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Dual-transmitter neurons: functional implications of co-release and co-transmission

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Co-transmission, the ability of a neuron to release multiple transmitters, has long been recognized in selected circuits. However, the release of multiple primary neurotransmitters from a single neuron is only beginning to be appreciated. Here we consider recent examples of co-transmission as well as co-release — the packaging of multiple neurotransmitters into a single vesicle. The properties associated with each mode of release greatly enhance the possible action of such neurons within circuits. The functional importance of dual- (or multi-) transmitter neurons extends beyond actions on postsynaptic receptors, due in part to differential spatial and temporal profiles of each neurotransmitter. Recent evidence also suggests that the dual-transmitter phenotype can be dynamically regulated during development and following injury or disease.

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Introduction

Neurotransmitter phenotype has long been recognized as a hallmark of neuronal identity. The classical view was that each neuron releases a single neurotransmitter, leading to the 'one neuron, one transmitter' hypothesis [1], formalized by John Eccles as Dale's Principle [2]. However, we now know that many neurons throughout the brain are capable of releasing two or more neurotransmitters [3–5]. For simplicity, in this review we refer to these cells as 'dual' transmitter neurons. Some of the first evidence for such dual- (or multi-) transmitter neurons was noted in 1979, when Jan *et al.* reported a very slow synaptic potential in a subset of sympathetic neurons that accompanied the well-established cholinergic transmission (Figure 1) [6]. This slow potential was mediated by a peptide, LHRH (luteinizing hormone-releasing hormone), indicating that the presynaptic neuron released a neuroactive peptide as well as acetylcholine [6,7]. Such co-transmission, defined broadly as the release of multiple neurotransmitters from a single neuron, has been reported for many neuromodulators including ATP, neuroactive peptides, neurotrophic factors and even ions such as Zn^{2+} [8–15]. Recent evidence, however, suggests that neurons can co-transmit not only neuromodulators but also multiple primary neurotransmitters including fast-acting neurotransmitters, monoamines and acetylcholine [16–20].

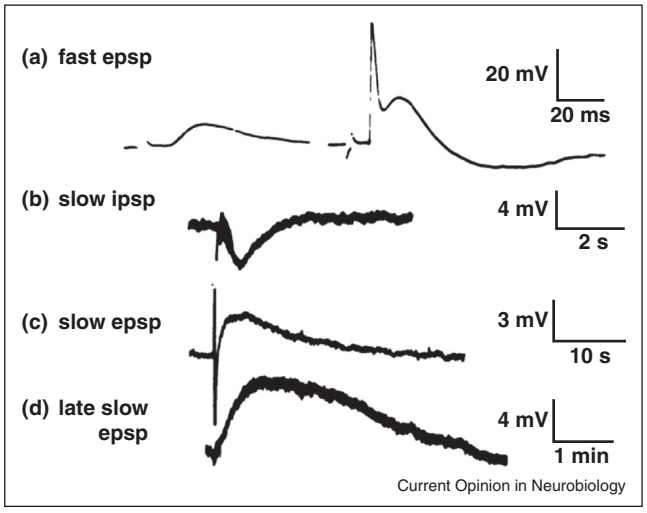
Although dual-transmitter neurons are found throughout the brain, the functional significance of co-transmission on neuronal circuits has been difficult to dissect. This difficulty arises, in part, because in addition to activating postsynaptic receptors, co-released neurotransmitters can modulate presynaptic and postsynaptic responses and even modulate the packaging of other neurotransmitters into synaptic vesicles [4]. Additionally, each neurotransmitter may be differentially released in time and space, thereby complicating analysis. A consideration of all these parameters is necessary to understand how dual-transmitter neurons alter the computational capabilities of neuronal circuits. Of note, the functional importance of co-transmission has been better described in select invertebrate systems, where each transmitter can differentially enhance the ability of the circuit to participate in multiple computational tasks [21–24]. Here we focus on recent studies of dual-transmitter neurons, including the mechanisms governing the release of multiple neurotransmitters, and the functional importance of co-transmission and co-release on circuit function in the mammalian CNS.

Co-release versus co-transmission

The release of multiple neurotransmitters from a single neuron does not necessarily imply co-release, that is that two or more neurotransmitters are packaged into a single population of synaptic vesicles (Figure 2a). Co-transmission can be more broadly defined as the release of multiple neurotransmitters from non-overlapping pools of synaptic vesicles (Figure 2b). The distinction between co-release and co-transmission is important because each mode of release can have different potential impacts on circuit function. For example, release from different sets of vesicles can be differentially regulated by differential Ca^{2+} sensitivity or the spatial segregation of vesicles (Figure 2b).

Recent work by Tritsch and colleagues provides an elegant example of *co-release* in the ventral tegmental

Figure 1



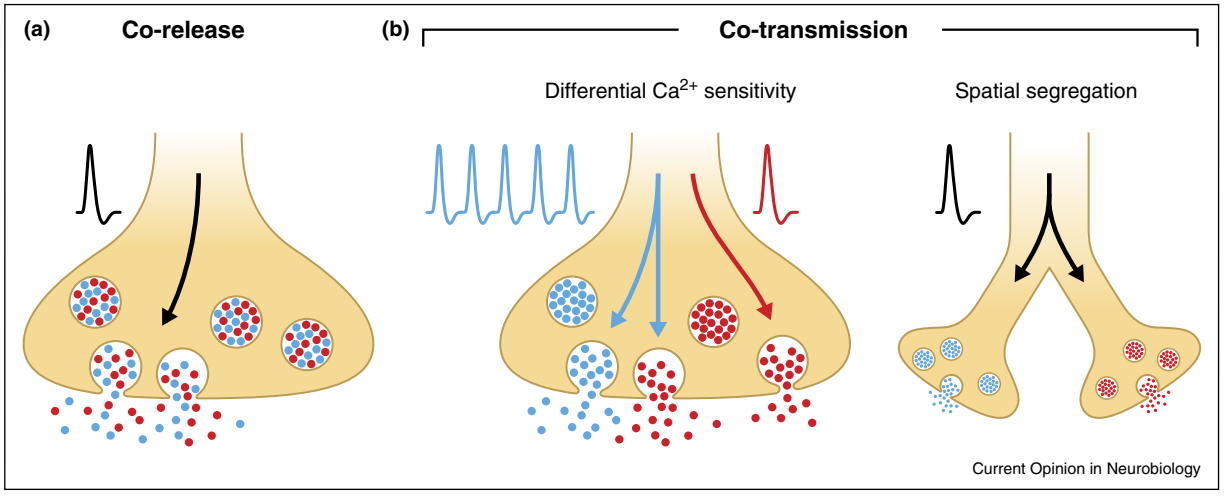
Co-transmission in the sympathetic nervous system. Stimulation of a sympathetic ganglion neuron revealed **(a)** a fast, nicotinic EPSP (left) and higher stimulation evoked an action potential (right). (**b and c)** Such stimuli can also elicit a slow muscarinic IPSP **(b)** or EPSP **(c)** depending on the stimulation conditions. **(d)** Strong stimulation generated a late slow EPSP consistent with co-transmission by the neuromodulator LHRH. Note timescale difference in each panel. Adapted from Figure 1 of Jan *et al.* [6].

area (VTA) [25••]. Their work indicates that dopaminergic neurons targeting striatal spiny neurons co-release GABA. Surprisingly, the conditional knockout of the vesicular GABA transporter (VGAT) [26–28], failed to eliminate GABA release [25••]. Instead, inhibition or conditional knockout of the vesicular monoamine transporter (VMAT2), which was thought to only package monoamines [29], completely eliminated GABA release [25••]. Although this work did not examine changes at the single vesicle level, the results indicate that GABA is a non-canonical substrate for VMAT2. Although the function of co-released GABA within the VTA circuitry has not yet been well characterized, the ability of VMAT2 to package a non-canonical substrate, such as GABA, into synaptic vesicles suggests that monoaminergic neurons expressing VMAT2 are capable of co-releasing GABA.

Co-transmission, on the other hand, has been demonstrated in a handful of circuits, including the retina where a subpopulation of starburst amacrine cells release acetylcholine (ACh) and GABA [30–32]. Consistent with a co-transmission phenotype, cholinergic synapses are uniformly distributed on the postsynaptic neuron, whereas GABAergic synapses are non-uniformly distributed [32]. Furthermore, the release of GABA and ACh has different Ca^{2+} sensitivities, suggesting that co-transmission in this circuit involves both spatial segregation of vesicles and differential release [32]. Ostensibly, these features allow single starburst amacrine cells to participate in distinct, yet related circuit functions.

Interestingly, the co-transmission phenotype can be confined to a subset of synaptic boutons in an individual neuron. For example, in cultured dopaminergic neurons, immunohistochemical studies suggest that most boutons contain vesicular glutamate and monoamine transporters,

Figure 2



Co-release and co-transmission are distinct modes of release: **(a)** with co-release, both neurotransmitters (mixed red and blue) are packaged into the same set of synaptic vesicles. Upon an action potential invading the presynaptic terminal, vesicles containing both neurotransmitters are released into the synaptic cleft. **(b)** In contrast, co-transmission requires neurotransmitters be sequestered into distinct populations of synaptic vesicles with differential release mediated by differential Ca^{2+} sensitivities (left panel). For example, a single action potential might release one set of vesicles (red), but multiple action potentials might be required to release both sets of vesicles (red and blue). Alternatively, co-transmission can rely on spatial segregation of vesicle populations to different boutons (right panel) in which case, unique information is transmitted to different postsynaptic targets.