The commentaries by Barnes [1], Geinisman [5], and Smith et al. [8] reflect the changing landscape in research on the neurobiological and cognitive effects of aging. All agree on several points. Frank neurodegeneration, which was formerly thought to cause cognitive impairment, does not occur among the principal neurons of the hippocampal formation. Instead, other changes within this circuitry are candidate substrates of brain aging. A current emphasis on the structural and functional integrity of synapses signals a shift away from a prior focus on neuron loss that has fundamental implications for understanding the process of brain aging. Further, all agree on the need for behavioral assessment as a background for neurobiological studies to determine the cognitive status of subjects in the study population.

Ultrastructural studies provide the most definitive assessment of structural change in synapses. Geinisman evaluates the available data on the aged hippocampus and finds that the results are largely inconclusive. The application of appropriate ultrastructural methods for determining synapse number using sufficient samples of behaviorally characterized subjects is needed to provide a solid quantitative basis for judging whether synaptic connections are preserved or diminished. His commentary, along with that of Barnes, also raises the broad issue of whether changes in synaptic function can be adequately captured by anatomical investigations. We fully agree with these cautionary comments. Our view, which we are confident is shared by the commentators, is that a comprehensive definition of the effects of aging on hippocampal circuitry will require multiple methodological approaches.

As noted by Geinisman, the careful application of methods for ultrastructural quantitation could fail to detect alterations in specific receptor proteins at synapses that lead to changes in functional connections, i.e., silent synapses. Barnes points to another example where anatomical studies alone provide limited information about the functional status of a circuit. Her physiological studies have shown that a decrease in synaptic input to the dentate gyrus from the entorhinal cortex in aged rats [1,3] is accompanied by increases in the strength of existing connections. We could add to these examples other findings from our own research. For example, we reported that the levels of metabotropic glutamate and muscarinic cholinergic receptor proteins in hippocampus are not different between young and aged rats or altered as a function of learning ability [4,6]. However, these receptors do not stimulate phosphoinositide turnover to the same extent in the aged rats as compared to the young rats, and the aged rats with the more severe learning deficits were those with more blunting of phosphoinositide turnover. That finding is consistent with other electrophysiological evidence that the response of aged hippocampal neurons to cholinergic stimulation is blunted [7] and further appears to indicate that the defect exists in the biochemical properties of cells that might not be reflected in morphological measures of synaptic connectivity.

Although functional studies are undoubtedly important, all would agree that such studies are informed by evidence concerning structural integrity or a lack thereof. The approach used in our report, while providing a method for assessing large global losses in synaptic integrity (a 50% loss in synaptophysin immunoreactivity has been observed in the hippocampus of AD patients [9]), falls far short of the detailed anatomical analysis that is ultimately needed. Our more limited goal was to assess whether any detectable loss of synaptic markers, using three different target proteins, would be evident in a rigorously quantitative assay using an ample number of young and aged behaviorally characterized rats. Acknowledging the limitations of this approach, the data support the interpretation that there is no substantial overall change in synaptic markers in the hippocampus of the aged rat, with or without cognitive impairment.

Our method of using protein extracted from the total hippocampus could indeed obscure changes limited to a circumscribed region of this structure, e.g., decreases confined to dorsal hippocampus as noted by Geinisman or decreases confined to a specific connectional zone. The
benefits of maintaining anatomical resolution are clearly
illustrated by Smith et al. describing their recent study that
examined synaptophysin immunoreactivity using quanti-
tative confocal scanning microscopy [8]. When the anato-
mical subregions within the hippocampus were separately
analyzed, changes were found with respect to age and cog-
nitive status. Most notably, only the immunoreactivity in
zones of the dentate gyrus molecular layer and the CA3
region that receive innervation from layer II neurons of
entorhinal cortex was significantly correlated with the se-
verity of cognitive impairment in the aged rats. At the same
time, it is notable that they found no effect of age and no
relationship with learning impairment when their data were
collapsed across all hippocampal regions (somewhat com-
parable to immunoblotting of total hippocampal protein).
Given that the current report and the study by Smith et al.
used the same study population of aged animals with a
common behavioral characterization, the complementary
methods provide largely converging results. Both methods
indicate that widespread loss of synaptic markers is not
evident but that more regionally localized changes do exist.
It would be informative to evaluate other markers such as
those used in our study with the methods of Smith et al.; a
decrease in synapse numbers in a connectional zone would
be expected to concomitantly decrease a number of different
synaptic proteins. Such studies, notwithstanding their short-
comings, may converge to provide a useful guide for ultra-
structural analysis. As noted by Geinisman, a major chal-
lenge in ultrastructural studies is selection of appropriate
areas for analysis. Thus, it would be of interest to assess the
prediction that effects of aging on synaptic integrity would
be greatest in those regions where synaptic markers were
decreased relative to comparison regions where no changes
were observed.

In closing this discussion, we think it is important to note
that no disagreement exists in the commentaries about the
utility of the animal model that is the subject of these
investigations. The pioneering work of Barnes [2] indicated
that assessment of spatial cognition in rats could provide a
sensitive window into aging of the hippocampal system.
The rapid development of neurobiological methods and
findings from basic research on the hippocampal system are
being incorporated into the use of this animal model, which
continues to provide one of the most informative settings for
research on cognitive aging.

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