

# Miniscope-based blood vessel imaging enables a novel assay for testing migraine therapeutic concepts

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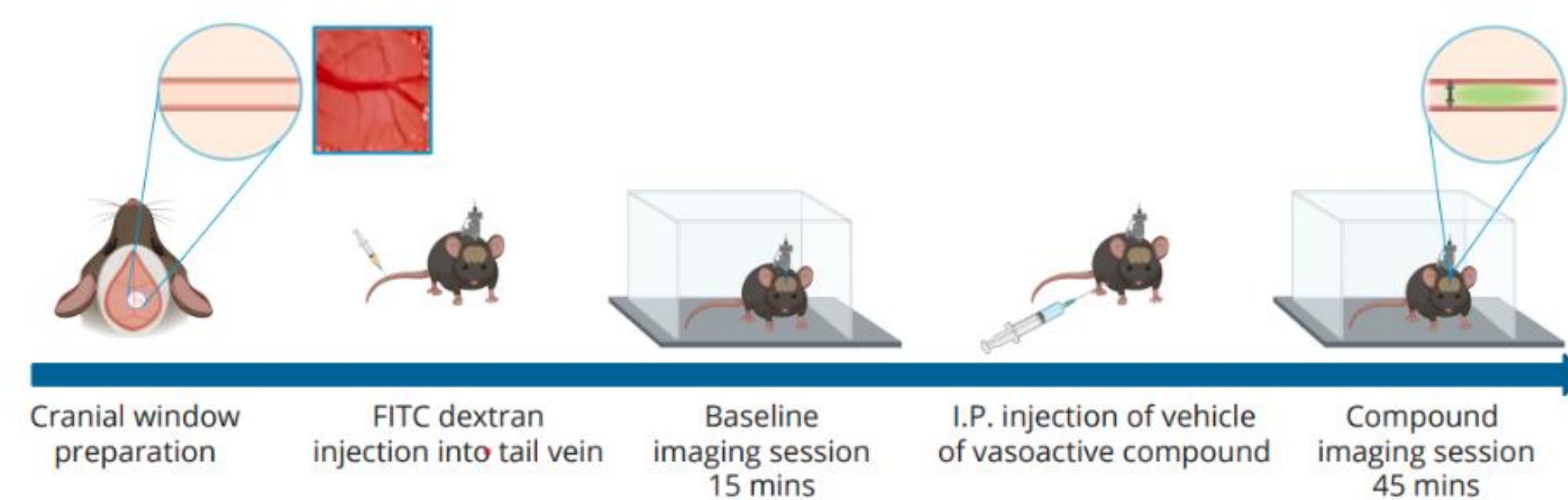
## Background

Neurovascular dynamics play an important role in brain health and CNS disorders like migraine, but precisely whether cerebrovascular reactivity in migraine is the disease's cause or consequence remains an active area of research that warrants further attention.

The current study delves into the importance of dorsal meningeal vessels and intermediate vessels, whose dilation is frequently linked to the onset of migraine episodes. Within this context, we introduce a novel preclinical tool and associated methods to evaluate vascular changes in vivo by testing vasoactive compounds such as Levcromakalim, Nitroglycerin (NTG) and the neuropeptides Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and vasoactive intestinal peptide (VIP), to replicate migraine-like conditions and assess their effects on vasodilation.

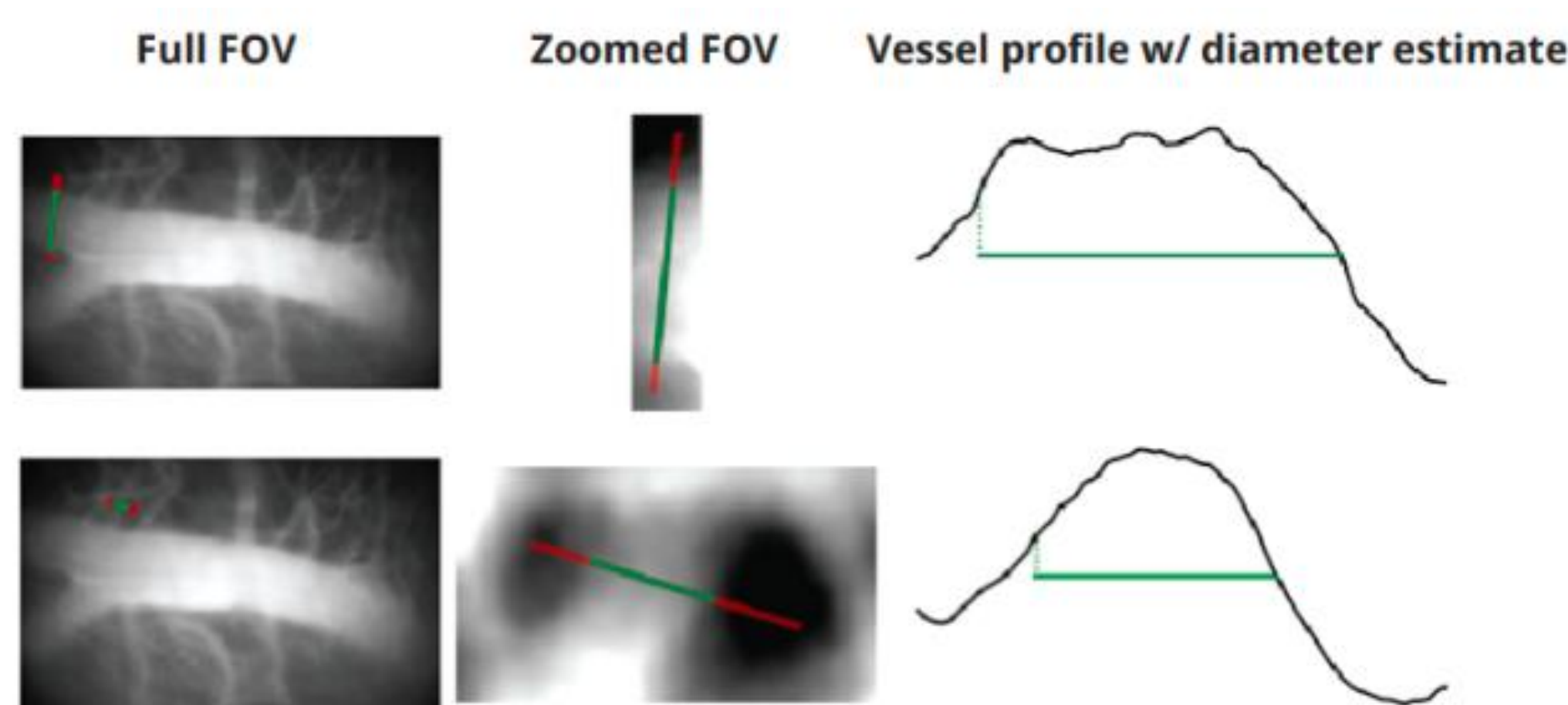
## Experimental Design

### Workflow of migraine-like vascular assay



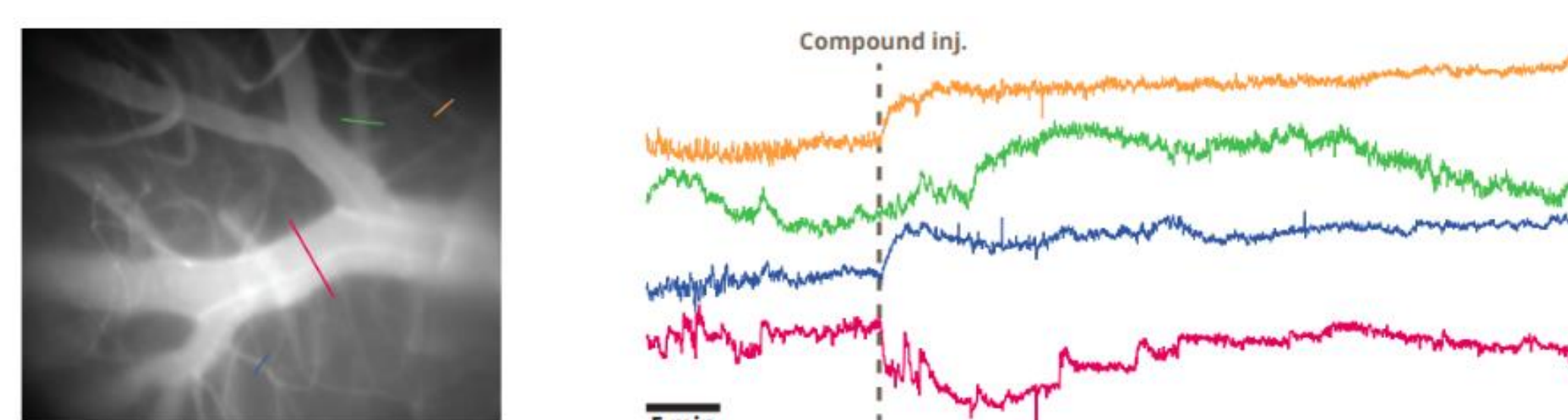
To enable optical access to dorsal meningeal vessels and its secondary and tertiary branches, we have been using one-photon miniaturized microscopes ('miniscopes'), which enables high spatio-temporal resolution of blood vessels permitting longitudinal measurements of vessel diameter and/or red blood cell velocity in freely behaving, unanesthetized mice.

### Algorithmic diameter estimation



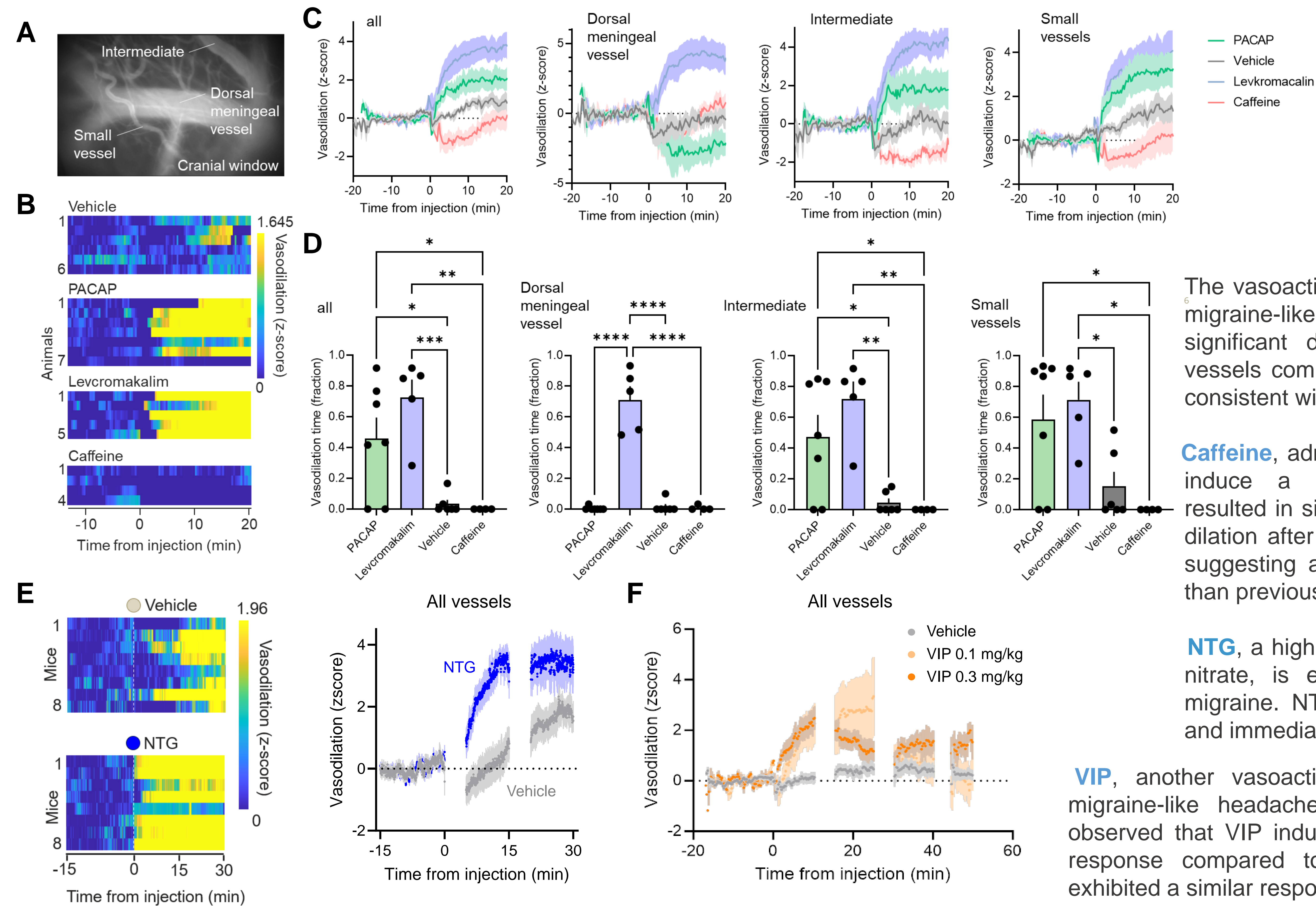
A newly developed algorithm accurately determines diameter for both small and large vessels by estimating the distance between its edges, which are identified by calculating the halfway point between the global maximum and the local minimum on each side of the vessel's pixel intensity profile.

### Example image and diameter estimation



Together with our collaborators at Lundbeck, we have applied the capabilities of this platform to develop an in vivo assay for screening therapeutic compounds in mechanistic migraine-like hypersensitivity models in mice.

## Time course of vasoactive responses



**Levcromakalim**, a drug known to consistently elicit migraine-like attacks in people with migraine, resulted in a significant dilation of dorsal meningeal vessels, across all tested animals, showing similarity to what is seen in the clinical challenge.

The vasoactive peptide, **PACAP**, that can elicit migraine-like attacks, resulted in variable but significant dilation of the dorsal meningeal vessels compared to vehicle control injections, consistent with published data.

**Caffeine**, administered as a negative control to induce a vasoconstriction response, also resulted in significant dorsal meningeal vessels dilation after a brief period of vasoconstriction, suggesting a more complex effect of caffeine than previously reported.

**NTG**, a highly permeable and lipophilic organic nitrate, is extensively used as a model of migraine. NTG administration induces a clear and immediate increase in vasodilation.

**VIP**, another vasoactive peptide, that can also elicit migraine-like headaches in humans, was tested. We observed that VIP induced a rapid but brief vasodilation response compared to PACAP. All vessel categories exhibited a similar response profile to VIP.

## Conclusions and Future perspectives

These data and establishment of the methods lay the groundwork for a miniscope-based assay for efficacy assessment of novel migraine therapeutic with a neurovascular component. Our ambition is to successfully show the relationship between peptide mediated vasodilation and trigeminovascular activation, as well as behavioural changes. We will, therefore, continue to use these methods to evaluate other potential mechanisms and therapeutic targets implicated in migraine pathophysiology, such as PACAP, CGRP, nitric oxide (NO), and monoacylglycerol lipase signalling, as well as to assess standard-of-care migraine treatments and novel drug strategies.

