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LETTER

The immature brain needs GABA to be excited and hyper-excited

The perspective article 'Immature brains don't need GABA to get "hyper"-excited', Goaillard (2010) highlights the paper of Shao & Dudek (2009) who suggested that seizures generated in 'immature' CA3 slices by $GABA_A$ receptor blockers do not depend on the excitatory action of GABA, but on other mechanisms including intrinsic parameters, release properties, recurrent synaptic connections, etc. From this work centred on epilepsies, Goaillard challenges whether GABA excites immature neurons at all, as suggested two decades ago and repeatedly confirmed. It is important to stress that Dudek and Shao conducted their study on CA3 neurons recorded from third week animals when the developmental shift of GABA has already occurred thereby mixing two different issues: the developmental sequence and the effects of seizures. Indeed, GABA can excite adult epileptic neurons because of intracellular chloride accumulation (Cohen *et al.* 2002). Indeed, Goaillard challenges the concept that GABA excites immature neurons (at an earlier age) relying on the study of Rheims *et al.* (2009) who claimed that the depolarizing action of GABA is an artifact due to energy deprivation in slices where glucose is the sole metabolic source. Although they never measured metabolism, these authors suggested that to drive a sufficient mitochondrial ATP production, glucose alone is not adequate but needs additional substrates contained in the maternal milk such as ketone bodies, lactate and pyruvate. This issue is particularly relevant since most people working on slices around the world uses brain tissues obtained from neonatal or juvenile animals and glucose as the only energy supplier. However, three recent studies from different laboratories (Kirmse *et al.* 2010; Ruusuvuori *et al.* 2010; Tyzio *et al.* 2011), using a variety of different approaches such as invasive and non-invasive electrophysiological or imaging techniques (including dynamic multi-photon recordings to simultaneously visualize hundreds of neurons), have challenged these results. Thus, in newborn rats, ketonic body metabolites (KBMs) did not affect GABA signalling including correlated network activity such as giant depolarizing potentials (GDPs), a hallmark of developmental circuits (Tyzio *et al.* 2011). Similarly, fluorescence imaging of immature cells of the upper cortical plate in slices from mice revealed that GABA-mediated somatic calcium transients, which require the activation of $GABA_A$ receptors and voltage-dependent calcium channels, were unaffected by KBMs, indicating that this energy-substrate-enriched artificial cerebrospinal fluid (ACSF) was unable to influence the direction of GABA action (Kirmse *et al.* 2010). The addition to glucose of physiological concentrations of pyruvate and lactate (150 μM and 1.5 mM, respectively, with a rodent pyruvate/lactate ratio being 1:10 as in humans) or KBMs does not alter GABA reversal or GDPs (Tyzio *et al.* 2011). In contrast, pyruvate at 5 mM concentrations (40-fold higher than normal) does shift GABA actions to hyperpolarizing as shown by Rheims *et al.* (2009) and Holmgren *et al.* (2010), but this has little to do with energy. Indeed, Kaila and coworkers (Ruusuvuori *et al.* 2010) have compellingly shown that weak acids like lactic acid affect the intracellular pH leading to acidosis, thus directly reducing the intracellular chloride and shifting the direction of GABA action. The authors measured mitochondrial membrane potential (a direct signature of the metabolic state of neurons) and the intracellular pH in CA3 hippocampal neurons in neonatal slices. They showed that withdrawal of glucose (with or without L-lactate) but not addition of L-lactate to glucose containing ACSF modified the mitochondrial membrane potential. Furthermore, non-metabolized weak acids, including lactic acid or propionic acid, produced a similar acidification as L-lactate and abolished GDPs, confirming that their actions are mediated by acidification and not metabolism. Also, increasing the CO_2 level to acidify neurons, GDPs are readily blocked, indicating that the effects of weak acids have nothing to do with changes in oxidative energy metabolism but are directly mediated by the intracellular acidification to which GABA action is extremely sensitive (Ruusuvuori *et al.* 2010).

It is worth noting that, beside the depolarizing action of GABA, other factors may contribute to enhance network excitability in neonatal brain, including the low expression of Kv7.2 and Kv7.3 channels responsible for I_m (Safiulina *et al.* 2008) and the expression of the persistent Na^+ current (I_{nap}) that tends to promote spike generation by GABA (Valeeva *et al.* 2010). Interestingly, intrinsic bursting activity, which plays an instructive role in GDP generation, is more intense and regular in neonates than in adults. Inhibiting Kv7.2/7.3 channels with linopiridine further increased intrinsic neuronal excitability and GDP generation suggesting that these channels, although at a low level, are already expressed in neonate animals. However, application of linopiridine in the presence of the NKCC1 blocker bumetanide (known to shift GABA action from the depolarizing to the hyperpolarizing direction) failed to rescue GDPs, further indicating that the depolarizing action of GABA is essential for enhancing neuronal excitability and GDP generation.

It is worth noting that in young neurons seizures produce, as in adults, a shift of GABA from the hyperpolarizing to the depolarizing direction and the reduced capacity of immature neurons to remove chloride will contribute to exacerbate the effects of seizures (Khalilov *et al.* 2003). Therefore, although other parameters are possible as suggested by Shao and Dudek, their importance depends on a better understanding of the dynamic regulation of chloride in health and disease.

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