Journal Club

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Illuminating Cholinergic Microcircuits in the Neocortex

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Review of von Engelhardt et al. (http://www.jneurosci.org/cgi/content/full/27/21/5633)

Distinct subpopulations of neocortical interneurons are critical for coordinating ensemble of neuronal activities. However, their diverse morphological and electrophysiological properties have hindered a full understanding of their respective role in cortical microcircuits. With the notable exception of glutamatergic stellate cells, interneurons are mostly GABAergic. However, a subpopulation of these cells also express choline acetyl transferase (ChAT). Presently, it is not clear whether ChAT+ interneurons release acetylcholine (ACh). Although the effect of locally released ACh remains enigmatic, cortical neurons presumably receive a cholinergic innervation from locally projecting ChAT+ cells as well as the subcortical nucleus basalis magnocellularis. To better understand the role of intracortical cholinergic neurons in neocortical circuitry, von Engelhardt et al. (2007), in their recent article in *The Journal of Neuroscience*, characterized ChAT+ cortical neurons. The authors used a reporter gene approach based on a modified bacterial artificial chromosome (BAC), and generated a transgenic mouse line expressing the enhanced green fluorescent protein (EGFP) under the control of the ChAT promoter [von Engelhardt et al. (2007), their Fig. 1 (http://www.jneurosci.org/cgi/content/full/27/21/5633/F1)]. EGFP+ cells were found only in brain areas known to contain cholinergic neurons such as the nucleus basalis and pedunculopontine tegmental nucleus [von Engelhardt et al. (2007), their Fig. 2 (http://www.jneurosci.org/cgi/content/full/27/21/5633/F2), Table 1 (http://www.jneurosci.org/cgi/content/full/27/21/5633/T1)]. In the neocortex, most EGFP+ neurons had a bipolar morphology with their somata in layer II/III, although there were some multipolar EGFP+ cells in deeper layers. ChAT immunofluorescence revealed that, in brain areas showing strong ChAT immunoreactivity such as in spinal cord, >96% of EGFP+ cells also express ChAT. In contrast, in the neocortex where cells exhibited lower ChAT immunoreactivity, 74% of EGFP+ neurons were ChAT+ and only 42% of ChAT+ neurons also expressed EGFP. This discrepancy may be attributable to the lack of some genetic regulatory elements in the BAC. Because ChAT is expressed in some cortical interneurons (Bayraktar et al., 1997; Cauli et al., 1997), the authors investigated the expression of interneuronal markers in cortical EGFP+ cells. Ninety-five and 77% of EGFP+ neurons were vasoactive intestinal peptide (VIP) and calretinin (CR) immunoreactive, respectively [von Engelhardt et al. (2007), their Fig. 3 (http://www.jneurosci.org/cgi/content/full/27/21/5633/F3)]. Parvalbumin, cholecystokinin, somatostatin, and calbindin immunoreactivity were found in a lower percentage of EGFP+ cells (<14%).

Next, the authors examined the morphological and electrophysiological properties as well as the synaptic connectivity of bipolar EGFP+ cells. These cells typically exhibited vertically oriented dendritic and axonal arbors with axons extending mainly to layers IV and V [von Engelhardt et al. (2007), their Fig. 6 (http://www.jneurosci.org/cgi/content/full/27/21/5633/F6)]. Although a subset of these cells exhibited a regular firing pattern after threshold current injection, the majority showed a "stuttering firing" profile (i.e., clusters of action potentials intermingled with unpredictable periods of silence). These findings are reminiscent of previous reports describing an irregular firing profile in vertically oriented bipolar interneurons that coexpressed VIP, CR, and ChAT (Cauli et al., 1997; Porter et al., 1998). Whole-cell paired-recording stimulation of neighboring pyramidal neurons and interneurons evoked EPSPs and IPSPs in 8 and 15% of EGFP+ cells, respectively. Surprisingly, neither cholinergic postsynaptic potentials nor changes in excitability were observed in pyramidal cells or interneurons after firing of nearby EGFP+ cells [von Engelhardt et al. (2007), their Fig. 7 (http://www.jneurosci.org/cgi/content/full/27/21/5633/F7)]. In fact, only two putatively postsynaptic interneurons of 226 tested cells (including pyramidal cells) exhibited IPSPs after firing of EGFP+ cells. Although this result is consistent with the

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lack of GAD67 in bipolar EGFP+ cells [von Engelhardt et al. (2007), their Fig. 4 (http://www.jneurosci.org/cgi/content/full/27/21/5633/F4)], previous studies in rats demonstrated coexistence of GAD67, GAD65, and GABA with ChAT in bipolar VIP+ interneurons (Bayraktar et al., 1997; Cauli et al., 1997; Porter et al., 1998). The absence of a cholinergic phenotype is quite unexpected given the numerous cholinergic effects described previously. Although a defect in the maturation of the cholinergic signaling or an abnormally high acetylcholinesterase activity in acute slices provide possible explanations, the results suggest that pyramidal cells and GABAergic interneurons may not be the main target of ChAT+ interneurons. However, GABAergic VIP+ interneurons are known to be activated by nicotinic agonists (Porter et al., 1999). Thus, ChaT+ neurons themselves may be one possible target of EGFP-ChAT+ neurons (Fig. 1), although these cells also receive cholinergic input from the basal forebrain (Cauli et al., 2004). Paired recording between EGFP+ cells would help clarify this issue and determine whether EGFP+ cells are electrically connected, as has been shown for some bipolar interneuron populations (Venance et al., 2000; Hestrin and Galarreta, 2005). Endothelial cells that express muscarinic receptors are an additional potential target of EGFP+. Indeed, blood vessels receive innervation from VIP+ interneurons and cholinergic neurons from nucleus basalis (Cauli et al., 2004) (Fig. 1). ChAT+ neurons would therefore be ideally situated to regulate cerebral blood flow in response to neuronal activity.

Although no direct synaptic partner of ChAT+ cells was established, several experiments support the release of ACh by EGFP+ neurons. Specifically, activation of EGFP+ neurons produced a small but significant increase in the frequency of spontaneous EPSCs (sEPSC) in pyramidal cells during EGFP+ cell firing [von Engelhardt et al. (2007), their Fig. 8 (http://www.jneurosci.org/cgi/content/full/27/21/5633/F8)]. In contrast, the amplitude of sEPSCs remained constant, as did the frequency of sIPSCs. The sensitivity of sEPSC increase to hexamethonium, a blocker of nicotinic receptors, suggests that ACh released by EGFP+ neurons enhanced glutamate release through the activation of nicotinic receptors.

Although, the authors focused exclusively on local inputs to and outputs from cholinergic neurons, extracortical input to ChAT+ neurons would also be worth examining. Bipolar VIP+ interneurons receive input from thalamocortical neurons (Staiger et al., 1996) and intracortical ChAT+ cells are innervated by serotoninergic neurons from the raphe nuclei (Cauli et al., 2004). Thus, intracortical cholinergic neurons may play a crucial role in the integration of subcortical and intracortical pathways. The transgenic mouse model developed by von Engelhardt et al. (2007) provides a powerful tool to study the role of ChAT+ interneurons in cortical networks. Clearly, additional work is necessary to establish such an integrative role of intracortical cholinergic interneurons; however, the findings of von Engelhardt et al. (2007) represent a stepping stone in illuminating the cholinergic functions in cortical information processing.

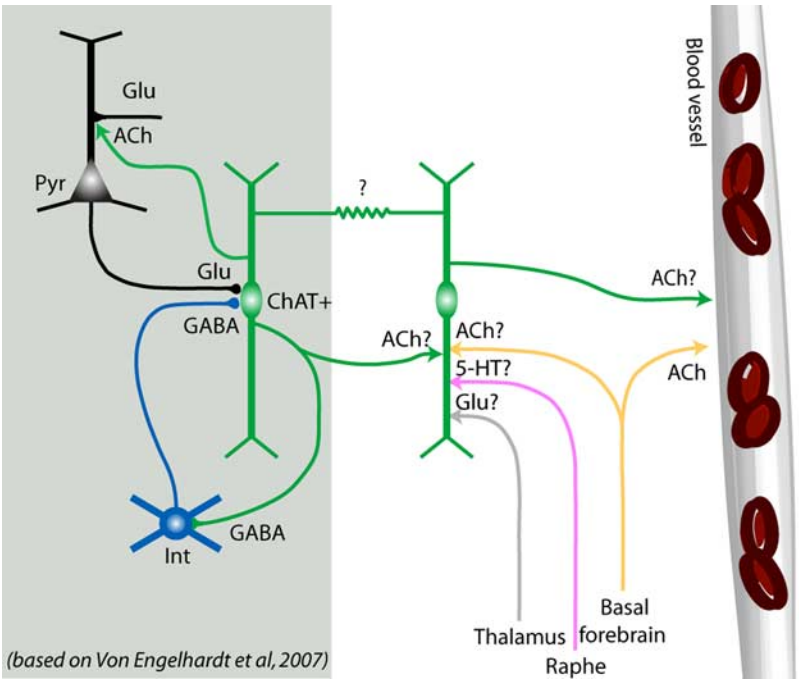

Figure 1. Cholinergic interneurons in the neocortical network. Scheme showing connections between EGFP+–ChAT+ neurons (green) with pyramidal cells (Pyr; black) and other interneurons (Int; blue) as reported by von Engelhardt et al. (2007) (gray box). Input and output of VIP+ neurons that may also apply to ChAT+ neurons are also represented (white background) with the putative connections labeled with question marks. The jagged line between two EGFP+ neurons represents electrical coupling.

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