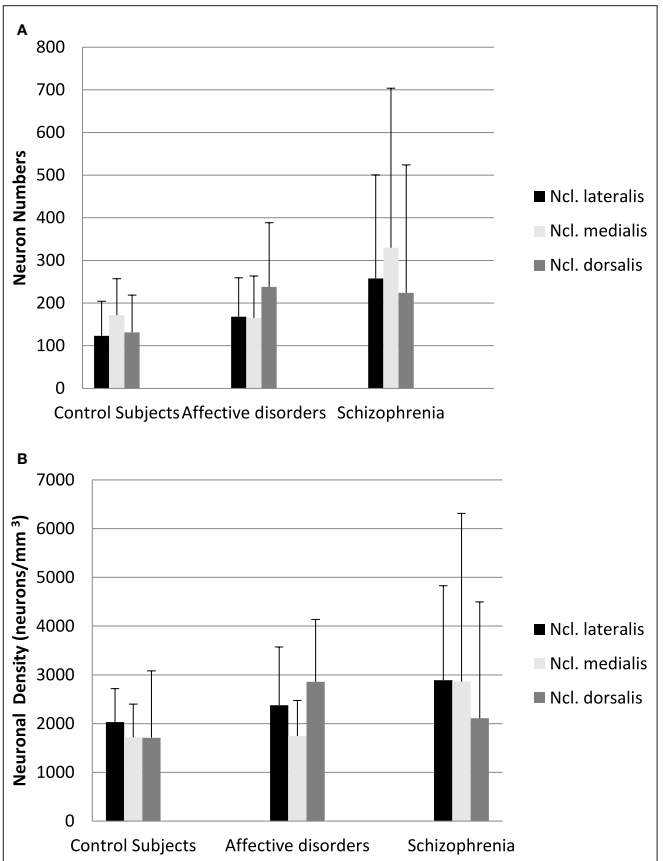

FIGURE 2 | (A) Numbers of calretinin-immunoreactive neuron in the septal nuclei of patients with schizophrenia, patients with affective disorders, and healthy control subjects. The data are presented as the mean and standard deviation. **(B)** Densities of calretinin-immunoreactive neuron in the septal nuclei of patients with schizophrenia, patients with affective disorders, and healthy control subjects. The data are presented as the mean and standard deviation.

Discussion

The present study demonstrates for the first time that there are no alterations in the density and number of calretinin-immunoreactive neurons in the lateral, medial, and dorsal septal nuclei of patients with schizophrenia or patients with affective disorders compared to healthy control subjects.

Although some studies demonstrated significant changes in calretinin-immunoreactive neurons in schizophrenia and bipolar disorder compared to control subjects in brain regions such as the dentate and the dorsolateral prefrontal gyri (Oh et al., 2012; Walton et al., 2012), the majority of studies of calretinin-immunoreactive neurons in schizophrenia in brain areas such as the hippocampus, prefrontal, and cingulate cortices, and the mammillary bodies (Zhang and Reynolds, 2002; Zhang et al., 2002; Bernstein et al., 2007; review by Eyles et al., 2002) and in affective disorders in the cingulate cortex (Cotter et al., 2002; see **Table 3**) are consistent with our present findings.

A novel interaction between calretinin and AMPA [(S)-2 amino-3-(3-hydroxy-5-methyl-4-isoazolyl)-propionic-acid] has been proposed as a potential target for the development of new antipsychotic therapeutics for schizophrenia (Siekmeier and vanMaanen, 2013). However, new drugs that affect this pathway should be developed with caution because the

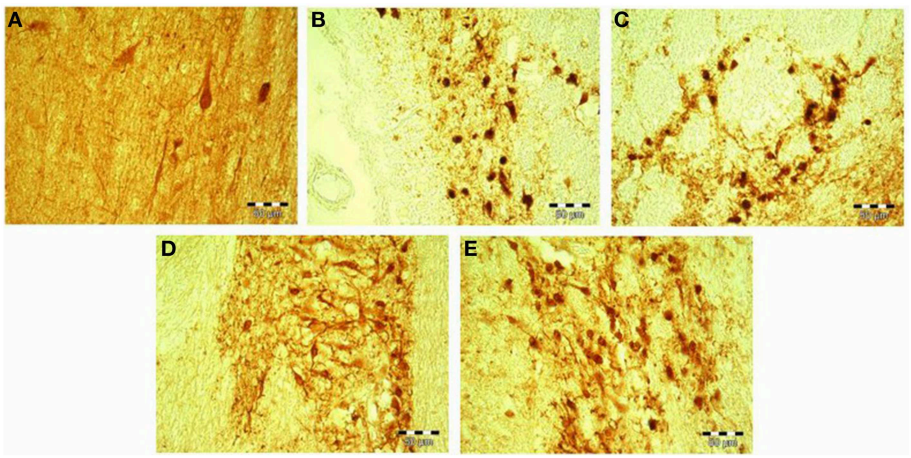

FIGURE 3 | (A–E) Calretinin-immunopostive neurons in the Ncl. medialis of a healthy control subject **(A)**; Ncl. medialis of a patient with major depressive disorder **(B)**; Ncl. lateralis of a patient with major depressive disorder **(C)**; Ncl. medialis of a patient with schizophrenia **(D)**; and Ncl. medialis of a healthy control subject **(E)**. Scale bars correspond to 50 μm. Ncl., nucleus.

GABA-potentiating drug vigabatrin, an irreversible inhibitor of GABA-transaminase, induces alterations in GAD67, GAD65, parvalbumin, and calbindin levels in certain brain regions, including the hippocampus and cerebral cortex (Levav-Rabkin et al., 2010). Increased density of GAD65/67-immunoreactive neuropil suggests a GABAergic hyperactivity in the hippocampus and might compose a risk factor for suicidal behavior in affective disorders (Gos et al., 2009). Increased densities of GAD65/67-positive neurons in the dorsolateral prefrontal and superior temporal cortices and in the hippocampus have been observed among patients with major depressive disorder compared with control subjects and patients with bipolar disorder as well as in the orbitofrontal cortex among patients with major depressive disorder and bipolar disorder compared to control subjects (Bielau et al., 2007) and in the posterior subiculum and parahippocampal gyrus in treated patients with chronic schizophrenia compared with control subjects (Schreiber et al., 2011) Further, a reduced level of GAD67 protein in the prefrontal cortex has been observed in major depressive disorder compared with control subjects (Karolewicz et al., 2010), and a reduction in GAD67 along with parvalbumin-positive cells of the dorsal hippocampus has been observed in a rat model of schizophrenia (Dickerson et al., 2014). Calbindin/GAD67-positive and calretinin/GAD67-positive neurons are much more involved in pathological processes in brain diseases, including schizophrenia, than are neurons with GAD65 (Rocco et al., 2015). Alterations in GABAergic interneurons and minicolumns in the neocortex are characteristic of neuropathological diseases such as schizophrenia (Raghanti et al., 2010). Moreover, the dorsolateral prefrontal cortex, with its glutamatergic and GABAergic populations, has been the focus of recent schizophrenia research (Lewis et al., 2005; Hoftman and Lewis, 2011). Increased levels of GABA have been observed in prefrontal cortices in unmedicated patients with schizophrenia (Kegeles et al., 2012). Reduced numbers of parvalbumin-immunoreactive neurons in the hippocampus, prefrontal and frontal cortices, and mammillary bodies have been reported in patients with schizophrenia compared with control subjects (Beasley and Reynolds, 1997; Lewis et al., 2001; Reynolds and Beasley, 2001; Reynolds et al., 2001, 2002, 2004; Beasley et al., 2002; Zhang and Reynolds, 2002; Zhang et al., 2002; Bernstein et al., 2007; see **Table 3**) and in an animal model of schizophrenia (Reynolds et al., 2004; Penschuck et al., 2006; Harte et al., 2007; Bissonette et al., 2014). In addition, a significant decrease in parvalbumin-immunoreactive neurons in the entorhinal cortex of patients with bipolar disorder compared with control subjects has been observed (Pantazopoulos et al., 2007).

Increased oxidative stress and changes in antioxidant systems, such as decreased glutathione, in schizophrenia compromise the integrity of parvalbumin interneurons in the ventral hippocampus (Steullet et al., 2010).

Upregulation of parvalbumin in the prefrontal cortex during adolescence due to pre- and postnatal disturbances has been observed (Caballero et al., 2014). Age-related changes have been observed in the calbindin-, calretinin-, and parvalbumin-immunoreactive neurons of the human cerebral cortex (Bu et al., 2003) and in the parvalbumin-immunoreactive neurons of the medial and lateral geniculate nuclei of rhesus macaques (Gray et al., 2013). Nicotine enhances GABAergic and serotonin synaptic transmission in the medial septum (Wu et al., 2003; Aznar et al., 2005; DuBois et al., 2013).

Methylphenidate, which is used to treat children suffering from attention deficit hyperactivity disorder (ADHD), causes an increase in calretinin neurons in the medial septum and in the vertical limb of the diagonal band of Broca (MS/VDB) of rats (García-Avilés et al., 2015). Furthermore, cannabis abuse results in decreased expression of GAD67 in the parvalbumin-containing interneurons of the prefrontal cortex in a rat model of schizophrenia (Zamberletti et al., 2014). Prenatal lead exposure results in a loss of parvalbumin-interneurons co-labeled with GAD67 protein in specific brain regions such as medial prefrontal cortex, striatum, and hippocampus but to increased activity of

TABLE 3 | Post-mortem studies of calretinin-, calbindin-, and parvalbumin-immunoreactive neurons in patients with schizophrenia, bipolar patients, and patients with major depressive disorder compared to control subjects.

Authors	Sample sizes	Brain areas	Results
Daviss and Lewis, 1995	*N* = 10 schizophrenia patients	Prefrontal cortical areas 9 and 46	Calbindin ↑ (schizophrenia patients compared to control subjects)
	N = 5 control subjects	Neuronal cell density	Calretinin ↔
Beasley and Reynolds, 1997	*N* = 18 schizophrenia patients *N* = 22 control subjects	Prefrontal cortex Neuronal cell density	Parvalbumin ↓ (in layers III, IV of schizophrenia patients compared to control subjects)
Kalus et al., 1997	*N* = 5 schizophrenia patients	Anterior cingulate cortex	Nissl ↔ (total neuronal density)
	N = 5 control subjects	Neuronal cell density Somal profile density	Parvalbumin ↑ (soma profile density in layers Va and Vb in schizophrenia patients compared to control subjects)
Woo et al., 1997	*N* = 15 schizophrenia patients *N* = 15 control subjects	Prefrontal cortex (areas 9 and 46) Occipital cortex (area 17) Neuronal cell density Somal size	Parvalbumin ↔
Holt et al., 1999	*N* = 10 schizophrenia patients	Total striatum	Choline acetyltransferase↓ (in the total striatum and most prominent in the ventral striatum in schizophrenia patients compared to control subjects)
	N = 9 control subjects	Neuronal cell density	Calretinin ↓ (in the total striatum and most prominent in the caudate nucleus in schizophrenia patients compared to control subjects)
Reynolds and Beasley, 2001	*N* = 18 schizophrenia patients	Prefrontal cortex	Parvalbumin ↓ (in layers III, IV of schizophrenia patients compared to control subjects)
	N = 22 control subjects	Relative density of neurons	Calretinin ↔
Beasley et al., 2002	*N* = 15 schizophrenia patients	Neuronal cell density	Parvalbumin ↓ (in layer III of schizophrenia patients to control subjects)
	N = 15 bipolar patients		Calbindin ↓ (in layers II, III and V of schizophrenia patients compared to control subjects and layer V of bipolar patients compared to control subjects; by comparing individual laminar densities between groups (correction for multiple comparisons) only a reduction in layer II in schizophrenia patients compared to control subjects)
	N = 15 patients with major depressive disorder dorsolateral prefrontal cortex (Broadmann area 9)		Calretinin ↔
Cotter et al., 2002	*N* = 15 schizophrenia patients	Anterior cingulate cortex	Calretinin ↔
	N = 15 bipolar patients	Neuronal cell density	Parvalbumin ↔
	N = 15 patients with major depressive disorder	Neuronal cell size	Calbindin ↔
	N = 15 control subjects		
Reynolds et al., 2002	*N* = 15 schizophrenia patients	Entorhinal cortex Prefrontal cortex	Parvalbumin ↓ (schizophrenia patients compared to control subjects)
	N = 15 bipolar patients	Neuronal cell density	Calbindin ↔
	N = 15 patients with major depressive disorder		Calretinin ↔
	N = 15 control subjects		

(Continued)

TABLE 3 | Continued

Authors	Sample sizes	Brain areas	Results
Zhang and Reynolds, 2002	*N* = 15 schizophrenia patients *N* = 15 bipolar patients *N* = 15 patients with major depressive disorder	Hippocampus Relative cell density of neurons Neuronal body size	Calretinin ↔ Parvalbumin ↓ (cell density in male schizophrenia patients and bipolar patients (CA 1) compared to control subjects; neuronal body size ↓ in schizophrenia patients and bipolar patients compared to control subjects)
Zhang et al., 2002	*N* = 15 schizophrenia patients *N* = 15 control subjects	Hippocampus Neuronal cell density Neuronal cell size	Parvalbumin ↓ (neuronal cell density) Calretinin ↔
Danos et al., 2003	*N* = 12 schizophrenia patients *N* = 14 control subjects	Anteroventral thalamic nucleus (AN) Neuronal cell density	Parvalbumin ↓ (parvalbumin-immunoreactive-thalamocortical projection neurons in the left and right AN in schizophrenia patients compared to control subjects)
Tooney and Chahl, 2004	*N* = 6 schizophrenia patients *N* = 6 control subjects	Prefrontal cortex Relative density of neurons Somal size of neurons	Calretinin ↔ Calbindin ↔ Parvalbumin ↔
Chance et al., 2005	*N* = 12 schizophrenia patients *N* = 12 control subjects	Planum temporale Neuronal cell density	Calbindin ↔
Wheeler et al., 2006	*N* = 9 schizophrenia patients *N* = 9 control subjects	Posterior cingulate cortex Visual cortex Neuronal cell density	Calbindin ↔
Bernstein et al., 2007	*N* = 15 schizophrenia patients *N* = 15 control subjects	Mammillary bodies Neuronal cell number	Parvalbumin projection neurons ↓ (schizophrenia patients compared to control subjects) Calretinin ↔ GAD ↔
Pantazopoulos et al., 2007	*N* = 10 schizophrenia patients (SZ) *N* = 10 bipolar patients (BP) *N* = 16 control subjects (CS)	Entorhinal cortex (EC) Neuronal cell number Neuronal density Soma size	Parvalbumin ↓ (neuronal density and cell number in bipolar patients (BP) compared to control subjects (CS)) (neuronal density ↓ of the superficial layers of the lateral and caudal EC in BP compared to CS) (neuronal density ↓ of the superficial layers of the caudal EC in SZ compared to CS)
Rajkowska et al., 2007	*N* = 14 patients with major depressive disorder (MDD) *N* = 11 control subjects	Dorsolateral prefrontal cortex (dlPFC) Orbitofrontal cortex (ORB) Neuronal cell density Neuronal cell size	Calbindin ↓ in cell density and size in dlPFC in patients with MDD compared to control subjects a trend for reduction in parvalbumin and calbindin-immunoreactive neurons in cell density and cell size in ORB in patients with MDD compared to control subjects
Konradi et al., 2011a	*N* = 13 schizophrenia patients *N* = 20 control subjects	Hippocampus Neuron number	Parvalbumin ↓ Somatostatin ↓
Konradi et al., 2011b	*N* = 14 bipolar patients *N* = 18 control subjects	Hippocampus Neuron number	Parvalbumin ↓ (CA4, CA1) and somatostatin ↓ (CA1) (bipolar patients compared to control subjects)

(Continued)

TABLE 3 | Continued

Authors	Sample sizes	Brain areas	Results
Wang et al., 2011	*N* = 11 patients with schizophrenia *N* = 13 patients with type 1 bipolar disorder *N* = 15 control subjects	Caudal entorhinal cortex (EC) Subiculum Parasubiculum Neuronal density	Parvalbumin ↓ and somatostatin ↓ (in the caudal EC and parasubiculum of bipolar and schizophrenia patients compared to control subjects Calbindin ↔
Oh et al., 2012	*N* = 15 patients with schizophrenia *N* = 15 bipolar patients *N* = 15 patients with major depressive disorder *N* = 15 control subjects	Dorsolateral prefrontal cortex (dlPFC) Neuronal cell density Neuronal cell size	Calretinin ↓ (in layer I in patients with major depressive disorder compared to control subjects) A significant correlation between reduced density of calretinin-immunoreactive in the dlPFC of patients with major depressive disorder and lower density or size of glial cells and pyramidal neurons in subjects from the Stanley Neuropathology Consortium
Walton et al., 2012	*N* = 15 control subjects *N* = 15 schizophrenia patients *N* = 15 bipolar patients *N* = 15 patients with major depressive disorder	Hippocampus Neuronal cell number	Calretinin ↑ (in the dentate gyrus of schizophrenia patients and bipolar patients compared to control subjects)

the subcortical dopaminergic system and intensified locomotor response to cocaine in a rat model of schizophrenia (Stansfield et al., 2015).

A major limitation of the present study is the small numbers of healthy control subjects, patients with schizophrenia, and patients with affective disorders. It was not possible to determine the numbers of smokers and non-smokers in our study's population, a minor drawback of the study. The psychotropic medication in patients with schizophrenia and patients with affective disorders was only recorded during the last 3 months before the patients' lives.

Our findings of no significant differences in the density and number of immunoreactive calretinin neurons in the medial, lateral, and dorsal septal nuclei in patients with patients with schizophrenia, patients with affective disoders, and healthy control subjects should be interpreted as a preliminary result. Future research examining the distribution of calretinin neurons in the septal nuclei of patients with schizophrenia or patients with affective disorders should utilize larger sample sizes. Forthcoming investigations should also focus on the distribution and the densities of parvalbumin-immunoreactive neurons in the septal nuclei of patients with schizophrenia and patients with affective disorders compared with healthy control cases.

Evolutionary Trade-off of Calretinin and Schizophrenia

An evolutionary perspective on why calretinin is affected in the neurodevelopmental disorder schizophrenia provides some insight into this disorder. While several evolutionary theories have been proposed for the persistence of schizophrenia (Davis et al., 1991; Adriaens, 2008; da Silva Alves et al., 2008). Although it is impossible to verify these theories using ancestral hominin remains because brain tissue cannot be fossilized. Numerous hypotheses have been proposed for the superior cognitive abilities of *Homo species*, and these have been largely based on the threefold increase of the human neocortex during the Pleistocene period (2 Ma–13 kya). However, much of this research has focussed on brain anatomy in relation to cerebral volume size rather than neuro-hormonal regulation. It has been established that GABAergic interneurons are crucial in the diverse activities of pyramidal cells (Hendry et al., 1987; Zaitsev et al., 2009). From an evolutionary perspective, GABA has been shown to have an inhibitory function in the nervous systems of both vertebrates and invertebrates (Fiorillo and Williams, 1998; Gou et al., 2012). Although, it has been established that there exists a relationship between glutamatergic pyramidal neurons and GABAergic interneurons (of which calretinin-positive neurons are a subtype of GABAergic interneurons; Radonjic et al., 2014), this relationship is not well-understood in relation to the development of schizophrenia in humans.

Evolution of the Neurotransmitters Gamma-aminobutyric Acid and Glutamate and their Receptors

A recent theory hypothesizes that there may have been positive Darwinian selection in the modification of interneuron populations in humans, leading to cognitive specializations in *Homo species* (Sherwood et al., 2010). Current studies indicate that there is an evolutionary continuity between human and non-human primates, as well as changes in subcortical descending projections during human evolution (Rilling et al., 2008; Sherwood et al., 2010). Calretinin-positive interneurons are the most abundant GABAergic neurons in primates (Hladnik et al., 2014). Interestingly, anatomical and neurohormonal changes to the brain during human evolution may have informed changes in glutamatergic and GABAergic processes in interneurons, thereby possibly altering calretinin regulation in the hippocampus and in key motor centers. The development of obligate bipedalism required a host of morphological and neuro-hormonal changes: CNS, metabolic and cardiovascular responses due to sustained running, swivel hips, slow-twitch muscles, plantar arches and longer femurs, as well as modification

of eccrine glands (Mattson, 2012). A recent theory contends that the advent of endurance hunting from *Homo erectus* onwards mediated thermo-regulatory changes in the dopaminergic system (Previc, 2002) and that such changes influenced neuro-hormonal regulation (Mattson, 2012). This theory also states that endurance hunting demanded retention of geographical areas and mnemonic recall in hippocampal areas to maximize resource acquisition (Mattson, 2012; Brisch et al., 2014). It could be suggested that the increase in physical activity levels (PAL) from *Homo erectus* onwards, may have caused an evolutionary trade-off in which higher metabolic demands in ancestral hominins may have come at an evolutionary cost of making GABA interneurons more vulnerable. Even slight physiologic variations in brain temperature may alter neuron composition and function (Andersen and Moser, 1995). Research indicates that GABA in the preoptic area and anterior hypothalamus (POAH) acts in heat regulation (Ishiwataa et al., 2005), while hyperthermia may increase hippocampal excitability and decrease GABA regulation in pyramidal cells (Qu et al., 2007; Qu and Leung, 2009). Therefore, selective pressures informing human brain evolution may have come at a cost of altering calretinin regulation of GABAergic hippocampal-interneurons, which may have contributed to the development of schizophrenia in humans.

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