Scientifica PatchScope Pro: an integrated calcium-imaging and patch-clamp system suitable for selecting specific subsets of neurons for electrophysiology recordings

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Introduction

Scientifica PatchScope Pro 3000

The PatchScope Pro is the first fully integrated system for patching cultured cells, providing a complete electrophysiology setup with a small footprint, consisting of:

- Inverted phase-contrast fluorescence microscope
- Motorised PatchStar micromanipulators
- Motorised XY stage
- Motorised objective changer
- Motorised brightfield/fluorescence selection
- Motorised 'return to cell of interest'

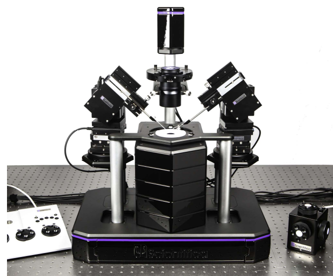
This system is suitable for a wide range of electrophysiological experiments and fluorescence imaging, with a focus on pharmacological studies.

Remote-control functionality: The PatchScope Pro offers the distinct advantage of remote control, which allows management of the entire rig without manual intervention. The fully motorised system is controlled through Scientifica's LinLab Software or range of fingertip control options, ensuring complete stability and reproducibility, improving data quality.

The Motorised Objective Changer enables seamless switching between two objectives, while the motorised fluorescence feature allows remote selection of brightfield luminescence or fluorescence filter set. The small footprint alongside full remote-control capability is ideal for effective shielding against ambient light (using blackout materials) allowing high quality fluorescence measurements to be obtained in standard laboratory lighting.

Selecting cells of interest: The integration of the motorised stage with Scientifica's LinLab Software allows cells of interest to be identified and mapped for further studies. For example, XY co-ordinates of cells identified using fluorescent markers or response to ligands using calcium-imaging can be stored for subsequent electrophysiological recordings.

PatchScope Pro 3000 fully integrated patch clamp and imaging platform



Pilot study using PatchScope Pro to target subsets of sensory neurons

The transient receptor potential (TRP) family includes a large number of nonselective Ca^{2+}-permeable cation channels such as TRPV1, TRPA1 and TRPM8, implicated in the detection or transduction of sensor stimuli[1].

TRPM8 depolarises somatosensory neurons in response to cold (<28 °C)[1,2]. In addition to being activated by changes in temperature, TRPM8 is also gated by chemical agonists that elicit sensation of cold such as menthol[3,4]. In rat, TRPM8 is detected in 10-20% of dorsal root ganglia (DRG)[5,6]. More specifically, menthol elicits afferent impulses in cold-sensitive C and Aδ DRG fibres[4].

TRPM8 is a voltage-dependent channel activated by membrane depolarisation and shows a pronounced outward rectification that arises from the rapid and voltage-dependent closure of the channel at negative voltages[2].

TRPM8 temperature sensitivity is modulated by the transmembrane voltage, changes in ambient temperature (cooling) or application of agonists inducing a leftward shift of the activation curve, resulting in channel activity at more physiological voltages[2].

Scientifica PatchScope Pro allows for identification of specific subpopulations of neurons by measuring cells' responses to specific ligands using calcium imaging. This provides an advantageous way of recording electrophysiological properties of small subpopulations of neurons, such as TRPM8 expressing sensory neurons, using single integrated platform.

Material and methods

Primary Cell Culture

Rat DRG neurons (P15-P21) were isolated and cultured at optimal densities on glass coverslips. Experiments were performed 24 (day 1) and 48 hours (day 2) after seeding.

Test Compounds

Supramaximal concentrations of capsaicin, cinnamaldehyde and menthol were applied to identify and target subpopulations of neurons expressing different TRP channels. In particular, TRPV1 channels were activated by 10 µM capsaicin, TRPA1 by 30 µM cinnamaldehyde and TRPM8 by 500 µM menthol.

Calcium (Ca^{2+}) Imaging Recordings

Ca^{2+} imaging recordings were conducted using Scientifica PatchScope Pro. Prior to experiments cells were loaded with Fluo-4 AM (4 µM) and incubated at 37°C for 30 minutes.

Fluorescence signals were background corrected and fluorescence levels (F) normalised against prestimulus fluorescence level (F_0) to yield F/F_0 values. A minimum of two-fold increase in Ca^{2+} responses, after agonist application, was used as a cutoff.

Electrophysiology

Whole-cell patch clamp recordings were performed using Scientifica PatchScope Pro. External solution, ECS, contained (in mM): 135 NaCl, 4.7 KCl, 1 $MgCl_2$, 10 HEPES, 10 Glucose, 1 $CaCl_2$. Internal solution, ICS, contained (in mM): 130 NaCl, 10 HEPES, 1 $MgCl_2$, 5 EGTA, 5 Mg-ATP, 0.3 Na-GTP. In current clamp mode, following recording of a stable resting membrane potential (RMP), 200 µM menthol was applied for a minimum of 10 seconds to elicit responses. Menthol responses were identified when the change in RMP (ΔRMP) was greater than 3 mV (ΔRMP > 3 mV). In voltage clamp mode, DRG neurons were held at -60 mV. Once a stable baseline current was recorded, 200 µM menthol was applied for a minimum of 10 seconds to elicit responses. A minimum increase of 40 pA in elicited current was used as a cutoff.

Results

Isolated rat dorsal root ganglia (rDRG) culture

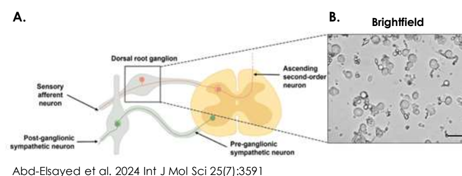


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Figure 1: The image shows the location of rDRG in relation to the spinal cord **(A)** and representative image of isolated rDRG neurons 24 hours after seeding **(B)**. Scale bar 50 µm.

Targeting TRP channels in rDRG neurons using Ca^{2+} imaging

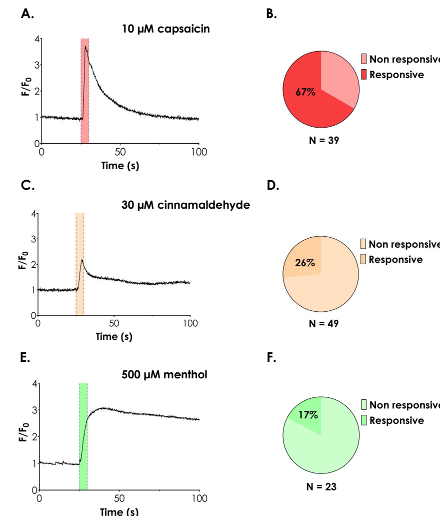

Figure 2: Representative fluorescence tracings show Ca^{2+} responses after TRPV1 **(A)**, TRPA1 **(C)** and TRPM8 **(E)** channel activation using selective agonists: 10 µM capsaicin, 30 µM cinnamaldehyde and 500 µM menthol (5 s application). Percentage of neurons responding to TRPV1 **(B)**, TRPA1 **(D)** and TRPM8 **(F)** channel agonists. Data were collected on day 1. (N = 2 fields of view for each agonist from 2 DRG preparations).

Verification of TRPM8 Ca^{2+} imaging responses using repeat applications of menthol

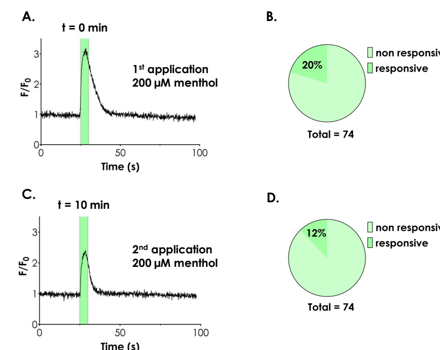

Figure 3: Representative fluorescence traces show Ca^{2+} responses after first **(A)** and second **(C)** application of 200 µM menthol (t = 0 and 10 minutes respectively). Percentages of responding neurons to first **(B)** and to second **(D)** application of 200 µM menthol are shown. Data were collected on day 1. (N = 3 fields of view for each agonist from 1 DRG preparation).

Whole-cell current clamp and voltage clamp recordings of pre-identified TRPM8 positive rDRG neurons

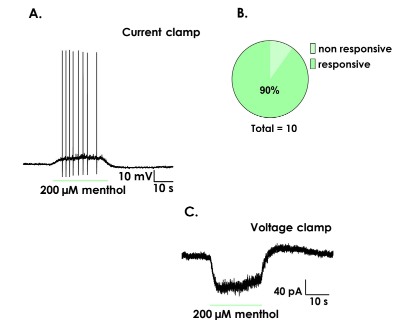

Figure 4: TRPM8 positive DRG neurons were previously identified using Ca^{2+} imaging technique. Representative current clamp recording show RMP depolarisation followed by repetitive firing induced by application of 200 µM menthol **(A)**. Total percentages of patch-clamped neurons responding to application of 200 µM menthol **(B)**. Representative trace showing increase in current at -60 mV upon application of 200 µM menthol **(C)**. Data were collected on day 1 and day 2. (N = 10 fields of view from 5 DRG preparations).

Conclusions

PatchScope Pro was successfully used to identify different subpopulations of sensory neurons expressing TRP channels (TRPV1, TRPA1 and TRPM8) using Ca^{2+} imaging technique.

Proportion of sensory neurons expressing TRPV1, TRPA1 and TRPM8 reflected data in literature[5,6,8].

Calcium responses to repeated compound applications of 200 µM menthol were verified in TRPM8 positive DRG neurons.

Lastly, we successfully patched and recorded pre-identified TRPM8 positive DRG neurons, using current clamp and voltage clamp mode. Further optimisation for pharmacological studies combining Ca^{2+} imaging and electrophysiology may be required.

References

1. Chuang *et al.* 2004 *Neuron* 43:8559-869
2. Voets *et al.* 2004 *Nature* 430:748-754
3. Caterina *et al.* 1997 *Nature* 389:816-824
4. McKemy *et al.* 2002 *Nature* 416:52-58
5. Peier *et al.* 2002 *Cell* 108:705-715
6. Dhaka *et al. The Journal of Neuroscience* 28(3):566-575
7. Abd-Elsayed *et al.* 2004 *Int J Mol Sci* **25**(7):3591
8. David Julius 2013 *Annu Rev Cell Dev Biol* 29:355384