Cytoarchitecture of the Entorhinal Cortex in Schizophrenia

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Objective: *The purpose of this study was to determine whether schizophrenia is associated with abnormalities in neuronal migration in the entorhinal cortex.* *Method:* *Nissl-stained sections through three cytoarchitectonic subdivisions of the entorhinal cortex were examined in postmortem brain specimens from 10 schizophrenic subjects and 10 matched normal comparison subjects.* *Results:* *No qualitative differences in cytoarchitecture were observed between the schizophrenic and comparison subjects.* *Conclusions:* *These findings do not replicate previous reports of cytoarchitectural disturbances in the entorhinal cortex of schizophrenic subjects and thus fail to support the hypothesis of abnormal neuronal migration in schizophrenia.*
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Structural imaging and postmortem studies have documented a decrease in the size of the medial temporal lobe in subjects with schizophrenia. Located within this region is the entorhinal cortex, a critical relay station between the hippocampus and cortical association regions. The entorhinal cortex has been considered to be an important site of pathology in schizophrenia because of, in part, reported abnormalities in the arrangement of neurons in the superficial layers of this region (1, 2). The apparent displacement of layer II neurons to deeper cortical layers in some schizophrenic subjects has been considered to be a consequence of abnormal neuronal migration, an interpretation that has had substantial influence on hypotheses of schizophrenia as a neurodevelopmental disorder.

However, recent studies have demonstrated that the normal cytoarchitecture of the human entorhinal cortex is quite complex and that the organization of neurons in each cortical layer differs substantially across subdivisions of the entorhinal cortex (3, 4). Because these differences may reflect the connectivity and functional role of each entorhinal cortex subdivision, it is important to determine which subdivisions exhibit abnormalities in schizophrenic subjects. Consequently, we examined three cytoarchitectonic subdivisions of the entorhinal cortex in brain specimens from matched groups of schizophrenic and normal comparison subjects.

METHOD

Postmortem brain specimens were obtained from the left temporal lobe of six male and four female schizophrenic subjects and from seven male and three female normal comparison subjects. All specimens were obtained through the Allegheny County Coroner's Office (5). For each subject, consensus DSM-III-R diagnoses were made by an independent panel of experienced clinicians who used information obtained from clinical records and structured interviews with surviving relatives, as previously described (5). Written informed consent was obtained from the next of kin. The mean age (49.9 years, SD=11.3) and postmortem interval (13.7 hours, SD=6.3) of the schizophrenic subjects did not differ from that of the comparison subjects (51.3 years [SD=13.6], and 11.4 hours [SD=5.1], respectively). The schizophrenic group consisted of the following subtypes: chronic paranoid (N=2), chronic undifferentiated (N=3), residual (N=1), schizoaffective (N=3), and not specified (N=1). Brain specimens from an additional 11 normal comparison subjects (mean age=45.1 years [SD=17.4], postmortem interval=5.3 hours [SD=2.4]) were also examined. The purpose of this group of subjects was to determine how consistently the cytoarchitectonic features of the different subdivisions of the entorhinal cortex were preserved across normal individuals. No neuropathological abnormalities were detected in any of the 31 subjects.

For each case, the left medial temporal lobe was blocked coronally, immersed in 4% paraformaldehyde in phosphate buffer for 48 hours, washed in sucrose solutions, and then sectioned (40 μm) on a cryostat. Every 10th section was mounted, stained for Nissl substance with thionin, and coded. Sections from each case were examined to determine the location and appearance of the cytoarchitectonic subdivisions of the human entorhinal cortex by using previously published criteria (3, 4, 6). Specifically, we sought to identify the olfactory,

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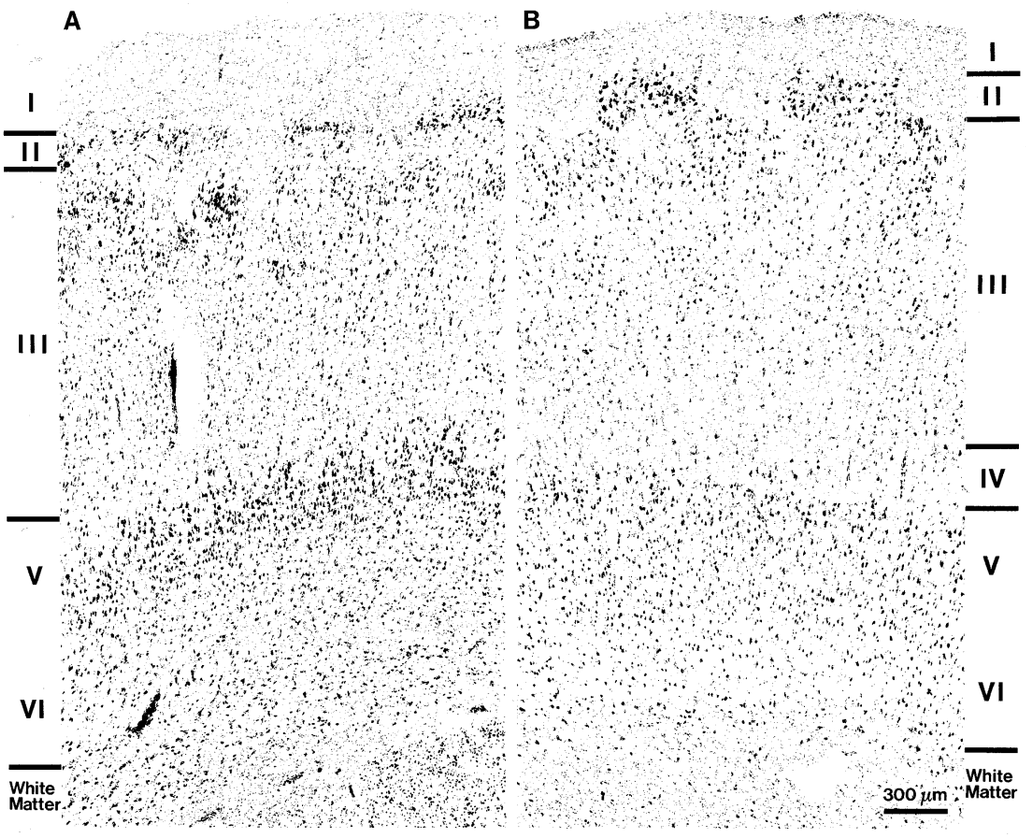
FIGURE 1. Nissl-Stained Sections Through Olfactory (A) and Rostral (B) Subdivisions of Human Entorhinal Cortex of a Normal Comparison Subject[a]



[a]Note the substantial differences in the cytoarchitectonic features of these two adjacent regions, particularly in layers II and III.

rostral, and intermediate subdivisions that together comprise the rostral half of the entorhinal cortex, the portion of the entorhinal cortex in which cytoarchitectural abnormalities have been observed in previous studies (1, 2). Tissue sections were examined by both investigators, and for the matched schizophrenic and comparison subjects, these analyses were done blind to diagnosis and case number. Particular attention was given to the presence of features such as "heterotopic" neuronal clusters and distortions in the shape of sulci, which have been previously identified as abnormal in schizophrenic subjects (1, 2, 7). In most cases, the observations of both investigators were identical. Any disagreements were resolved by joint examination of the tissue.

RESULTS

Examination of the additional 11 normal subjects confirmed the substantial regional heterogeneity in the cytoarchitecture of the human entorhinal cortex that has been described in previous studies (3, 4, 6). Although the cytoarchitectonic differences across subdivisions involved all cortical layers, the arrangement of neurons in layers II and III in particular differed across entorhinal cortex subdivisions in each subject. For example, in the olfactory subdivision, layer II neurons were small and typically arranged in a thin band. Located below this band were clumps of darkly stained neurons in the most superficial portion of layer III. The cell-sparse zone between the neurons in layers II and III gave the appearance of a double row of cells (figure 1, part A). In the adjacent rostral subdivision, layer II was composed of clusters of cells separated by wide, cell-sparse zones. Immediately below layer II, neurons in superficial layer III were also clustered into clumps that were not in register with the layer II islands (figure 1, part B). In the more caudal intermediate region, layer II neurons were larger and arranged in very distinct, island-like clusters, whereas layer III neurons were diffusely distributed. The intermediate region possessed the cytoarchitectonic features generally described as typical of the entorhinal cortex (4).

Examination of the coded sections from the schizophrenic and matched comparison subjects revealed similar findings. The typical features of olfactory, rostral, and intermediate regions were readily identified in all 20 subjects. Neurons in layers II and superficial III were arranged in the pattern characteristic of each subdivision in every subject, and layer II neurons were not

obviously displaced in any of the schizophrenic subjects. None of the schizophrenic or comparison subjects exhibited abnormal invaginations or convolutions of the cortical surface, although the intrarhinal sulcus did appear to be particularly deep in four of the schizophrenic and two of the comparison subjects.

DISCUSSION

This study did not reveal any evidence of substantial or unique abnormalities in the cytoarchitecture of the entorhinal cortex in schizophrenic subjects and thus failed to replicate previous reports of qualitative cytoarchitectural disturbances in the entorhinal cortex in schizophrenia. These differences across studies may reflect differences in the subject populations. For example, in contrast to the community-based study group used in this report, Arnold et al. (2) studied six subjects from the Yakolev Collection, each of whom previously had undergone prefrontal lobotomy.

Such differences in case selection may also contribute to our failure to replicate the observations of Jakob and Beckmann (1), although consideration of the methodology used in their study suggests an alternative explanation. Jakob and Beckmann described the presence of a "disordered double row of neurons" in the superficial layers of schizophrenic subjects; however, this feature is characteristic of layers II and III in the olfactory region of normal brains (see figure 1, part A). Since these investigators selected tissue sections from each case on the basis of distance from an external landmark rather than on cytoarchitectural features, they may have inadvertently compared different entorhinal cortex subdivisions in at least a subset of their schizophrenic and comparison subjects. This interpretation is supported by observations that the volume of the medial temporal lobe is decreased in schizophrenia (8), which suggests that the typical location of entorhinal cortex subdivisions relative to external landmarks may be altered in schizophrenic subjects. Furthermore, differences in the deep cortical layers of the schizophrenic and comparison subjects illustrated by Jakob and Beckmann suggest that more rostral regions (e.g., olfactory, as in figure 1, part A) were examined in the schizophrenic subjects, whereas more caudal areas (e.g., rostral or intermediate, as in figure 1, part B) were studied in the comparison subjects.

The results of the present study do not confirm the presence of cytoarchitectural abnormalities in the entorhinal cortex as a common feature of schizophrenia, which highlights the importance of comparing the same entorhinal cortex subdivisions across schizophrenic and normal comparison subjects. Another research group, which carefully identified subdivisions of the entorhinal cortex, was also unable to find evidence of cytoarchitectural abnormalities in schizophrenic subjects (9). Together, these studies do not support the hypothesis that schizophrenia is associated with an abnormality in the migration of cortical neurons in the entorhinal cortex. However, they do not exclude the presence of other types of disturbances in the neural circuitry of the entorhinal cortex in schizophrenia.

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