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Kissing or fused since some time

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I am particularly interested in the data published by Brown et al. (2010) in their paper entitled "Vesicular release of glutamate utilizes the proton gradient between the vesicle and the synaptic cleft". The paper presents a decrease in transmitter output when the extracellular pH is acidified. The authors interpret their observation in terms of interference with the pH gradient from the vesicular lumen and the bulk extracellular medium when the vesicles are assumed to be "kissing", which is when vesicles are believed to open to the extracellular milieu without completely fusing with the presynaptic plasma membrane. However, vesicular kisses have been seriously challenged (Chen et al., 2008; Granseth et al., 2009) and another interpretation may be drawn from the data.

Exocytosis was originally proposed to support the brevity of presynaptic transmitter output that account for postsynaptic transient responses. Indeed fast freezing electron microscopy and membrane labeling techniques demonstrated that exocytosis is involved in presynaptic activity, hence, the opening of the vesicular lumen is now automatically assumed to be the mechanism that releases the transmitter. However, membrane labeling techniques fail to show as many vesicular fusions than there are elementary transmission recorded. Consequently it is commonly assumed that the pore may also open only transiently without the vesicle fusing according to the "kiss-and-run" hypothesis. This assumption, although challenged at fast synapses (Chen et al., 2008; Granseth et al., 2009), is imposed by the conviction that the sole possible release mechanism is the opening of the fusion pore. Paradoxically, the authors also recognized "*that diffusion of* [the transmitter] *glutamate through the pore would be too slow to generate the rapid rise in glutamate in the cleft to produce the time-course of synaptic currents.*" This means that the opening of the pore is still thought to occur but not sufficient for fast release. The authors continue: "*Therefore, it is likely that glutamate is expelled from synaptic vesicles to provide transmission with the observed time course…*" Yet, if the opening of the pore is not sufficient for fast release, and if another mechanism needs to expel the transmitter wherever it comes from, then why the opening of a vesicle would necessarily precede immediately each release? There is in fact no direct evidence either that the transmitter is released from the vesicular lumen or that release happens just after the pore opens. This is a logical and common opinion but still pure hypothesis.

Alternatively there is direct evidence that presynaptic vesicular traffic has a continuous constitutive role since this traffic incorporates vesicular glycolipids and glycoproteins such as gangliosides, synaptotagmin and SV2 proteoglycan in the presynaptic membrane where they bind other intersynaptic extracellular matrix components such as laminin (see Vautrin, 2010 for review) to form what was called the synaptomatrix (Vautrin, 2009). This constitutive traffic depends on the synaptic activity and is likely to control synaptic contact size and efficacy (Matz et al., 2010). Furthermore, evidence was provided that the transmitter can be held under a non-diffusible form in the matrix of presynaptic vesicles (Reigada et al., 2003) and that it can be held by the synaptomatrix at the presynaptic surface (Vautrin et al., 2000). Thus it is not clear whether the transmitter is released as soon as the vesicle comes in "kissing" position, or later, some time after it has completely fused and has incorporated its lumenal matrix into the synaptomatrix.

The author are wrong when stating that "*there would be no role for an* H^+ gradient if release occurred by full fusion". Actually, it becomes increasingly clear that in many cell types the role of H^+ pumps is not limited to acidifying the lumen of intracellular organelle but also to function at the cell surface where it generates a juxtacellular unstirred layer of H^+ (Harvey, 2009). After presynaptic vesicle fusion, vesicular H^+ pumps and vesicular transmitter transporters (Fei et al., 2008) are in position to acidify the synaptomatrix and to support a surface accumulation of transmitter respectively. The authors point to the fact that exocytosis alone is not sufficient for fast release and recognize that an extra step is required to let the transmitter free to interact with the postsynaptic receptors. The voltage dependent calcium channels that activate release and the Ca^{2+} sensor synaptotagmin (Yao et al., 2010) are integral parts of the synaptomatrix suggesting that the Ca^{2+} microdomain activates directly the release from the synaptomatrix without activating extemporaneously exocytosis (Vautrin, 2010).

What is clear from the Authors' data is that reducing the pH gradient between the extracellular space and the site wherever the released transmitter comes from reduces the transmitter output. What is not clear is whether this interference affects the gradient between the lumen of "kissing" vesicles and the bulk milieu or between the synaptomatrix and the bulk milieu. Neither is clear whether it is the extrusion of transmitter or the available transmitter that accounts for the reduced output. Synaptic vesicle status at the time of transmission remains unclear since there is still no direct morphological evidence that a vesicular pore opens just prior the release. In all experiments there is only a general relationship between vesicular traffic and presynaptic activity. Most interestingly, both, the increase in bulk milieu acidity (the Authors' Figure 1A and Figure 3A) and blockade of the H^+ pumps using FCCP (Figure 5A), induced similar progressive asymptotic rundowns of the transmitter output. (Bafilomycin differs from FCCP as it acts on the pumps from inside the vesicles and acts only when vesicle turnover is activated; see Cavelier and Attwell, 2007). Knowing that accumulated glutamate levels in the synaptic vesicle are not maintained in the absence of active transport (Carlson and Ueda, 1990), the

similar time course of the rundowns when blocking the pumps on all the vesicles (by ATP depletion with FCCP) or acting from the outer surface of the membrane (using acidic extracellular medium) suggests that in both case the same immediately releasable pool is depleted and that this pool is accessible from the surface. Therefore, the data may not be solely interpreted in terms of relative proportion of kissing and fusing vesicles since it is entirely consistent with a permanent surface accessibility of the pool of immediately releasable transmitter (which has already been shown by Vautrin et al., 2000) for GABA; the maintenance of the pool of surface glutamate requiring an H^+ gradient at the presynaptic surface. The surface retention mechanisms of GABA is probably slightly different than for Glutamate as FCCP does not affect the GABA output until the vesicular traffic is activated (see Authors' Figure 8) possibly because GABA does not leak as much from the synaptomatrix than glutamate.

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