

DISSECTING THE REGIONAL ORGANIZATION OF WIRING AND VOLUME TRANSMISSION IN THE SEROTONERGIC SYSTEM

G. Gianni, M. Picchi, S. Nazzi, S. Migliarini, N. Barsotti, M. Pasqualetti

Department of Biology, University of Pisa, Italy

Serotonergic (5-HT) neurons give rise to a highly distributed projection system, regulating diverse physiological and behavioral functions. Beyond classical synaptic communication (wiring transmission, WT), serotonin also acts through volume transmission (VT), based on extrasynaptic release from axonal varicosities [1]. While this dual framework is widely accepted, its regional organization remains largely unresolved, reflecting the challenge in disentangling synaptic and non-synaptic components of serotonergic connectivity [2].

To address this issue, we investigated the organization of serotonergic projections in the mouse brain using complementary retrograde tracing approaches. A glycoprotein-deleted rabies virus, which infects neurons exclusively at presynaptic terminals, was used to selectively label 5-HT neurons establishing synaptic contacts in target areas (WT). In parallel, a pseudotyped rabies virus combined with a Cre-dependent helper adeno-associated virus enabled the retrograde labeling of 5-HT neurons independently of synaptic specializations, thus capturing the overall projecting population (WT + VT).

Comparative analyses across tracing strategies revealed the relative contribution of the two modalities, showing that dif-

ferent brain regions receive distinct proportions of WT- and VT-associated 5-HT inputs, and pointing to a heterogeneous and region-specific organization of serotonergic transmission.

In addition, ongoing analyses are addressing the molecular organization of 5-HT axonal varicosities using pre- and post-synaptic markers, to corroborate the viral tracing results and directly assess the presence and regional distribution of synaptic specializations at serotonergic release sites.

Taken together, these findings provide a first anatomical framework to dissect the regional balance between wiring and volume transmission in the mammalian serotonergic system, shedding light on how this signaling duality is spatially organized across the brain, with implications for understanding how the serotonergic system achieves its remarkable functional versatility.

References

1. Agnati LF, Zoli M, Strömberg I, Fuxe K. Intercellular communication in the brain: wiring versus volume transmission. *Neuroscience*. 1995 Dec;69(3):711-26.
2. Gianni G, Pasqualetti M. Wiring and volume transmission: an overview of the dual modality for serotonin neurotransmission. *ACS Chem Neurosci*. 2023 Dec 6;14(23):4093-4104.