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Increased neuronal cell number in the dorsal motor nucleus of the vagus in schizophrenia

Axer H, Bernstein H-G, Keiner S, Heronimus P, Sauer H, Witte OW, Bogerts B, Bär K-J. Increased neuronal cell number in the dorsal motor nucleus of the vagus in schizophrenia.

Objective: Recently, a reduction in efferent vagal regulation has been found in schizophrenic patients.
Methods: Therefore, the brainstems of nine schizophrenic patients and nine normal controls were stereologically analysed. The number of neurons using the optical fractionator method and nuclear volumes applying the Cavalieri principle was estimated in Nissl stained sections of the dorsal motor nucleus of the vagus (DMNV) and the hypoglossal nucleus.
Results: The neurons in the right DMNV were significantly increased in the schizophrenic group compared to normal controls ($p = 0.047$), while the volumes of the DMNV did not differ. In contrast, no such differences were found in the hypoglossal nucleus.
Conclusion: Although this pilot study is limited by its small sample size, the analysis of the solitarius–ambiguus–vagus system in schizophrenic patients is an interesting target in schizophrenia research. The most reasonable background for increased neuron numbers in the DMNV could be a system-specific neurodevelopmental disturbance in schizophrenia.

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Keywords: dorsal motor nucleus of the vagus; hypoglossal nucleus; schizophrenia; stereology; autonomic dysfunction; sudden cardiac death

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Introduction

Cardiovascular morbidity and an increased risk for sudden cardiac death are important health issues in schizophrenia research (1). Various physiological studies have revealed autonomic dysfunction in schizophrenia, assuming that this might play a role in the cascade of events. In particular, efferent parasympathetic modulation has been shown to be decreased in the acute (2) and the chronic stage (3). Furthermore, decreased baroreflex sensitivity (BRS) and increased QT variability have been described in the acute state of the disease (4). All autonomic measures altered in schizophrenia have been associated with an increased risk of arrhythmias and sudden cardiac death in other diseases (5,6).

In addition, the prevalence of metabolic syndrome among patients with schizophrenia is up to four times higher than in the general population (7). Metabolic syndrome is characterised by glucose homeostasis abnormalitites, dyslipidemia, hypertension and abdominal obesity (8). Although the development of metabolic syndrome in schizophrenia is associated with antipsychotic medication (9,10), it has been shown that drug-naïve patients with schizophrenia have impaired fasting glucose tolerance and have higher levels of plasma glucose, insulin and cortisol than healthy comparison subjects (11).

However, to date it is impossible to disentangle whether autonomic dysfunction is just caused by acute psychopathology (e.g. excessive arousal, anxiety), or whether functional–anatomical abnormalities of the autonomic nervous system also may contribute to dysautonomia in the disease. The intention of this study was therefore to investigate morphological alterations in autonomic brainstem nuclei, which influence cardiovascular and gastrointestinal functions as well. The dorsal motor nucleus of the vagus

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(DMNV) is one candidate in central autonomic circuitry, which acts predominately as an effector on gastrointestinal regulation.

While the ambiguus nucleus is an efferent parasympathetic control centre of the esophagus and the cardiorespiratory system (12), the great majority of neurons in the DMNV constitute parasympathetic cholinergic efferents to control the upper gastrointestinal tract (13). Most of these cells project to the myenteric plexus or the interstitial cells of Cajal. The vagal afferent information is processed by the nucleus tractus solitarius which in turn is highly connected with the DMNV and the ambiguus nucleus. However, in addition to its dominating gastrointestinal projections, the DMNV seems also to be involved in cardiovascular regulation (14–16). While the nucleus tractus solitarius is organised viscerotopically, the DMNV is organised in functional units innervating specific organs (17). Its neuronal population is morphologically inhomogeneous in soma size and shape (18).

Major projections to DMNV come from the nucleus tractus solitarius. GABAergic projections (GABA-A receptor subtype) influence the tonic vagal output, i.e. DMNV neurons maintain a spontaneous slow pacemaker-like activity. In contrast, glutamatergic and catecholaminergic projections act in a phasic excitatory or inhibitory manner and can activate specific gastrointestinal reflexes (13). Moreover, vagal circuitries can be modulated by descending central nervous system (CNS) projections (such as the medullary raphe through thyrotropin-releasing hormone and serotonin) or by blood-borne proteins and peptides (e.g. insulin, pancreatic polypeptide, peptide YY, tumour necrosis factor α) (19). Thus, different transmitter systems (e.g. cholinergic output and GABAergic input) influence neurons in the DMNV, which are also known to play a role in the pathophysiology of schizophrenia (20,21).

The hypothesis of this first exploratory pilot study is that the neuronal cell number in the DMNV is altered in schizophrenic patients as a morphological correlate of the autonomic and gastrointestinal dysfunction in schizophrenia. The anatomical localisation of the DMNV in the vicinity to the hypoglossal nucleus allows the stereological analysis of cell and volume estimates whereby the hypoglossal nucleus can be used as an internal control.

Materials and methods

Subjects

The brainstems of nine schizophrenic patients and nine matched normal controls were obtained from the new Magdeburg brain collection. All patients were diagnosed according to Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) criteria (22). Table 1 shows the demographic details of the subjects examined. Age did not differ. The post-mortem interval of the normal controls did not differ significantly between both groups (Mann–Whitney U test $p = 0.926$).

Histology. The tissue was removed after death and fixed *in toto* in 8% phosphate-buffered formaldehyde for at least 2 months ($\mathrm{pH} = 7.0$, $T = 15–20^{\circ}\mathrm{C}$). The brainstems were embedded in paraffin, serially

Table 1. Demographic details

	#	Age	Gender	Post-mortem interval (h)	Duration of illness (years)	Diagnosis
Schizophrenic group (SC)	1	51	m	12	22	Catatonic schizophrenia
	2	48	m	48	32	Residual schizophrenia
	3	34	m	5	2	Paranoid schizophrenia
	4	55	f	48	6	Paranoid schizophrenia
	5	58	m	24	25	Residual schizophrenia
	6	57	m	72	23	Residual schizophrenia
	7	51	m	48	28	Disorganised schizophrenia
	8	54	m	48	34	Residual schizophrenia
	9	61	f	24	3	Catatonic schizophrenia
	mean ± SD	52.1 ± 7.9	7:2 (m:f)	36.6 ± 21.5	19.4 ± 12.5	
Normal controls (CO)	1	50	f	72		
	2	54	m	24		
	3	63	m	48		
	4	64	m	36		
	5	52	f	24		
	6	64	f	24		
	7	65	f	24		
	8	63	m	48		
	9	47	m	24		
	mean ± SD	58.0 ± 7.1	5:4 (m:f)	36.0 ± 17.0		

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sectioned at 20 μm axially to the brainstem axis and mounted. A first section through the medulla oblongata was chosen randomly and every 50th section was stained with cresyl violet to allow an identification of the DMNV and the hypoglossal nucleus. Thus, the distance between these stained sections was 1 mm.

DMNV can be localised easily on histological brainstem sections as it is located laterally to the hypoglossal nucleus which lies just laterally to the midline of the dorsal caudal medulla oblongata, directly beneath the floor of the fourth ventricle. The hypoglossal nucleus contains multipolar motorneurons innervating the muscle of the tongue through the hypoglossal nerve. The nucleus intercalatus Staderini is interposed between both cell groups the DMNV and the hypoglossal nucleus.

The thickness of each section was determined with a 63× oil immersion objective, because of shrinkage of the slices after histological processing. The thickness of the section was determined by focusing the upper and lower surfaces of the section, and then subtracting the z-axis coordinate of the lower surface from that of the upper surface. The thickness of the section after the histological procedures was 18.9 ± 0.8 μm.

Stereology. All analyses were performed blind to diagnosis and other clinical information. The image analysis computer software Stereo Investigator 6 (MicroBrightField, Inc., Vermont, USA) was used for the stereological estimations. The areas of the studied nuclei were delineated according to the atlas of the human brainstem (23). The boundaries of the nuclear areas were delineated using the 5× objective and the volumes of the nuclei were estimated according to the Cavalieri principle (24).

Estimates of the total number of the cell in the DMNV and the hypglossus nucleus were determined using the optical fractionator method (24,25). Fractions of slabs of the nucleus under study were acquired by systematic random sampling and then from these a systematic random sampling of microscopic slides was derived on which the neuronal numerical density was estimated by the optical disector. Six slices of each subject were analysed.

For counting the cell numbers in the DMNV a fixed counting frame with 80×80 μm^2 and a sampling grid size of 100×100 μm^2 were used. Neuronal cells were identified, when they had a cytoplasm and a nucleus with a prominent nucleolus. The cells were counted according to the unbiased sampling rule (26) using the 63× oil lens when they came into focus within the counting frames. Cells were counted at a magnification of 63×. The nucleus was the unit for counting neurons. Guarding zones were 2 μm and the depth of the grid was 14 μm.

Slightly different sampling parameters were used for counting the multipolar motoneurons of the hypoglossal nucleus using a fixed counting frame with 70×70 μm^2 and a sampling grid size of 150×150 μm^2 at a magnification of 63×.

Statistics. The data were statistically analysed using SPSS for Windows 11.5 (SPSS Inc., Chicago, USA). The non-parametric two-tailed Mann–Whitney U test was used to compare the groups because the Kolmogorov–Smirnov test did not reveal a normal distribution of data. The level of significance was accepted for $p < 0.05$.

Results

In the hypoglossal nucleus multipolar motoneurons can be found, which innervate the musculature of the tongue. The DMNV neurons are morphologically characterised by a prominent nucleolus and a Nissl intense cytoplasm. All vagal neurons were counted in this study although these neurons differ in size and shape (18). Figure 1 shows an example of the histological images of the DMNV and the hypoglossal nucleus.

Estimated cell counts and volumes of the DMNV are shown in Table 2 and measurements of the hypoglossal nucleus are displayed in Table 3. The coefficient of error (Gundersen) (27) for the cell count of the DMNV was between 0.05 and 0.08 and for the cell count of the hypoglossal nucleus was between 0.10 and 0.12 (calculated by the fractionator program in Stereo Investigator software).

Statistical testing revealed a significant difference in neuronal numbers in the right DMNV of patients with schizophrenia and normal controls (cell count in the right DMNV $p = 0.047$). Figure 2 gives some examples to show the differences in neuronal cell numbers in the DMNV in the middle of the nucleus. Neuronal numbers in the left DMNV did not reach significance, but showed a similar tendency. In contrast, no such differences between groups were found in the cell count estimates of the hypoglossal nucleus.

Figures 3 and 4 show the box plots of cell numbers and volumes (in μm^3) of right and left DMNV and hypoglossal nucleus in the schizophrenic group ($n = 9$) and normal controls ($n = 9$). No differences in volumes of the DMNV and the hypoglossal nucleus could be shown.

No significant correlations (Pearson correlation coefficients) could be found between post-mortem intervals and estimated cell numbers and estimated

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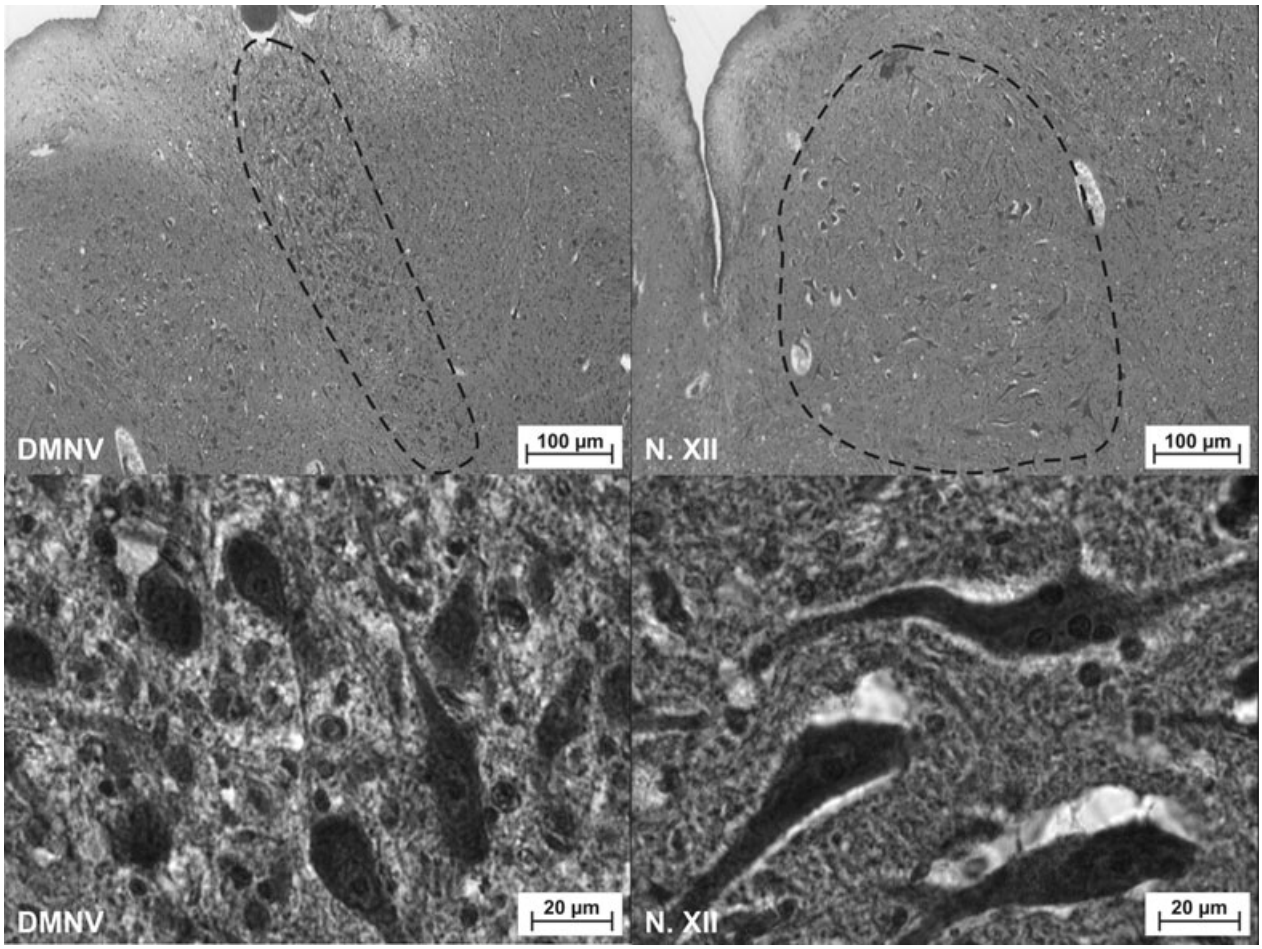

Fig. 1. Histology of the dorsal motor nucleus of the vagus (DMNV) and the hypoglossal nucleus (N. XII).

Table 2. Estimated cell numbers and volumes of the DMNV

		Right DMNV		Left DMNV	
		Cell estimate	Volume (μm^3)	Cell estimate	Volume (μm^3)
Schizophrenic group (SC)	Mean	22718	2.60×10^9	24050	2.47×10^9
	SD	7498	0.48×10^9	8720	0.42×10^9
Normal controls (CO)	Mean	16815	$2,30 \times 10^9$	17932	2.30×10^9
	SD	4755	0.65×10^9	7080	0.74×10^9
Mann–Whitney *U*	*Z*	−1.192	−1.192	−1.545	−0.751
	p	***0.047***	0.233	0.122	0.453

Table 3. Estimated cell numbers and volumes of the hypoglossal nucleus

		Right hypoglossus		Left hypoglossus	
		Cell estimate	Volume (μm^3)	Cell estimate	Volume (μm^3)
Schizophrenic group (SC)	Mean	11176	4.39×10^9	11988	4.47×10^9
	SD	2081	0.72×10^9	3007	0.69×10^9
Normal controls (CO)	Mean	12552	4.12×10^9	12438	4.35×10^9
	SD	2988	1.44×10^9	4273	1.62×10^9
Mann–Whitney *U*	*Z*	−0.619	−1.104	−0.177	−1.015
	p	0.536	0.270	0.860	0.340

nuclear volumes as well as between age and estimated cell numbers and estimated nuclear volumes.

Discussion

This study aimed to elucidate whether anatomical correlates of autonomic dysfunction can be found in brainstem sections of patients with schizophrenia. Interestingly, we found that the number of neurons in the DMNV to be significantly increased in the schizophrenic group compared to normal controls, while the volumes of the DMNV did not differ. However, there are several limitations of the study. Sample size was relatively small. The *p*-value of differences in neuron number in right DMNV between groups was modest, while in left DMNV significance was not reached, which mainly accounts to differences in variability. Therefore, conclusions have to be drawn with caution.

The DMNV has not been the topic of studies in schizophrenia research, although autonomic dysfunction was reported for various branches of

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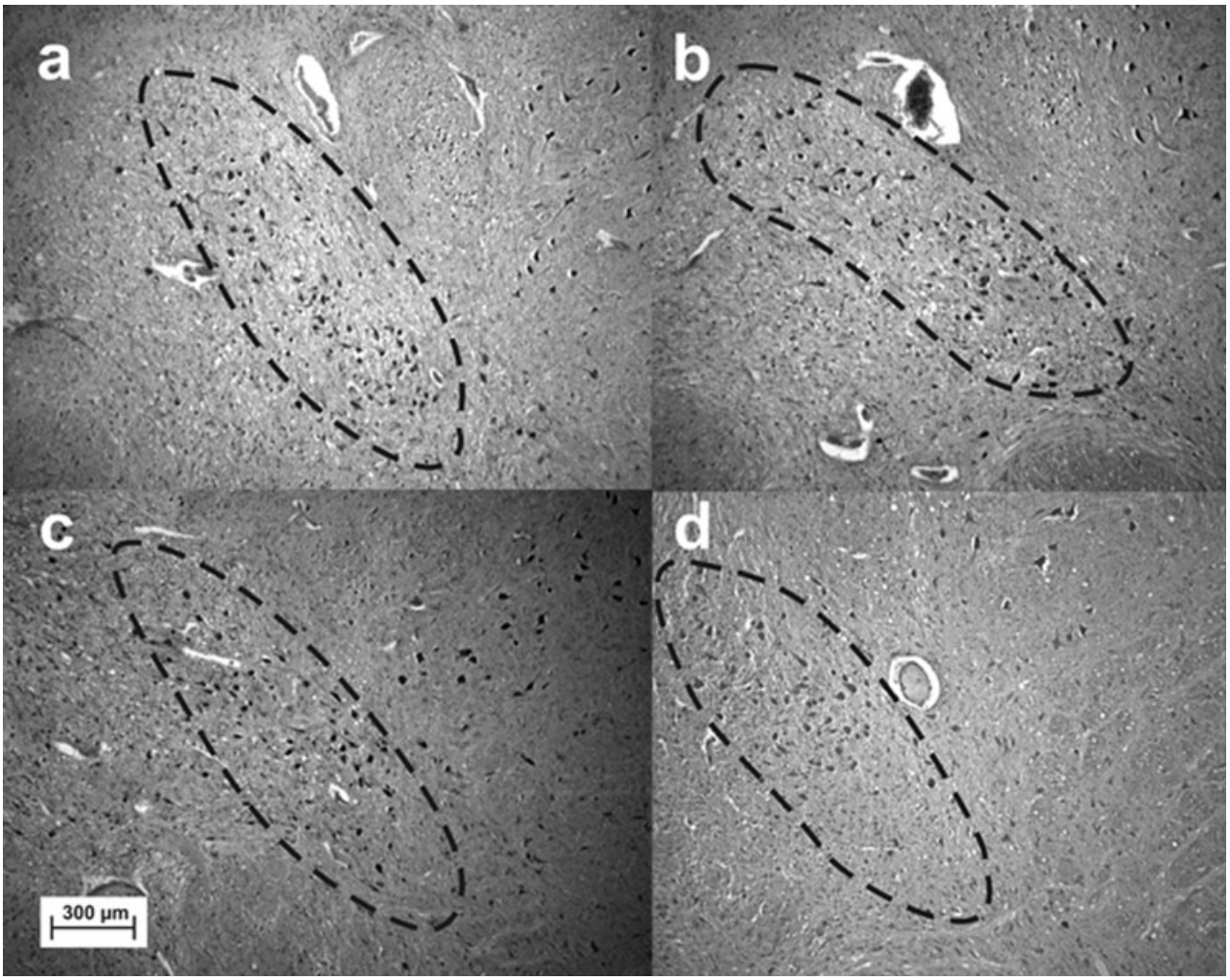

Fig. 2. Differences in number of nerve cells in DMNV (dashed lines) in comparable sections in the middle of the nucleus. (a) and (b) show examples from schizophrenic patients, (c) and (d) show examples from normal controls.

the peripheral vagal nerve (2,28). The difference in neuronal numbers in the right DMNV seems to be a specific finding since no changes were observed in the hypoglossal nucleus.

Many post-mortem stereological studies in schizophrenia found morphological changes in diverse hemispheric structures e.g. thalamus (29–31), basal ganglia (32), anterior cingulate cortex (33,34), prefrontal cortex (35,36) and other cortical areas (37).

In contrast, only few studies investigated brainstem structures in patients with schizophrenia. Most of these studies investigated the locus coeruleus, because the locus coeruleus comprises norepinephrine-containing neurons which belong to a widespread projection system to the cerebral cortex. Marner et al. (38) found an increased volume of the pigmented neurons in the locus coeruleus of subjects with schizophrenia, while no differences could be found in total cell counts. Craven et al. (39) found no significant differences regarding number and size of tyrosine hydroxylase-positive cells in the locus coeruleus.

The dorsal raphe nucleus projects widely upon forebrain regions, thus being a major source of serotonergic innervation of the prefrontal cortex and has been implicated in the pathophysiology of schizophrenia as well. However, an abnormality in number and size of serotonergic neurons in the dorsal raphe nucleus could not be found (40).

The pedunculopontine nucleus is a cholinergic part of the ascending reticular activating system and negative symptoms in schizophrenia seem to be linked to cholinergic overactivation (41). Although German et al. (42) could not detect differences in these regions using an antibody against choline acetyltransferase, Garcia-Rill et al. (43) found an increase of nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase positive neurons in the pedunculopontine nucleus and in the laterodorsal tegmental nucleus in patients with schizophrenia.

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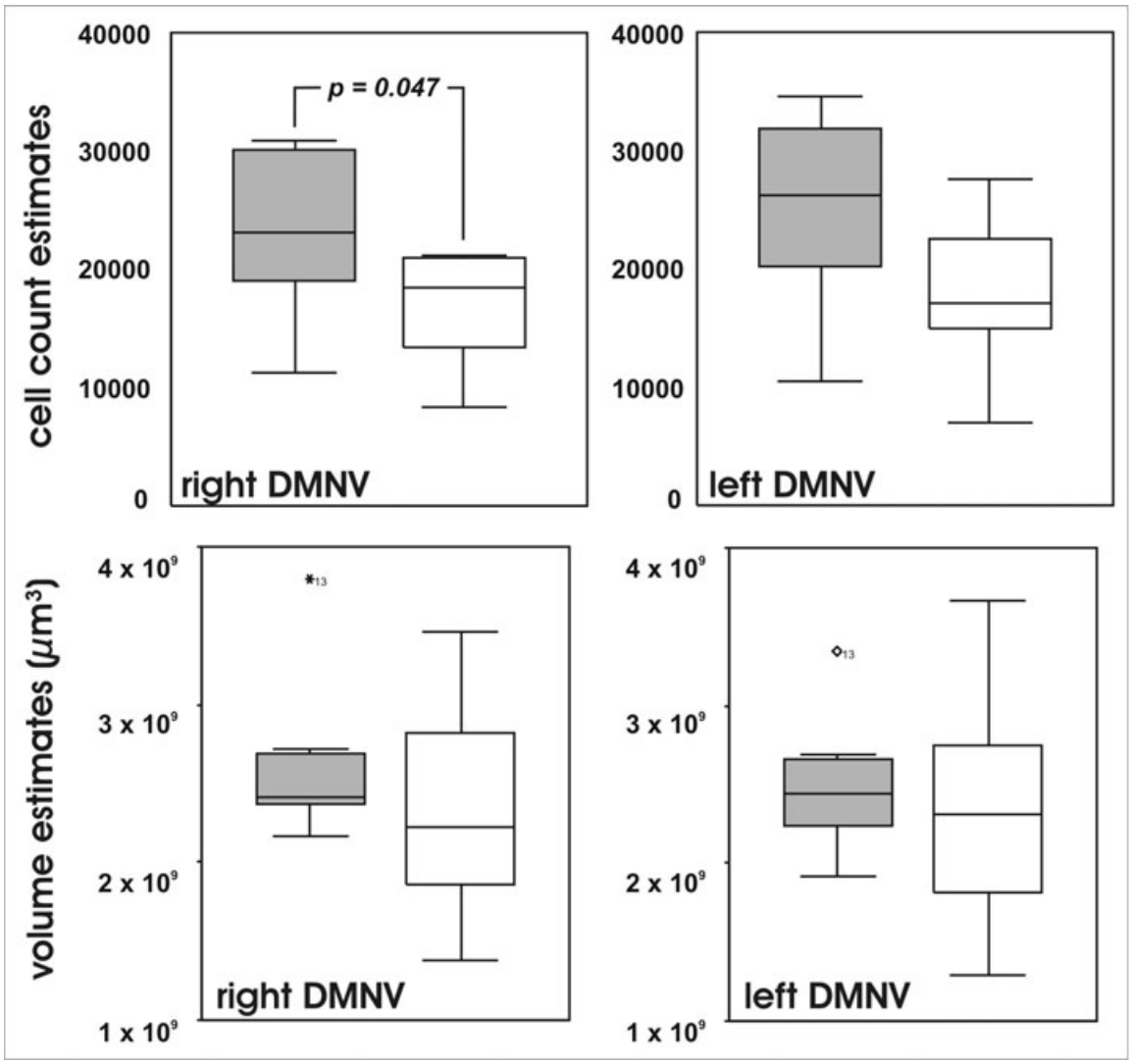

Fig. 3. Box plots of cell count estimates and volume estimates of the DMNV in the schizophrenic group (grey) and normal controls (white). Note that the differences in the cell counts in the right DMNV reached statistical significance.

Moreover, choline acetyltransferase has been found to be decreased in the mesopontine tegmentum in patients with schizophrenia (44) and was correlated to orientation and reasoning. Thus, these aspects point to an alteration in the cholinergic system in the brainstem in the disease. The finding of increased cholinergic neuron counts in the pedunculopontine nucleus in schizophrenia parallels our result of an increased neuronal cell count in the DMNV.

A possible reason for neuronal cell changes could also be chronic antipsychotic medication. Recently, it has been shown that chronic antipsychotic medication leads to smaller grey matter volume, lower glial cell number, and higher neuron density without a difference in total neuron number in antipsychotic-exposed monkeys (45). Furthermore, glial cell loss (33) may have an impact to increased neuron density, However, no difference was observed in the hypoglossus nucleus in our study. Thus, this seems to be unlikely the cause for the findings of this study.

The most reasonable background for increased neuronal numbers in the DMNV could be a neurodevelopmental disturbance, which also has been hypothesised to account for increased cell counts in the pedunculopontine nucleus (43). A prenatal disturbance may influence the formation and/or the dissolution of neurons in the DMNV. The development of DMNV begins at 9 weeks and subnuclear divisions within the DMNV appear at 15 weeks. During the period from 21 to 25 weeks mature organisation evolves (46). The structural development of DMNV occurs in parallel to functional maturation of the cardiovascular and gastric movements (47–49). Thus, this time interval of DMNV development (9–25 weeks of development) seems to be a sensitive period

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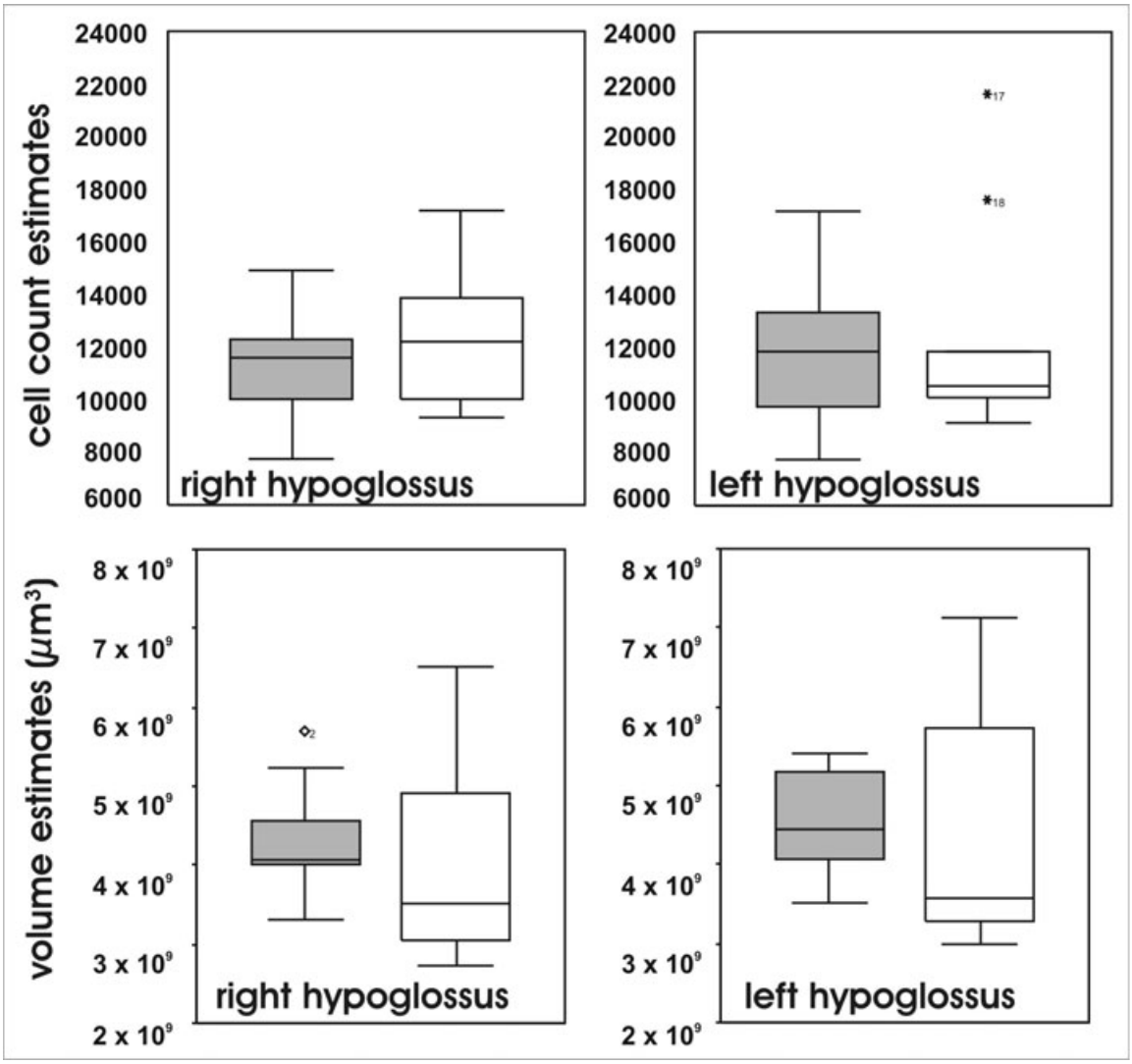

Fig. 4. Box plots of cell count estimates and volume estimates of the hypoglossus in the schizophrenic group (grey) and normal controls (white).

for neurodevelopmental disturbances to take place in DMNV.

Nyffeler et al. (50) found an increase of GABA-A-receptor expression in adult hippocampus and amygdala after maternal inflammation during fetal brain development. Thus, the analysis of differences in GABA-A receptor expression in the DMNV in schizophrenia could be an aim in future research. Moreover, some of the vagal neurons are known to contain nitric oxide synthetase (NOS) (51), which also may play a role in schizophrenia (52,53).

Whether the increase of neurons in DMNV in schizophrenia has functional consequences is not clear. Physiological studies of heart rate variability, BRS and cardiac output show a reduced vagal information flow and a loss of efferent vagal activity in unmedicated patients with schizophrenia (54,55). One could speculate that the increased neuronal cell count in the DMNV may be associated with insufficient output and ultimately with decreased peripheral vagal modulation in schizophrenia. Future studies need to address a possible link to impaired glucose regulation, metabolic syndrome and gastrointestinal dysfunction.

The low number of investigated subjects in this pilot study does not allow a generalisation of results, but justifies further research of the cholinergic system in schizophrenia. In particular, increase the sample size in line with the effect size estimates of this pilot project and to include further investigations into cell properties. Moreover, the solitarius–ambiguus–vagus system in the brainstem should be studied analysing distinct transmitter systems such as NOS, acetylcholine acetyltransferase or GABA-A.

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