

Fluorescence imaging of olfactory nerve by selective Nav1.7 sodium channel targeted Inhibitor (Hsp1a) in the diagnosis of smell disorders

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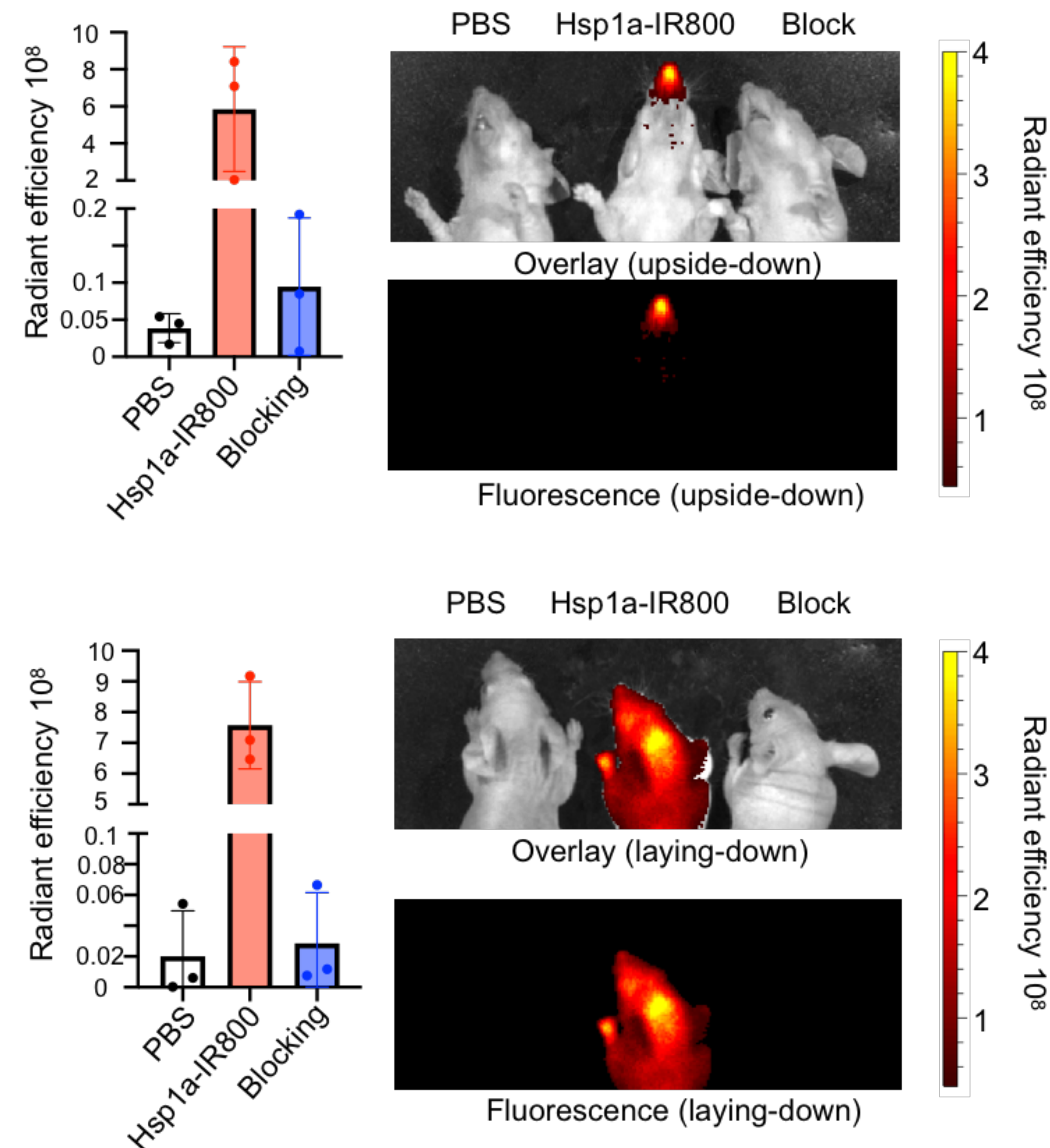
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Background

Anosmia/hyposmia affects 13.3 million people in the US alone according to the recent U.S. National Health and Nutrition Examination Survey (NHANES). Smell disorders have a profound impact on the quality of life, moreover, often result in anxiety and depression. Head and neck malignancies and the treatment provided are the major causes of smell and taste disorders. The majority of patients receiving radiotherapy or chemotherapy will experience different levels of smell disorders. As of today, there is no objective method to diagnose smell disorders. It is known that Nav1.7 sodium channels are abundantly expressed in the olfactory nerve, both in the epithelium and the olfactory bulb. Furthermore, patients with SCN9A gene loss of function, that encodes Nav1.7, additionally to insensitivity to pain, experience anosmia. Hsp1a is a recently discovered peptide with a potent selective Nav1.7 channel binding capacity. We analyzed the possibility to use Hsp1a conjugated to a fluorescent dye, to visualize normal olfactory nerve in a mouse model.

Methods

Athymic nude mice were intravenously injected with Hsp1a-IR800 (1nmol, 100μL PBS). As a control, mice were injected with PBS only, and block mice were injected with a combination of Hsp1a and Hsp1a-IR800. All mice were restraint using intraperitoneal ketamine/xylazine cocktail injection and in-vivo epifluorescence images obtained on IVIS Spectrum (PerkinElmer) using a filter set (excitation = 745 nm, emission = 810 nm). Images were taken 30 minutes after the tail vein injections. Animals were sacrificed and olfactory nerve/bulb, muscle, heart, spleen, kidney, liver images also obtained using IVIS imaging system. Autofluorescence was removed through spectral unmixing. Semiquantitative analysis of the Hsp1a-IR800 signal was conducted by measuring the average radiant efficiency (in units of the unit [p/s/cm²/sr]/[μW/cm²]) in regions of interest (ROIs) that were placed on the region of olfactory epithelium/bulb.



•Figure 1. Hsp1a-IR800 accumulation in olfactory epithelium/bulb. The figure describes in-vivo mice imaging with Hsp1a-IR800. Epifluorescence images of animals injected with PBS, Hsp1a-IR800 (1nmol, Hsp1a-IR800 in 100 μL PBS) and Hsp1a-IR800/Hsp1a formulation respectively. Images were taken 30 min after tail vein injection. Fluorescence intensity quantifications of the respective panels are on the left side.

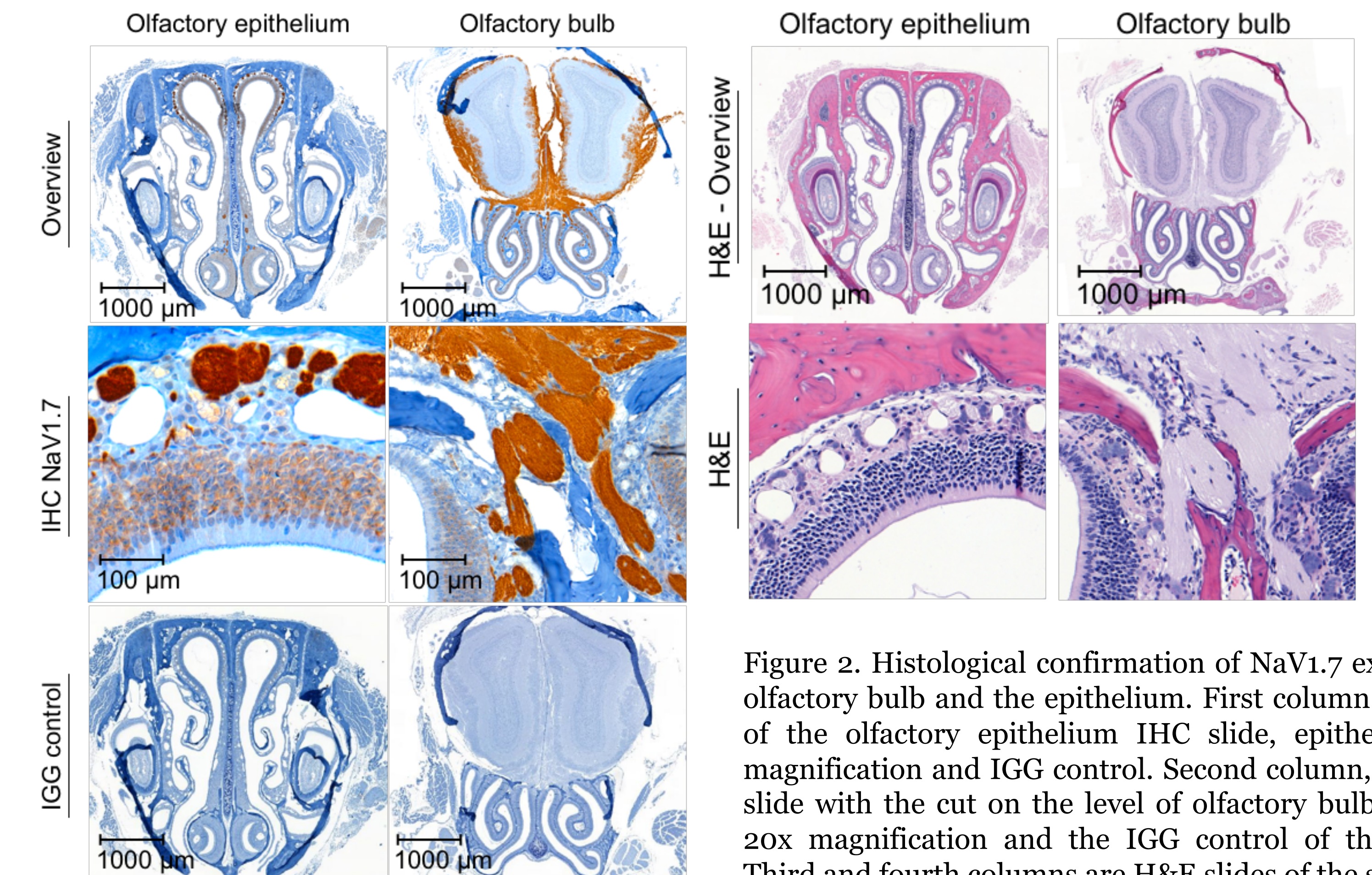


Figure 2. Histological confirmation of Nav1.7 expression of the olfactory bulb and the epithelium. First column is the overview of the olfactory epithelium IHC slide, epithelium with 20x magnification and IGG control. Second column, is the overview slide with the cut on the level of olfactory bulb, the slide with 20x magnification and the IGG control of the same region. Third and fourth columns are H&E slides of the same areas.

Results

On epifluorescence in-vivo images in mice receiving imaging agent, the area of olfactory epithelium/bulb was clearly visible. The radiant efficiency was significantly less in both mice injected with PBS and in mice injected with the unmodified peptide in combination with imaging agent(blocking) (Figure 1). We detected 150-fold increase in radiant efficiency compared to mice group injected with PBS and 61-fold increase compared to blocking mice. Ex-vivo, images showed a similar significant difference between mice groups of olfactory epithelium/bulb region. The heart and kidneys are the only internal organs with higher fluorescence signals comparing with control and block mice. To confirm the presence of Nav1.7 sodium channels in the olfactory system of the mice. We also performed IHC using Nav1.7 antibody, as seen in figure 2. Olfactory epithelium sensory neurons, axon bands, and olfactory bulb abundantly express Nav1.7 sodium channels.

Conclusions

We show that the imaging of mouse olfactory epithelium/bulb is possible, suggesting that labeled Hsp1a tracers hold promise in diagnosis of olfactory nerve disorders.