

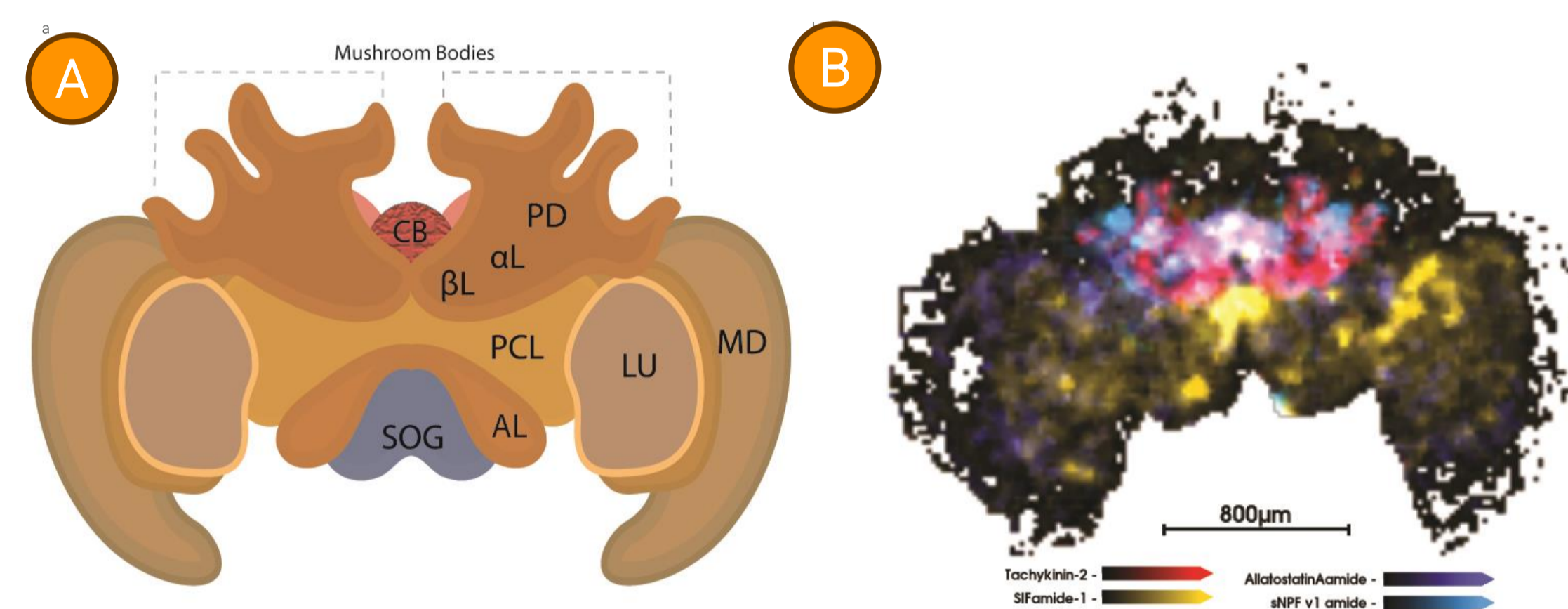
# Visualizing the Neuropeptide Distribution in the Common Eastern Bumble Bee using trapped ion mobility MALDI Imaging

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## Introduction

Neuropeptides are critical for brain, endocrine and exocrine function, yet characterization in-tissue remains a substantial challenge for MALDI Imaging. High abundance lipids and excess salt hinder the detection of neuropeptides due to ion suppression. Removing these interfering compounds provides an approach for the detection of neuropeptides within tissue. Trapped ion mobility spectrometry (TIMS) aids in post-ionization separation of neuropeptides of interest, allowing for the elucidation of numerous bioactive peptides in the common eastern bumble bee (Figure 1).



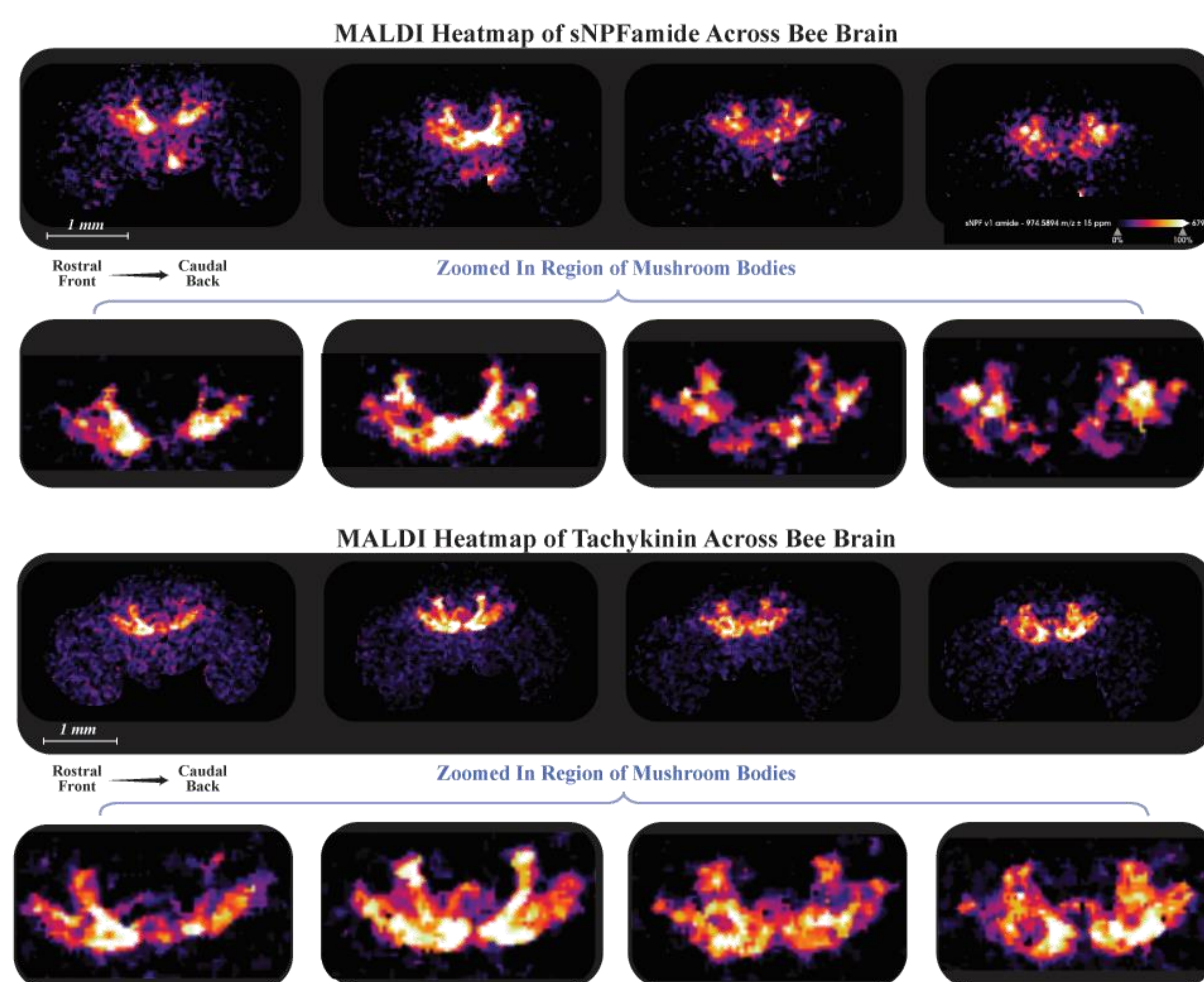
**Figure 1:** (A) Anatomical diagram showing the frontal plane of *Bombus impatiens* with mushroom bodies labeled. (B) Heatmap overlay from MALDI Imaging showing the intensity distribution of neuropeptides of interest, specifically tachykinin, allatostatin, SIF, and sNPF.

## Methods

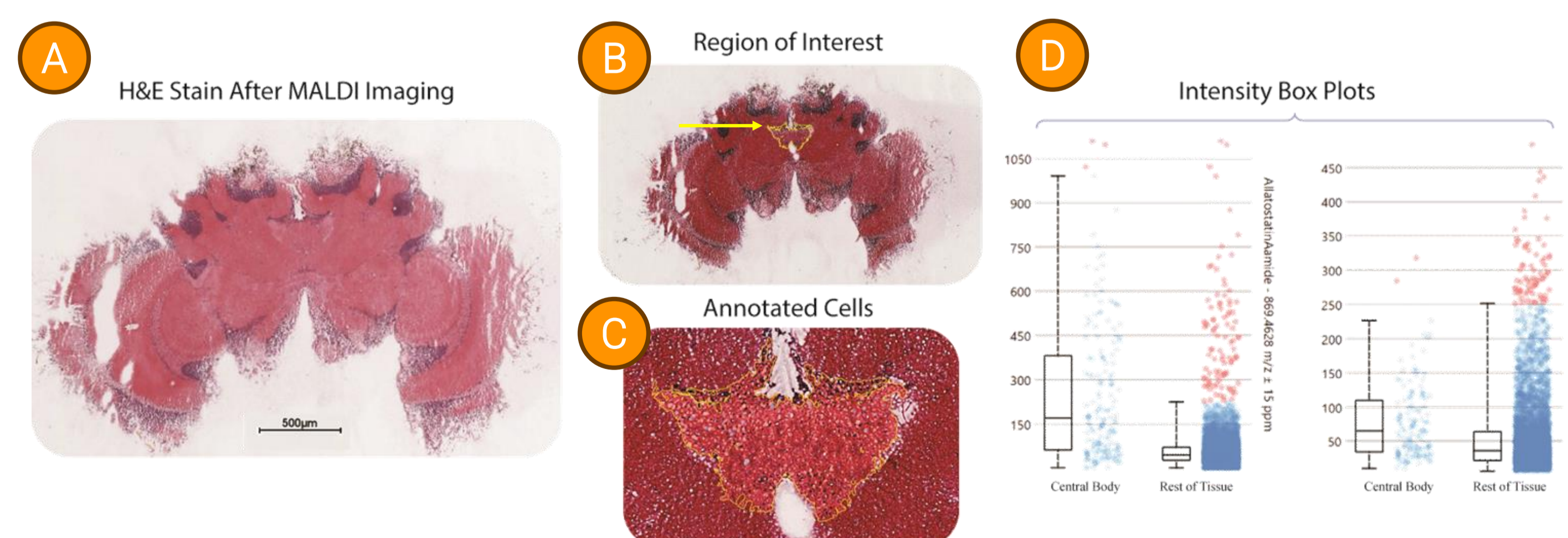
Bumble bee heads were sectioned at 10  $\mu\text{m}$  thickness before thaw-mounting onto IntelliSlides®. The tissue slides were washed twice for 10 s in 70% ethanol, followed by one 10 s washes in 95% ethanol with a 30 s drying time between each wash step. The MALDI matrix was 10 mg/mL  $\alpha$ -cyano-4-hydroxycinnamic acid and was sprayed on with an M5 sprayer (HTX, Chapel Hill, NC). MALDI Imaging data was acquired on a timsTOF flex MALDI-2 with TIMS on in positive ion mode at 20  $\mu\text{m}$  spatial resolution from 800-2500  $m/z$ , a 100 ms ramp time, a  $1/K_0$  range from 1.2–2.5, and 150 laser shots per pixel with a 10 kHz repetition rate.

## Results

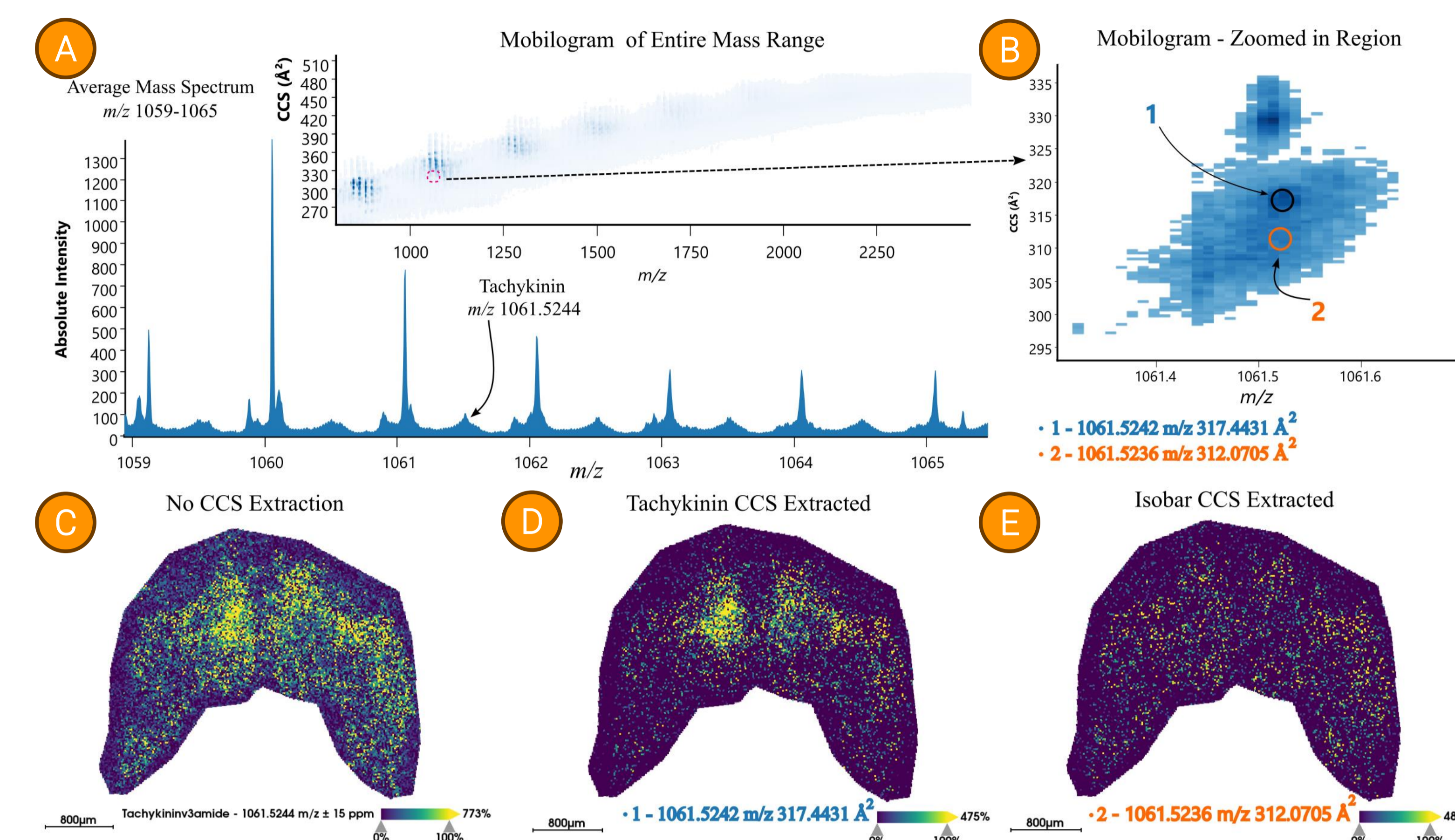
- The neuropeptide distribution and localization in the central body and mushroom bodies highlight clear brain ultrastructure (Figure 2).
- Multiplexing MALDI Imaging with histological techniques, such as hematoxylin and eosin (H&E) staining allows additional morphological data to be gained from the dataset (Figure 3).
- By extracting these neuropeptides by their collision cross section (CCS) values, interfering signals can be removed from the images to provide accurate spatial information (Figure 4).



**Figure 2:** Differential neuropeptide distribution across the central body and mushroom bodies. Four serial sections are shown.



**Figure 3:** Histological images and cell type annotation in QuPath®. (A) H&E stain of a serial section of the honey bee brain. (B) Region of interest defined in QuPath® for cell annotation. (C) Zoomed-in region from (B), indicated by the yellow arrow and outline. (D) Two intensity box plots showing the signal intensity for the central body region versus the remaining tissue area.



**Figure 4:** (A) Averaged mass spectrum and the corresponding mobilogram, with the tachykinin peptide indicated. (B) Zoomed-in mobilogram region showing an interfering contaminant (1) and tachykinin peptide (2). (C) MALDI image showing non-CCS extracted tachykinin  $m/z$  1061.5244, which included additional signal that can be observed outside of the mushroom bodies. (D) CCS extracted tachykinin removed additional signal and showed accurate tachykinin distribution throughout the mushroom bodies. (E) CCS extracted isobar at  $m/z$  1061.5236 showed additional signal that would have been incorporated into the MALDI image without ion mobility separation.

## Summary

Albeit, a small 'brain', the bumble bee contains  $\sim 1$  million neurons where the mushroom bodies are responsible for processing and encoding all the different sensory inputs. Performing a series of ethanol washes allowed the putative identification of numerous neuropeptides from the common eastern bumble bee. By extracting these neuropeptides by their collision cross section values interfering signals can be removed from the MALDI images to provide accurate spatial information.

## Conclusion

- Neuropeptide detection and spatial localization can be studied using the timsTOF flex.
- Extracting these neuropeptides by their collision cross section (CCS) values interfering signals can be removed from the MALDI images to provide accurate spatial information.

## Technology

### References

- Menzel, R. The honeybee as a model for understanding the basis of cognition. *Nat Rev Neurosci* 13, 758–768 (2012).
- Prataveira, Marcel et al. "MALDI imaging analysis of neuropeptides in the Africanized honeybee (*Apis mellifera*) brain: effect of ontogeny." *Journal of proteome research* vol. 13,6 (2014): 3054-64. doi:10.1021/pr500224b