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Letter to the editor

Reduction of GABA subunit theta-containing cortical neurons in schizophrenia

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Neuropathological abnormalities in patients with schizophrenia (SZ) include cortical thinning of the temporal and frontal regions (Walton et al., 2018; van Erp et al., 2018). Meta-analyses of imaging studies have shown that brain regions such as the insula, cingulate cortex and frontal cortex are most commonly identified as altered in SZ (Baiano et al., 2007). Interestingly, the anterior cingulate (ACC) and fronto-insular (FI) cortices contain a specialized type of neuron termed the von Economo neurons (VENs), implicated in complex social behavior (Nimchinsky et al., 1995; Allman et al., 2011). A reduced number of VENs is observed in early-onset SZ, and the VENs that remain show the presence of lysosomal aggregates (Brune et al., 2010; Krause et al., 2017). The reduced number of VENs and impaired social cognition found in SZ is in line with findings in neurodegenerative diseases where social cognition and behavior are altered (Seeley et al., 2006; Santillo et al., 2013).

Recent work has revealed that VENs are part of a larger cortical cell population predominantly present in the ACC and FI in human brain. This neuronal population is characterized by the expression of the $GABA_A$ receptor subunit theta (GABRQ) and comprises VENs and a subpopulation of Layer 5b pyramidal neurons. The selective vulnerability of VENs in bvFTD extends to the GABRQ-expressing pyramidal neurons, another indication that these neurons belong to the same population (Gami-Patel et al., 2019). Here, we investigate the presence of VENs and the GABRQ-expressing neuronal subpopulation in the ACC of SZ patients.

Post-mortem brain tissue was obtained from the Netherlands Brain Bank and the department of pathology, Amsterdam University Medical Centres, location VUmc, Amsterdam, The Netherlands. Donors with SZ ($n = 9$) had been diagnosed during life and reviewed for current diagnostic criteria by a psychiatrist (A.Do.) (Schultze-Lutter and Schimmelmann, 2014). The average estimated age of SZ onset was 39.1 years of age, varying from 26 years to 57 years old. Although social-emotional functioning was not mentioned specifically in the reports, for all patients increasing strenuous or absent social relations were reported. Age-matched healthy controls ($n = 15$) had no reported psychiatric or other neurological complaints. Donors with concomitant pathology related to dementia were excluded from the study. Clinical and demographic details are listed in Supplementary Table 1. All donors had given written informed consent for brain autopsy and use of brain tissue and clinical information for scientific research, in compliance with ethical and legal guidelines. Tissue from the right perigenual ACC (Vogt, 2016) were collected and total L5 neuronal numbers, VENs, and GABRQ-expressing neurons were assessed as described previously (Gami-Patel et al., 2019). Pearson correlation was used to assess correlation and differences in neuronal numbers between groups were assessed using one-way ANOVA with Tukey's post-hoc analysis.

Reduced GABRQ-immunoreactivity was noticed in SZ compared to control donors (Fig. 1A–F).There was no difference in Layer 5 (L5) surface area between control and SZ donors ($p = 0.07$; Fig. 1G). There was a significant reduction of 27% in total L5 pyramidal neuron population (mean controls = 3339: mean SZ = 2425; $p = 0.01$, Fig. 1H). In addition, a significant loss of 68% of VENs was observed in SZ compared to controls (mean number of VENs controls = 33.3, mean SZ = 10.44; $p = 0.01$, Fig. 1I). To control for the neuronal reduction in L5 in SZ, a ratio of VENs over total L5 neuronal population was calculated, for which a reduction was also found in donors with SZ ($p = 0.02$, Fig. 1J). In addition, the total GABRQ-expressing L5 neuronal population shows a highly significant reduction in numbers of neurons in donors with SZ (−56%: mean control = 356. Mean SZ = 288; $p < 0.001$, Fig. 1K) compared to control donors. The ratio of GABRQ-expressing neurons over total L5 neuronal population was also significantly reduced ($p = 0.01$, Fig. 1L). No correlation was observed between numbers nor ratio of VENs and GABRQ expressing neurons and age of death or disease duration.

Here we demonstrate that the numbers of GABRQ-expressing pyramidal neurons and VENs of the ACC are reduced more than half in SZ compared to controls. To correct for the overall neuronal reduction in L5 in SZ, we used the ratio of VENs or GABRQ-expressing population over the total neuronal L5 population, which also revealed a strong reduction in numbers of the VENs and GABRQ-expressing subpopulations in SZ. Brüne and colleagues identified a pattern of VENs reduction in SZ, where lower VEN numbers correlated with longer disease duration, and a significant reduction of VENs in early onset SZ (i.e. < 20 years) compared to controls (Brune et al., 2010). In our cohort, we only have

Abbreviations: VENs, von Economo neurons; ACC, anterior cingulate cortex; FI, frontoinsular cortex; GABRQ, $GABA_A$ receptor subunit theta; GABRE, $GABA_A$ receptor subunit epsilon; L5, layer 5.

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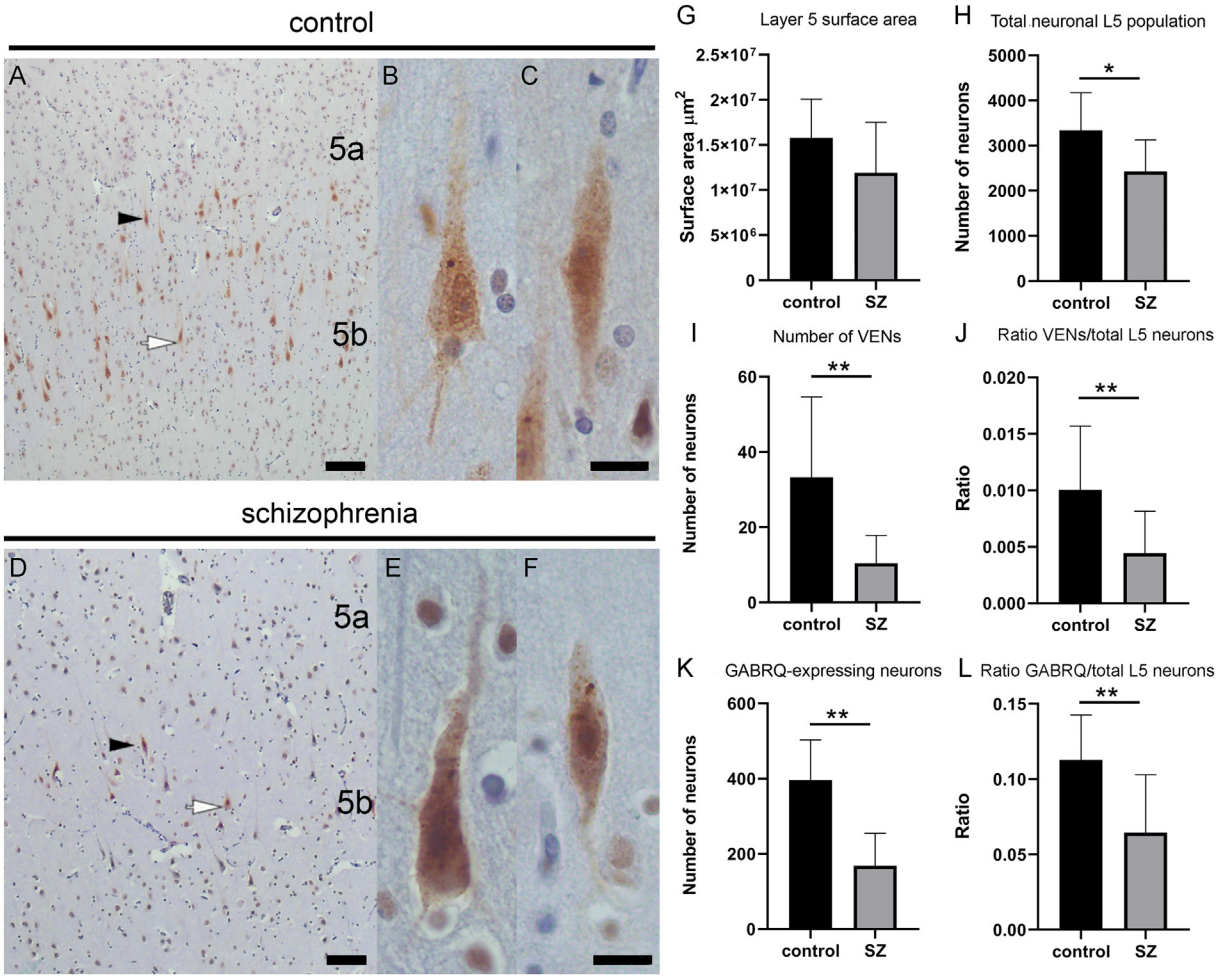

Fig. 1. Selective decrease of GABRQ-expressing neurons in L5 of the ACC in SZ. Representative examples of GABRQ immunoreactivity in Layer 5a and 5b of the ACC in control and SZ donors are shown. A higher number of GABRQ-positive neurons are observed in a control donor (A) existing of pyramidal (B) and VEN (C) neurons at high magnification, compared to schizophrenia (D,E,F). Black arrowheads (A,D), indicate VENs, white arrows (A,D), GABRQ-positive pyramidal neurons. Scale bar represents 250 µm (A,D) and 20 µm (B,C,E,F). No significant difference was seen in L5 surface area between SZ and control (G). A significant difference in the total number of L5 pyramidal (H) and VENs (I) was observed between control and SZ donors. The ratio of VENs over total neuronal populations reflects the significant selective reduction of VENs in SZ (J). Between control and SZ, there was a significant decrease in GABRQ positive neurons in SZ (K), which is reflected in a significant difference between the ratios of GABRQ neurons over total Layer 5 neurons (L).

donors with an onset after 20 years and see a significant reduction based on the ratio without differentiating in onset-subtypes. In addition, we observed no differences in reduction of neurons using the clinical defined cut-off of 40 years for early- and late-onset SZ (Howard et al., 2000). No correlation between disease duration and VEN density was observed, most likely because our SZ donors lived longer and had a relatively long disease duration.

Our findings indicate that VENs are part of a neuronal subpopulation of GABRQ-expressing neurons and also support the hypothesis that VENs and other related GABRQ-expressing neurons are involved in the pathogenesis of SZ. Loss of these neurons has been associated with impaired social-emotional processing in neurodegenerative diseases (Seeley, 2008; Santillo et al., 2013; Gami-Patel et al., 2019). This suggests that in SZ, VENs and GABRQ-expressing neurons are preferably linked to the negative symptoms in SZ including loss of empathy and diminished emotional response. Interestingly, the cortical GABRQ-containing GABA receptor is thought to have a unique stoichiometry in the human brain (Gami-Patel et al., 2019). This offers perspective for the targeted intervention of this specific neuronal cell population where compounds can be created to stimulate cell function in the remaining neurons using agonists, or by receptor-targeted drug delivery in order to stimulate neuronal activity. A targeted intervention can alleviate the negative symptoms related to social-emotional processing.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2020.11.037.

Contributors

A. Dijkstra and J. Hoozemans designed the study, A. Dijkstra and P. Gami-Patel collected the data. A. Rozemuller and M Bugiani were involved in sample collecting and pathological diagnosis, and Y. Pijnenburg and A. Dols were involved in clinical evaluation of the donors. G. Smit was involved in study content and all authors were involved in critical revision of the manuscript for important intellectual content.

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Declaration of competing interest

No conflict of interest to declare.

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