Likely effects of transmitter co-expression and diverse synaptic connectivity in the invertebrate model organism *Cupiennius salei*

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INTRODUCTION

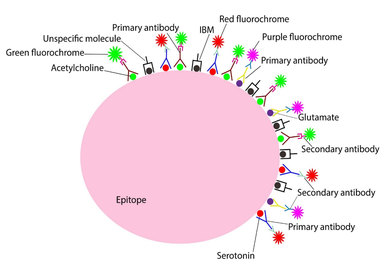
Over recent years numerous research undertakings have revealed that most if not all neurons co-express two or more neuroactive substances. Anatomical and functional studies have demonstrated a surprising variety of diverse co-expression patterns and electrophysiological responses that are evoked by biochemically diverse neuron populations. Here we present an invertebrate model system in which co-expression of different transmitters is a common occurrence.

Using immunohistochemistry and electron microscopy we demonstrate diverse biochemical neuron populations and demonstrate how the biochemical diversity in neurons can lead to an astoundingly diverse repertoire of physiological responses in a system that consists of a comparatively small number of neurons. This diversity may be generated due to the number of variables within the system, which include: (1) signal intensity, (2) number of biochemically diverse neurons that may encode for specific information such as sensory modality or location of the perceived stimulus, (3) varying synaptic interaction types, which may integrate different external and internal stimuli the animal is subjected to, (4) postsynaptic receptor types, (5) plasticity of transmitter expression/release and formation of new synapses. On a mathematical level these variables provide a potentially vast repertoire of different physiological responses to external and internal stimuli utilizing a comparable small number of neurons.

METHODS

Experimental animals: *Cupiennius salei* females were used in this study due to the fact that they are easy to breed, cost-effective, and have efferent network in slit sense organs that is likely phylogenetically related to the human vestibular cochlear system.

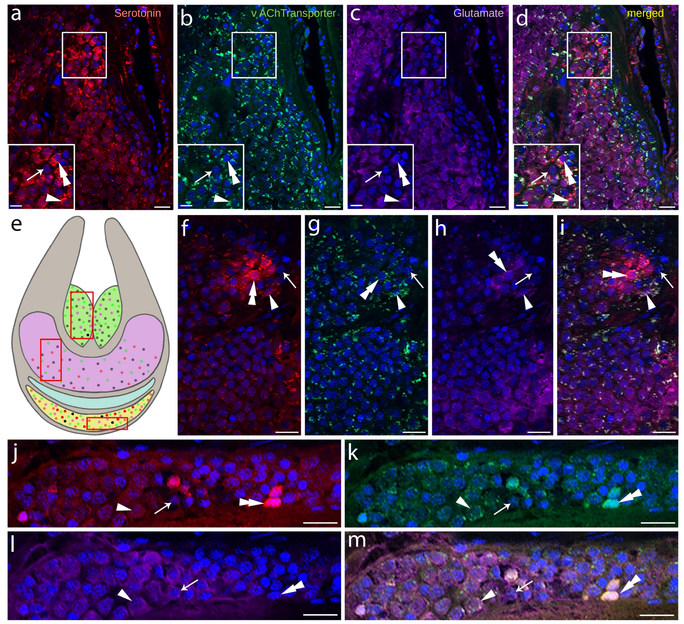
- In-situ Hybridization
- Electron microscopy
- Western blot analysis
- Three-dimensional reconstruction
- Immunohistochemistry



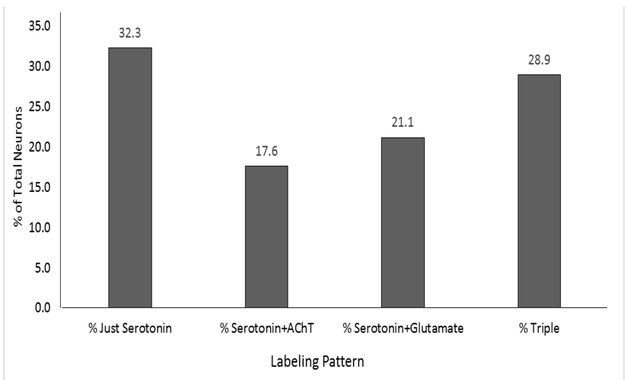
Visual description of immunolabelling

RESULTS

Examples of morphologically and biochemically diverse neuron populations

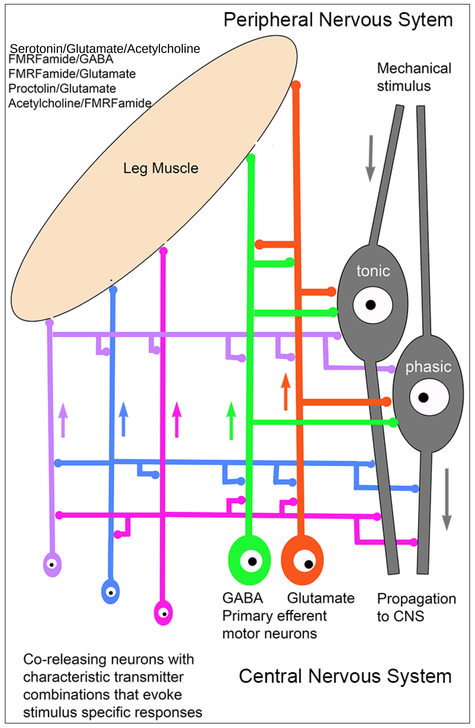


Expression of serotonin (*red*), acetylcholine (*green*), and glutamate (*purple*) in the visual region of the central nervous system. Image E is a schematic representing the distribution of neurons in the visual ganglia (images a-d), the dorsal cell layer (f-i), and the posterior cell layer (j-m). Scale bars: a-d: 20µm, a-d insets: 10µm, f-m: 20µm.

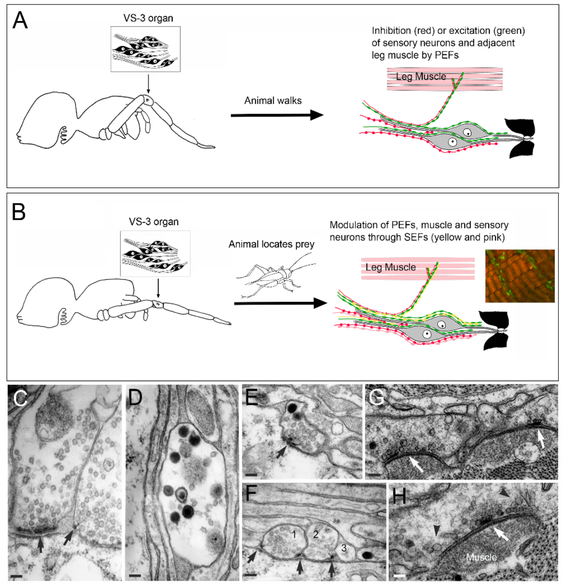


Overall percentage of serotonergic neurons that displayed only serotonin labeling, only co-expression with acetylcholine, only co-expression with glutamate, and triple labeling for all measured transmitters. Percentages represent the combined averages across all regions of the brain and of all sized neurons.

Neuronal networks and synaptic connectivity at mechanosensory neurons in C. salei



Synaptic connectivity of efferent innervation on spider mechanosensory neurons and adjacent leg muscle. Both mechanosensory afferent neurons (*grey*) and leg muscle are innervated by morphologically distinct, biochemically diverse (*top left*) efferent neurons (*purple, pink, blue, green and orange neurons*). Based on ultrastructural investigations the efferent neurons can be distinguished based on large neurons that form major synaptic contacts onto both muscle and sensory neurons (*green and orange neurons*) and small modulatory neurons that are distinguished by predominantly mixed presynaptic vesicle populations suggesting that these neurons co-express two or more neuroactive compounds (*purple, blue, pink neurons*). The synaptic connectivity shown here is based on extensive serial sectioning and three-dimensional reconstruction at the ultrastructural level. Our working hypothesis is that the activation of the small, co-expressing neurons is initiated by sensory stimuli and enables the animals to initiate behavioral responses to external and internal stimuli. We propose that different transmitter receptors are expressed at sensory neurons and leg muscle and that the transmitters have opposing effects. *Question marks* indicate the functional questions that will be addressed in future undertakings.



Working hypothesis for the efferent innervation of VS-3 sensory neurons and adjacent leg muscle in *C. salei*. (A) VS-3 neurons are innervated by primary efferent fibers that have inhibitory (GABA, *green fibers*) and excitatory (glutamate, *red fibers*) function, thus allowing rapid up- or downregulation of sensitivity during normal walking behavior. For the sake of clarity we have only drawn one walking leg and omitted the SEFs in this schematic drawing. **(B)** As the spider detects relevant stimuli such as prey, the sensitivity of the VS-3 neurons may be modulated allowing the animal to locate prey while altering the sensitivity of the sensory neurons to prevent excessive excitation caused by slow leg movements towards the prey. We propose that the SEFs (*yellow and pink fibers*) exert these modulatory functions and that this modulation is also effective at the adjacent leg muscle where it may initiate the typical slow and fast leg movements in spiders during hunting (*photo inset in B*: efferent innervation of leg muscle adjacent to the VS-3 organ show the same labeling and morphological characteristics as efferent innervation along VS-3 neurons). **(C-H)** Electron micrographs of efferent synapses onto VS-3 neurons and adjacent muscle tissue. **(C)** Large presynaptic fibers (proposed PEFs) with predominantly homogenous vesicle populations form prominent synaptic contacts onto the sensory neurons and each other (*arrows*) and have been shown to contain GABA. **(D-F)** Smaller efferent fibers contain mixed vesicle populations and form complex synaptic interactions onto sensory neurons (E). In (F) three efferent fibers (1-3) form dyadic synapses onto the sensory neuron. Fiber 1 is presynaptic to a glia cell (*left arrow*) and the sensory neuron (*middle arrow*). Efferent neuron 2 forms a dyadic synapse onto fiber 3 and the sensory neuron (*right arrow*). **(G, H)** Neuromuscular junctions adjacent to VS-3 neurons reveal diverse synapses (*arrows*) with mixed vesicle populations along the muscle. *Arrowheads* in H show large electron-lucent vesicles in the periphery of the presynaptic active zone. Scale bar: (*C-F*) 50 nm; (*G, H*) 80 nm.

CONCLUSIONS

Sensory neurons in the invertebrate model organism *C. salei* are innervated by a small network of biochemically diverse efferent neurons

The combination of biochemically diverse neurons and their complex synaptic interaction patterns are the foundation for a potentially vast number of different electrophysiological response patterns that may be generated within the mechanosensory neurons

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