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Stereological approaches to dementia research using human brain tissue

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ABSTRACT

The relationship between the clinical features of dementia disorders and the resultant changes in underlying neuropathological mechanisms has long been of interest to researchers working in the field of neurodegenerative disorders. The majority of neuropathological research in dementia has utilized semi-quantitative analysis of protein inclusions, which have defined the hallmark histological features of the conditions. However, the advent of three-dimensional stereological techniques has enabled unbiased and fully quantitative assessment of brain tissue. The present review focuses on studies that have used these techniques to elucidate important relationships between neuropathological changes and clinical features and, in doing so, revealed important mechanistic insights into the pathophysiology of dementia disorders.

1. Introduction

Understanding the neuropathological correlates of the neurodegenerative dementias has remained a subject of intense interest and scrutiny over many decades. Neurodegenerative processes involve cascades of pathological events that culminate in the progressive and irreversible dysfunction and destruction of neurons and synapses, spreading in a stereotypical fashion through regions of the brain, defining its clinical presentation (Jellinger, 2001, 2012). The spectrum of dementing neurodegenerative disorders are usually bound by a common pathological factor, namely the presence of aberrant misfolded intra- and extracellular deposits of native proteins (Hardy and Gwinn-Hardy, 1998; Hyttinen et al., 2014). The neuron populations and brain areas affected are generally defined by the protagonist protein, thus characterizing clinical symptomatology (Taipa et al., 2012).

In large, the findings that have defined the major neuropathological markers associated with neurodegenerative dementias have stemmed from semi-quantitative 'two-dimensional' methods. Indeed, all major staging criteria in neurodegenerative dementias recommend single section sampling of markers in specific anatomical regions deemed vulnerable to pathological changes in the brain and brainstem (Braak and Braak, 1995; Montine et al., 2012; Thal et al., 2002; Braak et al., 2004; Mckeith et al., 2005). Whilst this method is widely used the classification of diseases in post-mortem tissue, this approach is undermined by factors arising from bias in the interpretation of three-dimensional structures and markers on two-dimensional sections taken from one part of a particular reference area. Stereological analysis, on the other hand, describes a methodological approach that provides a three-dimensional interpretation of structures based on observations made on two-dimensional sections, representing the 'gold standard' for the unbiased assessment of the structural components of the brain. This means that the strict sampling protocol that stereology requires is often at odds with the need for the rapid and efficient classification of cases, where a balance must be met between diagnostic and research requirements.

Despite the difficulties in incorporating stereological protocol into analysis of pathological changes, the inherent benefits of the approach have encouraged researchers to seek out the often subtle changes to the human brain across the various dementing disorders. This review draws together a plethora of diverse stereological studies that have mapped morphological changes in the brain, such as in neuronal populations or vascular integrity, with ante-mortem clinical and/or post-mortem pathological correlates.

2. The benefits of stereological analysis in post-mortem human brain tissue analysis

The problems arising from two-dimensional analysis, including issues surrounding the 'reducing fraction', widely used 'correction' formulae, such as that devised by Abercrombie, and the 'reference

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trap', arising from the use of density as a proxy measure of number, have been well documented elsewhere (Clarke, 1992; Hedreen, 1998) and beyond the scope of this review. However, despite the inherent difficulties in conducting three-dimensional analysis in post-mortem human brain tissue, well-designed and executed stereological studies have been performed. The findings emanating from such studies have yielded novel, interesting and, importantly, accurate, findings in field of dementia research.

A properly conducted design-based stereological study should be free of methodological ambiguity, requiring meticulous planning and preparation prior to commencing any experiment. Firstly, a clearly defined reference volume (V_{ref}) should be delineated for sampling in its entirety. The entire reference space can be systematically uniformly randomly sampled in the *z*-axis within serially sectioned structures or from blocks of tissue of a defined thickness. In the case of larger structures, such as neocortical regions, blocks of tissue may be acquired and sections taken at precisely defined intervals (e.g. 5 mm–1 cm) (Fabricius et al., 2013; Pelvig et al., 2008). For smaller structures, such as brain nuclei or glands, serial sectioning may be more appropriate. In both cases, a uniform, random starting point should be selected within the first interval, followed by a complete series of sections at equally spaced intervals through the entire V_{ref}. Uniform, systematic sampling should also be applied to the V_{ref} on sections in the *x* and *y* axes, through the use of a probe relevant to the parameter measured of a specific size and depth, placed randomly and uniformly through the entire reference space at intervals, reflecting the desired level of accuracy of the population estimate from taking coefficient of error (CE) levels into account. The V_{ref} may be calculated using Cavalieri's formula:

$$V = T \times a(\mathrm{p}) \times \sum P,$$

where *T* is the slab thickness or intersectional distance, $a(\mathrm{p})$ is the area per point, and $\sum P$ is the sum of points hitting the reference area.

Using the V_{ref}, calculated from Cavalieri's formula, one may then estimate the total number, length or surface area of objects per V_{ref} using a suitable probe. Most of the studies included in this review have used the optical disector approach (Gundersen et al., 1988) for the calculation of total neuron number, which uses the following formula:

$$N_v = \frac{\sum^{p-} Q^-}{P \times V}$$

where N_v is the numerical density, $p-$ is the disector samples, Q^- is the *Q*-weighted number of objects counted, *P* is the total number of disectors, and *V* is the disector volume.

A number of studies reviewed have also employed the physical disector approach (Sterio, 1984) to calculate synapse (Scheff et al., 2013, 2011) and neuron (Pakkenberg et al., 1991) number. Here, pairs of thin sections are taken at intervals through the V_{ref} and the objects observable in the first, but not second, section of the pair are counted. This approach is useful when objects are large relative to section thickness, lending itself particularly well to images acquired from electron microscopy (Scheff et al., 2013, 2011).

Alternatively, some studies reviewed (Joelving et al., 2006; Piguet et al., 2011) have employed the optical fractionator (Gundersen et al., 1988). Here, the total number of objects can be calculated by combining the optical disector with a systematic uniform sampling scheme, the fractionator. The optical fractionator estimates the total number of particles (*N*) of the V_{ref} obtained by multiplying the reciprocals of the fractions with the total particle count ($\Sigma Q-$) per brain structure obtained within the optical disectors:

$$N = \mathrm{ssf}^{-1} \times \mathrm{asf}^{-1} \times \mathrm{tsf}^{-1} \times \sum Q^{-1},$$

where ssf is the section sampling fraction, asf is the sampling fraction, tsf is the thickness sampling fraction and $\sum Q^-$ is the total number of objects counted within the disector.

As most post-mortem tissue is cut in a non-random fashion in a vertical, uniform random manner, length and surface area measures could potentially be biased as a result of their orientation in the brain. For example, in the measurement of neuronal white matter tracts, the relationship between probe and the structure may be highly anisotropic, leading to inaccurate estimates of length. As such, reviewed studies examining the length of structures, e.g. neuropil threads (Giannakopoulos et al., 2007) have employed an intrinsically isotropic probes such as the cycloid or spherical 'space ball' (Mouton et al., 2002) probes.

3. Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent form of age-related dementia, accounting for approximately 70% of cases (Reitz and Mayeux, 2014). Clinically, AD is marked by memory loss and impaired reasoning with a gradual onset and a progressive course (Mckhann et al., 2011). Neurofibrillary tangles of phosphorylated tau protein within neurons and extracellular plaques consisting of amyloid-β protein are considered to be the characteristic neuropathological findings in AD upon post-mortem histological examination (Montine et al., 2012). Neurofibrillary tangles and amyloid-β plaques are encountered at pathological predilection sites at early and pre-symptomatic stages, prior to stereotypical sequences of accumulation and deposition in vulnerable brain regions (Stratmann et al., 2015)

The hallmark pathological lesions in AD are also typically found in the elderly non-demented (Tomlinson et al., 1968; Price et al., 2009; Von Gunten et al., 2010; Schneider et al., 2009), which has prompted speculation that AD is linked to senescent mechanisms in the brain (Herrup, 2010; Yankner et al., 2008). To investigate the link between senescence and AD, stereological studies have evaluated patterns of neuronal loss with aging in the absence of dementing symptoms, and compared these findings to neuronal loss typically observed in AD. West et al. (1994) used the point counting method and optical disector probe to estimate neuronal number in subregions of the hippocampal formation and found age-related reductions in neurons in the CA4 and subiculum in controls ranging from 13 to 101 years. However, CA1 neuronal loss was found to be distinct to AD cases and not found in normal aging cases, suggesting specific patterns of neuronal loss in AD and that progressive aging alone cannot fully explain the pathological changes of AD (West et al., 1994).

Several staging schemes have illustrated the pathological progression of the characteristic lesions of AD (Braak et al., 2006; Thal et al., 2002; Josephs et al., 2014). Stereological designs have been used to study sites that are affected at early stages of the pathological process, often by comparing cases that had different levels of cognitive ability prior to death. These studies have often benefitted from excellent clinical information that can be compared to any observed cytoarchitectonic changes that may occur in tandem with cognitive dysfunction. However, some degree of caution must be exercised when comparing clinical information obtained during life with post-mortem findings. AD is marked by progressive deterioration meaning that control of the interval between the last clinical assessment and death is of great importance.

Assessment of whole brain hemispheres at systematic intervals from slabs using region point counting with the Cavalieri principle to determine volume of the neocortex and central grey matter (basal ganglia, thalamus, hypothalamus and substantia