TECHNICAL COMMENT

NEUROSCIENCE

Comment on “Principles of connectivity among morphologically defined cell types in adult neocortex”

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(Research Article, 27 November 2015, aac9462) describe detailed experiments that substantially add to the knowledge of cortical microcircuitry and are unique in the number of connections reported and the quality of interneuron reconstruction.The work appeals to experts and laypersons because of the notion that it unveils new principles and provides a complete description of cortical circuits.We provide a counterbalance to the authors’ claims to give those less familiar with the minutiae of cortical circuits a better sense of the contributions and the limitations of this study.

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There is no doubt that the data described in Jiang et al. (1) will be a valuable source for descriptions of connectivity between defined neurons.

Some of the authors' observations are new, some confirm previous observations, and some are at odds with previous studies. Because no single method used to identify cortical connections (such as paired recordings used by Jiang et al.) can provide a definitive description of the connectivity between specific identified cell types, the observations that conflict with previous results must be further explored. The limitations of this study must be recognized, and further experiments must be conducted to unambiguously resolve these issues.

Because Jiang et al. present a large and complex data set and the literature on cortical circuits is very extensive, it is beyond the scope of this Comment to delve into all of the issues that are raised. We highlight some key limitations of the study, as well as limitations of other studies, to exemplify what challenges lie ahead. The primary method used was to simultaneously record intracellularly fromup to eight neurons in cortical brain slices. Each cell is sequentially activated while recording from others to test for possible connections. By recording from many neurons at once, there is a combinatorial explosion in the numbers of cell pairs that can be sampled, allowing for the generation of a very large sample (more than 11,000 pairs in Jiang et al.). However, many connections are cut during the preparation of brain slices. The effects can be enormous and depend on slice thickness, the distance of recorded cells from the slice surface, and the distances between recorded cells (2). All of these parameters were biased toward connection loss—slices were thin, cells were close to the surface (within 15 to 60 mm), and to fit eight electrodes in the recording space, sampled cells were far apart. These factors result in a failure to detect connections and, accordingly, the paper detected no connections whatsoever between layer 5 excitatory neurons and extremely few between layer 2/3 excitatory neurons (only 1.8% connectivity rate). These numbers contrast with numerous published studies, and the authors suggest that other studies used slices from immature animals and that connections are eliminated as animalsmature. The authors fail to cite or address previous studies from adult animals [e.g., (3)] in which connections were assessed using sharp electrode recordings (which sample deeper cells) and thicker slices that revealed connection rates comparable to those in younger animals (typically at least 10 to 20% connected pairs).

Moreover, not all connections are cut equally, and the loss of connectivity depends on the trajectory of the axon of different cell types and the plane of sectioning, as well as laminar location and distance (2). Therefore, the connectivity matrix reported by Jiang et al. is likely scaled down and distorted.

This is just one example of a discrepancy with published studies and a single example of a limitation of the methods used; not all discrepancies can be so easily resolved based on the published literature. Other limitations include the use of current clamp to measure synaptic responses, which will bias conclusions of connectivity depending on the input resistance of the postsynaptic cells and will favor large connections, and the exclusive usage of potassium gluconate–based intracellular solution, which is associated with severe space-clamp problems (4). Further experiments using complementary approaches and direct comparisons across ages will be necessary to resolve discrepancies between this study and the published literature.

Concerning the identification and classification of cell types, community efforts to arrive at a unified nomenclature (5) have not resulted in complete agreement. However, there is consensus that multiple parameters must be measured and used. Jiang et al. defined their cells primarily based

onmorphological reconstructions. The computational methods for classification were not confirmed with external validators provided by different methods. This is necessary because, by definition, any classification algorithm used will generate a classification, regardless of whether it is real.

Using these methods, the authors claim to have discovered several “new cell classes,” but it is not clear that their groupings really represent distinct groups or are new. They imply, for instance, that, other thanMartinotti cells, no other interneuron types were previously described in layer 5. This is at odds with publications reporting vasointestinal peptide (VIP) bipolar interneurons in layer 5 [e.g., (6) and earlier references therein] and the studies on fast-spiking parvalbumin (PV) basket cells in this layer [recently reviewed by (7)]. The authors redefine and rename interneuron types resulting in claims of novel cell classes and improper acknowledgment of much of the literature. For instance, their new cell types in layer 5—including the shrub cell (SC), the horizontally elongated cell (HEC), and the VIP-expressing bitufted cell (BTC)—appear to correspond to previously described interneurons: (i) SCs resemble small and nest basket cells (8); (ii) HECs resemble layer 5a fast-spiking basket cells that have an axonal arbor that is horizontally focused in the narrow layer 5a [e.g., (9, 10)]; and (iii) the VIPexpressing BTCs likely correspond to the VIP bipolar interneurons previously described in layers 2 to 6 (6). A more careful comparison to the cell types described in the literature and analysis should be performed before it is concluded that a new cell type has been discovered. Last, given the large sample presented by Jiang et al., it is surprising that several well-documented interneuron types, such as cholecystokinin (CCK) basket cells and g-aminobutyric acid–releasing (GABAergic) projection cells (11, 12), were missed, although their representation is likely much higher than 1 in 1000. This suggests that the selection of types to categorize and illustrate might have been biased.

Contrary to the authors’ claim that they have provided “the most complete wiring diagram of neocortical microcircuits to date,” their description is incomplete, partly arbitrary, and also not definitive. The completeness of their description does not rival the accumulated knowledge from decades of studies on cortical connectivity using a diverse range of complementary and powerful techniques. It ignores cortical layers 4 and 6 and focuses largely on the diversity of inhibitory neurons in layers 2/3 and 5, with little consideration for the diversity of excitatory neuron types, and there is no consideration of gap-junction coupling, a major feature of the connectivity between interneurons. We hope that our colleagues will recognize that this study adds to a growing body of knowledge about the cell types and connectivity of the cerebral cortex, but we are far from finished.

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