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The emergence of neural activity and its role in the development of the enteric nervous system

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ABSTRACT

The enteric nervous system (ENS) is a vital part of the autonomic nervous system that regulates many gastrointestinal functions, including motility and secretion. All neurons and glia of the ENS arise from neural crest-derived cells that migrate into the gastrointestinal tract during embryonic development. It has been known for many years that a subpopulation of the enteric neural crest-derived cells expresses pan-neuronal markers at early stages of ENS development. Recent studies have demonstrated that some enteric neurons exhibit electrical activity from as early as E11.5 in the mouse, with further maturation of activity during embryonic and postnatal development. This article discusses the maturation of electrophysiological and morphological properties of enteric neurons, the formation of synapses and synaptic activity, and the influence of neural activity on ENS development.

Introduction

The enteric nervous system (ENS) consists of a network of neurons and glia embedded within the wall of the intestine that is essential for regulating gastrointestinal motility (Furness, 2012; Gershon, 1998). The ENS is a unique division of the peripheral nervous system as it is capable of functioning autonomously, without input from extrinsic sources. There are a large number of enteric neurons (similar to the number of neurons in the spinal cord), which can be separated into several sub-classes by their morphology, chemical coding and electrophysiological properties (Brookes, 2001). Proper development of enteric neurons and their circuits is vital to ensure normal intestinal function. Congenital gastrointestinal diseases such as Hirschprung disease result from impaired ENS development (Heanue and Pachnis, 2007).

The development of each part of the nervous system comprises a series of overlapping stages, including proliferation, migration, neuronal differentiation (including neurotransmitter specification, morphology and axon guidance), and synaptogenesis. There is now abundant evidence for neuronal and network activity involving different ion channels, neurotransmitters and receptors at early stages during the development of the central nervous system (CNS) (Spitzer, 2006). This early electrical activity plays essential roles in a variety of processes, including proliferation, migration, neuronal differentiation, axon pathfinding and the refinement of connections in the CNS (Ben-Ari and Spitzer, 2010; Blankenship and Feller, 2010; Borodinsky et al., 2012; Spitzer, 2006). However, while extensive research over the last two decades has made remarkable progress in our understanding of the genetic and molecular control of ENS development (Gershon, 2010; Hao and Young, 2009; Heanue and Pachnis, 2007; Laranjeira and Pachnis, 2009; Sasselli et al., 2012; Young, 2008), very little information was available about the development and role of neuronal activity in the developing gut until recently. Recent studies have shown that the ENS is one of the first parts of the nervous system to exhibit electrical activity, and that the activity of early-differentiating neurons influences the development of those that differentiate later. Moreover, at least in the mouse, maturation of the ENS occurs over a protracted period and extends well into postnatal stages. This review discusses the development of neural activity in the gut, the maturation of electrophysiological properties of enteric neurons and the formation of synapses between neurons and target cells. We also review the role of neural activity in the development of the ENS.

Development of enteric neurons and neural activity

The neurons and glia of the ENS arise from the neural crest. In the mouse, enteric neural crest cells (ENCCs) enter the foregut at

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E9.5 and migrate rostro–caudally in chains to colonize the entire GI tract by E14.5 (Kapur et al., 1992; Young et al., 2004). By E10.5, 10–20% of ENCCs already express pan-neuronal markers including Hu, neurofilament-M, peripherin, neuron-specific enolase, Tuj1 and PGP9.5 (Baetge and Gershon, 1989; Young et al., 1999). In zebrafish, ENCCs migrate from the hindbrain to the vicinity of the gut around 36 h post-fertilization (h.p.f.). They then migrate longitudinally as two parallel chains of cells along the GI tract and subsequently turn circumferentially around the gut to achieve full colonization by 96 h.p.f. (Shepherd et al., 2004). Similar to the mouse, zebrafish ENCCs begin to express the neuronal marker Hu early in development, around 48 h.p.f. (Holmberg et al., 2003; Wallace et al., 2005).

Differentiation of enteric neurons

Unlike in the developing CNS, there are no distinct proliferative zones in the developing ENS where proliferating progenitors and precursors give rise to postmitotic neurons. Instead, enteric neurons at E10.5 and E11.5 are dispersed amongst non-neuronal ENCCs (Fig. 1). The factors that induce a sub-population of ENCCs to commence neuronal differentiation are unknown. A number of soluble factors produced by the gut mesenchyme are known to promote enteric neuron differentiation, including glial cell line-derived neurotrophic factor (GDNF), bone morphogenetic proteins (BMPs) and neurotrophin-3 (Chalazonitis et al., 1994, 1998, 2004; Heuckeroth et al., 1998). As cells expressing pan-neuronal markers are intermingled with non-neuronal ENCCs, it is likely that only a sub-population of ENCCs is competent to respond to the environmental cues promoting neuronal differentiation, perhaps by expressing sufficient levels of the appropriate receptors or components of signal transduction pathways. Transcription factors are also likely to play a role, and it has been proposed that a balance between the expressions of *Sox10*, which is involved in the maintenance of multi-lineage progenitors, and *Ascl1* (formerly known as *Mash1*), which promotes neuronal differentiation and suppresses *Sox10* expression, is important for determining ENCC fate (Okamura and Saga, 2008). The transcription factor Hand2 is also important for the differentiation of enteric neurons as reduction or ablation of *Hand2* expression results in a reduced number of enteric neurons (D'Autreaux et al., 2011, 2007; Hendershot et al., 2007; Lei and Howard, 2011). In particular, *Hand2* is important for the differentiation of subtypes of enteric neurons that are immunoreactive for neuronal nitric oxide synthase (nNOS) and vasoactive intestinal peptide (D'Autreaux et al., 2011; Hendershot et al., 2007). In developing dorsal root ganglia (DRG), NOTCH-mediated lateral inhibition between neighboring cells plays a key role in fate determination (Wakamatsu et al., 2000). However, whilst conditional perturbation of NOTCH signaling in ENCCs results in major defects in gliogenesis, there are only minor defects in neurogenesis (Taylor et al., 2007). It is also possible that neuronal differentiation is linked to the number of cell cycles undergone; however, unlike CNS and DRG neurons, cell cycle exit does not always precede neuronal differentiation in the ENS, as a subpopulation of ENCCs that expresses pan-neuronal markers at E10.5–E12.5 is also proliferative (Lei and Howard, 2011; Teitelman et al., 1981; Young et al., 2005). An important future area of study is to examine what factors determine the timing of initial fate specification and differentiation of individual ENCCs. Recent studies have shown that endogenous neural activity and neurotransmitter release influence neuronal differentiation, as discussed below.

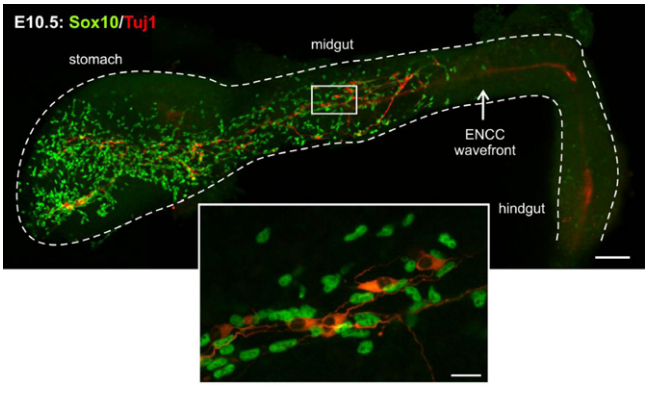

Fig. 1. Neuronal differentiation commences at early stages of ENS development. Wholemount preparation of E10.5 mouse gut processed for immunoreactivity against Sox10 (*green*) to identify non-neuronal ENCCs and Tuj1 (*red*) to identify enteric neurons. The boxed section is magnified in the inset. Scale bars = 100 μm, and 20 μm (inset). Note: there is non-specific staining in the lumen of the hindgut.

Early neural activity is not an "all-or-nothing" event

Studies of the generation of neurons from human embryonic stem cells have revealed that cells expressing pan-neuronal markers can be electrically inactive (Moe et al., 2005), and for many years it was unclear whether the cells expressing pan-neuronal markers during early ENS development are electrically active. In a study by Howard et al. (1995), trunk neural crest cells isolated from stage 13 quail embryos and cultured for 7–8 days in vitro were found to exhibit fast inward and delayed outward currents resembling voltage-dependent Na^+ and K^+ currents, respectively, in response to depolarization. Interestingly, these responses were observed in both "neuronal" and "non-neuronal" cells, as characterized by their morphology. In a recent study using short-term (24 h) cultures of dissociated E11.5 mouse gut, spontaneous intracellular Ca^{2+} ($[Ca^{2+}]_i$) transients were found to occur both in cells expressing pan-neuronal markers and in non-neuronal cells. However, only the former responded to electrical field stimulation (Hao et al., 2011). Moreover, patch-clamp recordings have shown that ENCCs expressing pan-neuronal markers include cells that are (i) electrically inactive and do not respond to depolarization; (ii) cells that exhibit graded active potentials (GAPs), an immature form of electrical response; and (iii) cells that fire action potentials (APs), many of which have similar properties to APs in adult enteric neurons (Fig. 2) (Hao et al., 2012). The three types of responses to imposed depolarization in E11.5 enteric neurons most likely represent cells at

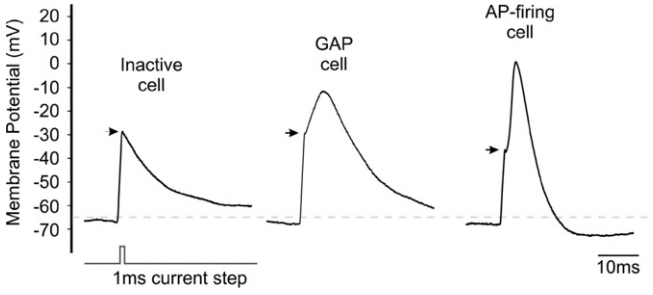

Fig. 2. Some early enteric neurons are electrically excitable. Responses to depolarization of cells expressing neuronal markers from the gut of E11.5 mice that had been cultured for 4–12 h. All responses were recorded from a baseline membrane potential of approximately −65 mV. Cells were stimulated with a 1 ms-duration depolarizing current. An initial passive increase in membrane potential was elicited in all cells (arrowheads). Three types of responses were observed: "inactive cells" that did not respond with an active rise in membrane potential; "GAP cells" that exhibited an immature, but active response; and "AP-firing cells", which fired all-or-nothing APs. Reproduced with permission from Hao et al., 2012.

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