Into Great Silence without VGLUT3

Gudrun Ahnert-Hilger[1] and Reinhard Jahn[2,*]
[1]AG Functional Cell Biology, Institute for Integrative Neuroanatomie, Center for Anatomy, Charité-Universitätsmedizin Berlin, 10115 Berlin, Germany
[2]Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, 37077 Göttingen, Germany
*Correspondence: rjahn@gwdg.de
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The vesicular glutamate transporters VGLUT1 and VGLUT2 fill synaptic vesicles with glutamate, an essential prerequisite for glutamatergic transmission in the CNS. In contrast, the third isoform, VGLUT3, is not confined to glutamatergic neurons, and its function has remained enigmatic. In this issue of *Neuron*, Seal et al. show that mice lacking VGLUT3 are profoundly deaf and exhibit nonconvulsive seizures.

Synaptic vesicles store neurotransmitters and release them by Ca^{2+}-dependent exocytosis upon stimulation. Transmitter loading of synaptic vesicles is achieved by vesicular transmitter transporters that utilize the proton electrochemical gradient across the vesicle membrane as an energy source. For glutamate, the major excitatory transmitter in the mammalian CNS, three structurally related vesicular transporters have been discovered, termed VGLUT1, VGLUT2, and VGLUT3 (Edwards, 2007). VGLUT1 and -2 are confined to bona fide glutamatergic neurons and have a largely complementary distribution that appears to cover virtually all glutamatergic neurons in the mammalian CNS. Knockouts of either VGLUT1 or VGLUT2 lead to a loss of glutamatergic transmission from the affected neurons, thus establishing these VGLUTs as the key determinants for a glutamatergic phenotype. Not surprisingly, the deletion mutants suffer from severe pathological symptoms (Fremeau et al., 2004; Wojcik et al., 2004) or die at birth (Moechars et al., 2006; Wallen-Mackenzie et al., 2006).

VGLUT3, however, does not fit into this scheme. While it resembles the other VGLUTs in its ability to transport glutamate, it is mainly expressed in neurons that are traditionally thought to be specific for other classical (i.e., nonpeptide) transmitters, such as serotonin, dopamine, GABA, and acetylcholine (Fremeau et al., 2004). Apparently, there are only very few neurons of unclear function that utilize VGLUT3 as their exclusive vesicular transporter, and thus the physiological role of this transporter has hitherto remained enigmatic.

In this issue of *Neuron*, Seal and coworkers (Seal et al., 2008) have established a mouse mutant lacking VGLUT3, with surprising results. These mice are deaf due to a loss of transmission between the sensory *hair* cells in the inner ear and the afferent sensory neurons, establishing VGLUT3 as the essential vesicular transporter in these cells. Furthermore (and equally fascinating), they find that VGLUT3-deficient mice exhibit nonconvulsive seizures, suggesting a modulatory role of VGLUT3 in the network function of interneurons.

The inner hair cells of the organ of Corti are the genuine sensory cells of the inner ear, which ultimately convert sound into auditory nerve activity. They respond to mechanical stimuli by the opening of stretch-activated ion channels, resulting in depolarization. As is typical for neurons releasing transmitter in response to graded potentials, inner hair cells possess ribbon synapses. Depolarization causes influx of Ca^{2+} ions, which leads to the release of glutamate via exocytosis of synaptic vesicles. Glutamate then activates AMPA receptors on the dendrites of the postsynaptic bipolar sensory neurons (for review see Moser et al., 2006; Ottersen et al., 1998).

Outer hair cells also depolarize upon mechanical excitation and use glutamate as transmitter (Ottersen et al., 1998). However, in contrast to inner hair cells, they receive only few afferent contacts, and the function of afferent signaling is unclear. Outer hair cells primarily serve as mechanical cochlear amplifier (reviewed by Fettiplace and Hackney, 2006). Their function can be conveniently assayed by recording the otoacoustic emissions in the outer ear canal. VGLUT3 knockout mice are completely deaf, although the organ of Corti and its neuronal connections are perfectly developed, and the function of the outer hair cells is not impaired. Seal et al. show that it is exclusively the inner hair cells that are affected, in line with the observation that in the inner ear VGLUT3 is exclusively expressed in these cells.

VGLUT3 extends the list of proteins needed for sensory signal transduction in the inner ear. Deafness due to loss of transmitter release from inner hair cells is also observed in mice lacking otoferlin, an integral membrane protein carrying six calcium-binding C2 domains (Roux et al., 2006). Like VGLUT3, expression of otoferlin is mostly confined to inner hair cells in the organ of Corti. Otoferlin resides on synaptic vesicles and interacts with neuronal SNAREs in a Ca^{2+}-dependent manner, resembling the neuronal Ca^{2+} sensors synaptotagmin I and II. Thus, it is possible that otoferlin functionally substitutes for synaptotagmins (that are absent from inner hair cells) and serves as the primary Ca^{2+} receptor that couples depolarization-induced influx of Ca^{2+} to rapid exocytosis of synaptic vesicles (Roux et al., 2006). Transmitter release from inner hair cells is also perturbed (but not abolished) in mouse mutants lacking the central portion of the ribbon-associated protein bassoon. Bassoon is necessary to anchor the ribbons to the active zone (Khimich et al., 2005). Similar to many additional genes shown to be required for inner ear function, however, bassoon expression is not confined to the organ of Corti but rather is

a general component of active zones in CNS synapses.

While the deafness of the VGLUT3 KO mice is due to the essential role of VGLUT3 in loading synaptic vesicles of the inner hair cells with neurotransmitter, their seizures cannot be easily explained by a primary defect in subsets of glutamatergic neurons. Rather, it is possible that this phenotype is effected by the loss of VGLUT3 from neurons whose primary transmitter is different from glutamate.

For many years it was assumed that a single neuron only releases a single neurotransmitter (referred to as "Dale's principle"). During the last decades it was recognized that the principle couldn't be maintained in a strict sense. It is well established that neuropeptides are coreleased with many classical transmitters and, furthermore, that ATP is released together with acetylcholine and monoamines from the same vesicle pool. More recently, corelease of GABA and glycine from the same neuron has been established (Jonas et al., 1998), which is explained by the fact that both amino acids use the same vesicular transporter (VGAT) (see Edwards, 2007, for review) . However, it is highly controversial whether the same neuron can simultaneously release excitatory and inhibitory neurotransmitters under physiological conditions (see e.g., Gutierrez and Heinemann, 2006; Uchigashima et al., 2007). Thus, the key question is whether expression of VGLUT3 in inhibitory neurons signifies corelease of glutamate and GABA, particularly when considering that such corelease has been documented in cultured GABAergic neurons expressing VGLUT1 (Takamori et al., 2000).

VGLUT3 KO mice display interictal activity and rare seizures. At present, it is unclear which of the various neuronal populations expressing VGLUT3 are primarily responsible for this phenotype. Intriguingly, these seizures are reminiscent of the epileptiform activity observed under certain pathological conditions, which involves hypersynchronization of GABAergic interneurons (D'Antuono et al., 2004). Thus, it is conceivable that the seizures are caused by the lack of glutamate corelease from subpopulations of GABAergic interneurons.

Presently, one can only speculate about the function of coreleasing an excitatory and inhibitory transmitter from the same neuron. If both transmitters were released from the same synapses and activating ionotropic receptors, the result of this direct antagonism would be a major reduction in input resistance, effectively short-circuiting the membrane. However, the rules of the game may be more similar to those governing the corelease of peptides and classical transmitters. Thus, it is possible that (1) the sites of release are not identical, i.e., that the transmitters are released from different subsets of synapses or from dendrites and synapses, respectively; (2) receptors exhibit different affinities or are differentially distributed at the pre- and postsynaptic membrane, respectively; (3) the receptor types involved are different, e.g., one transmitter activating slow metabotropic, the other one fast ionotropic receptors, thus avoiding direct antagonism. All of these mechanisms would add another layer of regulation in the fine-tuning of neuronal networks. It is to be expected that further analysis of the VGLUT3-deficient mice will be instrumental in shedding new light on these fascinating questions.

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