

Schizophrenia and anteroventral thalamic nucleus: selective decrease of parvalbumin-immunoreactive thalamocortical projection neurons

Peter Danos^{a,*}, Bruno Baumann^a, Hans-Gert Bernstein^a, Michael Franz^a,
Renate Stauch^a, Georg Northoff^a, Dieter Krell^a, Peter Falkai^b,
Bernhard Bogerts^a

^aDepartment of Psychiatry, University of Magdeburg, Leipziger Str. 44, 39120 Magdeburg, F.R.G.

^bDepartment of Psychiatry, University of Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, F.R.G.

Received 21 July 1997; revised 17 November 1997; accepted 4 December 1997

Abstract

This study was designed to examine possible anatomical changes of thalamocortical circuits in schizophrenics. Previous immunocytochemical studies have shown that parvalbumin, a calcium-binding protein, occurs in thalamocortical projection neurons, but not in GABAergic interneurons in the anteroventral thalamic nucleus (AN). Using parvalbumin-immunocytochemistry we investigated the densities of thalamocortical projection neurons in the AN of schizophrenic cases ($n = 12$) and controls ($n = 14$). The densities of all neurons in the AN were estimated by Nissl-staining. The majority of thalamocortical projection neurons in AN were identified by parvalbumin-immunoreaction. Significantly reduced densities of thalamocortical projection neurons were estimated in the right ($P = 0.003$) and left AN ($P = 0.018$) in schizophrenic subjects. The densities of all neurons in right and left AN were also diminished in schizophrenics; however, these decreases did not reach statistical significance. The reductions of parvalbumin-positive thalamocortical projection neurons were not correlated with the length of disease, this finding supporting the neurodevelopmental etiology of structural abnormalities in schizophrenia. © 1998 Elsevier Science Ireland Ltd.

Keywords: Post-mortem studies; Limbic thalamus; Thalamic projecting neurons; Calcium-binding proteins

*Corresponding author. Tel.: +49 391 6714204; fax: +49 391 6715223; e-mail: peter.danos@medizin.uni-magdeburg.de

1. Introduction

The central pathophysiological feature in schizophrenia seems to be more a disturbed circuitry involving different brain regions than a disturbance in just one brain region (Carlsson and Carlsson, 1990). Recent studies with positron emission tomography (Siegel et al., 1993; Buchsbaum et al., 1996; Andreasen et al., 1996) and magnetic resonance imaging (Andreasen et al., 1994) suggest abnormalities of the thalamocortical circuitry as one of the crucial disturbances in schizophrenia. However, an analysis of thalamic neurons which send their axons to the cortex as the anatomical substrate of the thalamocortical circuitry has yet not been performed.

The anterior nuclear thalamic group is dominated by a large anteroventral nucleus. This nucleus is sometimes called the nucleus anterior principalis (Hassler, 1959). The anteroventral nucleus of the thalamus (AN), a pear-shaped structure surrounded by the fibers of the internal medullary lamina (Fig. 1A–D), is reciprocally connected with the hippocampal region and the cingulate cortex (Amaral and Cowan, 1980; Armstrong, 1990), two regions which have been shown to be involved in schizophrenia (Benes et al., 1991; Cleghorn et al., 1992; Benes, 1993; Noga et al., 1995; Fletcher et al., 1996).

Furthermore, in positron-emission-tomography (PET) studies, a diminished metabolic activity was measured in the anterior regions of the thalamus in schizophrenia (Buchsbaum et al., 1996).

The major aim of the present study was to determine the densities of thalamocortical neurons of AN in schizophrenic and control subjects. In the cerebral cortex, parvalbumin-containing neurons are subsets of GABAergic interneurons (DeFelipe, 1993); however, parvalbumin-immunoreactive neurons in the thalamus are known to be projection neurons: anatomical studies in monkeys, using combined immunocytochemical anterograde and retrograde tracing techniques, have shown that parvalbumin is a marker for thalamic relay neurons projecting to the cortex (Jones and Hendry, 1989; Molinari et al., 1995). Immunocytochemical studies in the human striate and visual cortices (Blümcke et al., 1990; Cao et

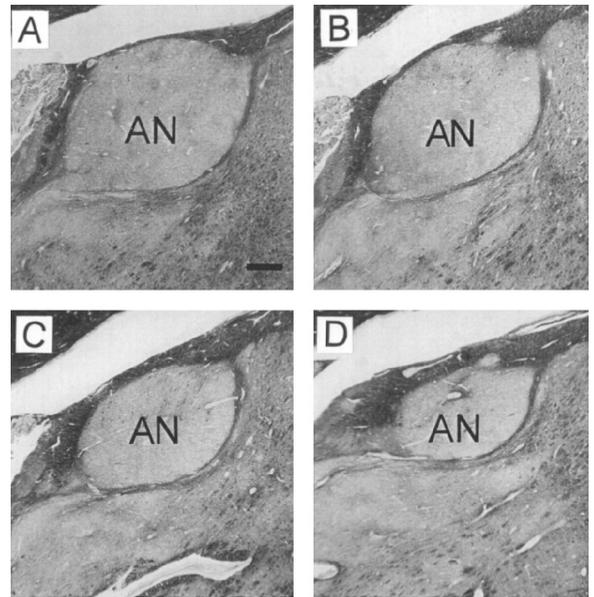


Fig. 1. Low power photomicrograph with a sequence of four sections through the anteroventral thalamic nucleus, in antero-posterior order (A–D), and stained with a combined Nissl- and myelin-staining. Scale bar = 0.75 mm.

al., 1996), and studies in human cingulate and temporal cortices (del Rio and DeFelipe, 1994; Kalus and Senitz, 1996) also suggest that thalamocortical neurons from association nuclei, including the AN, are also parvalbumin-immunoreactive.

2. Methods

2.1. Subjects

Post-mortem brain tissue of 12 schizophrenics and 14 matched controls were used for the present study (Table 1). All brains were obtained from the new Düsseldorf/Magdeburg brain collection. Patients and controls died between the years 1986 and 1993. There were no significant differences (Student's *t*-tests, chi-square analysis) in possible confounding variables between the schizophrenics and the controls. Matching criteria were (mean \pm S.D.) age (schizophrenics = 54.0 ± 9.3 years, controls = 53.1 ± 8.9 years; $P = 0.81$), gender (schizophrenics = six males, six females,

controls = eight males, six females; $P = 0.71$), post-mortem interval (schizophrenics = 35.3 ± 13.4 h, controls = 29.6 ± 13.9 h; $P = 0.30$), fixation interval (schizophrenics = 7.1 ± 2.9 months, controls = 5.9 ± 4.3 months; $P = 0.42$) and shrinkage factors (schizophrenics = 2.26 ± 0.25 , controls = 2.08 ± 0.19 ; $P = 0.13$). Shrinkage factors were determined for each brain before and after dehydration and embedding of tissue. Volume shrinkage factors were calculated using the formula: $VF = (A1/A2)^{3/2}$ (VF, volume shrinkage factor; A1, cross-sectional area before processing of tissue; A2, cross-sectional area after processing of tissue).

Only patients with well-preserved and extensive

clinical records were selected for this study. The mean duration of illness was 21.6 ± 7.3 (mean \pm S.D.) years. Schizophrenic subjects were diagnosed as chronic schizophrenia according to DSM-III-R (295.62), since it has been observed that in the majority of cases schizophrenic subtypes alternate during a longer period of illness (Deister and Marneros, 1993). Brains with reports of substance abuse, dementia, or neurological illness and chronic terminal diseases known to affect the brain (i.e. chronic liver, kidney, heart and lung diseases, cancer, cortisol treatment) were excluded. Patients were excluded if they were older than 65 years, or had a lifetime history of serious head trauma. All patients had received

Table 1
Demographic and histological data of schizophrenic patients and control subjects

Case/sex/age (years)	Post-mortem interval (h)	Fixation time (months)	Cause of death
Control subjects			
1/m/56	30	7.0	Acute pancreatitis
2/m/50	72	3.0	Myocardial infarction
3/m/64	30	8.0	Ruptured aortic aneurysm
4/f/52	24	3.0	Heart failure
5/m/47	24	3.0	Respiratory insufficiency
6/m/38	19	2.5	Heart failure
7/m/56	24	1.5	Heart failure
8/f/64	24	1.5	Peritonitis
9/m/46	24	9.5	Heart failure
10/m/61	24	17.0	Heart failure
11/f/38	24	7.5	Pulmonary embolism
12/f/65	48	9.5	Ruptured aortic aneurysm
13/f/65	24	3.0	Heart failure
14/m/54	24	6.5	Pulmonary embolism
Schizophrenic subjects			
1/m/49	24	12.0	Heart failure
2/m/65	48	7.0	Respiratory insufficiency
3/m/46	48	7.0	Pulmonary embolism
4/f/53	48	3.5	Myocardial infarction
5/m/51	48	9.5	Ileus
6/m/39	16	10.0	Cardiac arrhythmia
7/f/52	24	4.5	Suicide by drowning
8/f/40	48	6.5	Sepsis
9/f/55	48	9.5	Suicide by overdose of medication
10/m/61	24	3.0	Pneumonia
11/f/62	24	9.0	Gastrointestinal hemorrhage
12/f/56	24	3.5	Myocardial infarction

neuroleptic treatment at many times during their disease. Control brains without a history of neuropsychiatric disorders were obtained from the same pathological institutes, or medical examiner's officers.

2.2. Tissue processing

The histological procedure has been described in a previous article (Bogerts et al., 1990). Briefly, brains were embedded in paraplast and cut into 20- μm thick whole brain coronal serial sections. The stereotactic coordinates described by Schaltenbrand and Bailey (1959) were used as demarcation for the anterior and posterior pole and the edges of the AN (Fig. 1). Sections were then used for Nissl-staining or for parvalbumin-immunoreaction. Sections were collected in cold phosphate buffer, 0.1 mol/l (pH 7.4) mounted on gelatin-coated slides, and air dried for 24 h. Sections were incubated overnight with antibody to parvalbumin (mouse monoclonal antibody, SWANT Bellinzona, AB 235, Switzerland). The primary antibody was visualized by using a peroxidase-conjugated secondary antibody (antibody to mouse immunoglobulin G) and developed with H_2O_2 -3,3'-diaminobenzidin-tetrahydrochloride (DAB) (Vector Laboratories, Burlingame, CA). This monoclonal antibody has been shown to stain the parvalbumin protein specifically (Celio, 1990). The specificity of the parvalbumin immunostaining was investigated by treating the sections by the immunocytochemical methods described above, with the primary antibody being omitted. None of the sections processed in this fashion exhibited specific immunoreactivity.

2.3. Morphometric analysis

The numerical densities and soma sizes of Nissl-stained neurons and parvalbumin-immunoreactive neurons were measured separately in AN of the right and left hemispheres of schizophrenics and controls with a computer-assisted system. For the measurements of neuron densities and neuron soma sizes, a $200\times$ magnification was used. Neurons were measured in six $424\ \mu\text{m}\times 424\ \mu\text{m}$ counting squares in a

section in the right and left AN, respectively. Previous studies have described that the density of neurons in human AN is homogenous along the whole rostrocaudal length of the axis (Armstrong, 1990). However, in order to confirm this, we performed Nissl-staining and parvalbumin-staining of five sections from the anterior to the posterior part of the whole AN in both sides (right and left) of five brains. A repeated measures multivariate analysis of variance (MANOVA) was then performed in both staining procedures with the densities in the different levels as within-subjects factors. No significant differences in the neuron densities throughout the different levels of the AN could be measured in the group of Nissl-stained neurons in the right ($F = 1.3$, $df = 4$, $P = 0.42$) or left AN ($F = 0.20$, $df = 4$, $P = 0.89$) or in the group of parvalbumin-stained neurons in the right ($F = 1.48$, $df = 4$, $P = 0.28$) or left AN ($F = 0.80$, $df = 4$, $P = 0.57$).

The counting areas were systematically positioned with the following procedure: the first counting area was placed in the middle of the diagonal axis which connects the two poles of AN. The poles on the coronal sections are located where the internal medullary lamina splits in the two main fiber bundles. Adjacent squares were positioned along this diagonal axis below and above the first field. All neuronal profiles counted were 6 μm or more in diameter. A Kappatechnik (Kappatechnik, Gleichen, F.R.G.) video camera module attached to a Leica DM RB microscope (Leica, Gießen, F.R.G.) and Digitrace software (Imatec, Neufahrn, F.R.G.) were used for the measurement of neuronal densities.

Numerical densities in four cell populations (Nissl-staining, left and right hemisphere, parvalbumin-staining, left and right hemisphere) were estimated by counting the number of neuronal profiles per counting area, numbers were then corrected according to the Abercrombie (1946) correction for nuclear diameters (range 14.0–14.5 μm in the four cell populations) formula and section thickness (20 μm), and the estimated density of numerical densities per cubic millimetre was determined. The computer software also calculated the area of the soma profiles neuron diameter. For each case, data from the six count-

ing areas were summed.

The number of neurons sampled per case was 290.6 ± 76.7 (mean \pm S.D.). Results were expressed in terms of positive neurons per square mm. All measurements were performed under blind conditions by two trained independent (R.S, M.F) raters. The intraclass correlation coefficients for reliability, calculated according to Fleiss (1986), ranged for the four neuronal populations (Nissl-staining, right and left hemisphere; parvalbumin-staining, right and left hemisphere) between 0.94 and 0.97. For the statistical analysis neuronal counts performed by R.S. were used.

Analysis of variance (SPSS/PC + 4.0) was performed with diagnosis (schizophrenic subjects or controls) as a between factor and confounding variables as covariates (age, gender, duration of illness, post-mortem interval, fixation interval and shrinkage factor) to compare the neuron densities and the soma sizes in both groups. Correlation analyses (Pearson correlation coefficients) between the neuron population densities, the soma sizes and the confounding variables were also performed.

3. Results

PV-immunoreaction stained 62–43% of Nissl-stained AN neurons in the right and left hemisphere of schizophrenics and controls (see Table 2).

Analysis of variance revealed no significant differences in soma sizes of Nissl-stained neurons and parvalbumin-immunoreactivity between the two groups. There were no significant differences in soma sizes (in μm^2) of Nissl-stained neurons in left AN (145.5 ± 32.9) or right AN (147.7 ± 30.8) of schizophrenics in comparison to soma sizes in left AN (161.2 ± 32.7 ; $F = 0.96$, $P = 0.49$) and right AN (150.2 ± 28.7 ; $F = 1.30$, $P = 0.33$) in the control group. Soma sizes of parvalbumin-stained neurons in left AN (154.9 ± 16.6) and right AN (171.0 ± 24.1) in schizophrenic subjects were not significantly different from soma sizes in left AN (153.0 ± 37.5 , $F = 1.6$, $P = 0.21$) or right AN (155.9 ± 31.3 ; $F = 0.71$, $P = 0.66$) in control subjects. These results show that differences in cell size do not confound measures of numerical density. Pearson correlations revealed no significant correlations between duration of illness and densities of parvalbumin-immunoreactive neurons in the right AN ($r = -0.16$; $P = 0.61$) or left AN ($r = -0.39$; $P = 0.20$). Further correlation analyses revealed no significant correlations between neuron densities, soma sizes and confounding variables.

After adjustment for the confounding variables (ANOVA), the densities of parvalbumin-immunoreactive neurons were significantly reduced in left AN ($P = 0.018$) and right AN ($P = 0.003$) in schizophrenic subjects (Table 2, Fig. 2C and D). The densities of Nissl-stained neurons were

Table 2

Densities of all AN neurons (Nissl-staining) and of thalamocortical projection neurons (parvalbumin staining) of 12 schizophrenic subjects and 14 control subjects^a. Densities are given as means \pm S.D. neurons per cubic millimetre

	Schizophrenics	Controls	Diff. (%)	Analysis (df = 7)	
				F	P
All neurons					
Left	5662.2 ± 792.9	6664.3 ± 1301.4	- 15.1	1.73	0.16
Right	5728.0 ± 747.2	6586.5 ± 939.4	- 13.1	1.49	0.23
Thalamocortical neurons					
Left	2425.0 ± 892.2	3687.8 ± 960.1	- 34.2	3.35	0.018
Right	2648.6 ± 882.1	4145.6 ± 739.4	- 36.0	4.95	0.003

^aDifferences between schizophrenic subjects and control subjects in neuronal densities were examined by analysis of variance (ANOVA) with age, sex, illness duration, post-mortem interval, fixation interval and shrinkage factors as covariates.

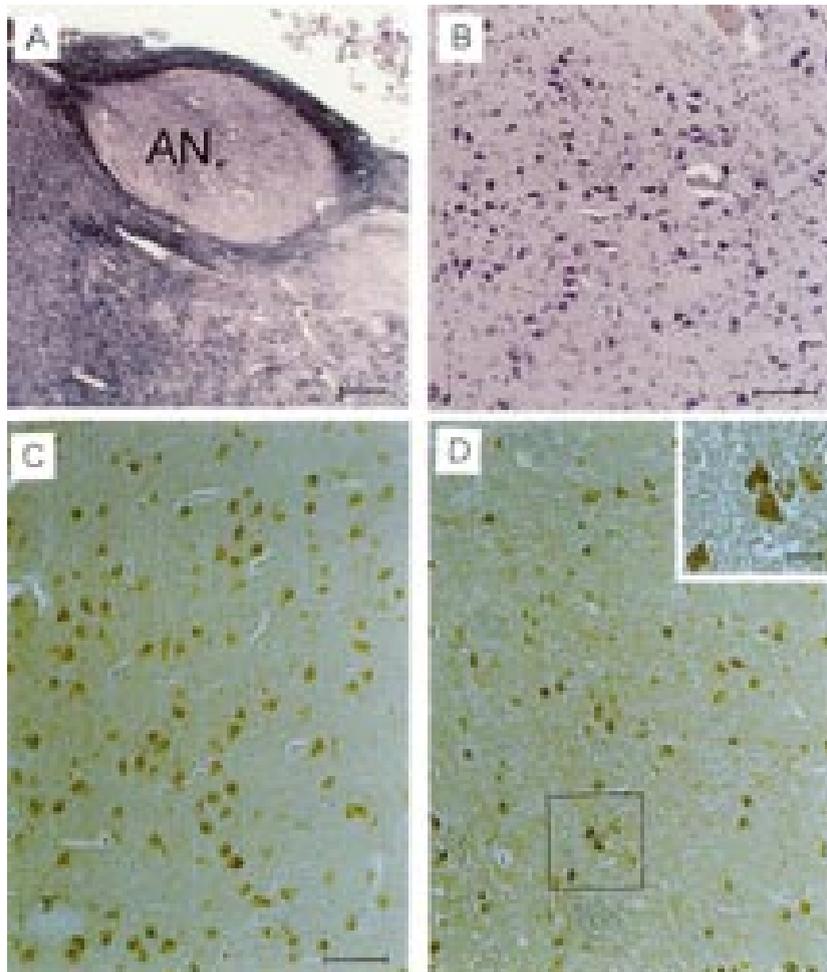


Fig. 2. (A) Low power photomicrograph of a coronar section through the anteroventral nucleus of the thalamus (AN) stained with a combined Nissl- and myelin-staining. (B) Higher magnification of AN with densely packed neurons (Nissl-staining). (C, D) Parvalbumin-stained cell bodies of presumptive thalamocortical projection neurons in AN of a control subject (C) and of an age and sex-matched schizophrenic subject (D). The density of PV-positive thalamocortical neurons is significantly reduced in schizophrenics. Inset at top right of (D) shows an example of neurons stained for PV. Scale bars, 0.75 mm in (A); 100 μ m in (B) through (D); and 25 μ m in inset of (D).

decreased in right and left AN in schizophrenic subjects; however, these decreases were not significant (Table 2).

4. Discussion

Our results suggest a selective decrease of parvalbumin-immunoreactive (PV) cortically projecting neurons in the right and left anteroventral

nucleus of the thalamus (AN) in schizophrenic subjects. Previous studies in monkeys have shown that in thalamic nuclei parvalbumin-immunoreaction stains selectively neurons projecting to cortical regions (Jones and Hendry, 1989). However, in humans, the percentage of parvalbumin-positive thalamocortical projection neurons in AN within all thalamocortical projection neurons had not yet been established.

In the present study, 62–43% of all AN neu-

rons were PV-positive. Anatomical studies have shown that projecting neurons in AN represent 72–52% of all AN neurons (Braak and Weinel, 1985; Hunt et al., 1991), which means that in the present study the majority of thalamocortical projection neurons were stained by parvalbumin. The observed decreases of PV-positive thalamocortical projection neurons were selective, since the decreases of the densities of all neurons in AN did not reach statistical significance.

The following methodological limitations could have influenced the present results. It should be emphasized that the method which we performed for the measurement of neuron densities is a relative estimate and not an absolute calculation of the number of Nissl-stained neurons and of parvalbumin-immunoreactive neurons. However, in human studies differences in neuronal densities between controls and a group of patients can be accurately determined when comparing tissue that is similarly processed (Mathern et al., 1995).

Another methodological limitation could be seen in the possibility that PV also stains GABAergic interneurons in AN. However, immunocytochemical animal studies in guinea pig (De Biasi et al., 1994) and monkey (Jones and Hendry, 1989) have shown that GABAergic interneurons in AN are PV-negative.

The reduced densities of PV-positive neurons in schizophrenics could also be due to the influence of neuroleptic medication in these patients, since all patients have been treated with neuroleptics for a long time period.

Neuroleptic medication could have had a toxic effect on PV-positive neurons or could have influenced parvalbumin expression in neurons. In the case of a toxic effect on PV-positive neurons, one might expect a relationship between the duration of illness and the densities of PV-positive neurons, since the duration of neuroleptic treatment corresponds approximately to the duration of illness. However, in our study, no significant correlations were found between the densities of PV-positive neurons and the duration of illness.

It appears possible that the observed decrease of PV-positive neurons in AN of schizophrenics represents just a neuroleptic-dependent decrease of synthesis of PV in these neurons, since it has been shown that expression of PV can be altered

in an activity-dependent manner (Jones, 1993). Experimental data about an influence of neuroleptics on the expression of parvalbumin in neurons are not available at present.

If neuroleptics or other unknown factors only suppressed the expression of parvalbumin in the neurons, then the densities of Nissl-stained neurons should be unchanged. However, in the present study, we found a substantial, but not yet significant, reduction of the densities of Nissl-stained neurons. Therefore, although alterations in the neuronal parvalbumin content due to neuroleptic medication cannot be excluded, our findings suggest that the observed reduced densities of PV-positive neurons in AN of schizophrenics are a result of a neuronal loss.

The hypothesis that the reduced densities of PV-positive neurons in the anteroventral nucleus are due to a loss of neurons is also consistent with the results of a study of Pakkenberg (1990) who found a pronounced loss of neurons in the mediodorsal thalamic nucleus in schizophrenic subjects.

It also appears possible, that the observed decreased densities of thalamocortical neurons in schizophrenics could be due to an enlarged AN in schizophrenics. However, previous studies have found a reduction of the thalamus in schizophrenia (Andreasen et al., 1990, 1994), and a significantly decreased volume of the mediodorsal thalamus in untreated and in neuroleptic-treated schizophrenics (Pakkenberg, 1992). Furthermore, a recent MRI study measured a decreased area of the left anterior thalamus in schizophrenia (Buchsbaum et al., 1996). Therefore, it is unlikely that the present data of decreased PV-positive projection neurons in the AN of schizophrenics are due to an enlarged volume of these nuclei.

In a recent report, Buchsbaum et al. (1996) have found diminished metabolic activity in the anterior regions of the thalamus in schizophrenia. These findings are consistent with our findings of decreased density of neurons in the anterior nuclei, since it is known that glucose metabolism is positively correlated with neuronal densities (McGeer et al., 1990). Furthermore, another PET study has found a decrease in glucose metabolism in both sides of the AN but not in the posterior thalamus after withdrawal of medication with haloperidol in schizophrenic patients (Holcomb et

al., 1996), suggesting that the antipsychotic effect of haloperidol is mediated by thalamocortical circuits involving the AN.

In the present study we have found no significant correlations between the densities of thalamocortical neurons and age or duration of illness. This finding is consistent with the neurodevelopmental hypothesis of schizophrenia (Weinberger, 1995).

What link may exist between schizophrenic symptoms and anatomical disturbances in the anteroventral thalamus? The AN belongs to the limbic PAPEZ-circuit, a circuit which has been supposed to represent the neural substrate of schizophrenia (Bogerts, 1989). However, the functions in which the AN is involved are still not precisely known. Anatomical studies, comparing the relative size of monkey's AN with the human AN, have found a relative enlargement of this nucleus in human brain, these data suggesting that the AN nucleus may be particularly important in modulating high-order cortical processing in the human brain.

Behavioral works have shown that the AN modulates states of alertness and attack (MacDonnell and Flynn, 1968). Furthermore, this nucleus is also involved in the semantic memory system (Kopelman, 1995) and attention (Rousseaux et al., 1991), cognitive functions that are known to be impaired in schizophrenia (McKenna et al., 1994; Goldberg and Weinberger, 1995).

Beasley and Reynolds (1997) reported a decreased number of PV-positive neurons in the prefrontal cortex of schizophrenics; however this finding could not be replicated in a recent study by Woo et al. (1997). Daviss and Lewis (1995) have found an increase in local circuit neurons which contain calbindin, a calcium-binding protein, in the prefrontal cortex of schizophrenics. Therefore, the role of calcium-binding proteins in the etiology of schizophrenia is still inconclusive.

Based on animal studies about the neurotoxic effect of *N*-methyl-D-aspartate glutamate receptor antagonists in the posterior cingulate cortex, it has been recently proposed (Olney and Farber, 1995) that schizophrenic symptoms may be due to disturbances of the glutamatergic system. Since it is known that thalamocortical projections are glu-

tamatergic (Gigg et al., 1992; Kharazia and Weinberg, 1994), the present findings also strengthen the hypothesis of impaired function of the glutamatergic system in schizophrenia.

In conclusion, the present results provide anatomical evidence for a selective involvement of thalamocortical projection neurons in the etiology of schizophrenia. Further neuroanatomical studies on projection neurons within the limbic system are needed in order to elucidate the complex neuropathological basis of schizophrenia.

Acknowledgements

This research report was supported by the Stanley Foundation and by the Deutsche Forschungsgemeinschaft.

References

- Abercrombie, M., 1946. Estimation of nuclear population from microtome sections. *Anatomical Record* 94, 239–247.
- Amaral, D.G., Cowan, W.M., 1980. Subcortical afferents to the hippocampal formation in the monkey. *Journal of Comparative Neurology* 189, 573–591.
- Andreasen, N.C., Arndt, S., Swayze, V., Cizadlo, T., Flaum, M., O'Leary, D., Ehrhard, J.C., Yuh, W.T., 1994. Thalamic abnormalities in schizophrenia visualized through magnetic resonance image averaging. *Science* 266, 294–298.
- Andreasen, N.C., Ehrhardt, J.C., Swayze, V.W. II, Alliger, R.J., Yuh, W.T.C., Cohen, G., Ziebell, S., 1990. Magnetic resonance imaging of the brain in schizophrenia. *Archives of General Psychiatry* 47, 35–44.
- Andreasen, N.C., O'Leary, D.S., Cizadlo, T., Arndt, S., Rezal, K., Boles Ponto, L.L., Watkins, G.L., Hichwa, R.D., 1996. Schizophrenia and cognitive dysmetria: a positron-emission tomography study of dysfunctional prefrontal-thalamic-cerebellar circuitry. *Proceedings of the National Academy of Sciences of the United States of America* 93, 9985–9990.
- Armstrong, E., 1990. Limbic thalamus: anterior and mediodorsal nuclei. In: Paxinos, G. (Ed.), *The Human Nervous System*. Academic Press, San Diego, pp. 469–482.
- Beasley, C.L., Reynolds, G.P., 1997. Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. *Schizophrenia Research* 24, 349–355.
- Benes, F.M., 1993. Relationship of cingulate cortex to schizophrenia and other psychiatric disorders. In: Vogt, B.A., Gabriel, M. (Eds.), *Neurobiology of Cingulate Cortex and Limbic Thalamus*. Birkhäuser, Boston, pp. 581–605.
- Benes, F.M., McSparren, J., Bird, E.D., SanGiovanni, J.P., Vincent, S.L., 1991. Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. *Archives of General Psychiatry* 48, 996–1001.

- Blümcke, I., Hof, P.R., Morrison, J.H., Celio, M.R., 1990. Distribution of parvalbumin immunoreactivity in the visual cortex of old world monkeys and humans. *Journal of Comparative Neurology* 301, 417–432.
- Bogerts, B., 1989. Limbic and paralimbic pathology in schizophrenia: interaction with age and stress related factors. In: Schulz, S.C., Tamminga, C.A. (Eds.), *Schizophrenia: Scientific Progress*. Oxford University Press, New York, pp. 216–227.
- Bogerts, B., Falkai, P., Haupts, M., Greve, B., Ernst, St., Tapernon-Franz, U., Heinzmann, U., 1990. Post-mortem volume measurements of limbic system and basal ganglia structures in chronic schizophrenics. *Schizophrenia Research* 3, 295–301.
- Braak, H., Weinel, U., 1985. The percentage of projection neurons and local circuit neurons in different nuclei of the human thalamus. *Journal für Hirnforschung* 26, 525–530.
- Buchsbaum, M.S., Someya, T., Teng, C.Y., Abel, L., Chin, S., Najafi, A., Haier, R.J., Wu, J., Bunney, W.E., Jr., 1996. PET and MRI of the thalamus in never-medicated patients with schizophrenia. *American Journal of Psychiatry* 153, 191–199.
- Cao, Q.L., Yan, X.X., Luo, X.G., Garey, L.J., 1996. Prenatal development of parvalbumin immunoreactivity in the human striate cortex. *Cerebral Cortex* 6, 620–630.
- Carlsson, M., Carlsson, A., 1990. Schizophrenia: a subcortical neurotransmitter imbalance syndrome? *Schizophrenia Bulletin* 16, 425–432.
- Celio, M.R., 1990. Calbindin D-28k and parvalbumin in the rat nervous system. *Neuroscience* 35, 375–475.
- Cleghorn, J.M., Franco, S., Szechtman, B., Kaplan, R.D., Szechtman, H., Brown, G.M., Nahmias, C., Garnett, E.S., 1992. Toward a brain map of auditory hallucinations. *American Journal of Psychiatry* 149, 1062–1069.
- Daviss, S.R., Lewis, D.A., 1995. Local circuit neurons of the prefrontal cortex in schizophrenia: selective increase in the density of calbindin-immunoreactive neurons. *Psychiatry Research* 59, 81–96.
- De Biasi, S., Arcelli, P., Spreafico, R., 1994. Parvalbumin immunoreactivity in the thalamus of guinea pig: light and electron microscopic correlation with gamma-aminobutyric acid immunoreactivity. *Journal of Comparative Neurology* 348, 556–569.
- DeFelipe, J., 1993. Neocortical neuronal diversity: chemical heterogeneity revealed by co-localization studies of classic neurotransmitters, neuropeptides, calcium binding proteins and cell surface molecules. *Cerebral Cortex* 3, 273–289.
- Deister, A., Marneros, A., 1993. Long-term stability of subtypes in schizophrenic disorders: a comparison of four diagnostic systems. *European Archives of Psychiatry and Clinical Neuroscience* 242, 184–190.
- del Rio, M.R., DeFelipe, J., 1994. A study of SMI 32-stained pyramidal cells, parvalbumin-immunoreactive chandelier cells, and presumptive thalamocortical axons in the human temporal neocortex. *Journal of Comparative Neurology* 342, 389–408.
- Fleiss, J.C., 1986. *The Design and Analysis of Clinical Experiments*. Wiley, New York.
- Fletcher, P.C., Frith, C.D., Grasby, P.M., Friston, K.J., Dolan, R.J., 1996. Local and distributed effects of apomorphine on fronto-temporal function in acute unmedicated schizophrenia. *Journal of Neuroscience* 16, 7055–7062.
- Gigg, J., Tan, A.M., Finch, D.M., 1992. Glutamatergic excitatory responses of anterior cingulate neurons to stimulation of the mediodorsal thalamus and their regulation by GABA. *Cerebral Cortex* 2, 477–484.
- Goldberg, T.E., Weinberger, D.R., 1995. Thought disorder, working memory and attention: interrelationships and the effects of neuroleptic medications. *International Clinical Psychopharmacology* 10 Suppl 3, 99–104.
- Hassler, R., 1959. Anatomy of the thalamus. In: Schaltenbrand, G., Bailey, P. (Eds.), *Introduction to Stereotaxis with an Atlas of the Human Brain*, vol. 1. Thieme Verlag, Stuttgart, pp. 230–290.
- Holcomb, H.H., Cascella, N.G., Thaker, G.K., Medoff, D.R., Dannals, R.F., Tamminga, C.A., 1996. Functional sites of neuroleptic drug action in the human brain: PET/FDG studies with and without haloperidol. *American Journal of Psychiatry* 153, 41–49.
- Hunt, C.A., Pang, D.Z., Jones, E.G., 1991. Distribution and density of GABA cells in intralaminar and adjacent nuclei of monkey thalamus. *Neuroscience* 43, 185–196.
- Jones, E.G., 1993. GABAergic neurons and their role in cortical plasticity in primates. *Cerebral Cortex* 3, 361–372.
- Jones, E.G., Hendry, S.H.C., 1989. Differential calcium binding protein immunoreactivity distinguishes classes of relay neurons in monkey thalamic nuclei. *European Journal of Neuroscience* 1, 222–246.
- Kalus, P., Senitz, D., 1996. Parvalbumin in the human anterior cingulate cortex. Morphological heterogeneity of inhibitory interneurons. *Brain Research* 729, 45–54.
- Kharazia, V.N., Weinberg, R.J., 1994. Glutamate in thalamic fibers terminating in layer IV of primary sensory cortex. *Journal of Neurosciences* 14, 6021–6032.
- Kopelman, M.D., 1995. The Korsakoff syndrome. *British Journal of Psychiatry* 166, 154–173.
- MacDonnell, M.F., Flynn, J.P., 1968. Attack elicited by stimulation of the thalamus and adjacent structure of cats. *Behaviour* 31, 185–202.
- Mathern, G.W., Babb, T.L., Pretorius, J.K., Leite, J.P., 1995. Reactive synaptogenesis and neuron densities for neuropeptide Y, somatostatin and glutamate decarboxylase immunoreactivity in the epileptogenic human fascia dentata. *Journal of Neuroscience* 15, 3990–4004.
- McGeer, E.G., McGeer, P.L., Harrop, R., Akiyama, H., Kamo, H., 1990. Correlations of regional postmortem enzyme activities with premortem local glucose metabolic rates in Alzheimer's disease. *Journal of Neuroscience Research* 27, 612–619.
- McKenna, P.J., Mortimer, A.M., Hodges, J.R., 1994. Semantic memory and schizophrenia. In: David, A.S., Cutting, J.C. (Eds.), *The Neuropsychology of Schizophrenia*. Lawrence Erlbaum, Hillsdale, NJ, pp. 163–178.

- Molinari, M., Dell'Anna, M.E., Rausell, E., Leggio, M.G., Hashikawa, T., Jones, E.G., 1995. Auditory thalamocortical pathways defined in monkeys by calcium-binding protein immunoreactivity. *Journal of Comparative Neurology* 362, 171–194.
- Noga, J.T., Aylward, E., Barta, P.E., Pearlson, G.D., 1995. Cingulate gyrus in schizophrenic patients and normal volunteers. *Psychiatry Research: Neuroimaging* 61, 201–208.
- Olney, J.W., Farber, N.B., 1995. Glutamate receptor dysfunction and schizophrenia. *Archives of General Psychiatry* 52, 998–1007.
- Pakkenberg, B., 1990. Pronounced reduction of total neuron number in mediodorsal thalamic nucleus and nucleus accumbens in schizophrenics. *Archives of General Psychiatry* 47, 1023–1028.
- Pakkenberg, B., 1992. The volume of the mediodorsal thalamic nucleus in treated and untreated schizophrenics. *Schizophrenia Research* 7, 95–100.
- Rousseaux, M., Kassiotis, P., Signoret, J.L., Cabaret, M., Petit, H., 1991. Syndrome amnésique par infarctus restreint du thalamus antérieur droit. *Revue Neurologique (Paris)* 147, 809–818.
- Schaltenbrand, G., Bailey, P. (Eds.), 1959. *Introduction to Stereotaxis with an Atlas of the Human Brain*, vol. 2. Thieme Verlag, Stuttgart, pp. 37–39.
- Siegel, B.V., Buchsbaum, M.S., Bunney, W.E., Jr., Gottschalk, L.A., Haier, R.J., Lohr, J.B., Lottenberg, S., Najafi, A., Nuechterlein, K.H., Potkin, S.G., Wu, J., 1993. Cortical-striatal-thalamic circuits and brain glucose metabolic activity in 70 unmedicated male schizophrenic patients. *American Journal of Psychiatry* 150, 1325–1336.
- Weinberger, D.R., 1995. From neuropathology to neurodevelopment. *Lancet* 346, 552–557.
- Woo, T.U., Miller, J.L., Lewis, D.A., 1997. Schizophrenia and the parvalbumin-containing class of cortical local circuit neurons. *American Journal of Psychiatry* 154, 1013–1015.