deviations in the interpretation and organization of speech. In a number of studies, failures in linguistic processing have been demonstrated at the levels of semantic, syntactic and discourse structure. Schizophrenia, a condition which apparently occurs in all societies with approximately the same incidence, may best be understood as an anomaly of the function which is most characteristically human – language.

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LETTERS TO THE EDITOR

How should brain nuclei be delineated? They don't need to be!

Gahr[1] reviewed three common methods to delineate brain areas in tissue sections: the cytoarchitectural, the connectional and cytochemical delineation. He focussed on the HVC (higher vocal center) nucleus of songbirds. He showed that the cytoarchitectural, cytochemical and projection properties of the same HVC brain area change independently both during development and in adulthood, and concluded that a combination of the three delineation methods may give new insights into neural plasticity and the dynamics of brain parcelation in general.

Insight into neural plasticity, however, does not depend on delineation of brain areas, but on estimation of total neuron numbers and glial cells. Total cell numbers of any brain nucleus with circumscript boundaries can be easily determined by multiplying the mean neuronal density with the volume of the nucleus[2–6]. The nucleus volume can be estimated by Cavalieri's principle[7]: multiply the sum of the cross-sectional areas of equidistant sections throughout the whole extent of the nucleus with the intersection distance. When more than ten sections are investigated and more than 100 cells are sampled the coefficient of error of the volume is less than 5%, which is negligible to the coefficient of variation of the group mean[8,9]. Unfortunately, in Gahr's review[1] no statement on total neuron number or total volume of the HVC nucleus has (or could have?) been made.

Delineation of brain structures with indistinct boundaries, however, such as the human basal nucleus of Meynert is impossible and, consequently, so is the volume, but total neuron numbers can still be estimated in normal controls and in disease[10] by using a systematic sampling design also known as the fractionator[2,3,6,11]. Again, the coefficient of error of the estimate is below 5% (Refs 10,11).

Techniques such as Cavalieri's principle and the fractionator form part of a set of tools for obtaining quantitative information about three-dimensional structures, based

on observations made on two-dimensional tissue sections[2–7]. This integrated set of precise tools is called stereology and may measure volume, area, length and number of arbitrarily shaped, sized and orientated particles in an efficient and unbiased manner. 'Particles' means anything that can be unambiguously identified from the set of profiles produced by a section through them, for example, fibers, somata, nuclei, nucleoli, synapses, receptors. 'Efficient' means 'with a low variability after spending a moderate amount of time' and 'unbiased' means 'without systematic deviation of the true value'[2,3,8,9]. When, as in the cytoarchitectural Nissl delineation of the HVC nucleus, the boundaries are defined on a fluctuating cell-size criterion, one may not interpret this phenomenon as 'dynamics of brain parcelation'[1]. Instead, one should stop delineating, as the self-evident requirement of unambiguous identification of the cells of interest is not fulfilled any longer.

It can not be stressed enough that anyone who is involved in quantitative neuroscience should be aware of the powerful tools of stereology. Only then will true insight into neurobiologic processes, neural plasticity and clinicopathologic correlations emerge.

Oscar Vogels
Dept of Neurology,
University Hospital Nijmegen,
PO Box 9101, Nijmegen 6500 HB,
The Netherlands.

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In a thoughtful review, Gahr discusses the limitations of relying on Nissl stains when attempting to define brain nuclear boundaries for volume estimations[1]. Nissl stains do indeed reflect cell activity, but are widely used to delimit brain structures. Therefore it is important to be reminded that activity and structure do not always co-vary. Gahr illustrates this from his own work on the canary (*Serinus canaria*) song system, where he reported that the boundaries of the vocal control nucleus HVC (higher vocal center), that change seasonally when defined based on Nissl stains, do not appear to change when they are defined based on cells expressing immunoreactive estrogen receptors, or based on cells within HVC that project to another nucleus, area X. Since the publication of Gahr's influential paper on this issue in 1990, at least seven studies[2–8], from four different laboratories, concerning adult songbirds of three different species have compared the boundaries of HVC and other song nuclei using multiple measures to delineate nuclear boundaries (for example, neurotransmitter and hormone receptor autoradiography[3–5], peptide immunohistochemistry[7,8], tract-tracing methods[2,3,9] and enzyme stains[9]). These studies have compared males and females[4,5,7,8] as well as male birds in different hormonal[3], photoperiodic[6,9] and seasonal conditions[2]. All of them, with one exception[2], have found congruence between boundaries defined by Nissl stains and other markers.

Gahr[1] also suggests that there is a disagreement about the boundaries of sexually dimorphic nuclei in the preoptic region of Japanese quail (*Coturnix coturnix japonica*). In actuality, these disagreements are related to nomenclature issues, not issues of boundary delineation. A variety of histochemical markers have been identified that define the boundaries of the sexually dimorphic preoptic medial nucleus in a way that agrees with Nissl stains[10]. In the case of immunoreactive aromatase, volume reconstructions have been completed that reflect sex differences and testosterone-induced changes in concordance with studies based on Nissl-defined boundaries[11]. We agree with the main premise of Gahr's paper, that measuring nuclear boundaries with various histological markers is informative. However, we should like to stress that studies of the avian brain by a variety of investigators have clearly established that nuclear boundaries based on Nissl stains generally parcel the brain in a manner that is in agreement with other histological characterizations. Dr Franz Nissl provided us with a widely used method over 100 years ago that will continue to be useful for the identification of changes in brain functioning.

Gregory F. Ball
Dept of Psychology,
Behavioral Neuroendocrinology Group, Johns Hopkins University,
Baltimore, MD 21218, USA.

Jacques Balthazart
Laboratorie de Biochimie, Unité de Recherches en Neuroendocrinologie du Comportement, Universite de Liège,
Liège, Belgium.

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Gahr states that the borders of brain nuclei should be delineated using a combination of different methods, since different methods might provide a different judgement of where the borders of a nucleus lie[1]. Although we are in agreement with this basic thesis, we disagree strongly with Gahr's claim that delineation of the telencephalic nucleus HVC (higher vocal center) in songbirds changes depending on whether cytoarchitectural, cytochemical or projection properties of HVC neurons are used as the criterion for judging the borders of this nucleus. We and others have demonstrated that HVC clearly changes volume as a function of season or hormone treatment, regardless of whether the borders of HVC are judged by the distribution of Nissl-stained neurons, estrogen- or androgen-accumulating cells, neuropeptide expression, neurotransmitter enzymes and receptors, or projection neurons within HVC (Refs 2–8). Thus, although Gahr stresses that Nissl staining can give a different picture of the borders of HVC relative to these other methods based on his own work, he does not cite the numerous studies that have failed to replicate this result.

Altnough the borders of HVC are apparently not different for different staining criteria, the borders of another song-control nucleus are. Once again, however, relevant papers are not cited by Gahr. Thalamic inputs to the telencephalic song-control nucleus IMAN (lateral magnocellular nucleus of the anterior neostriatum) define a region different from what we (and others) had originally delineated as the borders of this nucleus based on Nissl criteria[9]. However, the original finding that IMAN undergoes substantial regression was supported by the demonstration that