Human iPSCs RealDRG™ : a translatable assay for screening peripheral analgesics

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INTRODUCTION

- Opioids are effective analgesics but highly addictive (↑ morbidity and mortality).
- Alternative approach = target peripheral nociceptors (sensitised in neuropathic pain).
- Pre-clinical animal studies often lack translatability.
- We present a new-approach methodology (NAM) utilising human induced pluripotent stem cells (hiPSCs).
- RealDRG™ (Anatomic, Minneapolis, MN) sensory neurons are generated using directed differentiation with primal ectoderm intermediate.
- We validate our translatable quantitative assay for peripherally acting analgesics.

METHODS

Multi-electrode array (MEA)

Maestro Pro MEA plate reader, (Axion Biosystems)

Spontaneous and agonist- or temperature-evoked extracellular action potentials.

Ca^{2+} imaging

Opera Phenix microscope with pipettor, (Perkin Elmer)

Agonist-evoked Ca^{2+} responses were examined in RealDRG loaded with calcium indicator.

Manual patch-clamp (MPC)

Axon 700B amplifier/1332A digitizer interface (Molecular Devices)

Action potential trains were recorded and Tetrodotoxin (TTx)-insensitive Na^+ currents isolated.

For all methods, plates or coverslips were coated with Poly-L-Ornithine Solution and iMatrix-511 SILK. RealDRG™ hiPSCs (Anatomic, Minneapolis, MN) were plated according to manufacturers' instructions. Cells were maintained for 5 weeks (37 °C, 10% CO) with 50% media exchange every 2 days.

RESULTS

1. Functional validation of key analgesic target expression

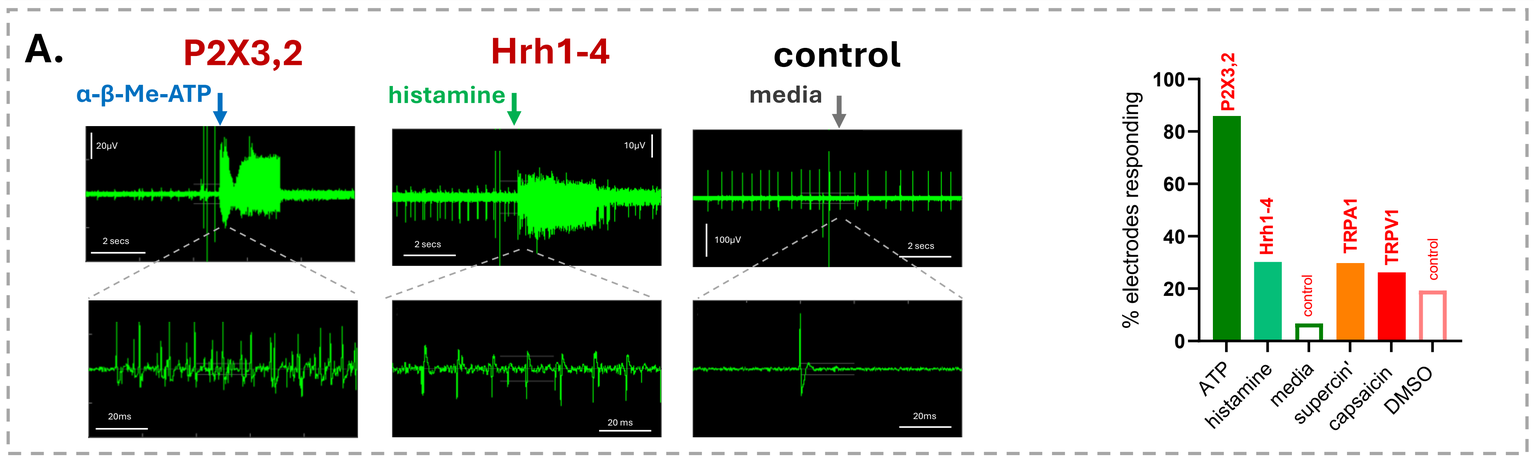

Figure 1. Validated analgesic targets shown in red. ***A,*** example MEA traces showing responses to α-β-Me-ATP (1μM), histamine (100μM) and media controls, magnified waveforms below. ***RHS***, bar chart showing % of cells responding to each agonist. ***B,*** example Ca^{2+} imaging traces showing responses to capsaicin, WS12 and CIM0216 (all 1μM). ***RHS,*** (as above). ***C,*** example MPC voltage traces showing single (LHS) and repetitive action potential firing (RHS) at days in vitro (DIV) 14 and 21 respectively. ***D,*** example time course (LHS) and current trace (RHS) showing partial inhibition of peak Na^+ current with TTx ± suzetrigine (both 100nM).

2. Ionic mediators of spontaneous local field potentials

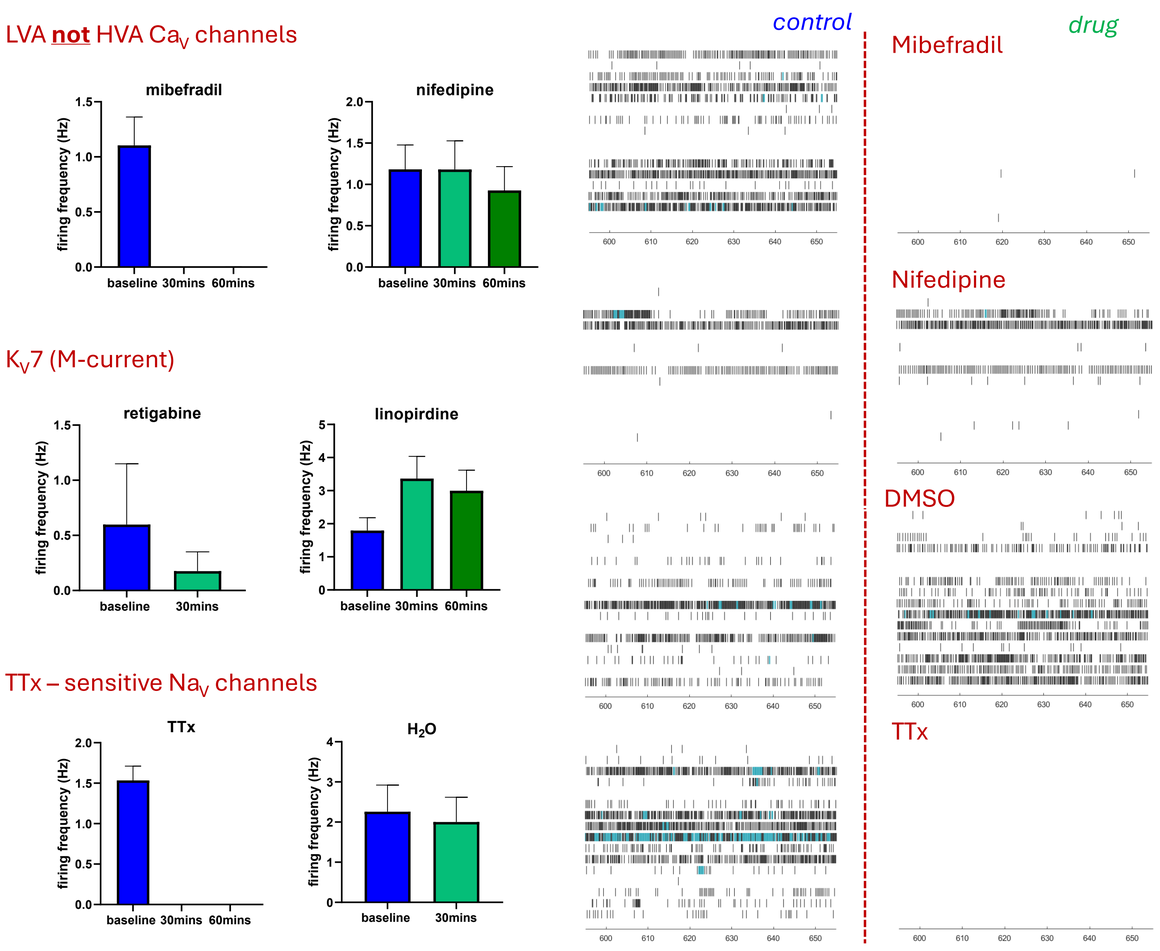

Figure 2. LHS, Bar charts of MEA data showing average spontaneous firing frequencies and effects of 30 or 60mins treatment with retigabine, linopirdine and nifedipine (all 10μM), mibefradil (3μM), tetrodotoxin (100nM) or H_20 control. ***RHS,*** raster plots showing effects of mibefradil, nifedipine, DMSO (3:1000) or TTx (60mins) on neural spike timing, from representative MEA wells.

3. Successful identification of peripherally acting analgesics

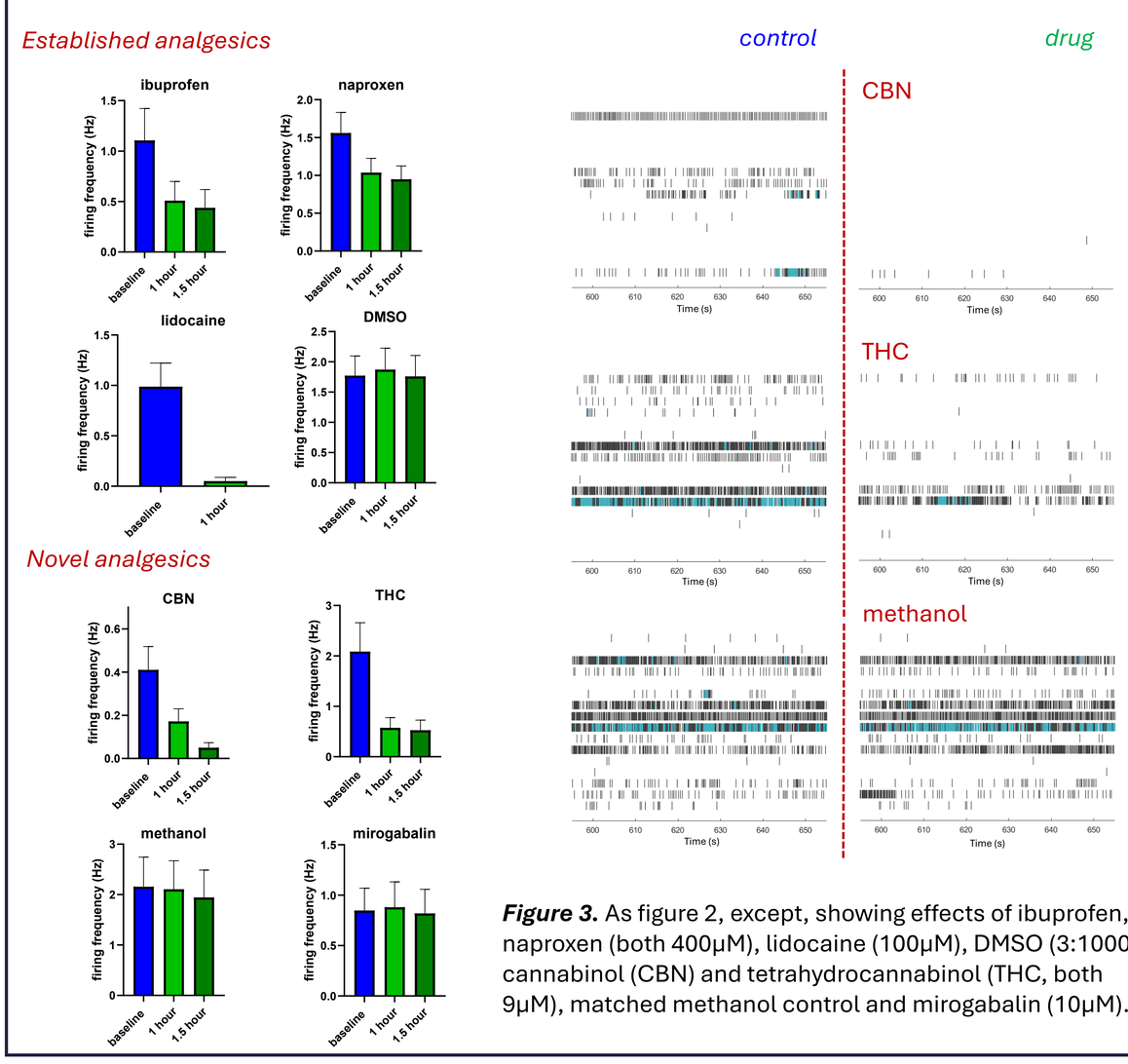

Figure 3. As figure 2, except, showing effects of ibuprofen, naproxen (both 400μM), lidocaine (100μM), DMSO (3:1000), cannabinol (CBN) and tetrahydrocannabinol (THC, both 9μM), matched methanol control and mirogabalin (10μM).

DISCUSSION AND CONCLUSIONS

- RealDRG hiPSC sensory neurons functionally express many key ion channels commonly targeted in analgesic drug development
- MEA recording of spontaneous firing-frequency provides a quantitative readout which is sensitive to peripherally acting clinical analgesics.
- This NAM provides a translatable alternative/adjunctive approach to pre-clinical animal studies in analgesic drug-development pipelines.