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last updated 2021-05-07

Scoring Centriole Migration in Olfactory Epithelium

The purpose of this protocol is to standardize the scoring method for assessing the status of centriole migration in cryosections of olfactory epithelium with immunofluorescent staining. These methods were developed in discussion with Jenn Wang and are also partly derived from the literature, especially the image shown to the right (Mulvaney & Heist, 1971).

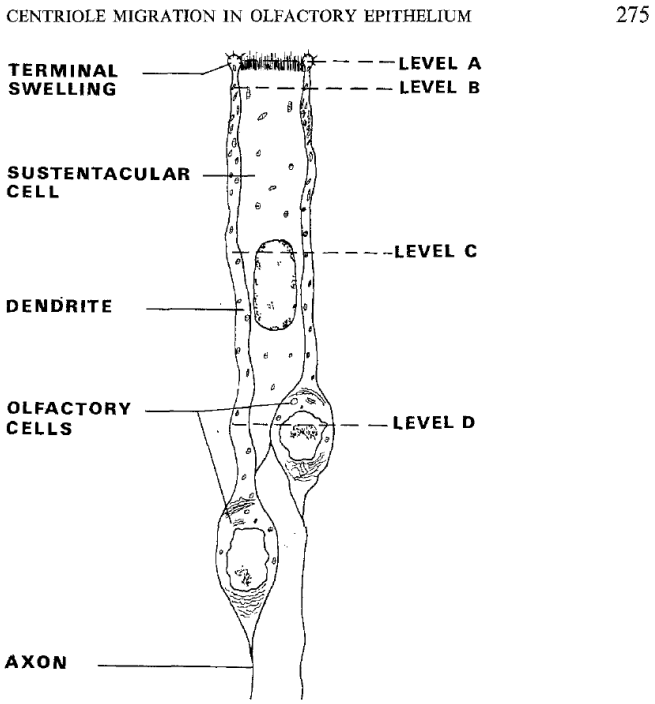

FIG. 1. Drawing of olfactory epithelium showing tissue levels at which centriole migration and sensory cell density data were taken. Level A, Terminal Swelling; Level B, Dendritic; Level C, Midway Between Olfactory Nucleus and Terminal Swelling; Level D, Nuclear.

full citation: Mulvaney, B. D., & Heist, H. E. (1971). Centriole migration during regeneration and normal development of olfactory epithelium. *Journal of Ultrasructure Research*, *35*(3–4), 274–281. https://doi.org/10.1016/S0022-5320(71)80157-0

Materials

- SlideBook (full version or reader will work)
- image files (should be acquired to include all correctly-oriented olfactory epithelium in the section, not just areas with migrating centrioles. Areas lacking migrating centrioles will still need to be included in the scoring for basal length.)

Procedure

1. Open the file of interest in SlideBook.
2. Create a corresponding spreadsheet (see template).
3. For each region (for example, OE3), a stack should have been acquired to include all migrating centrioles within the field of view. You may open a maximum projection of the region.
4. Use the ruler tool (Home > Tools > drop-down under the arrow allows you to select a ruler) to measure the **basal lamina length**. This can be defined as a line connecting the most basal nuclei in the epithelium.
    a. Note 1: The epithelium is often curved. If this is the case, approximate the length of the curve using a sum of line segments.

   b. Note 2: The image may include some olfactory epithelium in which the plane of sectioning and imaging is not orthogonal to the apical and basal surfaces. Only score regions where the plane of sectioning is approximately orthogonal, and omit regions that are at a very different angle.
   c. Note 3: Make sure that the regions being scored for basal length match the regions scored for centrioles.
5. Identify migrating centriole groups. To count, they must:
   a. visually appear to be more than a centrosome. (You will likely not be able to count a group of more than 4 centrioles at this resolution. The goal here is not to count centrioles but just to exclude Sus cell centrosomes.)
   b. be longer in the apical-basal dimension than in the side-to-side dimension. (Again, exact measurements aren't important. We want to exclude any progenitor cell centrioles that aren't migrating, including cells which might have died and started to delaminate. Their centrioles tend to be in rounder clusters.)
6. Record the number of centrioles in the **subapical compartment**. These are centrioles that are apical to the bottom of the nucleus of the nearest Sus cell. If you're having trouble deciding if a centriole group is subapical or apical, you can open the stack and check the phalloidin staining in the specific slices containing the group of interest.
7. Record the number of centrioles in the **basal compartment**. These are centrioles that are basal to the bottom of the nearest Sus cell nucleus.
8. Record the number of non-apical centriole **groups that were excluded** due to their shape. The goal here is to check if centriole groups are accumulating at a point prior to migration, so progenitor cells' centriole clusters should be counted in this category.
9. Save your spreadsheet and repeat with the other conditions or timepoints.

To the right is a diagram of the scoring method.