Volume, neuron density and total neuron number in five subcortical regions in schizophrenia

Pawel Kreczmanski,1,2 Helmut Heinsen,3 Valentina Mantua,1,6,7 Fritz Woltersdorf,4 Thorsten Masson,5 Norbert Ulfig,4 Rainald Schmidt-Kastner,1 Hubert Korr,1,2,5 Harry W. M. Steinbusch,1,2 Patrick R. Hof8 and Christoph Schmitz1,2

1Department of Psychiatry and Neuropsychology, Division of Cellular Neuroscience, Maastricht University, 2European Graduate School of Neuroscience (EURON), Maastricht, The Netherlands, 3Morphological Brain Research Unit, University of Wuerzburg, Wuerzburg, 4Department of Anatomy, University of Rostock, Rostock, Germany, 5Department of Anatomy and Cell Biology, RWTH Aachen University, Aachen, 6Department of Psychiatry, Section of Clinical Neuropharmacology, Institute of Psychiatry, Kings College London, London, UK, 7Department of Psychiatric Sciences and Psychological Medicine, Psychiatric Clinic III, University of Rome La Sapienza, Rome, Italy and 8Department of Neuroscience, Mount Sinai School of Medicine, New York, NY, USA

Correspondence to: Dr Christoph Schmitz, Department of Psychiatry and Neuropsychology, Division of Cellular Neuroscience, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands
E-mail: c.schmitz@np.unimaas.nl

Several studies have pointed to alterations in mean volumes, neuron densities and total neuron numbers in the caudate nucleus (CN), putamen, nucleus accumbens (NA), mediodorsal nucleus of the thalamus (MDNT) and lateral nucleus of the amygdala (LNA) in schizophrenia. However, the results of these studies are conflicting and no clear pattern of alterations has yet been established in these subcortical regions, possibly due to differences in quantitative histological methods used as well as differences in the investigated case series. The present study investigates these subcortical regions in both hemispheres of the same post-mortem brains for volume, neuron density and total neuron number with high-precision design-based stereology. The analysed case series consisted of 13 post-mortem brains from male schizophrenic patients [age range: 22–64 years; mean age 51.5 ± 3.3 years (mean ± SEM)] and 13 age-matched male controls (age range: 25–65 years; mean age 51.9 ± 3.1 years). A general linear model multivariate analysis of variance with diagnosis and hemisphere as fixed factors and illness duration (schizophrenic patients) or age (controls), post-mortem interval and fixation time as covariates showed a number of statistically significant alterations in the brains from schizophrenic patients compared with the controls. There was a reduced mean volume of the putamen [−5.0% on the left side (l) and −4.1% on the right side (r)] and the LNA (l: −12.1%, r: −17.6%), and a reduced mean total neuron number in the CN (l: −10.4%, r: −10.2%), putamen (l: −8.1%, r: −11.6%) and the LNA (l: −15.9%, r: −16.2%). These data show a previously unreported, distinct pattern of alterations in mean total neuron numbers in identified subcortical brain regions in a carefully selected sample of brains from schizophrenic patients. The rigorous quantitative analysis of several regions in brains from schizophrenic patients and matched controls is crucial to provide reliable information on the neuropathology of schizophrenia as well as insights about its pathogenesis.

Keywords: amygdala; design-based stereology; schizophrenia; striatum; thalamus

Abbreviations: ABNA = accessory basal nuclei of the amygdala; BNA = basal nuclei of the amygdala; CGM = cortical grey matter; CN = caudate nucleus; LNA = lateral nucleus of the amygdala; MDNT = mediodorsal nucleus of the thalamus; NA = nucleus accumbens


Introduction

Schizophrenia is a devastating neuropsychiatric disorder that presents with a variety of cognitive disturbances in attention, working memory, verbal production and response monitoring and inhibition. Equally diverse are the putative mechanisms which translate deficits into schizophrenic pathology, and the anatomical substrates of...
both the deficits and the pathology (Andreasen et al., 1999).
Modern imaging techniques have indicated various discrete morphological alterations in the brain from schizophrenic patients. Based on meta-analyses, there is now a strong body of evidence in favour of an enlargement of the lateral ventricles (Harrison, 1999; Wright et al., 2000; Shenton et al., 2001; Kasai et al., 2002), as well as of a reduction of the total volume of the cerebral cortex (MDNT (Pakkenberg, 1990; Popken et al., 1997), the mediodorsal nucleus of the thalamus (Bogerts, 1984; Beckmann and Lauer, 1997; Lauer and Beckmann, 1997), the mediodorsal nucleus of the thalamus (MDNT) (Pakkenberg, 1990; Popken et al., 2000; Young et al., 2000; Byne et al., 2002; Cullen et al., 2003; Dorph-Petersen et al., 2004; Danos et al., 2005) and the amygdala (Bogerts, 1984). However, many of the reported data are divergent if not mutually contradictory and no clear pattern of histological alterations has yet been established in schizophrenia. Such discrepancies can be explained by methodological differences, investigated parameters and particularly the available samples of brains from schizophrenic patients and controls (Harrison, 1999).

Post-mortem studies have revealed subtle neuropathological abnormalities in several cortical regions in schizophrenia such as the prefrontal, entorhinal and anterior cingulate cortices (for review see Harrison, 1999; Taminga et al., 2000; Selemon, 2001), as well as in subcortical regions such as the caudate nucleus (CN), putamen and nucleus accumbens (NA) (Bogerts, 1984; Beckmann and Lauer, 1997; Lauer and Beckmann, 1997), the mediodorsal nucleus of the thalamus (MDNT) (Pakkenberg, 1990; Popken et al., 2000; Young et al., 2000; Byne et al., 2002; Cullen et al., 2003; Dorph-Petersen et al., 2004; Danos et al., 2005) and the amygdala (Bogerts, 1984). However, many of the reported data are divergent if not mutually contradictory and no clear pattern of histological alterations has yet been established in schizophrenia. Such discrepancies can be explained by methodological differences, investigated parameters and particularly the available samples of brains from schizophrenic patients and controls (Harrison, 1999).

A possible solution to this situation is the analysis of several regions in brains from schizophrenic patients and matched controls with state-of-the-art quantitative histological techniques. We have used this approach recently in investigations of mean cell spacing abnormalities in the neocortex (Casanova et al., 2005) and capillary length densities in the frontal cortex (Kreczmanski et al., 2005) in a carefully selected sample of post-mortem brains from male schizophrenic patients and age/gender-matched controls. In the present study, we have continued the analysis of these brains by investigating five subcortical regions [CN, putamen, NA, MDNT and lateral nucleus of the amygdala (LNA)] with a high-precision design-based stereology approach for possible alterations in volumes, neuron densities and total neuron numbers. The analyses were performed under the hypothesis that a distinct pattern of histological alterations would emerge from these subcortical areas known to be involved in the neuropathology of schizophrenia.

Material and methods

Brain specimens

This study was performed on the same post-mortem brains (both hemispheres) from 13 male schizophrenic patients [mean age 51.9 ± 3.3 years; mean post-mortem interval (time between death and autopsy) 27.5 ± 6.0 h; mean fixation time 912 ± 372 days; data given as mean ± SEM] and from 13 age-matched male controls (mean age 51.9 ± 3.1 years; mean post-mortem interval 23.7 ± 3.8 h; mean fixation time 247 ± 53 days) that were investigated in our previous studies for mean cell spacing abnormalities in the neocortex (Casanova et al., 2005) and for capillary length densities in the frontal cortex (Kreczmanski et al., 2005). The age of the patients, illness duration, clinical diagnoses, causes of death, the post-mortem interval and the fixation time are summarized in Table 1. The schizophrenic patients did not differ from the controls with respect to mean age (Student’s two-tailed t-test; \( P = 0.946 \)), mean post-mortem interval (\( P = 0.581 \)) and mean fixation time (\( P = 0.089 \)). All schizophrenic cases had been patients either in German university hospitals or in German State psychiatric hospitals (six hospitals including one university hospital in which some control patients were also treated). Records from autopsy (including a summary of the medical history) were available for all schizophrenic cases and all controls. All pathologists involved in the autopsies were instructed by H.H. and adhered to identical handling and processing conditions of the brains. All schizophrenic patients met the Diagnostic Statistical Manual, 4th revision (DSM-IV) and International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10) diagnostic criteria. The clinical notes were assessed by two experienced clinical psychiatrists to ensure that the brains from the controls were free from psychopathology, and for clear evidence that the diagnosis of schizophrenic patients was concordant with DSM-IV criteria for schizophrenia. The mean age at onset was 22.9 ± 1.5 years. Exclusion criteria for both schizophrenic patients and controls comprised neurological problems that required intervention or interfered with cognitive assessment (e.g. stroke with aphasia), history of recurrent seizure disorder, history of severe head injury with loss of consciousness, diabetes mellitus with free plasma glucose >200 mg/dl and history of self-administered intoxication. Schizophrenic patients and controls were similar in terms of the ethnic backgrounds. However, they were not fully matched for socioeconomic status and education, which would have placed severe constraints on our sample. Moreover, all schizophrenic patients were subjected to long-term treatment with typical neuroleptics (because of the fact that most of the patients were not hospitalized throughout the duration of their illness, and, therefore, the clinical records did not cover fully the entire medication histories, it was not possible to calculate lifetime medication exposures). In all of the cases, autopsy was performed after consent was obtained from a relative according to the laws of the Federal Republic of Germany. The use of these autopsy cases for scientific investigations as outlined here has been approved by the relevant institutional review boards. Brains were fixed by immersion in 10% formalin (one part commercial 40% aqueous formaldehyde in nine parts H2O) prior to histological processing.

Tissue processing

The brainstem with the cerebellum was separated from the forebrain at the level of the rostral pons, and the hemispheres were divided medially. Then, both hemispheres were cut into serial 600–700 μm-thick coronal sections as previously described (Heinsen and Heinsen, 1991). Briefly, the hemispheres...
of the other cases and were thus excluded from the calculation of the mean post-mortem intervals. b The volume of the CGM of autopsy. Accordingly, the post-mortem intervals between death and autopsy of these patients cannot be compared with the corresponding conditions for all brains (except for brain C7 that was embedded in celloidin instead of gelatin).

Investigated brain regions

The CN, putamen, NA, MDNT and LNA were investigated for their volume, total number of neurons and neuron density. Delineations of these regions were performed according to established criteria in the literature (CN, putamen and NA: Brockhaus, 1942; Lauer and Heinsen, 1996; Holt et al., 1999; Lauer et al., 2001; MDNT: Dewulf, 1971; Hirai and Jones, 1989; Jones, 1997; Heinsen et al., 1999; Popken et al., 2000; Dorph-Petersen et al., 2004; LNA: Braak and Braak, 1983; Sims and Williams, 1990; Sorvari et al., 1996; Schumann and Amaral, 2005). In the case of the MDNT, the magnocellular, parvocellular and densocellular regions as well as the caudodorsal subdivision were included in the delineations, as also done by Popken et al. (2000), Byne et al. (2002) and Dorph-Petersen et al. (2004) (Fig. 1).

In addition, the volumes of the cortical grey matter (CGM), the basal and accessory basal nuclei of the amygdala (BNA and ABNA) were determined. Delineations of the BNA and ABNA also followed the criteria established by Braak and Braak (1983), Sims and Williams (1990), Sorvari et al. (1996) and Schumann and Amaral (2005).
Fig. 1 Photomicrographs of 600 μm-thick frontal sections of the right hemisphere from the control C1 (60 years old; A–I) and from the schizophrenic patient S13 (64 years old; K–S), showing the striatum (A, D, G, K, N, Q), the amygdala (B, E, H, L, O, R) and the thalamus (C, F, I, M, P, S). In D–F and N–P, respectively, the borders of the CN, putamen (P), NA (D, N), ABNA, BNA and LNA (E, O), and MDNT (F, P) are indicated. The high-power photomicrographs showing neurons in the NA (G, K), LNA (H, L) and MDNT (I, M) are representative of the magnification at which the stereological estimates were obtained. Scale bar = 23 mm in A–C and Q–S, 10 mm in D–F and N–P, and 50 μm in G–M.
The boundaries of the investigated brain regions were identified on all sections showing the corresponding region using an Olympus SZX9 stereo microscope (Olympus; Tokyo, Japan) and were marked on the back side of the glass slides with a felt-tip pen. Identification and delineation of boundaries was performed by H.H. (CGM, CN, putamen), V.M. (NA), T.M. (MDNT) and F.W. (LNA, BNA and ABNA). Cross-validation (and, if necessary, slight modifications of the delineations) was performed by C.S. (CGM, CN, putamen) and C.S., H.H. and P.R.H. (all other brain regions).

**Stereological analyses**

Stereological analyses were performed with a stereology workstation, consisting of a modified light microscope [Olympus BX50 with PlanApo objective 1.25× [numerical aperture (N.A.) = 0.04]] and UPlanApo objectives 10× (N.A. = 0.4), 20× (oil; N.A. = 0.8) and 40× (oil; N.A. = 1.0); Olympus, Tokyo, Japan], motorized specimen stage for automatic sampling (Ludl Electronics; Hawthorne, NY, USA) CCD colour video camera (HV-C200AMP; Hitachi, Tokyo, Japan) and stereology software (Stereoinvestigator; MicroBrightField, Williston, VT, USA).

Volumes of brain regions were analysed using Cavalieri’s principle [Cavaliere, 1635; see also Fig. 2 in Schmitz and Hof (2005)], by determining the projection area of a given brain region on each section showing this region, summing the data from all sections, and multiplying this value with the interval of selecting sections for staining with galloycinin (two or three; see above) and the average actual section thickness after tissue processing [determined with the calibrated fine adjustment knob of an Olympus BH microscope and a Planapo objective (40×; N.A. = 1.0) as described in Heinzen et al. (1994)]. The projection areas of the CGM were determined with point counting [Gundersen and Jensen, 1987; see also Fig. 1 in Schmitz and Hof (2005)] as already described in Kreczmanski et al. (2005).

In contrast, the projection areas of the subcortical regions were determined by tracing their boundaries on each section on video images displayed on the monitor of the stereology workstation.

Total neuron numbers were evaluated with the optical fractionator [West et al., 1991; see also Figs 1 and 4 in Schmitz and Hof (2005)]. All neurons whose nucleus top came into focus within unbiased virtual counting spaces distributed in a systematic-random fashion throughout the delineated regions were counted. Then, total neuron numbers were calculated from the numbers of counted neurons and the corresponding sampling probability. Details of the counting procedure for all investigated brain regions are summarized in Table 2.

In addition, the nearest neighbour distance distribution functions were determined for the neurons in the lateral nucleus of the right amygdala with the nearest neighbour method of the Stereoinvestigator software. For each neuron in the lateral nucleus of the right amygdala that was counted with the unbiased virtual counting spaces, the neuron positioned closest to the counted neuron was detected [as explained in Schmitz et al. (2002)]. Then, the distance between these neurons was calculated in three dimensions. From these individual nearest neighbour distances, the nearest neighbour distance distribution function in the lateral nucleus of the right amygdala was calculated for each investigated brain.

Finally, neuron densities were calculated individually for each subcortical region as the ratio of the total neuron number in and the volume of this region.

**Statistical analysis**

For both schizophrenic patients and controls, mean and standard error of the mean were calculated for all investigated variables (except for the nearest neighbour distance distribution functions), separately for the left and the right hemispheres. Comparisons between schizophrenic patients and controls were performed using generalized linear model multivariate analysis (MANOVA), with diagnosis and hemisphere as fixed factors and the following variables as covariates: (i) the adjusted illness duration of the schizophrenic patients (calculated as individual age at death minus age at onset plus the mean age at onset of all schizophrenic patients) or the age of the controls, respectively, (ii) the post-mortem interval and (iii) the fixation time [note that use of the actual individual illness duration of the schizophrenic patients instead of the adjusted ones as covariate would have caused invalid results of the MANOVA model because there was no illness duration of the controls, and the mean illness duration of the schizophrenic patients was significantly different from the mean age of the controls (Student’s two-tailed t-test; P < 0.001) whereas the mean adjusted illness duration was not (P = 0.974)]. For each investigated variable, all investigated brain regions were tested simultaneously. Post hoc tests in the analyses of covariance were performed with linear regression analysis. In all analyses an effect was considered statistically significant if its associated P-value was <0.05. Calculations were performed using SPSS (Version 12.0.1 for Windows, SPSS, Chicago, IL, USA).

The nearest neighbour distance distribution functions of the neurons in the lateral nucleus of the right amygdala from the schizophrenic patients and the controls were graphically analysed with empirical distribution function (EDF) plots as explained in detail in Schmitz et al. (2002). The computer simulations necessary to perform these EDF plot analyses were carried out with Microsoft Visual Basic (version 6.0; Microsoft, Redmond, WA, USA); graphical analysis was done with GraphPad Prism (version 4.00 for Windows; GraphPad Software, San Diego, CA, USA).

**Photography**

Photomicrographs shown in Fig. 1A–F and N–S were produced by digital photography using the stereology workstation described above. On average, ~120 images were captured for the composite in each Fig. 1A–C and Q–S, and 16 images for the composite in each Fig. 1D–F and N–P. These images were made into one montage using the Virtual Slice module of the Stereoinvestigator software. Photomicrographs shown in Fig. 1G–M were produced by digital photography using an Olympus DP 70 digital camera attached to an Olympus AX 70 microscope and cell software (version 2.3; Soft Imaging System, Münster, Germany). The final figure was constructed using Corel Photo-Paint v.11 and Corel Draw v.11 (Corel, Ottawa, Canada). Only minor adjustments of contrast and brightness were made, without altering the appearance of the original materials.
Fig. 2 Volumes of CGM (A, I, R), CN (B, K, S), putamen (P; C, L, T), NA (D, M, U), MDNT (E, N, V), BNA (F, O, W), ABNA (G, P, X) and LNA (H, Q, Z) in both hemispheres of the brains from 13 male schizophrenic patients (S; open bars in A–H, and dots in R–Z) and 13 age-matched controls [C; closed bars in A–H, and dots in I–Q; squares in case of the results obtained for the single brain embedded in celloidin (C7)]. In A–H, data are shown as mean and standard error of the mean for the left (l) and right (r) hemispheres from schizophrenic patients (S–l and S–r) and controls (C–l and C–r). In I–Z, individual data for the left hemispheres (closed dots or squares, respectively) and right hemispheres (open dots or squares, respectively) from controls (I–Q) and schizophrenic patients (R–Z) are shown as a function of the control patients’ age (or the illness duration of the schizophrenic patients, respectively). *P < 0.05 for the fixed factor Diagnosis in general linear model MANOVA.
Three-dimensional reconstructions

Reconstructions of LNA, BNA and ABNA in the right hemispheres were performed by digitally photographing close-up views of serial sections of the temporal lobe enclosing the amygdaloïd complex with a digital single lens reflex camera (Fuji FinePix S2 Pro; Fuji Photo Film Co., Tokyo, Japan) and a 50 mm macro objective (1:2.8; Sigma, Kanagawa, Japan). Then the stacked photos were imported into Amira software (version 3.1; Mercury Computer Systems; San Diego, CA, USA). The serial sections were aligned manually in the editor window of Amira with the contours of the parahippocampal gyrus, fusiform gyrus, and the surface of the anterior perforated substance serving as reference structures in the alignment of the individual sections. This coarse alignment was supplemented by a computer-assisted fine-tuned automatic alignment. Afterwards, the outlines of the LNA, BNA and ABNA were identified and manually traced in the image segmentation editor of Amira. With these outlines and the average section thickness, the software generated surface views of the investigated nuclei of the amygdala. Irregular contours were smoothed by additional editors.

Table 2 Details of the stereological counting procedures

<table>
<thead>
<tr>
<th></th>
<th>CN</th>
<th>P</th>
<th>NA</th>
<th>MDNT</th>
<th>LNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obj.</td>
<td>40x</td>
<td>40x</td>
<td>40x</td>
<td>20x</td>
<td>40x</td>
</tr>
<tr>
<td>B (μm²)</td>
<td>10000</td>
<td>10000</td>
<td>3600</td>
<td>15298</td>
<td>3600</td>
</tr>
<tr>
<td>H (μm)</td>
<td>30</td>
<td>30</td>
<td>20</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>D (μm)</td>
<td>3000</td>
<td>3000</td>
<td>600</td>
<td>800</td>
<td>600</td>
</tr>
<tr>
<td>Σ OD</td>
<td>171</td>
<td>176</td>
<td>461</td>
<td>626</td>
<td>767</td>
</tr>
<tr>
<td>Σ Q⁻¹</td>
<td>755</td>
<td>761</td>
<td>499</td>
<td>1071</td>
<td>1110</td>
</tr>
<tr>
<td>CEₚₑₑₚ(n)</td>
<td>0.036</td>
<td>0.036</td>
<td>0.048</td>
<td>0.031</td>
<td>0.031</td>
</tr>
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</table>

Table 3 Results of statistical analysis (P-values) with generalized linear model MANOVA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Brain region</th>
<th>A/ID</th>
<th>PMI</th>
<th>Fix</th>
<th>D</th>
<th>H</th>
<th>D × H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>CGM</td>
<td>0.129</td>
<td>0.609</td>
<td>0.023</td>
<td>0.008</td>
<td>0.392</td>
<td>0.601</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>0.143</td>
<td>0.973</td>
<td>0.961</td>
<td>0.429</td>
<td>0.436</td>
<td>0.860</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.082</td>
<td>0.004</td>
<td>0.169</td>
<td>0.020</td>
<td>0.782</td>
<td>0.929</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>0.600</td>
<td>0.013</td>
<td>0.572</td>
<td>0.299</td>
<td>0.394</td>
<td>0.842</td>
</tr>
<tr>
<td></td>
<td>MDNT</td>
<td>0.581</td>
<td>0.025</td>
<td>0.574</td>
<td>0.161</td>
<td>0.225</td>
<td>0.546</td>
</tr>
<tr>
<td></td>
<td>BNA</td>
<td>0.353</td>
<td>0.851</td>
<td>0.422</td>
<td>0.042</td>
<td>0.404</td>
<td>0.926</td>
</tr>
<tr>
<td></td>
<td>ABNA</td>
<td>0.515</td>
<td>0.463</td>
<td>0.416</td>
<td>0.858</td>
<td>0.053</td>
<td>0.497</td>
</tr>
<tr>
<td></td>
<td>LNA</td>
<td>0.900</td>
<td>0.628</td>
<td>0.462</td>
<td>0.006</td>
<td>0.988</td>
<td>0.520</td>
</tr>
<tr>
<td>Neuron density</td>
<td>CN</td>
<td>0.156</td>
<td>0.617</td>
<td>0.727</td>
<td>0.866</td>
<td>0.627</td>
<td>0.676</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.929</td>
<td>0.893</td>
<td>0.834</td>
<td>0.393</td>
<td>0.897</td>
<td>0.559</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>0.554</td>
<td>0.204</td>
<td>0.118</td>
<td>0.735</td>
<td>0.209</td>
<td>0.955</td>
</tr>
<tr>
<td></td>
<td>MDNT</td>
<td>0.424</td>
<td>0.188</td>
<td>0.800</td>
<td>0.210</td>
<td>0.781</td>
<td>0.978</td>
</tr>
<tr>
<td></td>
<td>LNA</td>
<td>0.126</td>
<td>0.892</td>
<td>0.507</td>
<td>0.786</td>
<td>0.835</td>
<td>0.389</td>
</tr>
<tr>
<td>Total neuron number</td>
<td>CN</td>
<td>0.047</td>
<td>0.861</td>
<td>0.297</td>
<td>0.011</td>
<td>0.764</td>
<td>0.932</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.062</td>
<td>0.012</td>
<td>0.129</td>
<td>0.005</td>
<td>0.792</td>
<td>0.707</td>
</tr>
<tr>
<td></td>
<td>NA</td>
<td>0.046</td>
<td>0.856</td>
<td>0.089</td>
<td>0.200</td>
<td>0.893</td>
<td>0.933</td>
</tr>
<tr>
<td></td>
<td>MDNT</td>
<td>0.818</td>
<td>0.555</td>
<td>0.975</td>
<td>0.419</td>
<td>0.529</td>
<td>0.698</td>
</tr>
<tr>
<td></td>
<td>LNA</td>
<td>0.170</td>
<td>0.608</td>
<td>0.898</td>
<td>0.015</td>
<td>0.591</td>
<td>0.926</td>
</tr>
</tbody>
</table>

The alignment of the individual sections. This coarse alignment was supplemented by a computer-assisted fine-tuned automatic alignment. Afterwards, the outlines of the LNA, BNA and ABNA were identified and manually traced in the image segmentation editor of Amira. With these outlines and the average section thickness, the software generated surface views of the investigated nuclei of the amygdala. Irregular contours were smoothed by additional editors.
Neuron number in schizophrenia

Rostral-caudal view Caudal-rostral view Dorsal-ventral view

C11 S13

Fig. 3 Reconstructions of the LNA (green), BNA (red) and ABNA (magenta) in the right hemisphere from the control C11 (60 years old) and from the schizophrenic patient S13 (64 years old) in rostral-caudal view (left column), caudal-rostral view (middle column) and dorsal-ventral view (right column). Compared with the volume of the LNA from C11 (set to 100%), the relative volume of this nucleus was 71.2% in S13. For the BNA, the corresponding values were 76.9% (S13/C4) and for the ABNA, 62.8%. Scale bar = 1 cm.

With respect to the mean neuron density in the investigated subcortical regions, there were no significant differences between the schizophrenic patients and the controls (Fig. 4). In contrast, the schizophrenic patients had a significantly reduced mean total neuron number in the CN \[l: -10.4\%, r: -10.2\%; F(1) = 7.088, \ P = 0.011\], the putamen \[l: -8.1\%, r: -11.6\%; F(1) = 8.733, \ P = 0.005\] and the LNA \[l: -15.9\%, r: -16.2\%; F(1) = 6.498, \ P = 0.015\] compared with controls (Fig. 5).

Furthermore, no significant differences between the left and the right hemispheres were found for the investigated variables in both schizophrenic and control cases \(P > 0.05\) for the fixed factor Hemisphere in all MANOVAs performed.

The post-mortem interval had a significant effect on the volume of the putamen \([F(1) = 9.553, \ P = 0.004]\) and the total neuron number in this brain region \([F(1) = 6.899; \ P = 0.012]\) (see Fig. S1 in the Supplementary online material). A significant effect was also found for the control patients’ age (or the illness duration of the schizophrenic patients, respectively) on the total neuron number in the CN \([F(1) = 4.197, \ P = 0.047]\) and the NA \([F(1) = 4.224, \ P = 0.046]\). However, post hoc linear regression analysis revealed only a significant, positive correlation between the illness duration and the total neuron number in the left CN in the brains from the schizophrenic cases \([r^2 = 0.213, F(1,10) = 2.704, \ P = 0.131]\). It can be therefore concluded that the alterations in mean volumes and mean total neuron numbers found in the investigated subcortical regions in the brains from the schizophrenic patients were not caused by the control patients’ age (or the illness duration of the schizophrenic patients, respectively), the post-mortem interval or the fixation time of the brains.

The nearest neighbour distance distribution function analysis of neurons in the lateral nucleus of the right amygdala showed no distinct differences between the schizophrenic patients and the controls (see Fig. S2 in the Supplementary online material).

Finally it should be mentioned that the results obtained for the single brain embedded in celloidin (C7) showed no systematic deviation from the results obtained for the other brains (Figs 2, 4 and 5).

Discussion

The present study revealed a distinct pattern of subtle neuropathological alterations in a carefully selected sample of brains from schizophrenic patients. This previously unrecognized set of alterations comprises reduced volumes of the total CGM, putamen and LNA as well as reduced total neuron numbers in the CN, putamen and LNA (note that the analysis of neuron densities showed no differences between schizophrenic patients and controls). Reports in the literature of reduced volumes of the NA and MDNT as well as reduced total neuron numbers in these subcortical regions in schizophrenia could not be confirmed. Furthermore, no correlation between the illness duration of the schizophrenic patients and the observed neuropathological alterations was found. The results from our control cases are in line with previous studies (summarized in Tables 4 and 5). The results obtained for the schizophrenic patients can be compared with data in the literature as discussed below (see also Tables 4 and 5).

In addition, a significant decrease in the total CGM volume \(9.3\%; \text{averaged for the left and right hemispheres}) was found when comparing the schizophrenic patients with the controls, in agreement with several reports in the literature (e.g. Zipurski et al., 1992, 1997; McCarley et al., 1999; Shenton et al., 2001).

Clinical neuroimaging studies have shown an enlargement of the striatum in relation to treatment with typical neuroleptics (Chakos et al., 1994; Shenton et al., 2001; Lang et al., 2004). This is in contrast to the results of a post-mortem study by Beckmann and Lauer (1997) who reported no differences in mean volumes of the CN and the putamen when comparing schizophrenic patients treated with typical neuroleptics to controls (\(-0.3\%\) and \(+1.9\%, \text{respectively}\); \(P > 0.05\)) as well as to a report by Bogerts (1984) who found a trend towards reduced mean volumes of the CN \(\text{\(-4.5\%\); \(P > 0.05\)}\) and the putamen \(\text{\(-4.1\%\);\)
when comparing schizophrenic neuroleptic-naïve patients with controls. Notably, several clinical neuroimaging studies reported a decrease in volume of the striatum in drug-naïve schizophrenic patients [reviewed in Shenton et al. (2001)], as well as reduced basal ganglia volumes after switching to olanzapine in schizophrenic patients chronically treated with typical neuroleptics (Chakos et al., 1994; Frazier et al., 1996; Lang et al., 2004). Accordingly, increased volume of the striatum in schizophrenia as found in certain clinical neuroimaging studies might reflect a specific, currently unknown action of typical neuroleptics that is reversible by switching to atypical neuroleptics or during histological processing.

In a post-mortem study, Beckmann and Lauer (1997) compared nine schizophrenic patients with nine matched controls, and found an increased total neuron number in the CN [P<0.05 only for the right hemisphere (difference: +17.5%) but not for the left hemisphere (difference: +15.8%)] and a trend towards an increased total neuron number in the putamen (+9.8%; P>0.05) in the brains of the schizophrenic patients. This is in contrast to the finding of reduced total neuron numbers in the CN (−10.2%) and putamen (−9.9%) in the brain of the schizophrenic patients in the present study. The reason for this discrepancy is unknown and might be related to differences in the stereological analysis [the number of

Fig. 4 Neuron density in CN (A, F, L), putamen (P; B, G, M), NA (C, H, N), MDNT (D, I, O) and LNA (E, K, P) in both hemispheres of the brains from 13 male schizophrenic patients (S; open bars in A–E, and dots in L–P) and 13 age-matched controls (C; closed bars in A–E, and dots in F–K; squares in case of the results obtained for the single brain embedded in celloidin (C7)]. In A–E, data are shown as mean and standard error of the mean for the left (l) and right (r) hemispheres from schizophrenic patients (S-l and S-r) and controls (C-l and C-r). In F–P, individual data for the left hemispheres (closed dots or squares, respectively) and right hemispheres (open dots or squares, respectively) from controls (F–K) and schizophrenic patients (L–P) are shown as function of the control patients age (or the illness duration of the schizophrenic patients, respectively).
unbiased virtual counting spaces used to estimate total neuron numbers was considerably lower in the study of Beckmann and Lauer (1997) than in the present study, implying a considerably higher variation in estimated total neuron numbers (Schmitz, 1998; Schmitz and Hof, 2000, 2005)].

Pakkenberg’s (1990) early report of a reduction in the mean volume of the NA in schizophrenia by 42% (and in
Table 4: Reports in the literature of estimated mean volumes of and estimated total neuron numbers in four subcortical regions in the brains of controls and schizophrenic patients (averaged for left and right hemispheres)

<table>
<thead>
<tr>
<th>Source</th>
<th>CN</th>
<th>P</th>
<th>NA</th>
<th>LNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume data for control cases (all values in mm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>2212</td>
<td>2713</td>
<td>204</td>
<td>414</td>
</tr>
<tr>
<td>1</td>
<td>2043</td>
<td>2681</td>
<td>522</td>
<td>...</td>
</tr>
<tr>
<td>2</td>
<td>2565</td>
<td>2979</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>3</td>
<td>...</td>
<td>...</td>
<td>i47</td>
<td>...</td>
</tr>
<tr>
<td>4</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>5</td>
<td>...</td>
<td>...</td>
<td>452</td>
<td>...</td>
</tr>
<tr>
<td>Volume data for schizophrenic patients (all values in millions)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>1</td>
<td>-9.1% (ns)</td>
<td>-4.6%</td>
<td>-8.8% (ns)</td>
<td>-15.0% (ns)</td>
</tr>
<tr>
<td>2</td>
<td>-4.5% (ns)</td>
<td>-4.1% (ns)</td>
<td>+1.2% (ns)</td>
<td>...</td>
</tr>
<tr>
<td>3</td>
<td>...</td>
<td>+10.5% (ns)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>4</td>
<td>...</td>
<td>...</td>
<td>-42.0% (ns)</td>
<td>...</td>
</tr>
<tr>
<td>Total neuron number data for control cases (all values in millions)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>6</td>
<td>32.7</td>
<td>35.4</td>
<td>2.43</td>
<td>4.43</td>
</tr>
<tr>
<td>4</td>
<td>28.2</td>
<td>...</td>
<td>3.00</td>
<td>...</td>
</tr>
<tr>
<td>5</td>
<td>...</td>
<td>...</td>
<td>2.93</td>
<td>4.00</td>
</tr>
<tr>
<td>Total neuron number data for schizophrenic patients (all values in millions)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>6</td>
<td>-10.2% (ns)</td>
<td>-99%</td>
<td>-2.1% (ns)</td>
<td>-16.0% (ns)</td>
</tr>
<tr>
<td>...</td>
<td>+16.6% (ns)</td>
<td>+98% (ns)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>4</td>
<td>...</td>
<td>...</td>
<td>-49.5% (ns)</td>
<td>...</td>
</tr>
</tbody>
</table>

CN = caudate nucleus; P = putamen; NA = nucleus accumbens; LNA = lateral nucleus of the amygdala. 1 = Bogerts (1984); 2 = Lauer and Beckmann (1997); 3 = Lauer et al. (2001); 4 = Pakkenberg et al. (1990); 5 = Schumann and Amaral (2006); 6 = Beckmann and Lauer (1997). *Including the NA; **Considering an average shrinkage factor of 0.53 reported in this study; ***Considering an average shrinkage factor of 0.53 reported in this study; ****Considering an average shrinkage factor of 0.47 reported in this study; *****Including the NA. **P < 0.05 only for the right hemisphere (difference: +17.5%) but not for the left hemisphere (difference: +15.8%), ns = not significant (i.e. P > 0.05). **P < 0.05.

The mean total neuron number in this nucleus by ~50%) should be taken cautiously, as was already discussed by Lauer et al. (2001). Pakkenberg’s (1990) data are also in contrast to Bogert’s (1984) finding of almost no difference in mean volumes of the NA between brains from 14 schizophrenic patients and 13 controls (+1.2%; P > 0.05). The discrepancy between the results of Pakkenberg (1990), those of Bogerts (1984), Lauer et al. (2001) and the present study might be due to differences in stereological design.

The MDNT has been the focus of several studies applying design-based stereological techniques over the last 20 years (Table 5). Pakkenberg (1990), Popken et al. (2000) and Byne et al. (2002) reported a reduced mean volume and a reduced mean total neuron number in the MDNT in the brains of schizophrenic patients compared with controls (studies with positive outcome; PO studies), whereas Cullen et al. (2003), Kemether et al. (2003), Dorph-Petersen et al. (2004), Danos et al. (2005) and the present study could not confirm these findings (studies with negative outcome; NO studies). Differences in study design may again explain this discrepancy (details are provided in Table 5). First, the PO studies had on average smaller sample sizes than the NO studies (PO studies: 9.3 schizophrenic patients versus 7.7 controls; NO studies: 21.2 versus 25.4); second, except in the study by Dorph-Petersen et al. (2004), in all NO studies, both left and right MDNTs were investigated, whereas in the PO studies, only either the left or the right MDNTs were analysed, and third, the average age of the schizophrenic patients and controls was higher in the PO studies than in the NO studies (PO studies: 65 years (schizophrenic patients) versus 65 years (controls); NO studies: 49 versus 51 years). However, small sample sizes and additional, confounding pathologies (in this case possible age-related neurodegeneration) have been identified as major potential shortcomings in most studies addressing the neuropathology of schizophrenia (Harrison, 1999).

Meta-analyses of clinical neuroimaging studies found reductions in the mean volume of the amygdala in schizophrenic patients compared with controls in the range of 5–10% (Lawrie and Abukmeil, 1998; Nelson et al., 1998; Wright et al., 2000; see also Honea et al., 2005). Although these clinical neuroimaging studies did not differentiate between the nuclei of the amygdala, these data are in line with the results of the present study as well as with results from a post-mortem study by Bogerts (1984) who found a significantly reduced volume of the amygdala in schizophrenic patients compared with controls (−22%). In contrast, Heckers et al. (1990) and Chance et al. (2002) did not observe volume reductions of the amygdala in schizophrenia in post-mortem studies; the reason for this discrepancy is unknown. Nevertheless, Chance et al. (2002) suggested that there may be alterations in other morphological aspects of the amygdala such as cellular composition, as found in the present study.

It should be mentioned that several reports have suggested schizophrenia being associated with a disturbance of cerebral asymmetry (for review see Crow, 1990, 1997; Petty, 1999). This did not apply to the findings of the present study in which no significant differences between the left and the right hemispheres were found for the schizophrenic patients and the controls. This is in agreement with other studies of subcortical pathology in brain regions in a three-dimensional impression during microscopic inspection.
schizophrenia. For instance, a meta-analysis of clinical neuroimaging studies, in which a volume of 100% in the comparison group was assumed, found for patients with schizophrenia an overall volume of 94% in the left and right amygdala (Wright et al., 2000). Disturbance of cerebral asymmetry in schizophrenia might therefore be restricted to discrete cortical regions such as the dorsolateral prefrontal cortex (Cullen et al., 2006).

What then, in this context, does the pattern of rather subtle neuropathological alterations reported in the present study contribute to our understanding of the pathogenesis of schizophrenia? Generally, two non-exclusive hypotheses for the development of schizophrenia have been proposed, a neurodevelopmental one (Lieberman, 1999; Tsuang, 2000; Lewis and Levitt, 2002; Murray et al., 2004) and a neurodegenerative one (Ashe et al., 2001; Sawa and Snyder, 2002; Church et al., 2002). A neurodevelopmental origin for schizophrenia has become the prevailing pathogenic hypothesis in recent decades (Weinberger, 1987; Lewis and Lewitt, 2002; Murray et al., 2004). Specifically, genetic and non-genetic mechanisms are thought to interact, in as yet not understood ways, to affect the developing brain, resulting in a predisposition to schizophrenia (see, e.g. Schmidt-Kastner et al., 2006). Although several genes have been proposed as candidates for schizophrenia (for review see Davis et al., 2003; Harrison and Owen, 2003; Owen et al., 2004; Harrison and Weinberger, 2005; Kirov et al., 2005), genetic defects may not account for all aspects of the pathogenesis of schizophrenia (Kendler, 2005). There is no direct evidence for a neurodevelopmental or a neurodegenerative origin of the results of the present study. Yet, considering that the reductions in both volumes of brain regions (CGM, putamen, LNA) and total neuron numbers (CN, putamen and LNA) did not correlate with illness duration, it is tempting to speculate that such negative correlation is indicative of a neurodevelopmental deficit. Particularly in respect to the alterations found in the LNA, it is noteworthy that early postnatal lesions of the amygdala in rats lead to a combination of behavioural changes which share features with symptoms of schizophrenia, whereas adult amygdala lesions do not produce such changes (Wolterink et al., 2004). Furthermore, tract-tracing studies have shown that connections between the basalateral amygdala and the prefrontal cortex (including the anterior cingulate cortex) mature slowly during postnatal development in rats (Bouwmeester et al., 2002; Cunningham et al., 2002). A slow maturation of connections may also be important for the primate brain, because lesions of mesial temporal limbic structures, including the amygdala, in postnatal monkeys (but not in mature monkeys) are associated with abnormal function of the prefrontal cortex of adult animals (Bertolino et al., 1997). Accordingly, alterations in the prolonged maturation of connections between the amygdala and the prefrontal cortex have been hypothesized to be involved during the onset of schizophrenia in adolescence (Benes, 2003). Reduced total neuron numbers in the LNA based on a neurodevelopmental deficit

### Table 5

Reports in the literature of estimated mean volumes and estimated total neuron numbers in the MDNT in the brains of controls and schizophrenic patients (averaged for left and right hemispheres)

<table>
<thead>
<tr>
<th>Source</th>
<th>V (mm³)</th>
<th>NN (×10⁶)</th>
<th>n (S/C)</th>
<th>H</th>
<th>Sex</th>
<th>Age (S/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data for controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>360</td>
<td>1.81</td>
<td>12 (C)</td>
<td>l</td>
<td>6/6</td>
<td>62 (C)</td>
</tr>
<tr>
<td>2</td>
<td>863</td>
<td>3.48</td>
<td>6 (C)</td>
<td>l</td>
<td>5/1</td>
<td>64 (C)</td>
</tr>
<tr>
<td>3</td>
<td>70i</td>
<td>3.17</td>
<td>5 (C)</td>
<td>r</td>
<td>4/1*</td>
<td>75 (C)</td>
</tr>
<tr>
<td>4</td>
<td>680</td>
<td>3.88</td>
<td>27 (C)</td>
<td>l+r</td>
<td>14/13</td>
<td>71 (C)</td>
</tr>
<tr>
<td>5</td>
<td>675i</td>
<td>60 (C)</td>
<td>l+r</td>
<td>45/15</td>
<td>40 (C)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>916</td>
<td>7.29</td>
<td>9 (C)</td>
<td>l</td>
<td>6/3</td>
<td>54 (C)</td>
</tr>
<tr>
<td>7</td>
<td>956</td>
<td>6.22</td>
<td>18 (C)</td>
<td>l+r</td>
<td>10/8</td>
<td>53 (C)</td>
</tr>
<tr>
<td>8</td>
<td>569</td>
<td>3.79</td>
<td>13 (C)</td>
<td>l</td>
<td>13/0</td>
<td>52 (C)</td>
</tr>
<tr>
<td>Data for schizophrenic patients (compared to controls)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-28.9%*</td>
<td>-40.3%*</td>
<td>12/12</td>
<td>l</td>
<td>8/4</td>
<td>6/6</td>
</tr>
<tr>
<td>2</td>
<td>-78%*</td>
<td>-270%*</td>
<td>6/6</td>
<td>l</td>
<td>5/1</td>
<td>5/1</td>
</tr>
<tr>
<td>3</td>
<td>-15.0%*</td>
<td>-29.7%*</td>
<td>10/5*</td>
<td>r</td>
<td>4/6*</td>
<td>4/1*</td>
</tr>
<tr>
<td>4</td>
<td>+4.7% (ns)</td>
<td>+5.2% (ns)</td>
<td>21/27</td>
<td>l+r</td>
<td>11/10</td>
<td>14/13</td>
</tr>
<tr>
<td>5</td>
<td>-6.7% (ns)</td>
<td>-</td>
<td>41/60</td>
<td>l+r</td>
<td>32/9</td>
<td>45/15</td>
</tr>
<tr>
<td>6</td>
<td>+2.1% (ns)</td>
<td>-6.5% (ns)</td>
<td>11/9</td>
<td>l</td>
<td>7/4</td>
<td>6/3</td>
</tr>
<tr>
<td>7</td>
<td>-6.9% (ns)</td>
<td>-6.8% (ns)</td>
<td>20/18</td>
<td>l+r</td>
<td>10/10</td>
<td>10/8</td>
</tr>
<tr>
<td>8</td>
<td>-8.8% (ns)</td>
<td>+4.6% (ns)</td>
<td>13/13</td>
<td>l+r</td>
<td>13/0</td>
<td>13/0</td>
</tr>
</tbody>
</table>

V = volume; NN = total neuron number; n = number of analysed brains; H = hemisphere; S = schizophrenic patients; C = controls; M = male; F = female; l = left; r = right. * = without the schizophrenic patients and controls suffering from Alzheimer’s disease analysed in this study; i = with the schizophrenic patients and controls suffering from Alzheimer’s disease. Data of the present study are given boldfaced.
could play an important role in these pathological processes and deserves further investigation.

Deficiencies in limbic functions which many researchers believe are the cause of the productive symptoms of schizophrenia such as paranoia and psychosis (Alexander et al., 1990; Bogerts, 1997; Weinberger, 1997) must have another neuropathological basis than reductions in the total neuron number in the NA [as suggested by Thune and Pakkenberg (2000)]. Likewise, involvement of MDNT in positive symptoms of schizophrenia [as discussed by, e.g. Andreasen et al. (1994), Friston (1999) and Stephan et al. (2006)] must have another neuropathological basis than reductions in the total neuron number in this nucleus [as suggested by, e.g. Thune and Pakkenberg (2000)]. Abnormal anatomical connections (i.e. 'miswiring' of association fibres), impairments in synaptic transmission and plasticity or complex combinations of both are the most relevant candidates in this regard (Stephan et al., 2006).

Furthermore, the hyperdopaminergic state of the striatum is one of the most important aspects of the pathophysiology and treatment of schizophrenia, but the mechanisms leading to increased dopamine levels in the basal ganglia are more complex than initially thought (Abi-Dargham et al., 2000; Carlsson et al., 2001; Winterer and Weinberger, 2004). The small decrease in neurons found in the present study could be due to a prolonged neurodevelopmental perturbation affecting several processes in the striatum, including neurogenesis and the innervation by dopaminergic mesencephalic fibres. A simple mismatch between neuronal numbers and dopaminergic innervation, however, is unlikely to explain long-lasting effects on neurotransmission, because glutamatergic cortical inputs and transmitters of striatal interneurons also come into play. Finally, the reduced number of neurons in LNA found in the present study could be involved in emotional disturbance in schizophrenia. According to Yaniv et al. (2004), LNA is a secondary interface limited to relatively simple, unimodal conditioned stimulus features, whereas BNA serves as an amygdalar sensory interface for complex, configural stimulus information. On the other hand, at least in rat, LNA is reciprocally connected with both BNA and ABNA (Savander et al., 1997). Accordingly, a reduced number of neurons in the LNA could result in impaired function of BNA in schizophrenia, with potential influence on at least two functional systems. First, the BNA interacts with striatal and cortical motor circuits, allowing active response to emotional arousing stimuli which is impaired in schizophrenia (Aleman and Kahn, 2005). Second, impaired output of the basolateral amygdala (including LNA and BNA) to the central nucleus of the amygdala (CNA) could disturb information flow from the CNA to brainstem areas involved in controlling specific involuntary components of emotional reaction (such as autonomic and endocrine responses) that are also disturbed in schizophrenia (Aleman and Kahn, 2005). Notably, the reduced number of neurons in the LNA could even be involved in alterations of the dopaminergic system in schizophrenia. This is due to the fact that all subdivisions of the amygdala project to the ventral striatum and therefore can indirectly influence dopaminergic neurons through amygdalo-striato-nigral loops (Haber and Fudge, 1997; Fudge et al., 2002; Fudge and Emiliano, 2003).

In summary, the results of the present study support a neurodevelopmental deficit in certain subcortical regions in schizophrenia, primarily affecting the development of the basolateral amygdala and the dorsal striatum. Further studies are required to address other neuropathological alterations in the prefrontal cortex and the dopaminergic system in schizophrenia and their possible interactions with disturbances of the development of the basolateral amygdala and dorsal striatum.

Supplementary material
Supplementary material is available at Brain Online.

Acknowledgements
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Neuron number in schizophrenia

Brain (2007), 130, 678–692

691

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Lang DJ, Kopala LC, Vandorpe RA, Rui Q, Smith GN, Goghari VM, et al. Reduced basal ganglia volumes after switching to olanzapine in...
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