Background

Prior analysis of mammalian mucosal chemosensory brush cells have largely focused on those present in the gastrointestinal tract, respiratory tract, pancreas, gall bladder, and thymus. More recently brush cells in the lower urinary tract of mice have been identified that exhibit tufted morphology, express choline acetyltransferase, and possess chemosensory activity. Serotonin-expressing cells in the lower urinary tract have similar chemosensory potential given their association with nerve terminals in adult animals and have been termed paraneurones. When these cells initially appear in development and their spatial distribution throughout the lower urinary tract during organogenesis has been not been determined. We assessed the timing and distribution of these cells, referred to hereafter as "neuromodulatory cells" (NM) based on their expression of neurotransmitters. Using a combination of immunohistochemistry (IHC) and transgene expression we mapped the spatial distribution of these cells over the interval from 14 days post coitus (dpc) in the mouse through to early postnatal stages.

Methods

To determine the initial appearance of NM during organogenesis, we relied on IHC for serotonin (5-HT) and transgenes driven from Villin1 (Vil1) and Choline acetyl transferase (ChAT). We related the distribution of these cells to nerve terminals by staining for sensory nerve processes expressing Calcitonin Gene Related Peptide (CGRP) and the pan-neuronal marker TuJ1. Fetal tissues were collected in daily increments, fixed, cryo-sectioned and imaged by confocal microscopy. Whole mount imaging was accomplished through clearing in a modified CUBIC solution accompanied by confocal ribbon microscopy

Results

We observed that Vil1-Cre NMs largely colocalize with 5HT+ cells in both male and female mice. In contrast ChAT-EGFP+ cells label a distinct population that does not express 5-HT. Vil1-cre+ cells first appear in the mid region of the pelvic urethra and become more widely distributed along the urethra at later stages. Vil1-cre lineage traced NM cells only begin to exhibit 5-HT+ granules at 16.5dpc. Initially Vil1-cre cells are not associated with nerve terminals and become innervated by CGRP+ nerve processes several days after their initial appearance. In contrast ChAT-EGFP expressing NM cells were only observed at later fetal stages despite the presence of ChAT-EGFP+ nerve terminals throughout the bladder wall at earlier stages.

Conclusion

NM cells in the lower urinary tract are both heterogeneous in the genes they express (Vil1 versus ChAT) and in their temporal appearance during development. The timeline of regionality of NM cells we have established lays the groundwork for future analysis of the function and physiology of these populations.