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CrossTalk opposing view: Interstitial cells are not involved and physiologically important in neuromuscular transmission in the gut

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In the gut, smooth muscle bundles are separated by intramuscular spaces containing nerve fibres and interstitial cells including intramuscular interstitial cells of Cajal (ICC-IM), a subset of a family of ICC (Komuro 2006). Traditionally, smooth muscle cells (SMCs) are believed to transduce the action of neurotransmitters that cause muscle contraction or relaxation by a process called direct neuromuscular transmission (NMT). Neuro-ICC-IM and neuro-smooth muscle (SM) transmissions regulate functions of ICC and SMCs, respectively. Initially proposed by Imaizumi & Hanna (1969), Sanders and colleagues championed the idea that the smooth muscle responses to nerve stimulation required mandatory transduction by the ICC-IM (indirect NMT) (Burns *et al.* 1996; Ward *et al.* 2000). The role of the smooth muscles was considered simply to mount mechanical contraction or relaxation in response to electrical signals transduced in ICC-IM. It has also been suggested that both ICC-IM and SMCs transduced neural signals to the smooth muscle. According to one view, ICC-IM is involved only in certain situations (direct *or* indirect NMT) (Bhetwal *et al.* 2013; Klein *et al.* 2013). According to another, ICC-IM is involved in parallel with smooth muscles (direct *and* indirect NMT) (Groneberg *et al.* 2013). However, most of the available data continue to support the traditional view of direct NMT, not requiring ICC-IM (Goyal & Chaudhury 2010) (see Table 1).

The traditional model: direct NMT

The direct NMT is supported by the following: (1) close synapse-like contacts of motor nerve varicosities with smooth muscles (Komuro 2012), (2) the presence of nitrergic and cholinergic signalling molecules in the smooth muscles (Bhetwal *et al.* 2013; Cobine *et al.* 2014; Lies *et al.* 2014*a*), (3) the presence of nitrergic and cholinergic signalling in the smooth muscles (Wang *et al.* 1996; Zhang *et al.* 1998), (4) expected effects of agonists of neurotransmitters in isolated smooth muscle (Wang *et al.* 1996; Zhang *et al.* 1998), and (5) demonstration of active generation of nitrergic inhibitory junction potential in the SMC (He & Goyal 2012).

Mandatory role of ICC-IM: indirect NMT

An assumed lack of direct NMT led to the conclusion of a mandatory role of ICC-IM in nitrergic NMT. This conclusion was supported by the following: (1) an assumed lack of direct innervation of smooth muscle and presence of exclusive close synaptic contacts of nerve varicosities with ICC-IM (Sanders *et al.* 2014*a*), (2) the abundance of the nitric oxide sensitive-guanylate cyclase (NO-GC) in ICC-IM but not in smooth muscle (Lies *et al.* 2014*a*), and (3) the reported loss of nitrergic NMT in ICC-IM-deficient W/W^v mice (Burns *et al.* 1996). The mandatory role of cholinergic transmission was based on the reported loss of cholinergic NMT in ICC-IM deficiency (Ward *et al.* 2000; Klein *et al.* 2013).

However, as summarized below, there is substantial evidence against the mandatory role of the ICC in nitrergic NMT.

First, close synapse-like junctions between the nerves and ICC are also present between the nerves and the smooth muscles (Mitsui & Komuro 2002). However, true synapses with a synaptic cavity are present at neither neuro-ICC nor neuro-smooth muscle junctions (Komuro 2012).

Second, a key assumption in the model of the role of ICC-IM in NMT is that the gap junctions conduct electrical potentials from ICC-IM to smooth muscles (Sanders *et al.* 2014*a*). Although gap junctions have been identified, their functional efficiency in conducting electrical signals has been shown to be poor (Sibave *et al.* 2006; Daniel *et al.* 2007).

Third, NO-GC is present in the smooth muscle, ICC and $PDGFR\alpha^+$ fibroblast-like cells and abundance of NO-GC does not correlate with greater function. $PDGFR\alpha^+$ fibroblast-like cells have the highest concentration of NO-GC, yet they do not participate in NMT (Cobine *et al.* 2014; Lies *et al.* 2014*a*).

Forth, several studies have reported that nitrergic inhibitory junction potential (IJP) and smooth muscle relaxation are preserved in ICC-IM-deficient W/W^v mice and W^s/W^s rats (Sivarao *et al.*, 2001, 2008; Huizinga *et al.* 2008; Zhang *et al.* 2010). Most recently, Sanders and colleagues (2014*b*) suggested that the discrepant results may be due to an incomplete loss of ICC in the interrogated tissues. They reported that complete absence of ICC-IM was required for a loss of nitrergic relaxation. However, in the absence of ICC-IM, there is no barrier between neurally released neurotransmitter and SMCs, leaving direct NMT intact (Bhetwal *et al.* 2013; Klein *et al.* 2013). Therefore, the report of loss of nitrergic neurotransmission in the absence of ICC in W/W^v mice (Sanders 2014*b*) is difficult to reconcile.

Evidence against a mandatory role of the ICC cholinergic NMT is the failure to reproduce the reported loss of cholinergic NMT in W/W^v mice (Ward *et al.* 2000;

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Table 1. Proposed models of involvement of intramuscular interstitial cells of Cajal (ICC-IM) in NMT in the gut and experimental results supporting them

	Involvement of ICC-IM in NMT			
	None	Mandatory	Optional	Complementary
Type of NMT	Direct	Indirect	Direct or indirect (situational)	Direct and indirect (dual)
Proposers	Traditional	Burn *et al.* 1996; Ward *et al.* 2000	Klein *et al.* 2013; Bhetwal *et al.* 2013	Groneberg *et al.* 2013
Neurotransmitters involved	All transmitters	Nitrergic and cholinergic	Nitrergic and cholinergic	Nitrergic only
Lack of evidence for true synaptic innervation of the ICC-IM*	Consistent	Against	Against	Consistent
Evidence for direct innervation of SM*	Consistent	Against	Against	Consistent
Evidence for the presence of functional signalling molecules in SM*	Consistent	Against	Consistent	Consistent
Lack of evidence for effective conduction from ICC-IM to SM via gap junctions*	Consistent	Against	Against	Against
Origin of nitrergic inhibitory junction potential in SM*	Consistent	Against	Against	Consistent
Preserved nitrergic NMT in ICC deficiency*	Consistent	Against	Consistent	Consistent
Loss of nitrergic inhibition with presumed deletion of NO-GC from ICC*	Against?	Consistent	Against	Consistent
Preserved cholinergic NMT in ICC deficiency*	Consistent	Against	Against	Consistent

*See text for discussion of the evidence.

Zhang *et al.* 2011; Bhetwal 2013; Goyal 2013).

Optional role of ICC-IM: direct *or* indirect NMT

Klein *et al.* (2013) reported that 'ICC-specific' deletion of protein kinase G1 (P*rkg1*) abolished nitrergic NMT, but deletion of ICC left nitrergic neurotransmission intact. To explain these paradoxical findings the authors made an intriguing proposal, that when present ICC-IM are involved in NMT, but when ICC-IM is absent, direct NMT takes place. This model was based on the presence of assumed true neuro-ICC-IM synapses that can restrict the accessibility of the released nitric oxide (NO) to the smooth muscle (Beckett *et al.* 2005). However, no true synapses have been identified between ICC and smooth muscles (Komuro 2012; Goyal & Chaudhury 2013). Moreover, NO is a highly diffusible gas (diffusion constant 3300 $\mu^2\ s^{-1}$) (Lancaster 1997). Therefore, ICC-IM cannot restrict it to having effects on adjacent smooth muscles. A similar unsubstantiated model was used to explain the persistence of cholinergic responses in W/W^v mice (Bhetwal *et al.* 2013; Goyal 2013).

Complementary role of ICC-IM: direct *and* indirect NMT

For nitrergic NMT, Groneberg and colleagues (2013) reported that cell-specific knockdown of NO-GC in SMCs or ICC did not affect nitrergic relaxation. However, double knockdown of NO-GC in both SMCs and ICC abolished nitrergic NMT (Groneberg *et al.* 2013). Other studies have reported that incomplete deletion of NO-GC in ICC results in a dominant loss of nitrergic relaxation (Lies *et al.* 2014*b*; Groneberg *et al.* 2015). Moreover, Klein *et al.* 2013 has reported that partial deletion of Prkg1 abolishes nitrergic NMT, but partial deletion of ICC does not (Klein *et al.* 2013). It is not clear why incomplete deletion of NO-GC has a more profound effect on nitrergic NMT than incomplete deletion of ICC. It is possible that *cKIT-CreER*^{T2} mutants may also affect NO-GC in smooth muscles. Validation studies using immunohistochemistry are not sensitive enough to reveal changes in NO-GC in smooth muscles (Groneberg *et al.* 2015). Finally, a complementary role of ICC in NMT is difficult to comprehend in the absence of a functional gap junction that can transmit signals from the ICC to SMC (Sibave *et al.* 2006; Daniel *et al.* 2007). A complementary role of cholinergic NMT is also speculated but without any experimental data to support it (Groneberg *et al.* 2015).

Conclusion

Overall, available evidence is most consistent with the traditional model of direct NMT without the involvement of ICC-IM. Parallel neuro-ICC and neuro-SM transmissions may independently regulate the functions of the ICC-IM and SMCs, respectively. There is little evidence for an obligatory or optional involvement of ICC-IM in NMT. A complementary role and physiological importance of ICC-IM in nitrergic NMT remain speculative. Moreover, it is unlikely that ICC-IM would only be involved in nitrergic NMT while a variety of other neurotransmitters will use direct NMT.

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Additional information

Competing interests

None declared.