Distinct regulatory programs control ascending and descending 5-HT axonal architecture

Lauren J. Donovan and Evan S. Deneris

Department of Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH

Of the estimated 86 billion neurons in the human brain only about 400,000 make and use serotonin (5-HT) as a neurotransmitter. Yet, serotonin appears to modulate nearly all neural circuitry in the vertebrate CNS. Pervasive 5-HT signaling is made possible by the expansive axonal architecture issuing from this small group of neurons. 5-HT neurons generate two highly ramified topographically organized axonal subsystems, ascending and descending, that delivers the transmitter throughout the brain and spinal cord, respectively, to influence numerous behavioral and physiological processes. In contrast to our extensive knowledge of the intrinsic regulatory programs that govern the specification of these 5-HT neurons, little is known about the intrinsic regulators that enable the exuberant axonal growth of developing 5-HT neurons. The LIM-homeodomain (LIM HD) transcription factor (TF), Lmx1b, is a key intrinsic regulator of 5-HT neuron terminal differentiation through its activation of genes encoding 5-HT synthesis (Tph2), reuptake (Sert), and vesicular monoamine transport (VMAT2). Lmx1b deficient mice lack nearly all brain 5-HT. Notably, Lmx1b expression continues in all 5-HT neurons through fetal to early postnatal maturation stage during which 5-HT neurons build their axonal architectures. Its ongoing expression led us to hypothesize that Lmx1b regulates other genes responsible for ascending and descending 5-HT axonogenesis. To investigate this idea, we generated conditionally targeted mice (Lmx1bfl/fl;ePet- Cre;Ai9; Lmx1bCKO) by crossing Lmx1bflox/flox mice with transgenic mice expressing Cre recombinase specifically in newborn 5-HT neurons and mice carrying the Ai9 reporter to enable marking of ascending and descending 5-HT axons with Td-Tomato. Strikingly, despite normal numbers of 5-HT cell bodies, the spinal cord of Lmx1bCKO mice was nearly devoid of Td-Tomato+ axons from cervical to lumbar levels. Moreover, investigation of 5-HT terminal fields in the forebrain of Lmx1bCKO mice revealed nearly complete absence of Td-Tomato+ axons in the hippocampus, cortex, olfactory bulbs, and some hypothalamic regions. Further analyses suggest that 5-HT axons can initiate primary growth through the medial forebrain bundle between E12-E15 and reach the thalamus but then abruptly stop and fail to route to various forebrain structures. To define the Lmx1b regulated axonal transcriptome that governs serotonergic axonogenesis we are performing RNA-seq analyses with flow sorted Lmx1bCKO vs. Lmx1bCON ascending and descending 5-HT neurons. These studies will solve a major long-standing gap in understanding how the 5-HT transmitter system is generated.

Funding:
NIH awards:
P50 MH096972, R01 MH062723