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Stereological approaches to identifying neuropathology in psychosis

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Abstract

The challenges involved in identifying the neuropathological substrates of the clinical syndrome recognized as schizophrenia are well known. Stereological sampling provides a means to obtain accurate and precise quantitative estimates of components of neural circuits, and thus offers promise of an enhanced capacity to detect subtle alterations in brain structure associated with schizophrenia. In this review, we 1) consider the importance and rationale for robust quantitative studies of brain abnormalities in postmortem studies of schizophrenia, 2) provide a brief overview of stereological methods for obtaining such measures, 3) discuss the methodological details that should be reported to document the robustness of a stereological study, 4) given the constraints of postmortem human studies, suggest how to approach the limitations of less robust designs, and 5) present an overview of methodologically sound stereological estimates from postmortem studies of schizophrenia.

Keywords

Cell Count; Cell Volume; Post Mortem; Schizophrenia; Stereology

Introduction

The challenges involved in identifying the neuropathological correlates of the clinical syndrome recognized as schizophrenia have been evident since the time of Kraepelin. As in many areas of medical investigation, advances in understanding the neural substrates of schizophrenia are dependent on advances in technology that permit new types of measurements required to dissect complex disease processes. Indeed, the infamous proclamation that (1) “schizophrenia is the graveyard of the neuropathologist” has now turned into a field of opportunities due to advances in a number of methodologies. One of these, the introduction of stereological sampling, has made available the types of accurate and precise measures of neuronal features that are required to detect subtle alterations in the

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structure of brain circuits. The dynamic field of stereology includes a continuous development of new methods as well as refinements of existing methods and improvements in their application. A thorough review of all the stereological techniques available for postmortem brain research is beyond the scope of this article and we refer the reader to recent review texts (2–6). We provide the rationale for robust quantitative studies of the brain abnormalities in schizophrenia, a brief overview of how to obtain such robust measures using stereological methods, a discussion of the methodological details needed to document a robust stereological study design, and suggestions for how to approach the limitations of less robust designs.

The rationale for robust quantitative studies

Structural brain changes associated with schizophrenia are typically not pronounced; for example, subjects with schizophrenia have an average reduction in brain weight of only 2% (7). Because the disease effect on any given brain element may be small, and even if large may be heterogeneously distributed within the brain region of interest, identifying such structural brain alterations requires robust quantitative data. Also, structural findings may easily be misinterpreted if based on biased methods that depend upon assumptions which may not be verifiable. Thus, obtaining robust quantitative data depends upon the use of unbiased methods which guarantee that individual measures are accurate; that is, on average, they estimate the true value.

Understanding the importance of unbiased methods requires knowledge of the exact meaning of unbiasedness (accuracy) and precision—see Fig. 1. Importantly, unbiasedness is an inherent feature of the methodological design and cannot be proven from the data alone: in contrast, precision can be directly observed from the scatter of the final data. Furthermore, bias may differ across subject groups despite identical, blinded, parallel processing of tissue specimens. For example, a systematic difference in shrinkage was detected in sections from subjects with depression relative to control subjects (8). Moreover, adding more subjects to an unbiased study makes the accurate assessment of the true group mean more precise, whereas in a biased study, adding more subjects to the study just makes the group mean more precisely inaccurate. Thus, when possible to implement, an unbiased study design substantially increases the information value of a study.

So-called design-based stereological methods are based upon unbiased principles of sampling and guarantee accuracy of the data to the degree that the unbiased principles are successfully implemented. Thus, design-based stereological studies are ideal for detecting small effects in heterogeneous structures and enhance clarity in data interpretation.

Another strength of design-based stereology is that the most common stereological techniques for estimation of volume and cell number allow for assessment of the method precision by determination of the coefficient of error (*CE*) (Table 1). Assessment of the method *CE* and its subcomponents—i.e. assessing the amount of random error introduced by the various parts of the stereological procedure—allows for fine-tuning of the method for increased efficiency (i.e., can the number of sections and/or the number of cells or points counted be lowered without markedly decreasing the level of precision, and thus reduce effort and expense?). Comparing the method *CE* to the observed coefficient of variation (*CV*; Table 1) across data from a group allows for documentation of the robustness of measures; that is, the study should be executed such that the precision of the method is sufficiently large relative to the intrinsic biological variability so that the data can be interpreted in the context of the biological measure of interest (see also Table 1). As the biological variation often is relatively large across human subjects, and the differences between schizophrenia and comparison subjects typically are small, less precision within a

subject may be acceptable. In contrast, the combination of accurate methods and sufficient sample size are crucial to obtain a robust group average (9).

In summary, design-based unbiased stereological methods are needed to 1) avoid unverifiable assumptions; 2) obtain accurate estimates of group means required for both meaningful group comparisons within and across studies; and 3) allow for statistical analysis of the method precision via the *CE*.

Totals are more robust than densities

Design-based stereological methods tend to focus on total quantities, like estimates of total number of neurons in a region of interest. In contrast, published studies of schizophrenia frequently report measures of density, such as the number of neurons per unit volume, which can be challenging to interpret. Because a density is a ratio, a change in density means that the numerator and the denominator changed to a different degree—e.g. both could go up, but if the denominator increases to a greater degree, then the ratio goes down. Thus, using cell density as a proxy for cell number comes with the assumption of no change in reference volume across groups. This assumption may not hold and is in stereology referred to as “the reference trap” (10); that is, an observed increase in neuronal density could be due to an increased neuron number, but could also be due to a reduced reference volume with preserved neuron number, or due to a decrease in neuron number in combination with an even greater decrease in the reference volume. A classical example is the observations by Haug that brain tissue from young subjects shrunk more during processing than tissue from older subjects. This caused an apparent decrease in cell density as a function of age—an effect that in preceding studies had been misinterpreted as a decrease in total neuron number with age (11,12). Another example of the limitations of density data is evident from our study of antipsychotic-exposed monkeys. Antipsychotic exposure was associated with an increased density of neurons and unchanged glial density; however, the antipsychotic exposed monkeys actually had significantly smaller brain volumes, unchanged neuron number, and a reduced number of glia cells (13–15). Thus, using only the cell density measures would give the erroneous impression that antipsychotics induced a specific increase in neuron number. It is also important to note that no difference in density only means that the ratio between numerator and denominator did not change; that is, similar reductions of the volume and cell number within the region of interest will lead to an unchanged cell density. For example, we observed that both the volume and total neuron number of the primary visual cortex were 22–25% smaller in a cohort of subjects with schizophrenia, with preserved neuronal density and cortical thickness (16).

In addition, in order for density measures to be accurate, the region of interest must be 1) available for sampling in its entirety; 2) well-defined, with borders that are recognizable for the complete region of interest; and 3) sampled such that all parts have a known (typically uniform) probability of being assessed. In addition, the object being counted must be reliably identified. Consequently, measuring the density of well-stained cells in an arbitrary central section can give rise to misleading density measures since the position and content of the arbitrary section may differ across subject groups due to systematic shifts in gyration pattern or in the shape of the region of interest. Thus, accurate estimates of both cell numbers and densities require that the entire region of interest is available for sampling.

Obtaining robust estimates of number and volume

Reference volume estimation

The standard stereological method for estimating the volume of the region of interest is the Cavalieri method (4,17). To implement this method, the region of interest is first divided into slabs by parallel, equidistant, uniformly random cuts. Alternatively, the region of

interest may be sectioned exhaustively, and with a random start a systematic set of sections is selected (e.g. every 100th section with the first section selected uniformly randomly among the first 100 sections). Second, the transected area of the region of interest is assessed in each sectioning plane or section—typically estimated by point counts (i.e. by superimposing a randomly oriented and positioned point grid, with a known area per point, and counting the number of points hitting the region of interest). The volume V of the region of interest may then be estimated as:

$$V = T - a - \sum P_i \quad (1)$$

where T is the slab thickness or intersectional distance, a is the area per point, and P_i the number of points hitting the region of interest in the i 'th section which is summed across all section planes/sections. Here and elsewhere “:=” indicates that the entity at the left is estimated by the expression to the right. If the method is based upon slabs, is it important to avoid over or under projection at the edge of the slabs—it is the cut surface of the slab (either top or bottom cut surface consistently across all slabs) that should form the basis for the point count, not the projection of the slab. The Cavalieri estimator is very efficient, in most cases leading to very precise estimates when counting only 200–400 points total across 8–12 slabs or sections (17,18).

Cell number estimation

Two major sampling designs are used for the estimation of cell number. The fractionator method (19,20) counts all cells in a known fraction of the tissue and multiplies the count by the inverse sampling fraction to estimate the total. That is, the region of interest is split into a number of smaller pieces and then a systematic, uniformly random sample of these is selected. This “split and sample” approach is repeated until the final sample is of a manageable size, allowing the observer to perform a complete cell count. The cell count is typically done using disector probes (see below). For example, the tissue containing a region of interest is split into blocks and every second block is sampled uniformly randomly; the sampled blocks are sectioned exhaustively and every 20th section sampled with a random start among the first 20 sections; then 4% of the area of the selected sections are sampled systematically by a randomly positioned grid of unbiased counting frames (21); finally each frame is sampled through the middle third of its thickness by optical disectors. If 200 cells were counted in the final disector sample, the total cell number can be estimated as: $2 \times 20 \times 25 \times 3 \times 200 = 600,000$. In general, the total number, N , of cells in the region of interest is estimated as the product of the inverse sampling fraction for each sampling step multiplied with the cell count:

$$N = \frac{1}{bsf} \cdot \frac{1}{ssf} \cdot \frac{1}{ass} \cdot \frac{1}{hsf} \cdot \sum Q^- \quad (2)$$

where bsf , ssf , ass , hsf are the block, section, area, and height sampling fractions, respectively—see also Fig. 2. $hsf = 1$ if using physical disector probes (see below). The sum is across all sections of the disector count Q^- for each section. Importantly, the method is robust to processing induced shrinkage, and because the reference volume is not estimated, the region of interest needs not to have sharp boundaries—as long as all cells belonging to the region of interest are unambiguously recognized at the final level of counting (e.g. by a specific stain). Notice, the fractionator is not biased by an inhomogeneous distribution of cells in the region of interest—see Fig. 3. The observer is allowed to optimize the sampling at each level to reduce the method variability. The smooth fractionator is one such an

approach, which in many situations will improve the estimator precision substantially (22). See also the recently developed proportionator method (23,24).

A second approach to obtaining estimates of cell number is the $N_V \times V_{Ref}$ design, also known as a disector design. In this approach, total cell number is estimated as the product of the numerical density, N_V , of the cells and the reference volume V_{Ref} (i.e. the volume of the region of interest) (25). Typically, the latter is estimated by Cavalieri's method, while the former is estimated from cell counts in a uniformly random sample of the tissue.

The cell density N_V is estimated as (see description of physical and optical disector probes below):

$$N_V = \frac{\sum Q^-}{n \cdot BA \cdot (a/p) \cdot \sum P} \text{ using physical disector probes} \quad (3)$$

$$N_V = \frac{\overline{t_{Q^-}}}{BA} \cdot \frac{\sum Q^-}{h \cdot (a/p) \cdot \sum P} \text{ using optical disector probes} \quad (4)$$

where n is the number of sections separating the sampling and look up section including the sampling section (for adjacent sections in the pair $n = 1$), BA is the microtome or cryostat block advance (26), i.e. the setting of the calibrated sectioning device, a is the area of the counting frame, p is the number of points associated to the frame (often one using the upper right corner or the center point), P is the number of frame points hitting the region of interest across a section, Q^- the disector count for a section, h is the disector height, and $\overline{t_{Q^-}}$ is the number-weighted mean section thickness—see Fig. 2 and (26). The sums are across all sections for a subject.

The sampling could be performed as fractionator sampling with the important difference that exact sampling fractions need not be known. This approach is also more robust to lost tissue than the fractionator and gives more freedom in designing the tissue sampling (27–30). However, the region of interest must have well-defined, detectable boundaries, and any shrinkage (or swelling) between the estimation of V_{Ref} and N_V should be avoided or accounted for.

For both the fractionator and the $N_V \times V_{Ref}$ design the final steps of cell counting are done using the disector, the stereological probe for unbiased counting (25). The disector exists in two versions: the physical disector and the optical disector.

The physical disector (25) consists of two thin sections a known distance apart—hence the name, disector or two sections. An unbiased counting frame (21) is superimposed upon one of the sections (sampling section) and a cell is counted if it is sampled by the counting frame—i.e. wholly or partly inside and not touching the exclusion lines—and if not present in the other section (look-up section). This counting rule ensures that cell size, shape and orientation do not bias the count—see Fig. 2 in (31) for an illustration of the problem. Just counting in one section is biased since larger cells are more likely to be present in a given section than are smaller cells. The physical disector requires that all cells be observable in at least one section, i.e. that no cell is lost completely in the sectioning process. Accordingly, the distance between the sections should be small enough such that no cell can hide undetected between sections and such that profiles from neighboring cells can be correctly

distinguished. Typically, this means that section thickness should be maximally 25% of the diameter of the cells to be counted and that sections should be adjacent, although the combination of large cells and very thin sections means that sections do not need to be adjacent.

The physical disector is conceptually a simple approach but can be challenging to implement since it requires the alignment of two physical sections at high magnification. However, recent advances in computerized stereology with automatic alignment of sections seems to have given the method a renaissance (6).

The more popular optical disector (19,26) uses thick tissue sections and a high numerical aperture oil (or water) immersion objective that permits focusing through the z axis of the section and counting cells directly as they appear in an unbiased counting frame. By generating a stack of perfectly aligned thin focal planes, the optical disector solves the alignment problem of the physical disector. However, sufficient guard zones are needed to avoid counting near the top and bottom section surfaces where split, torn out, and damaged cells may bias the count. Thus, the mounted, stained, and coverslipped sections need to be thick enough to allow for sufficient guard zones and a disector ideally at least 10 μm high. The latter is advisable because the focal plane of a high numerical aperture, oil immersion objective is $\sim 0.5\mu\text{m}$ thick, and a substantial number of focal planes are recommended. The size of the guard zones and the amount of z-axis shrinkage (i.e., in the thickness of the section) depends especially upon the embedding media (26). Compared to the thin sections of the physical disector, the thick sections of the optical disector allow complete inspection of cells in 3D and facilitate the morphological identification of cell types.

Cell size estimation

Among the many stereological methods that can be used to estimate individual cell volumes, the nucleator (32), and the planar rotator (33,34) are used most commonly. The optical rotator (35), volume estimation from point sampled intercepts (36), the selector (37), and the invariator (38,39) might be of interest for the neuroscientist in special settings.

All of these methods for cell size estimation require sections which are not only random in position but also in orientation. Two main types of stereological sections fulfill these requirements: Isotropic Uniform Random (IUR) sections (40,41), and Vertical Uniform Random (VUR) sections (42). However, generating randomly rotated sections in the brain may be a challenge (29,30).

Other parameters

Although beyond the scope of this paper, stereological methods exist for assessing a range of other parameters such as axonal or dendritic length (43,44); capillary length and number (45); the surface of the brain (27,46); and number of synapses (47,48).

Stereology and immunohistochemistry

Quantitative studies based upon immunohistochemical techniques have distinct challenges (49), in addition to those associated with obtaining sensitive and specific detection of the antigen of interest in postmortem tissue (50). A special requirement for quantitative immunohistochemical studies based upon thick sections is that the labeling penetrates the sections in their full thickness. With incomplete penetration, sampling is biased and the bias may systematically differ depending on the diagnose group. When using the optical disector with immunohistochemistry, penetration of the labeling should be verified by a pilot calibration study where a sufficient number of cells (> 400) are sampled systematic uniformly random across a set of sections by recording their z-axis position and the local

section thickness (49). Plotting the number of labeled cells as a function of distance between the top and bottom of the section will reveal whether the label fully penetrated the sections as well as the required size of top and bottom guard zones. Both the absolute and relative plots are informative—see Fig 4 in (51).

If the label did not fully penetrate the section, a dip in the number of stained cells is seen in the center of the section. We have with success used fluorescent labeled secondary antibodies without the need for the tertiary cascade system for improved penetration of the stain (15). Another obvious solution is to use thin sections in a physical disector based design (6) since it does not depend on z-axis measures.

Documenting a robust study design

Well-written scientific papers are expected to include sufficient methodological details and references to allow the reader to judge the rigor of the study as well as to perform a replication study. For stereological studies, we recommend that the items listed in Table 2 be reported as discussed briefly below.

Definitions of the structure and region of interest

“If you cannot define it, you cannot quantify it.” This axiom captures the need to include clear definitions of the region and structures (e.g. cells) of interest. Representative photomicrographs or drawings can be helpful but the text must include descriptions of “nontextbook” parts of the region of interest where it may be difficult to define the boundary. It may even be that the very definition of the region of interest turns out to be a study in itself—see e.g. (52).

Explain the sampling design in details

As discussed above, unbiased estimation starts with unbiased—typically uniformly random—sampling of the region of interest. Therefore, a clear description of how the final sections were sampled from the region of interest is needed. Most often this is best done by including a diagram of the sampling procedure—especially if the design is more complicated than just exhaustive sectioning of the region of interest—see (16,29,30). The description should also include details about how the tissue was cut. For example, marked variations in slab thickness may reduce the precision of the final estimates (53,54), and an un-calibrated microtome or cryostat may unknowingly generate sections with an unexpected thickness and thus bias the final results.

Describe the microscopy setup employed

Several key issues are important to report. The optical disector probe requires the use of a high numerical aperture oil or water immersion objective and a linear encoder (microcator) to monitor the z-movement of the stage; how this was accomplished should be reported. Whether a stereological software package and a computerized microscope were employed should be described since it can influence the methodological strength and limitations of a study.

List all main constants and sampling parameters

Table 2 summarizes the test system constants and sampling parameters to be reported, including details of pilot calibration studies. Reporting these parameters is important for documenting the precision of the study as well as for serving as a guide for optimizing future studies of the region of interest. Notice that a pilot calibration study is typically warranted for documenting that the guard zone size and disector height were sufficient and optimal—see (49,55) as well as Fig. 4 in (51).

Report the precision of the estimates

If possible, the mean *CE* for each of the reported stereological estimates should be included and contrasted with the observed variation in the final data. This information documents the degree to which method imprecision influenced the final data and how optimized the methods were—i.e. did the authors count enough, too much or too little? It is important to report how the *CEs* were assessed since the methodology for assessing the *CE* of the various stereological estimators is a field of ongoing research.

Assess shrinkage

It is important to assess or at least discuss the potential impact of tissue shrinkage, as it is crucial for comparing size estimates across studies. For any size estimate, it is typically very difficult or impossible to completely correct for tissue deformation as shrinkage may differ across brain regions, across the various components of a given region (13,26), and across subject groups (8). However, documenting that only a moderate shrinkage was present and that the shrinkage did not on average differ across groups would form a robust basis for interpretation of the results. Also, it should be remembered that estimates of total cell numbers are robust to shrinkage if based upon rigorously implemented design-based stereological methods—most easily by using a fractionator approach (26).

Report results with enough detail

Ideally, the results should include means, observed coefficient of variation and corresponding coefficient of error for each group. Also, it is often a good idea to report data in scatter plots instead of histograms as “one dot per measure” efficiently allows the reader to judge not only the mean and spread but also potential clustering or presence of outliers in the data.

Report specific methodological limitations

It is important to report and discuss the potential impact of any deviation from the requirements of the used stereological methods. Examples of such limitations could be the use of sections without random orientation for cell size estimation or the impact of missing sections on cell number estimates. An illustration of both these issues can be found in (56).

Problems with under-reporting

Under-reporting may limit comparison to other studies. An example is the comparison of results of stereological studies of the human mediodorsal thalamic nucleus (MD) (56). We found that both total volume and total number of neurons of the MD differed substantially from study to study—see Fig. 11 in (56). A number of issues made it difficult to assess the cause of these differences: 1) the criteria for defining the region of interest as well as the neurons (including or excluding small neurons) probably differed among studies; 2) tissue and Nissl staining quality as well as the optics used may have limited the ability of some studies to distinguish small neurons from glial cells; 3) pilot calibration studies to determine and document sufficient size of the guard zones for the optical disectors were not used in most studies; 4) only our study used the number-weighted mean section thickness based version of the optical fractionator (26), which prevents biases due to uneven shrinkage in the section thickness; 5) none of the studies (including our own) kept track of total tissue shrinkage from fresh tissue.

Limitations of less robust designs

While it is advisable to use a stereological approach if possible, investigators may sometime have various reasons for not doing so—the use of biopsies or archival material not sampled

according to stereological principles are common reasons. However, in such cases it is still recommended to employ unbiased stereological principles as much as possible and then carefully declare and discuss the limitations to prevent overstating the strength of the conclusions. Readers are then able to decide to what extent they have confidence in the results of the study. For example, if the complete region of interest is not available for sampling—or only a few arbitrary sections are available—then any obtained estimate will only be valid for the sections examined—not the whole region of interest. This limitation has important implications: 1) it is not possible to obtain estimates of total numbers, leaving any density estimate vulnerable to “the reference trap” (10); 2) any estimate comes with the assumption that the region of interest is homogenous, i.e. the examined tissue is expected to look like what was not examined—an assumption that is difficult to impossible to verify without a complete stereological study. However, large differences across subject groups—e.g. a fivefold change in mean density of neurons per mm³ or spines per length of dendrite—within the examined tissue samples of a reasonably homogenous region strongly suggest the presence of a structural difference. Unfortunately, most often changes in postmortem samples from subjects with schizophrenia are much more subtle. Also, in developmental or disease studies, the reference volume may undergo substantial nonlinear changes as a function of age and/or disease state (due to in vivo size changes as well as differential shrinkage during tissue processing) thus efficiently limiting the interpretability of density data (e.g. neurons per mm³). However, comparison of the ratios of various densities—i.e. the number of boutons per neuron (instead of the density of boutons and neurons per mm³)—may be interpreted with some care assuming that the region of interest is reasonably homogenous.

Stereological postmortem studies of schizophrenia—a literature overview

It is beyond the scope of the current paper to give a comprehensive review of the existing literature of quantitative postmortem schizophrenia studies. However, we do provide a compilation of stereological postmortem studies of schizophrenia that fulfill the following basic criteria:

1. The studied region(s) of interest was anatomically well-defined.
2. The region(s) of interest was available in toto and was sampled in accordance with unbiased stereological principles—i.e. all parts of the complete region of interest had the same (or a known) probability of being assessed.
3. The final sample—the sections—were examined using unbiased stereological probes.
4. Stereological estimates of structural parameters were generated and reported.

For cell counting we required a true 3D count using disector probes—i.e. with guard zones when counting in (thick) single sections. For estimates of the volume of the region of interest (by Cavalieri’s principle), we did not require the individual section areas to be assessed by point counting but also accepted tracing and other forms of planimetry. For the probes that were employed, all relevant requirements had to be fulfilled, e.g. for the nucleator or rotator probes the sections had to be cut either IUR or VUR.

To identify relevant papers we searched PubMed (in March 2010) with a broad list of search terms leading to 1147 abstracts from which 319 papers of interest were selected. The papers were acquired and out of these 67 fulfilled our criteria. In addition, we included one reference (57) from a non-indexed journal. Thus, we are able to list stereological estimates from a total of 68 papers organized by brain region in Table 3–Table 6. Because total number estimates are the most robust, they are emphasized in the tables by bold text.

In the light of the current review, our stereological criteria for inclusion may appear lenient as we did not exclude papers based upon a general lack of methodological details. Thus, it is difficult to judge the robustness of some of the estimates in the lists due to methodological under-reporting. On the other hand, a range of otherwise informative quantitative postmortem schizophrenia studies did not make the list—e.g., several of our own studies assessing the size of neurons in various cortical regions using the nucleator probe were excluded due to incomplete sampling of the region of interest and sections that were not randomly rotated. Also, if a study reported several estimates, only the estimate(s) fulfilling above criteria was listed in the tables: e.g., if the reference volume was estimated by Cavalieri's principle and the neuron number by counting fractionator style but without guard zones (i.e., essentially by a 2D count) only the former estimate is included in the list.

Summary of stereological findings in postmortem schizophrenia studies

We have sought to present a fair list of sound stereological estimates—not a comprehensive record of quantitative findings of varying methodological quality. When reading Table 3–Table 6 it should be taken into account that we have included all incremental publications from progressing studies spanning several papers to facilitate completeness of the method description despite some redundancy in the results. On the other hand, a number of studies assessing the volume of various brain regions in schizophrenia did not fulfill our criteria due to subtle methodological issues, and were therefore not included in the tables although they do report informative results (e.g., refs. 58–61).

A range of brain regions have only been targeted by one or a few studies to date. However, for regions examined in multiple stereological studies, the current findings appear to converge on the following conclusions, although it is important to note that these provisional conclusions are not based on meta-analyses or other objective assessments of the data:

Cerebral hemispheres (Table 3): The findings support a tendency towards a slightly reduced cortical volume, without any major global deficits in cortical cell number. The field is moving toward more targeted studies of specific cell types in specific cortical areas, including laminar analyses. Although such studies have detected schizophrenia-related cortical changes, more studies are needed before a consensus can be reached.

Thalamus (Table 4): A number of earlier studies found reduced volume and total neuron number in the mediodorsal thalamic nucleus. However, these findings have not been consistently replicated in newer studies with larger sample sizes. The several studies that have targeted the anterior complex and the lateral geniculate nucleus have consistently failed to find any schizophrenia-related differences. In contrast, a number of studies have found reduced volume of the pulvinar in schizophrenia.

Limbic system (Table 5): A number of studies have found a reduced number of neurons in the lateral nucleus of the amygdala or in the nucleus accumbens. On the other hand, almost all studies of the hippocampus did not report schizophrenia-related differences.

Basal ganglia, cerebellum, and brainstem (Table 6): Several studies have not found general structural changes of the cerebellum in schizophrenia. The results of the numerous studies of the striatum and pallidum report conflicting findings of increased or decrease volume and/or number of neurons.

Conclusion

The use of unbiased sampling designs can enhance the ability to correctly detect subtle alterations in brain structure in schizophrenia and other diseases by providing robust

quantitative data. In reporting such studies, it is essential to describe the key elements of the experimental approach and to interpret findings in accord with what the design employed can, or cannot, tell you. Additional studies using such robust designs are needed to sensitively capture the nature of brain structural alterations in schizophrenia.

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Reference List

1. Plum F. Prospects for research on schizophrenia. 3. Neurophysiology. Neuropathological findings. *Neurosci Res Program Bull.* 1972; 10:384–388. [PubMed: 4663816]
2. Evans, SM.; Janson, AM.; Nyengaard, JR. Quantitative methods in neuroscience: A neuroanatomical approach. Oxford: Oxford University Press; 2004.
3. Baddeley, A.; Vedel Jensen, EB. Stereology for statisticians. Boca Raton: Chapman & Hall/CRC; 2005.
4. Howard, CV.; Reed, MG. Unbiased stereology. Three-dimensional measurement in microscopy. 2 ed.. Abingdon: Garland Science/Bios Scientific Publishers; 2005.
5. Schmitz C, Hof PR. Design-based stereology in neuroscience. *Neuroscience.* 2005; 130:813–831. [PubMed: 15652981]
6. Boyce, RW.; Dorph-Petersen, K-A.; Lyck, L.; Gundersen, HJG. Stereological Solutions for Common Quantitative Endpoints in Neurotoxicology. In: Bolon, B.; Butt, MT., editors. *Fundamental Neuropathology for Pathologist and Toxicologists: Principles and Techniques.* J. Wiley and Sons; 2010. In press
7. Harrison PJ, Freemantle N, Geddes JR. Meta-analysis of brain weight in schizophrenia. *Schizophr Res.* 2003; 64:25–34. [PubMed: 14511798]
8. Stockmeier CA, Mahajan GJ, Konick LC, Overholser JC, Jurjus GJ, Meltzer HY, et al. Cellular changes in the postmortem hippocampus in major depression. *Biol Psychiatry.* 2004; 56:640–650. [PubMed: 15522247]
9. Gundersen HJG, Østerby R. Optimizing sampling efficiency of stereological studies in biology: or 'Do more less well!'. *J Microsc.* 1981; 121:65–73. [PubMed: 7014910]
10. Brændgaard H, Gundersen HJG. The impact of recent stereological advances on quantitative studies of the nervous system. *J Neurosci Methods.* 1986; 18:39–78. [PubMed: 3540470]
11. Haug H, Kühl S, Mecke E, Sass N-L, Wasner K. The significance of morphometric procedures in the investigation of age changes in cytoarchitectonic structures of human brain. *J Hirnforsch.* 1984; 25:353–374. [PubMed: 6481152]
12. Haug H. History of neuromorphometry. *J Neurosci Methods.* 1986; 18:1–17. [PubMed: 3540464]
13. Dorph-Petersen K-A, Pierri JN, Perel JM, Sun Z, Sampson AR, Lewis DA. The influence of chronic exposure to antipsychotic medications on brain size before and after tissue fixation: A comparison of haloperidol and olanzapine in macaque monkeys. *Neuropsychopharmacology.* 2005; 30:1649–1661. [PubMed: 15756305]
14. Konopaske GT, Dorph-Petersen K-A, Pierri JN, Wu Q, Sampson AR, Lewis DA. Effect of chronic exposure to antipsychotic medication on cell numbers in the parietal cortex of macaque monkeys. *Neuropsychopharmacology.* 2007; 32:1216–1223. [PubMed: 17063154]
15. Konopaske GT, Dorph-Petersen K-A, Sweet RA, Pierri JN, Zhang W, Sampson AR, Lewis DA. Effect of chronic antipsychotic exposure on astrocyte and oligodendrocyte numbers in macaque monkeys. *Biol Psychiatry.* 2008; 63:759–765. [PubMed: 17945195]
16. Dorph-Petersen K-A, Pierri JN, Wu Q, Sampson AR, Lewis DA. Primary visual cortex volume and total neuron number are reduced in schizophrenia. *J Comp Neurol.* 2007; 501:290–301. [PubMed: 17226750]
17. Gundersen HJG, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. *J Microsc.* 1987; 147:229–263. [PubMed: 3430576]

18. Gundersen HJG, Jensen EBV, Kiêu K, Nielsen J. The efficiency of systematic sampling in stereology—reconsidered. *J Microsc.* 1999; 193:199–211. [PubMed: 10348656]
19. Gundersen HJG. Stereology of arbitrary particles. A review of unbiased number and size estimators and the presentation of some new ones, in memory of William R. Thompson. *J Microsc.* 1986; 143:3–45. [PubMed: 3761363]
20. West MJ, Slomianka L, Gundersen HJG. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat Rec.* 1991; 231:482–497. [PubMed: 1793176]
21. Gundersen HJG. Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. *J Microsc.* 1977; 111:219–223.
22. Gundersen HJG. The smooth fractionator. *J Microsc.* 2002; 207:191–210. [PubMed: 12230489]
23. Gardi JE, Nyengaard JR, Gundersen HJG. Automatic sampling for unbiased and efficient stereological estimation using the proportionator in biological studies. *J Microsc.* 2008; 230:108–120. [PubMed: 18387046]
24. Gardi JE, Nyengaard JR, Gundersen HJG. The proportionator: unbiased stereological estimation using biased automatic image analysis and non-uniform probability proportional to size sampling. *Comput Biol Med.* 2008; 38:313–328. [PubMed: 18163985]
25. Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the disector. *J Microsc.* 1984; 134:127–136. [PubMed: 6737468]
26. Dorph-Petersen K-A, Nyengaard JR, Gundersen HJG. Tissue shrinkage and unbiased stereological estimation of particle number and size. *J Microsc.* 2001; 204:232–246. [PubMed: 11903800]
27. Braendgaard H, Evans SM, Howard CV, Gundersen HJG. The total number of neurons in the human neocortex unbiasedly estimated using optical disectors. *J Microsc.* 1990; 157:285–304. [PubMed: 2332884]
28. Pakkenberg B, Gundersen HJG. Total number of neurons and glial cells in human brain nuclei estimated by the disector and the fractionator. *J Microsc.* 1988; 150:1–20. [PubMed: 3043005]
29. Dorph-Petersen K-A. Stereological estimation using vertical sections in a complex tissue. *J Microsc.* 1999; 195:79–86. [PubMed: 10444304]
30. Dorph-Petersen K-A, Delevich KM, Marcisin MJ, Zhang W, Sampson AR, Gundersen HJG, et al. Pyramidal neuron number in layer 3 of primary auditory cortex of subjects with schizophrenia. *Brain Res.* 2009; 1285:42–57. [PubMed: 19524554]
31. Dorph-Petersen K-A, Nyengaard JR, Gundersen HJG. A brief introduction to particle number estimation. *Acta Stereol.* 1998; 17:201–214.
32. Gundersen HJG. The nucleator. *J Microsc.* 1988; 151:3–21. [PubMed: 3193456]
33. Vedel Jensen EB, Gundersen HJG. The Rotator. *J Microsc.* 1993; 170:35–44.
34. Vedel Jensen EB, Gundersen HJG. The Rotator (Erratum). *J Microsc.* 1993; 170:282.
35. Tandrup T, Gundersen HJG, Vedel Jensen EB. The optical rotator. *J Microsc.* 1997; 186:108–120. [PubMed: 9183850]
36. Gundersen HJG, Jensen EB. Stereological estimation of the volume-weighted mean volume of arbitrary particles observed on random sections. *J Microsc.* 1985; 138:127–142. [PubMed: 4020857]
37. Cruz-Orive LM. Particle number can be estimated using a disector of unknown thickness: the selector. *J Microsc.* 1987; 145:121–142. [PubMed: 3553604]
38. Cruz-Orive LM. A new stereological principle for test lines in three-dimensional space. *J Microsc.* 2005; 219:18–28. [PubMed: 15998362]
39. Cruz-Orive, LM. Stereology: old and new. In: Capasso, V.; Aletti, G.; Micheletti, A., editors. *Proceedings of the 10th European Congress of ISS. The MIRIAM Project Series.* Bologna: ESCULAPIO Pub. Co.; 2009. p. 3–14.
40. Mattfeldt T, Mall G, Gharehbaghi H, Möller P. Estimation of surface area and length with the orientator. *J Microsc.* 1990; 159:301–317. [PubMed: 2243364]
41. Nyengaard JR, Gundersen HJG. The isector: a simple and direct method for generating isotropic, uniform random sections from small specimens. *J Microsc.* 1992; 165:427–431.

42. Baddeley AJ, Gundersen HJG, Cruz-Orive LM. Estimation of surface area from vertical sections. *J Microsc.* 1986; 142:259–276. [PubMed: 3735415]
43. Tang Y, Nyengaard JR. A stereological method for estimating the total length and size of myelin fibers in human brain white matter. *J Neurosci Meth.* 1997; 73:193–200.
44. Marner L, Pakkenberg B. Total length of nerve fibers in prefrontal and global white matter of chronic schizophrenics. *J Psychiatr Res.* 2003; 37:539–547. [PubMed: 14563386]
45. Løkkegaard A, Nyengaard JR, West MJ. Stereological estimates of number and length of capillaries in subdivisions of the human hippocampal region. *Hippocampus.* 2001; 11:726–740. [PubMed: 11811667]
46. Pakkenberg B, Gundersen HJG. Neocortical neuron number in humans: effect of sex and age. *J Comp Neurol.* 1997; 384:312–320. [PubMed: 9215725]
47. Witgen BM, Grady MS, Nyengaard JR, Gundersen HJG. A new fractionator principle with varying sampling fractions: exemplified by estimation of synapse number using electron microscopy. *J Microsc.* 2006; 222:251–255. [PubMed: 16872424]
48. Chen F, Madsen TM, Wegener G, Nyengaard JR. Repeated electroconvulsive seizures increase the total number of synapses in adult male rat hippocampus. *Eur Neuropsychopharmacol.* 2009; 19:329–338. [PubMed: 19176277]
49. Dorph-Petersen, K-A.; Rosenberg, R.; Nyengaard, JR. Estimation of number and volume of immunohistochemically stained neurons in complex brain regions. In: Evans, SM.; Janson, AM.; Nyengaard, JR., editors. *Quantitative Methods in Neuroscience: A neuroanatomical approach.* Oxford: Oxford University Press; 2004. p. 216-238.
50. Lewis DA. The human brain revisited: opportunities and challenges in postmortem studies of psychiatric disorders. *Neuropsychopharmacology.* 2002; 26:143–154. [PubMed: 11790510]
51. Dorph-Petersen K-A, Caric D, Saghaei R, Zhang W, Sampson AR, Lewis DA. Volume and neuron number of the lateral geniculate nucleus in schizophrenia and mood disorders. *Acta Neuropathol.* 2009; 117:369–384. [PubMed: 18642008]
52. Sweet RA, Dorph-Petersen K-A, Lewis DA. Mapping auditory core, lateral belt, and parabelt cortices in the human superior temporal gyrus. *J Comp Neurol.* 2005; 491:270–289. [PubMed: 16134138]
53. Baddeley A, Dorph-Petersen K-A, Vedel Jensen EB. A note on the stereological implications of irregular spacing of sections. *J Microsc.* 2006; 222:177–181. [PubMed: 16872416]
54. Ziegel J, Baddeley A, Dorph-Petersen K-A, Vedel Jensen EB. Systematic sampling with errors in sample locations. *Biometrika.* 2010; 97:1–13.
55. Andersen BB, Gundersen HJG. Pronounced loss of cell nuclei and anisotropic deformation of thick sections. *J Microsc.* 1999; 196:69–73. [PubMed: 10540259]
56. Dorph-Petersen K-A, Pierri JN, Sun Z, Sampson AR, Lewis DA. Stereological analysis of the mediodorsal thalamic nucleus in schizophrenia: Volume, neuron number, and cell types. *J Comp Neurol.* 2004; 472:449–462. [PubMed: 15065119]
57. Damgaard Nielsen R, Abitz M, Pakkenberg B. Neuron and glial cell numbers in the mediodorsal thalamic nucleus in brains of schizophrenic subjects. *Image Anal Stereol.* 2008; 27:133–141.
58. Lesch A, Bogerts B. The diencephalon in schizophrenia: evidence for reduced thickness of the periventricular grey matter. *Eur Arch Psychiatr Neurol Sci.* 1984; 234:212–219.
59. Bogerts B, Meertz E, Schönfeldt-Bausch R. Basal ganglia and limbic system pathology in schizophrenia. A morphometric study of brain volume and shrinkage. *Arch Gen Psychiatry.* 1985; 42:784–791. [PubMed: 4015323]
60. Falkai P, Bogerts B, Schneider T, Greve B, Pfeiffer U, Pilz K, et al. Disturbed planum temporale asymmetry in schizophrenia. A quantitative post-mortem study. *Schizophr Res.* 1995; 14:161–176. [PubMed: 7710997]
61. Brisch R, Bernstein H-G, Stauch R, Dobrowolny H, Krell D, Truebner K, et al. The volumes of the fornix in schizophrenia and affective disorders: a post-mortem study. *Psychiatry Res.* 2008; 164:265–273. [PubMed: 19022630]
62. Cruz-Orive LM, Geiser M. Estimation of particle number by stereology: an update. *J Aerosol Med.* 2004; 17:197–212. [PubMed: 15625812]

63. Cruz-Orive LM. A general variance predictor for Cavalieri slices. *J Microsc.* 2006; 222:158–165. [PubMed: 16872414]
64. García-Fiñana M, Keller SS, Roberts N. Confidence intervals for the volume of brain structures in Cavalieri sampling with local errors. *J Neurosci Methods.* 2009; 179:71–77. [PubMed: 19428514]
65. Pakkenberg B. Post-mortem study of chronic schizophrenic brains. *Br J Psychiatry.* 1987; 151:744–752. [PubMed: 3502800]
66. Pakkenberg B. What happens in the leucotomised brain? A post-mortem morphological study of brains from schizophrenic patients. *J Neurol Neurosurg Psychiatr.* 1989; 52:156–161. [PubMed: 2703834]
67. Heckers S, Heinsen H, Heinsen YC, Beckmann H. Limbic structures and lateral ventricle in schizophrenia. A quantitative postmortem study. *Arch Gen Psychiatry.* 1990; 47:1016–1022. [PubMed: 2133373]
68. Heckers S, Heinsen H, Heinsen Y, Beckmann H. Cortex, white matter, and basal ganglia in schizophrenia: a volumetric postmortem study. *Biol Psychiatry.* 1991; 29:556–566. [PubMed: 2054430]
69. Pakkenberg B. Total nerve cell number in neocortex in chronic schizophrenics and controls estimated using optical disectors. *Biol Psychiatry.* 1993; 34:768–772. [PubMed: 8292680]
70. Lauer M, Beckmann H. The human striatum in schizophrenia. I. Increase in overall relative striatal volume in schizophrenics. *Psychiatry Res.* 1997; 68:87–98. [PubMed: 9104756]
71. Highley JR, McDonald B, Walker MA, Esiri MM, Crow TJ. Schizophrenia and temporal lobe asymmetry. A post-mortem stereological study of tissue volume. *Br J Psychiatry.* 1999; 175:127–134. [PubMed: 10627794]
72. Highley JR, Esiri MM, McDonald B, Cortina-Borja M, Herron BM, Crow TJ. The size and fibre composition of the corpus callosum with respect to gender and schizophrenia: a post-mortem study. *Brain.* 1999; 122:99–110. [PubMed: 10050898]
73. Highley JR, Esiri MM, McDonald B, Roberts HC, Walker MA, Crow TJ. The size and fiber composition of the anterior commissure with respect to gender and schizophrenia. *Biol Psychiatry.* 1999; 45:1120–1127. [PubMed: 10331103]
74. McDonald B, Highley JR, Walker MA, Herron BM, Cooper SJ, Esiri MM, Crow TJ. Anomalous asymmetry of fusiform and parahippocampal gyrus gray matter in schizophrenia: A postmortem study. *Am J Psychiatry.* 2000; 157:40–47. [PubMed: 10618011]
75. Highley JR, Walker MA, Esiri MM, McDonald B, Harrison PJ, Crow TJ. Schizophrenia and the frontal lobes: post-mortem stereological study of tissue volume. *Br J Psychiatry.* 2001; 178:337–343. [PubMed: 11282813]
76. Lauer M, Senitz D, Beckmann H. Increased volume of the nucleus accumbens in schizophrenia. *J Neural Transm.* 2001; 108:645–660. [PubMed: 11478417]
77. Thune JJ, Uylings HBM, Pakkenberg B. No deficit in total number of neurons in the prefrontal cortex in schizophrenics. *J Psychiatr Res.* 2001; 35:15–21. [PubMed: 11287052]
78. Hof PR, Haroutunian V, Copland C, Davis KL, Buxbaum JD. Molecular and cellular evidence for an oligodendrocyte abnormality in schizophrenia. *Neurochem Res.* 2002; 27:1193–1200. [PubMed: 12462417]
79. Hof PR, Haroutunian V, Friedrich VL Jr, Byne W, Buitron C, Perl DP, Davis KL. Loss and altered spatial distribution of oligodendrocytes in the superior frontal gyrus in schizophrenia. *Biol Psychiatry.* 2003; 53:1075–1085. [PubMed: 12814859]
80. Kreczmanski P, Schmidt-Kastner R, Heinsen H, Steinbusch HWM, Hof PR, Schmitz C. Stereological studies of capillary length density in the frontal cortex of schizophrenics. *Acta Neuropathol.* 2005; 109:510–518. [PubMed: 15886994]
81. Kreczmanski P, Heinsen H, Mantua V, Woltersdorf F, Masson T, Ulfing N, et al. Volume, neuron density and total neuron number in five subcortical regions in schizophrenia. *Brain.* 2007; 130:678–692. [PubMed: 17303593]
82. Smiley JF, Rosoklija G, Mancevski B, Mann JJ, Dwork AJ, Javitt DC. Altered volume and hemispheric asymmetry of the superficial cortical layers in the schizophrenia planum temporale. *Eur J Neurosci.* 2009; 30:449–463. [PubMed: 19656176]

83. Pakkenberg B, Gundersen HJG. New stereological method for obtaining unbiased and efficient estimates of total nerve cell number in human brain areas. Exemplified by the mediodorsal thalamic nucleus in schizophrenics. *APMIS*. 1989; 97:677–681. [PubMed: 2765273]
84. Pakkenberg B. Pronounced reduction of total neuron number in mediodorsal thalamic nucleus and nucleus accumbens in schizophrenics. *Arch Gen Psychiatry*. 1990; 47:1023–1028. [PubMed: 2241504]
85. Pakkenberg B. The volume of the mediodorsal thalamic nucleus in treated and untreated schizophrenics. *Schizophr Res*. 1992; 7:95–100. [PubMed: 1355358]
86. Pakkenberg B. Leucotomized schizophrenics lose neurons in the mediodorsal thalamic nucleus. *Neuropathol Appl Neurobiol*. 1993; 19:373–380. [PubMed: 8278019]
87. Popken GJ, Bunney WE Jr, Potkin SG, Jones EG. Subnucleus-specific loss of neurons in medial thalamus of schizophrenics. *Proc Natl Acad Sci USA*. 2000; 97:9276–9280. [PubMed: 10908653]
88. Young KA, Manaye KF, Liang C-L, Hicks PB, German DC. Reduced number of mediodorsal and anterior thalamic neurons in schizophrenia. *Biol Psychiatry*. 2000; 47:944–953. [PubMed: 10838062]
89. Byne W, Buchsbaum MS, Mattiace LA, Hazlett EA, Kemether E, Elhakem SL, et al. Postmortem assessment of thalamic nuclear volumes in subjects with schizophrenia. *Am J Psychiatry*. 2002; 159:59–65. [PubMed: 11772691]
90. Danos P, Baumann B, Bernstein H-G, Stauch R, Krell D, Falkai P, Bogerts B. The ventral lateral posterior nucleus of the thalamus in schizophrenia: a post-mortem study. *Psychiatry Res*. 2002; 114:1–9. [PubMed: 11864805]
91. Cullen TJ, Walker MA, Parkinson N, Craven R, Crow TJ, Esiri MM, Harrison PJ. A postmortem study of the mediodorsal nucleus of the thalamus in schizophrenia. *Schizophr Res*. 2003; 60:157–166. [PubMed: 12591579]
92. Danos P, Baumann B, Krämer A, Bernstein H-G, Stauch R, Krell D, et al. Volumes of association thalamic nuclei in schizophrenia: a postmortem study. *Schizophr Res*. 2003; 60:141–155. [PubMed: 12591578]
93. Highley JR, Walker MA, Crow TJ, Esiri MM, Harrison PJ. Low medial and lateral right pulvinar volumes in schizophrenia: a postmortem study. *Am J Psychiatry*. 2003; 160:1177–1179. [PubMed: 12777280]
94. Dixon G, Harper CG. No evidence for selective GABAergic interneuron deficits in the anterior thalamic complex of patients with schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004; 28:1045–1051. [PubMed: 15380866]
95. Young KA, Holcomb LA, Yazdani U, Hicks PB, German DC. Elevated neuron number in the limbic thalamus in major depression. *Am J Psychiatry*. 2004; 161:1270–1277. [PubMed: 15229061]
96. Danos P, Schmidt A, Baumann B, Bernstein H-G, Northoff G, Stauch R, et al. Volume and neuron number of the mediodorsal thalamic nucleus in schizophrenia: a replication study. *Psychiatry Res*. 2005; 140:281–289. [PubMed: 16297604]
97. Byne W, Kidkardnee S, Tatusov A, Yiannoulos G, Buchsbaum MS, Haroutunian V. Schizophrenia-associated reduction of neuronal and oligodendrocyte numbers in the anterior principal thalamic nucleus. *Schizophr Res*. 2006; 85:245–253. [PubMed: 16730162]
98. Byne W, Fernandes J, Haroutunian V, Huacon D, Kidkardnee S, Kim J, et al. Reduction of right medial pulvinar volume and neuron number in schizophrenia. *Schizophr Res*. 2007; 90:71–75. [PubMed: 17141474]
99. Selemon LD, Begović A. Stereologic analysis of the lateral geniculate nucleus of the thalamus in normal and schizophrenic subjects. *Psychiatry Res*. 2007; 151:1–10. [PubMed: 17383740]
100. Young KA, Holcomb LA, Bonkale WL, Hicks PB, Yazdani U, German DC. 5HTTLPR polymorphism and enlargement of the pulvinar: unlocking the backdoor to the limbic system. *Biol Psychiatry*. 2007; 61:813–818. [PubMed: 17083920]
101. Byne W, Tatusov A, Yiannoulos G, Vong GS, Marcus S. Effects of mental illness and aging in two thalamic nuclei. *Schizophr Res*. 2008; 106:172–181. [PubMed: 18835520]

102. Chana G, Everall I, Landau S, Cotter D. Glial cell number and nuclear size in the mediodorsal thalamic nucleus (MDNT) in schizophrenia. *Schizophr Res.* 2008; 102:344–345. [PubMed: 18508240]
103. Kreczmanski P, Heinsen H, Mantua V, Woltersdorf F, Masson T, Ulfing N, et al. Microvessel length density, total length, and length per neuron in five subcortical regions in schizophrenia. *Acta Neuropathol.* 2009; 117:409–421. [PubMed: 19198859]
104. Bogerts B, Falkai P, Haupts M, Greve B, Ernst S, Tapernon-Franz U, Heinzmann U. Post-mortem volume measurements of limbic system and basal ganglia structures in chronic schizophrenics. Initial results from a new brain collection. *Schizophr Res.* 1990; 3:295–301. [PubMed: 2282334]
105. Heckers S, Heinsen H, Heinsen Y, Beckmann H. Morphometry of the parahippocampal gyrus in schizophrenics and controls. Some anatomical considerations. *J Neural Transm Gen Sect.* 1990; 80:151–155. [PubMed: 2317316]
106. Heckers S, Heinsen H, Geiger B, Beckmann H. Hippocampal neuron number in schizophrenia. A stereological study. *Arch Gen Psychiatry.* 1991; 48:1002–1008. [PubMed: 1747014]
107. Krimer LS, Herman MM, Saunders RC, Boyd JC, Hyde TM, Carter JM, et al. A qualitative and quantitative analysis of the entorhinal cortex in schizophrenia. *Cereb Cortex.* 1997; 7:732–739. [PubMed: 9408037]
108. Bernstein H-G, Stanarius A, Baumann B, Henning H, Krell D, Danos P, et al. Nitric oxide synthase-containing neurons in the human hypothalamus: reduced number of immunoreactive cells in the paraventricular nucleus of depressive patients and schizophrenics. *Neuroscience.* 1998; 83:867–875. [PubMed: 9483570]
109. Briess D, Cotter D, Doshi R, Everall I. Mamillary body abnormalities in schizophrenia. *Lancet.* 1998; 352:789–790. [PubMed: 9737293]
110. Chance SA, Highley JR, Esiri MM, Crow TJ. Fiber content of the fornix in schizophrenia: lack of evidence for a primary limbic encephalopathy. *Am J Psychiatry.* 1999; 156:1720–1724. [PubMed: 10553734]
111. Chance SA, Esiri MM, Crow TJ. Amygdala volume in schizophrenia: postmortem study and review of magnetic resonance imaging findings. *Br J Psychiatry.* 2002; 180:331–338. [PubMed: 11925356]
112. Walker MA, Highley JR, Esiri MM, McDonald B, Roberts HC, Evans SP, Crow TJ. Estimated neuronal populations and volumes of the hippocampus and its subfields in schizophrenia. *Am J Psychiatry.* 2002; 159:821–828. [PubMed: 11986137]
113. Highley JR, Walker MA, McDonald B, Crow TJ, Esiri MM. Size of hippocampal pyramidal neurons in schizophrenia. *Br J Psychiatry.* 2003; 183:414–417. [PubMed: 14594916]
114. Stark AK, Uylings HBM, Sanz-Arigita E, Pakkenberg B. Glial cell loss in the anterior cingulate cortex, a subregion of the prefrontal cortex, in subjects with schizophrenia. *Am J Psychiatry.* 2004; 161:882–888. [PubMed: 15121654]
115. Malidelis YI, Panayotacopoulou MT, van Heerikhuizen JJ, Unmehopa UA, Kontostavlaki DP, Swaab DF. Absence of a difference in the neurosecretory activity of supraoptic nucleus vasopressin neurons of neuroleptic-treated schizophrenic patients. *Neuroendocrinology.* 2005; 82:63–69. [PubMed: 16415596]
116. Berretta S, Pantazopoulos H, Lange N. Neuron numbers and volume of the amygdala in subjects diagnosed with bipolar disorder or schizophrenia. *Biol Psychiatry.* 2007; 62:884–893. [PubMed: 17698040]
117. Pantazopoulos H, Lange N, Baldessarini RJ, Berretta S. Parvalbumin neurons in the entorhinal cortex of subjects diagnosed with bipolar disorder or schizophrenia. *Biol Psychiatry.* 2007; 61:640–652. [PubMed: 16950219]
118. Bernstein H-G, Ernst T, Lendeckel U, Bukowska A, Ansorge S, Stauch R, et al. Reduced neuronal expression of insulin-degrading enzyme in the dorsolateral prefrontal cortex of patients with haloperidol-treated, chronic schizophrenia. *J Psychiatr Res.* 2009; 43:1095–1105. [PubMed: 19394958]
119. Schmitt A, Steyskal C, Bernstein H-G, Schneider-Axmann T, Parlapani E, Schaeffer EL, et al. Stereologic investigation of the posterior part of the hippocampus in schizophrenia. *Acta Neuropathol.* 2009; 117:395–407. [PubMed: 18777029]

120. Ranft K, Dobrowolny H, Krell D, Biela H, Bogerts B, Bernstein H-G. Evidence for structural abnormalities of the human habenular complex in affective disorders but not in schizophrenia. *Psychol Med.* 2010; 40:557–567. [PubMed: 19671211]
121. Beckmann H, Lauer M. The human striatum in schizophrenia. II. Increased number of striatal neurons in schizophrenics. *Psychiatry Res.* 1997; 68:99–109. [PubMed: 9104757]
122. Bernstein H-G, Krell D, Braunewell K-H, Baumann B, Gundelfinger ED, Diekmann S, et al. Increased number of nitric oxide synthase immunoreactive Purkinje cells and dentate nucleus neurons in schizophrenia. *J Neurocytol.* 2001; 30:661–670. [PubMed: 12118154]
123. Andersen BB, Pakkenberg B. Stereological quantitation in cerebella from people with schizophrenia. *Br J Psychiatry.* 2003; 182:354–361. [PubMed: 12668413]
124. Andersen BB, Fabricius K, Gundersen HJG, Jelsing J, Stark AK. No change in neuron numbers in the dentate nucleus of patients with schizophrenia estimated with a new stereological method - the smooth fractionator. *J Anat.* 2004; 205:313–321. [PubMed: 15447690]
125. Marner L, Søbørg C, Pakkenberg B. Increased volume of the pigmented neurons in the locus coeruleus of schizophrenic subjects. A stereological study. *J Psychiatr Res.* 2005; 39:337–345. [PubMed: 15804384]
126. Gundersen HJG. Stereology: the fast lane between neuroanatomy and brain function—or still only a tightrope? *Acta Neurol Scand Suppl.* 1992; 137:8–13. [PubMed: 1357908]

Fig. 1.

The concepts of bias and precision. Each dot represent a measurement or estimate, the crosses are the respective means. Top panel: The centers of the targets indicate the true value to be determined. An unbiased (accurate) estimator does on average not deviate from the true value, whereas a biased estimator systematically deviates from the truth. A precise estimator shows low variation among the results whereas an imprecise estimator shows larger variability. However, precision (reproducibility) does not guarantee accuracy. Hence the saying: “Precision is to tell the same story again and again. Accuracy is to tell the truth.” An unbiased estimator tells the truth on average even if imprecise (upper right target in top panel). Bottom panel: The degree of precision can be observed directly from the results.

However, it is not possible from the results to tell whether the study was unbiased or biased as the target value is typically unknown. Unbiasedness is an inherent feature of the study design and not observable from the data: top and bottom row in lower panel cannot be distinguished when the targets are removed. The figure is inspired by Fig. 2 in (126).

Fig. 2.

The basics of fractionator sampling for cell number. A) The region of interest is split into a number of blocks and a fraction of these is sampled. As every second block is sampled in the example, the block samplings fraction bsf is $\frac{1}{2}$ in the figure. B) Sections are sampled from each block with a constant section sampling fraction ssf . Sections could be sampled by exhaustive sectioning of all blocks and sampling; e.g. every 20th section with a random start among the first 20 sections (not shown). Alternatively, if the blocks are cut as slabs with a known thickness T , ssf can be calculated as the microtome block advance BA divided by T . C) The sections are subsampled in the area with a constant area sampling fraction asf —typically by a uniformly random grid of unbiased counting frames. asf is then calculated as

the ratio between the counting frame area a and the area A of the basic tile of the grid. D) When using optical disector probes, the sections are subsampled in the thickness. Often (especially when using cryostat or vibratome sections) the final section thickness varies due to an uneven shrinkage of the mounted section. This results in a varying local sampling fraction from frame to frame as illustrated here. Therefore, the height sampling fraction hsf should be calculated based upon the number-weighted mean section thickness $\overline{t_Q^-}$ as indicated, where t_i is the local section thickness and q_i^- the corresponding disector cell count of the i 'th frame. This approach takes the effect of any uneven section collapse into account and is therefore generally recommended when using the optical fractionator (26).

Fig. 3.

The fractionator is not biased by inhomogeneously distributed cells within the region of interest. In this very simplified example, the region of interest contains seven cells and is sampled uniformly random with a sampling fraction $sf = 1/2$; that is, the region of interest is divided into two samples, one of which is sampled at random with probability $1/2$ and the number of cells in the sample is assessed. Thus, the total number of cells in the region of interest is estimated as $1/sf$ multiplied by the number of cells in the sample. A) The cells are distributed rather homogeneously and the region of interest is split into two parts of approximately the same size. The two possible estimates of total cell number are calculated. The estimator is clearly unbiased as the mean of the two estimates is equal to the actual total

cell number. B) The cells are distributed inhomogeneously and the region of interest is split in two pieces of different size. Obviously, the mean of the two estimates still equals the true value. However, the precision is reduced—the two estimates differ more and the *CE* is increased—due to the inhomogeneity and uneven split. C) Same cell distribution as in B) but now the region of interest is cut into bars. Sample one, *S1*, consists of the odd bars while sample 2, *S2*, consists of the even bars. The estimator is still unbiased but the change in sampling strategy by making each sample systematic uniformly random across the region of interest efficiently eliminated the impact of the inhomogeneity. Thus, the estimator will always be unbiased (i.e., hit the true value on average) independently of the distribution of the cells and the actual way of splitting the region of interest, as long as the sample has been selected uniformly randomly (here by probability $\frac{1}{2}$). This simple example underscores: 1) the unbiasedness of systematic uniformly random sampling and 2) the actual implementation of the sampling has a major impact on the precision of the results.

Table 1

Main statistical terms in stereological designs

Term	Explanation
SD	Standard deviation.
$SEM = \frac{SD}{\sqrt{n}}$	Standard error of the mean for n independent observations.
$CV = \frac{SD}{mean}$	Coefficient of variation (standard deviation relative to the mean). Convenient for comparing variability across various measures as it has no units.
CV_{Obs}	Observed coefficient of variation across all subjects in a group.
CV_{Bio}	The biological coefficient of variation, i.e. the intrinsic, natural variability across subjects in a group.
CE	Coefficient of error. A measure of the methodological variability, i.e. the variability of independent replications of the stereological estimate within a subject. It is typically not possible to generate independent replications of the estimate as it would require "gluing the brain back together" and re-sampling. However, research into statistical methods for predicting the CE from a (single) set of stereological data is a field of rapid progress. See e.g. (17,18,53,54,62–64) and references therein.
$\overline{CE} = \sqrt{mean(CE^2)}$	Mean CE across the individuals in a group.
$CV_{obs}^2 = CV_{Bio}^2 + \overline{CE}^2$	The important relation between the observed CV , the inherent biological variability, and the random error of the stereological method. The relation only holds for unbiased methods. The CE may be broken down into various subcomponents such as the estimator variability due to the sectioning, and the count of points or cells. Ensuring that the mean CE is less than half the observed CV will in many cases mean that the method imprecision does not impair the observer's ability to draw conclusions. Then, if a lower SEM is required, more subjects need to be added to the study. See also (9).

Table 2

List of parameters required to document a typical stereological study

Definition of what to estimate	Whole region of interest in 3D Relevant structures such as specific cell types
Sampling design	Unbiased (typically systematic, uniformly random) sampling Sampling of entire region of interest Sampling of tissue across the region of interest Use diagrams and/or photo series to explain more advanced designs
Blocking, slicing, cutting of tissue	By free hand, guided, or by machine (of what kind)? Calibrated cryostat/microtome?
Microscopy system	Objectives—high numerical aperture oil or water immersion needed for optical disector Presence of microcator (linear encoder) Use of motorized stage Calibrated (with calibration slide, daily?) Software package including version number
Test system constants	Stepping distances in x-y Frame size, area per point, etc. Disector height Size of guard zones (top and bottom for disector) Final onscreen magnification
Pilot / calibration study	How many subjects, sections, cells, measures? Document sufficient guard zones Document penetration of stain Document linear z-axis shrinkage
Sampling parameters	Number of sections sampled per subject Number of cells (points, intersections, etc.) counted per subject (of each type) Final section thickness (measured how, at how many sites?), mean and <i>CV</i>
Precision—<i>CE</i>	Calculated how? In the final results compare <i>CE</i> to observed <i>CV</i>
Shrinkage	Approximate magnitude
Results	Ideally report data as scatter plots—i.e. one point per subject

Table 3

List of proper stereological estimates in post mortem schizophrenia studies of the **cerebral hemispheres** (excl. hippocampus, entorhinal cortex and cingulate gyrus)

1st Author / year	Region(s)	Schiz/Cont /Other^a	Estimates^b	Reported significant schiz. related diff.^c
Pakkenberg 1987 (65)	Right & left: hemispheres (H), cortex (C), white matter (W), ventricles (Vt), central grey (G)	29/30	V(H, C, W, Vt, G)	Decreased V: H, C, G Increased V: Vt
Pakkenberg 1989 (66)	Right & left: cortex (C), white matter (W), ventricles (Vt), central grey (G), leucotomy lesion (L)	19 ^d +26/20	V(C, W, Vt, G, L)	Decreased V: C (only leucotomy group), W, G
Heckers 1990 (67)	Right & left: hemispheres (H), lateral ventricle (L) incl. anterior (A), inferior (I), posterior (P) horn	20/20	V(H, L, A, I, P)	No differences
Heckers 1991 (68)	Right & left: hemispheres (H), cortex (C), white matter (W)	23/23	V(H, C, W) V _V (C/H)	No differences
Pakkenberg 1993 (69)	Right & left: neocortex (C) incl. frontal (F), temporal (T), parietal (P), and occipital (O) lobes	8/16	V(C, F, T, P, O) N(neuron - C, F, T, P, O) N _V (neuron - C, F, T, P, O)	Decreased V: C, P Increased N _V : T
Lauer 1997 (70)	Right & left: hemispheres (H)	9/9	V (H)	No differences
Highley 1999 (71)	Right & left temporal lobe: total grey (G) and white (W) matter, incl. Grey matter of temp. pole (P), inferior temp. gyrus (I), middle temp. gyrus (M), superior temp. gyrus (S)	29/27	V(G, W, P, I, M S)	Decreased V: G, W, P (females only), S (left side only) V(S): Reversed left-right asymmetry
Highley 1999 (72)	Corpus callosum (C) incl. rostrum (R); inferior (IG), superior (SG), and posterior (PG) genu areas; anterior (AM), middle (MM), and posterior (PM) midbody areas; isthmus (I); splenium (S)	20/21	N(nerve fibers - C, R, IG, SG, PG, AM, MM, PM, I, S) N _A (nerve fibers - C, R, IG, SG, PG, AM, MM, PM, I, S)	Decreased N (females only): C, IG, SG, PG, AM, MM, PM, I, S N_A(C, R, IG, SG, PG, AM, MM, I): reversed gender ratio (schiz: N_A(female)<N_A(male); cont: N_A(male)<N_A(female))
Highley 1999 (73)	Anterior commissure (AC)	17/20	N(nerve fibers - AC) N _A (nerve fibers - AC)	Decreased N _A : AC (females only)
McDonald 2000 (74)	Right & left: grey matter of fusiform gyrus (F)	31/27	V(F)	V(F): Reversed left-right asymmetry
Highley 2001 (75)	Right & left: frontal lobe grey (G) and white (W) matter incl. precentral (P), superior frontal (S), middle frontal (M) gyri, infero-orbital cortex (I); and cingulate gyrus (C)	24/28	V(G, W, P, S, M, I, C)	No differences
Lauer 2001 (76)	Right & left: hemispheres (H)	9/9	V (H)	No differences
Thune 2001 (77)	Right & left: Prefrontal cortex (P)	8/10	V(P) N(neuron - P) N _V (neuron - P)	No differences

1 st Author / year	Region(s)	Schiz/Cont /Other ^a	Estimates ^b	Reported significant schiz. related diff. ^c
Hof 2002 (78)^e	Right: layer 3 of BA 9 (L3), white matter (W) below BA9 in superior frontal gyrus	4/4	N _V (oligodendrocytes – L3, W)	Decreased N _V : L3, W
Hof 2003 (79)	Right: layer III of BA9 (L3), white matter (W) below BA9 in superior frontal gyrus	7/7	V(L3, W) N(oligodendrocytes – L3, W) N _V (oligodendrocytes – L3, W)	Decreased N: L3, W Decreased N _V : L3, W
Marner 2003 (44)	Right & left: cerebral white matter (C) incl. Prefrontal white matter (P)	8/9	V(C,P) L(myelinated nerve fibers – C, P) L _V (myelinated nerve fibers – C, P) D(myelinated nerve fibers – C, P)	Increased L _V : C
Kreczmanski 2005 (80)	Right & left: cortex (C)	13/13	V(C)	Decreased V: C
Dorph-Petersen 2007 (16)	Left: primary visual cortex (PV)	10/10	V(PV) N(neuron – PV) N _V (neuron – PV)	Decreased V: PV Decreased N: PV
Kreczmanski 2007 (81)	Right & left: cortex (C)	13/13	V(C) ^f	Decreased V: C
Dorph-Petersen 2009 (51)	Left: primary auditory cortex (PA) incl. all six cortical layers (1, 2, 3, 4, 5, 6)	12/12	V(PA, 1, 2, 3, 4, 5, 6) V _V (1, 2, 3, 4, 5, 6) N(neuron – 3) N _V (neuron – 3)	No differences
Smiley 2009 (82)	Right & left: planum temporale (PT)	19/18	V(PT)	No differences

^a Number of subjects in each diagnostic group: schizophrenia, control group, other diagnostic group(s). Subject numbers from several groups within same category are separated by “+” sign.

^b Stereological estimates fulfilling the inclusion criteria—see text. The cited papers may include important qualitative and quantitative findings not listed here. Estimate abbreviations: V volume; V_V volume fraction; N number; N_V numerical density; N_A numerical areal density; L length; L_V length density; D diameter. The relevant region(s) or cell type(s) are indicated in the parentheses using the abbreviations found in the “Region(s)” column.

^c Only differences reported as statistically significant for the schizophrenia group are listed. Abbreviations as in “Estimates” column.

^d Group of leucotomized subjects with schizophrenia.

^e Limited methods details.

^f Also reported in Kreczmanski 2005.

Table 4List of proper stereological estimates in post mortem schizophrenia studies of the **thalamus**

1 st Author / year	Region(s)	Schiz/Cont /Other ^a	Estimates ^b	Reported significant schiz. related diff. ^c
Pakkenberg 1988 (28)	Left: mediodorsal n. (MD)	8/10	V(MD) N(neuron – MD) N _V (neuron – MD) N _N (glia/neuron – MD)	Decreased V: MD Decreased N: MD
Pakkenberg 1989 (83)	Left: mediodorsal n. (MD)	12/12	V(MD) N(neuron – MD) N _V (neuron – MD)	Decreased V: MD Decreased N: MD Decreased N _V : MD
Pakkenberg 1990 (84)	Left: mediodorsal n. (MD)	12/12	V(MD) N(neuron, astrocyte, oligodendrocyte – MD) N _V (neuron, astrocyte, oligodendrocyte – MD)	Decreased V: MD Decreased N(neuron, astrocyte, oligodendrocyte): MD Decreased N_V(neuron): MD
Pakkenberg 1992 (85)	Right & left: mediodorsal n. (MD)	8 ^d +12/14	V(MD)	Decreased V: MD (untreated and treated)
Pakkenberg 1993 (86)	Left: mediodorsal n. (MD)	8 ^e +12/12	V(MD) N(neuron, astrocyte, oligodendrocyte – MD) N _V (neuron, astrocyte, oligodendrocyte – MD)	Decreased V: MD (leucotomized further decreased) Decreased N(neuron, astrocyte, oligodendrocyte): MD^f Decreased N _V (neuron): MD
Popken 2000 (87)	Left: ventral posterior medial n. (VPM), mediodorsal n. (MD) incl. parvocellular (P), densocellular (D), magnocellular (M) subregions	6/6	V(VPM, MD, P, D, M) N(neuron – VPM, MD, P, D, M) N _V (neuron – VPM, MD, P, D, M)	Decreased N: MD, P, D
Young 2000 (88)	Left: anteroventral- anteromedial n. (A), mediodorsal n. (MD)	8/8	V(A, MD) N(neuron – A, MD) N _V (A, MD)	Decreased V: MD Decreased N: A, MD
Byne 2002 (89)	Right: whole thalamus (T); anterior n. (A); mediodorsal n. (MD) incl. Parvocellular (PC), magnocellular (MC), and caudodorsal (CD) subregions; pulvinar (P); centro-median n. (CM)	10/5 (14/8) ^g	V(T, A, MD, PC, MC, CD, P, CM)	Decreased V: MD, PC, P CD, P, CM)
Danos 2002 (90)	Right & left: ventral lateral posterior n. (VLp)	8/8	V(VLp) N(neuron – VLp) N _V (neuron – VLp)	Decreased V: VLp (left side only) Decreased N: VLp (left side only)
Cullen 2003 (91)	Right & left: thalamus (T) incl. mediodorsal n. (MD)	21/27	V(T, MD) N(neuron – MD)	No differences
Danos 2003 (92)	Right & left: thalamus (T) incl. mediodorsal n. (MD), medial pulvinar n. (MP)	12/13 (T, MD) 12/11 (MP)	V(T, MD, MP)	Decreased V: T, MD (left side only), MP
Highley 2003 (93)	Right & left: medial (MP) and lateral (LP) pulvinar	23/25 ^h	V(MP, LP) N(neuron – MP, LP)	Decreased V (right side only): MP, LP Decreased N (right side only): LP
Dixon 2004 (94)	Right (& left) ⁱ : anterior	6/6	V(A)	No differences

1 st Author / year	Region(s)	Schiz/Cont /Other ^a	Estimates ^b	Reported significant schiz. related diff. ^c
thalamic complex (A)				
Dorph-Petersen 2004 (56)	Left: mediodorsal n. (MD)	11/9/12	V(MD) N(neuron type 1 & 2 – MD)	No differences
Young 2004 (95)	Right & left: anteroventral- anteromedial n. (A), mediodorsal n. (MD)	12/11/13+13 (A) 11/11/12+12 (MD)	V(A, MD) N(neuron – A, MD) N _V (neuron – A, MD)	No differences
Danos 2005 (96)	Right & left: mediodorsal n. (MD)	20/18	V(MD)	No differences
Byne 2006 (97)	Right: anterior principal n. (A)	23/12	V(A)	No differences
Byne 2007 (98)	Right: pulvinar (P) incl. medial (M) and inferior/lateral (IL) parts	13/10	V(P, M, IL)	Decreased V: P, M
Kreczmanski 2007 (81)	Right & left: mediodorsal n. (MD)	13/13	V(MD) N(neuron – MD) N _V (neuron – MD)	No differences
Selemon 2007 (99)	Right & left: lateral geniculate n.: magnocellular (M), and parvocellular (P) layers	15/15	V(M, P) N(neuron, glia – M, P)	No differences
Young 2007 (100)	Right & left: pulvinar (P)	12/15/11+11	V(P) N(neuron – P)	No differences
Byne 2008 (101)	Right & left: anterior principal n. (A), centeromedian n. (C)	14/14/13+15 (A) 12/14/13+14 (C)	V(A, C)	No differences
Chana 2008 (102)^j	Right & left: mediodorsal n. (MD)	14/15/15+14 ^k	V(MD) N(glia – MD) N _V (glia – MD)	No differences
Damgaard Nielsen 2008 (57)	Right & left: mediodorsal n. (MD) incl. magnocellular (M), parvocellular (P), and densocellular (D) regions.	9/8	V(MD, M, P, D) N(neuron type 1 & 2, glia – MD, M, P, D)	No differences
Dorph-Petersen 2009 (51)	Left: lateral geniculate n. (LGN) incl. magno- (M) and parvo- (P) cellular laminae and interlaminar regions (I)	9/7/13	V(LGN, M, P, I) N(neuron – LGN, M, P)	No differences
Kreczmanski 2009 (103)	Right & left: mediodorsal n. (MD)	13/13	L(microvessel – MD) L _V (microvessel – MD) L _N (microvessel /neuron – MD)	No differences

^aNumber of subjects in each diagnostic group: schizophrenia, control group, other diagnostic group(s). Subject numbers from several groups within same category are separated by “+” sign.

^bStereological estimates fulfilling the inclusion criteria—see text. The cited papers may include important qualitative and quantitative findings not listed here. Estimate abbreviations: V volume; V_V volume fraction; N number; N_V numerical density; N_N numerical fraction; L length; L_V length density; L_N mean length per cell. The relevant region(s) or cell type(s) are indicated in the parentheses using the abbreviations found in the “Region(s)” column.

^cOnly differences reported as statistically significant for the schizophrenia group are listed. Abbreviations as in “Estimates” column.

^dGroup of untreated subjects with schizophrenia.

^eGroup of leucotomized subjects with schizophrenia.

^fMean neuron number further decreased in the leucotomized schizophrenia group.

^gNumber of subjects in each group when including subjects with Alzheimer's type pathology. Results listed are for subjects without Alzheimer's type pathology.

^hMaximum subject numbers from ranges in manuscript. Exact number of subjects for each estimate not reported in the paper.

ⁱSchizophrenia group: six right; control group: four right & two left.

^jLimited methods details. "Right and left" is inferred from the use of sections generated in relation to Young 2004 from tissue from the Stanley Foundation Neuropathology Consortium which is also used by Selemon 2007, Young 2007, and Byne 2008.

^kTwo subjects were excluded but it is not revealed from which group(s).

Table 5

List of proper stereological estimates in post mortem schizophrenia studies of the **limbic system** incl. hippocampus, entorhinal cortex & cingulate gyrus

1 st Author / year	Region(s)	Schiz/Cont /Other ^a	Estimates ^b	Reported significant schiz. related diff. ^c
Bogerts 1990 (104)	Right & left: hippocampus (H), n. accumbens (A)	18/21	V(H, A)	Decreased V: H
Heckers 1990 (105)	Right & left parahippocampal gyrus (PG)	18/18	V(PG)	No differences
Heckers 1990 (67)	Right & left: hippocampus (H), amygdala (A)	20/20	V(H, A)	No differences
Pakkenberg 1990 (84)	Left: venteromedial (Vm) and lateral (L) parts of n accumbens, basolateral n. of amygdala (B)	8/10 (Vm) 8/8 (L) 5/5 (B)	V(Vm, L, B) N(neuron, astrocyte, oligodendrocyte – Vm, L, B) N _V (neuron, astrocyte, oligodendrocyte – Vm, L, B)	Decreased V: Vm, L Decreased N(neuron): Vm, L Decreased N(astrocyte): Vm, L Decreased N(oligodendrocyte): Vm, L
Heckers 1991 (106)	Right & left: hemisphere (He), hippocampus (H), pyramidal cell layer (P), white matter (W), subiculum (S), CA1, CA2/3, CA4.	13/13	V(He, H, P, W, S, CA1, CA2/3, CA4)	Decreased V: W (left side only)
Pakkenberg 1993 (86)	Left: venteromedial (Vm) part of n. accumbens	8 ^d +8/10	V(Vm) N(neuron, astrocyte, oligodendrocyte – Vm) N _V (neuron, astrocyte, oligodendrocyte – Vm)	Decreased V: Vm Decreased N(neuron, astrocyte – further decreased in leucotomized, oligodendrocyte)
Krimer 1997 (107)	Left: entorhinal cortex (E) incl. prorhinal (Pr), lateral (L), intermediate rostral (Ir), intermediate caudal (Ic) and sulcal (S) subregions – 6 individual cortical layers for each subregion	7/8	V(E, Pr, L, Ir, Ic, S – for each subregion: layer 1, 2, 3, 4, 5, 6)	No differences
Bernstein 1998 (108)^e	Right & left: central and caudal part of the paraventricular n. (P) of the hypothalamus.	10/13/8	N _V (NOS-IR neuron – P)	Decreased N _V : P (right side only)
Briess 1998 (109)^e	Right & left: mamillary bodies (MB)	9/6/2	V(MB) N(neuron – MB) N _V (neuron – MB)	Increased V: MB (left side only) Decreased N _V : MB
Chance 1999 (110)	Right & left: fornix (F)	29/33	A(F) N(nerve fiber – F) N _A (nerve fiber – F)	Increased N _A : F (men only)
McDonald 2000 (74)	Right & left: grey matter of parahippocampal gyrus (P)	31/27	V(P)	V(P): Reversed left-right asymmetry
Lauer 2001 (76)	Right & left: accumbens (A)	9/9	V(A) V _V (A/hemisphere)	Increased V _V : A
Chance 2002 (111)	Right & left. amygdala (A)	18/18	V(A)	No differences
Walker 2002 (112)	Right & left: hippocampus (H), dentate fascia (D), hilus (Hil), CA2/3, CA1, subiculum (S)	30/29	V(H, D, Hil, CA2/3, CA1, S) N(H, D, Hil, CA2/3, CA1, S)	No differences

1 st Author / year	Region(s)	Schiz/Cont /Other ^a	Estimates ^b	Reported significant schiz. related diff. ^c
			N _V (H, D, Hil, CA2/3, CA1, S)	
Highley 2003 (113)	Right & left hippocampus: hilus (Hil), CA2/3, CA1, subiculum (S)	13/16	v _N (pyramidal neuron – Hil, CA2/3, CA1, S)	No differences
Stark 2004 (114)	Right & left: BA 24, BA 32	12/14	V(BA24, BA32) N(neuron, glia – BA24, BA32) N _V (neuron, glia – BA24, BA32)	Decreased N(glia): BA24
Malidelis 2005 (115)^e	<i>f</i> Dorsolateral part (D) of the supraoptic n. of the hypothalamus	5/5	V(D)	No differences
Berretta 2007 (116)	Right & left amygdala: lateral (L), basal (B), accessory basal (AB), and cortical (C) subnuclei	16/12/10	V(L, B, AB, C) N(neuron – L, B, AB, C) N _V (neuron – L, B, AB, C)	Decreased N: L
Kreczmanski 2007 (81)	Right & left: n. accumbens (Ac); lateral n.(L), basal n (B), and accessory basal n (Ab) of the amygdala	13/13	V(Ac, L, B, Ab) N(neuron – Ac, L) N _V (neuron – Ac, L) d(neuron-neuron – L)	Decreased V: B, L Decreased N: L
Pantazopoulos 2007 (117)	Right & left: entorhinal cortex (E)	10/16/10	V(E)	No differences
Bernstein 2009 (118)^e	Right & left: paraventricular (P) and supraoptic (S) nuclei of the hypothalamus	14/14	N _V (neuron: IDE-IR: P, S)	No differences
Kreczmanski 2009 (103)	Right & left: n. accumbens (Ac); lateral n. of the amygdala (L)	13/13	L _V (microvessel – Ac, L) L(microvessel – Ac, L) L _N (microvessel /neuron – Ac, L)	No differences
Schmitt 2009 (119)^g	Right & left: posterior part of hippocampus regions: CA1, CA2/3, CA4, subiculum (S)	10/10	V(CA1, CA2/3, CA4, S) N(neuron, astrocyte, oligodendrocyte – CA1, CA2/3, CA4, S) N _V (neuron, astrocyte, oligodendrocyte – CA1, CA2/3, CA4, S)	Decreased: N(oligodendrocyte): C4 Decreased: N _V (oligodendrocyte): C4
Ranft 2010 (120)^e	Right & left: medial (M) and lateral (L) habenular nuclei	17/13/14	V(M, L) N(neuron – M) N _V (neuron – M)	No differences

^aNumber of subjects in each diagnostic group: schizophrenia, control group, other diagnostic group(s). Subject numbers from several groups within same category are separated by “+” sign.

^b Stereological estimates fulfilling the inclusion criteria—see text. The cited papers may include important qualitative and quantitative findings not listed here. Estimate abbreviations: V volume; V_V volume fraction; N number; N_V numerical density; N_A numerical area density; A area; L length; L_V length density; L_N mean length per cell; v_N mean cell volume; d nearest neighbor distance. The relevant region(s) or cell type(s) are indicated in the parentheses using the abbreviations found in the “Region(s)” column.

^cOnly differences reported as statistically significant for the schizophrenia group are listed. Abbreviations as in “Estimates” column.

^d Group of leucotomized subjects with schizophrenia.

^eLimited methods details.

^fSide(s) not listed in manuscript.

^gCell sampling using a disector height of 17 μ m in paraffin sections of a mean (SD) thickness of 18.9 μ m $\pm$ 1.2 μ m. I.e. very limited guard zones—almost a 2D count.

Table 6

List of proper stereological estimates in post mortem schizophrenia studies of the **basal ganglia, cerebellum & brainstem**

1 st Author / year	Region(s)	Schiz/Cont /Other ^a	Estimates ^b	Reported significant schiz. related diff. ^c
Pakkenberg 1988 (28)	Left: ventral pallidum (P)	8/10	V(P) N(glia – P) N _V (glia – P) N _N (glia/neuron – P)	No differences
Bogerts 1990 (104)	Right & left: internal pallidum (IP), external pallidum (EP), caudate n. (C), putamen (PU)	18/21	V(IP, EP, C, PU)	Decreased V: IP
Pakkenberg 1990 (84)	Left: ventral pallidum (P)	8/10	V(P) N(neuron, astrocyte, oligodendrocyte – P) N _V (neuron, astrocyte, oligodendrocyte – P)	No differences
Heckers 1991 (68)	Right & left: striatum (S), pallidum (P)	23/23	V(S, P)	Increased V: S (left side only), P (right side only)
Pakkenberg 1993 (86)	Left: ventral pallidum (P)	8 ^d +8/10	V(P) N(neuron, astrocyte, oligodendrocyte – P) N _V (neuron, astrocyte, oligodendrocyte – P)	Decreased N _V (neuron): P
Beckmann 1997 (121)	Right & left striatum (S): caudatus-accumbens (C-A), putamen (P)	9/9	N(neuron, glia) N _N (glia/neuron)	Increased N(neuron) (right side only): S, C-A
Lauer 1997 (70)	Right & left striatum (S): caudatus-accumbens (C-A), putamen (P)	9/9	V(S,C-A, P) V _V (S,C-A,P)	Increased V: S, C-A, P
Bernstein 2001 (122)^e	Right & left: dentate n. (DN) of the cerebellum	9/9/7	N _V (neuron, NOS-IR neuron, VILIP1-IR neuron – DN)	Decreased N _V (neuron): DN Increased N _V (NOS-IR neuron): DN
Andersen 2003 (123)	Cerebellum (C) incl. cortical grey matter (G) of anterior (A), posterior (P), and flocculonodular (F) lobes, anterior (AV) and posterior (PV) vermis, white matter (W), central nuclei (CN)	8/10	V(C, G, A, P, F, AV, PV, W, CN) N(purkinje, granule cells – C, A, P, F, AV, PV) S(C) t(molecular, granular – A, P, F, AV, PV) v _N (purkinje soma, nucleus – A, P, F, AV, PV)	No differences
Andersen 2004 (124)	Dentate nucleus (D) of cerebellum	8/8	V(D) N(neuron – D)	No differences
Marner 2005 (125)	Right & left: Locus coeruleus (LC)	9/10	V(LC) N(pigmented neuron – LC) v _N (pigmented neuron – LC)	Increased v _N (pigmented neuron): LC
Kreczmanski 2007 (81)	Right & left: caudate nucleus (C), putamen (P)	13/13	V(C, P) N(neuron – C, P) N _V (neuron – C, P)	Decreased V: P Decreased N: C, P
Kreczmanski 2009 (103)	Right & left: caudate	13/13	L _V (microvessel – C, P) L(microvessel – C, P) L _N (microvessel	No differences P)

1 st Author / year	Region(s)	Schiz/Cont /Other ^a	Estimates ^b	Reported significant schiz. related diff. ^c
	nucleus (C), putamen (P)		/neuron – C, P)	

^a Number of subjects in each diagnostic group: schizophrenia, control group, other diagnostic group(s). Subject numbers from several groups within same category are separated by “+” sign.

^b Stereological estimates fulfilling the inclusion criteria—see text. The cited papers may include important qualitative and quantitative findings not listed here. Estimate abbreviations: V volume; VV volume fraction; N number; NV numerical density; NN numerical fraction; S surface area; t laminar thickness; L length; LV length density; LN mean length per cell; vN mean cell volume. The relevant region(s) or cell type(s) are indicated in the parentheses using the abbreviations found in the “Region(s)” column.

^c Only differences reported as statistically significant for the schizophrenia group are listed. Abbreviations as in “Estimates” column.

^d Group of leucotomized subjects with schizophrenia.

^e Limited methods details.