Pain Relief Foundation Research Grant Project Report

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'Development of novel in vitro human induced pluripotent stem cell (hiPSC)-derived sensory-like neuronal model of orofacial neuropathic pain'

Background and importance of the project

Pain is usually triggered by injury. The aim of pain is to highlight the injured area, causing a change in its use to allow healing e.g. A sprained ankle is painful to walk on, this means we walk less, allowing the toe to heal. Neuropathic pain is pain which comes directly from nerves. Neuropathic pain commonly presents in nerves that have been injured previously, which despite healing continue over a long period of time to send out pain messages when they should not. Neuropathic pain felt in the head, face or mouth is called orofacial neuropathic pain. Due to its location orofacial pain affects essential social functions (e.g. eating and talking). Such social functions provide enjoyment of, and meaning to, life worsening the impact of this pain. Simple toothache is a good example demonstrating how a relatively small injury (e.g. small hole) in a tooth can cause severe pain and lead to considerable distress.

There remain many unanswered questions as to what causes long lasting orofacial neuropathic pain and there is no specific treatment which is guaranteed to help this type of pain. This study, funded by the Pain Relief Foundation has provided the first stage of development of a laboratory model of a cell which responds like a human nerve that has orofacial neuropathic pain. There is no model of this type currently available. Generating this model will allow detailed exploration of how and why the nerves are sending pain messages in this condition. This understanding offers potential in the future for the development of targeted treatments which could reduce or eliminate orofacial neuropathic pain. Wider benefits of this study include reducing or replacing the use of animals in orofacial pain research and the potential for benefit to any medical or pain condition which involves the incorrect functioning of nerves.

Project method

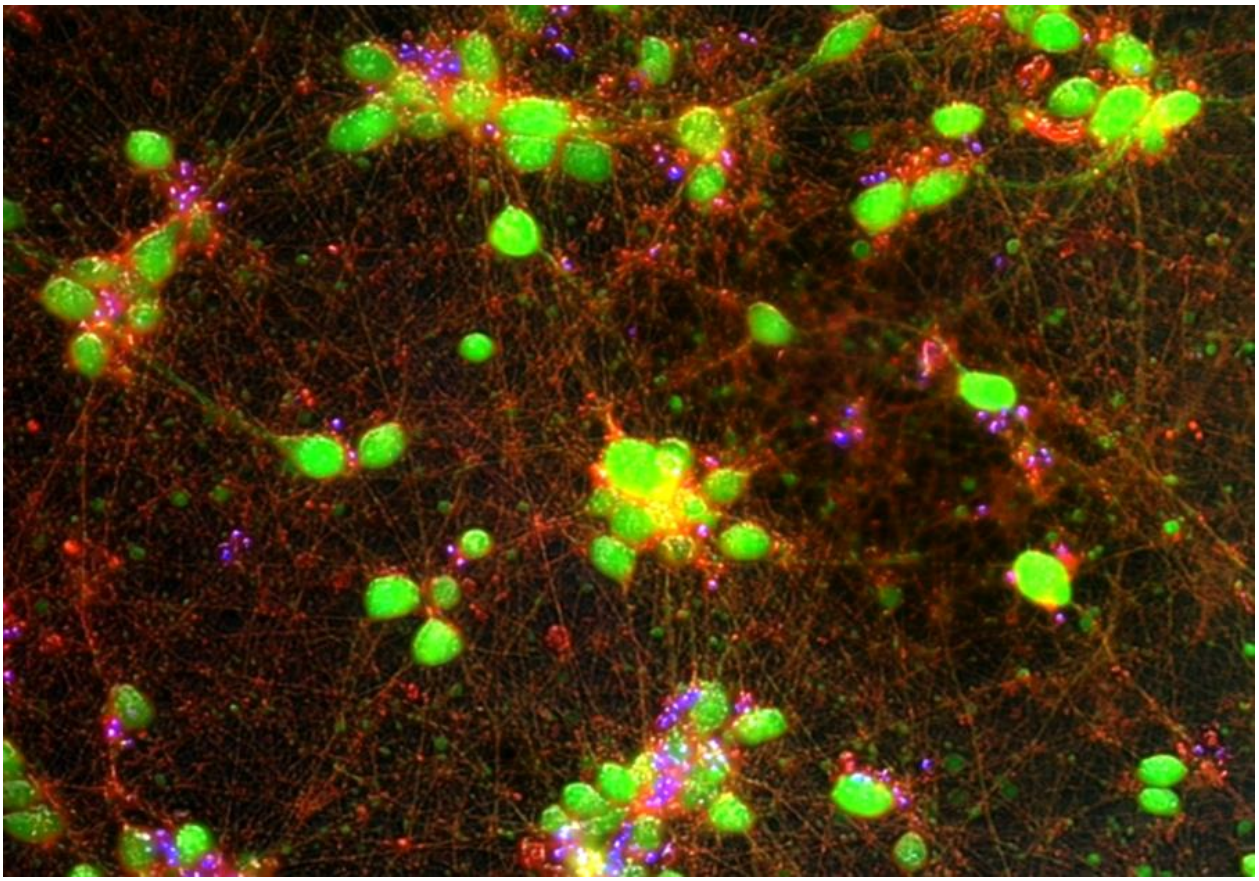
Skin cells from live human donors (human induced pluripotent cells) were purchased and treated with different compounds in the laboratory so they changed into pain-sensing nerve cells (sensory nerves). These pain-sensing nerve cells were treated with different combinations of substances that the body naturally releases when in pain (allogenic substances).

Our pain-sensing nerve cells were tested in the laboratory to see how they send messages and how the allogenic substance changed the way they send messages.

Primary outcomes

1. **The pain-sensing nerve cells developed from skin cells look like real sensory nerves**

Figure 1: Image shows pain sensing nerve cells grown in the project.
The shape, size and structure of the cells shown is representative of a real sensory nerve.
The green colour stains cells capable of working successfully (viability indicator), figure 1 shows that the pain-sensing nerve cells grown are viable.



2. The pain-sensing nerve cells developed from skin cells function like real sensory nerves

Figure 2 a) Spontaneous activity of the pain-sensing nerve cells grown in the project confirm activity
Exemplar trace of spontaneous neuronal activity of HD33n1 hiPSC-derived neurons.

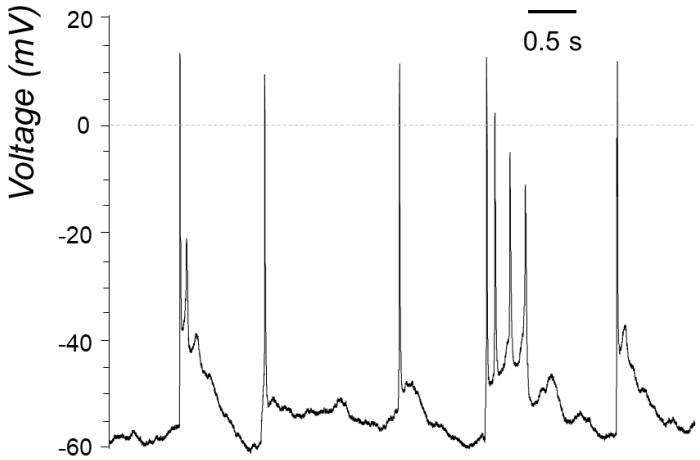
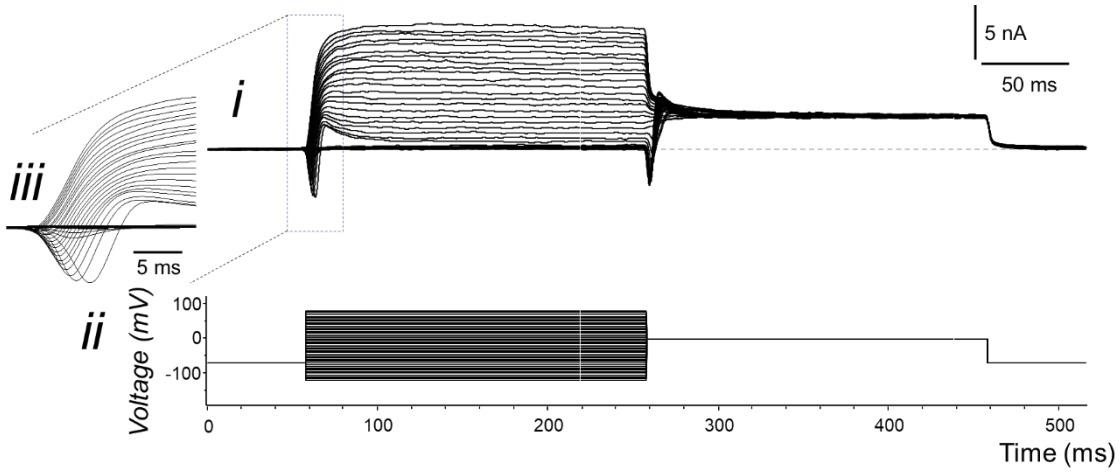


Figure 2 b) Electrical currents measured in the pain-sensing nerve cells developed in the project confirm activity
Exemplar family of whole-cell transmembrane ion currents in HD33n1 hiPSC-derived sensory-like neurons (i - upper) during the voltage-clamp step protocol (ii - lower); extraction of Na^+ current activation currents at the beginning of the voltage-step pulse (iii - middle).

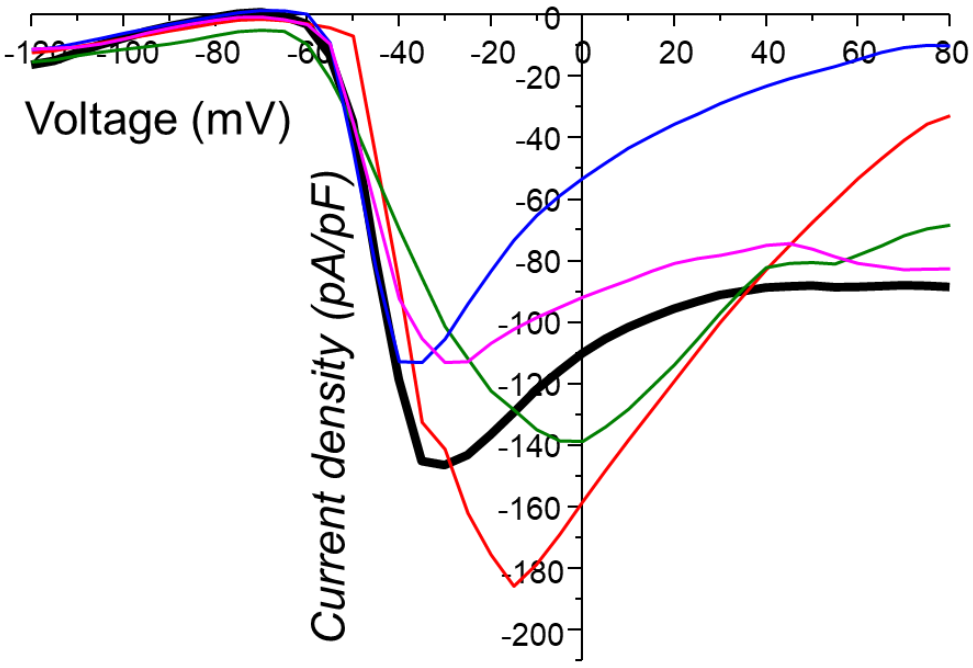


3. Combinations of allogenic substances applied to the developed pain sensing nerve cells induce changes in function

i. Mean current density-voltage characteristics for whole-cell voltage-gated Na^+ currents recorded in HD33n1 hiPSC-derived sensory-like neurons in Control and chronically administrated with different allogenic substances (PC1-4)

ii. Table displays mean peak Na^+ current density (pA/pF), standard error (SEM), number of cells (n) and voltage at peak Na^+ current (mV) of the corresponding groups

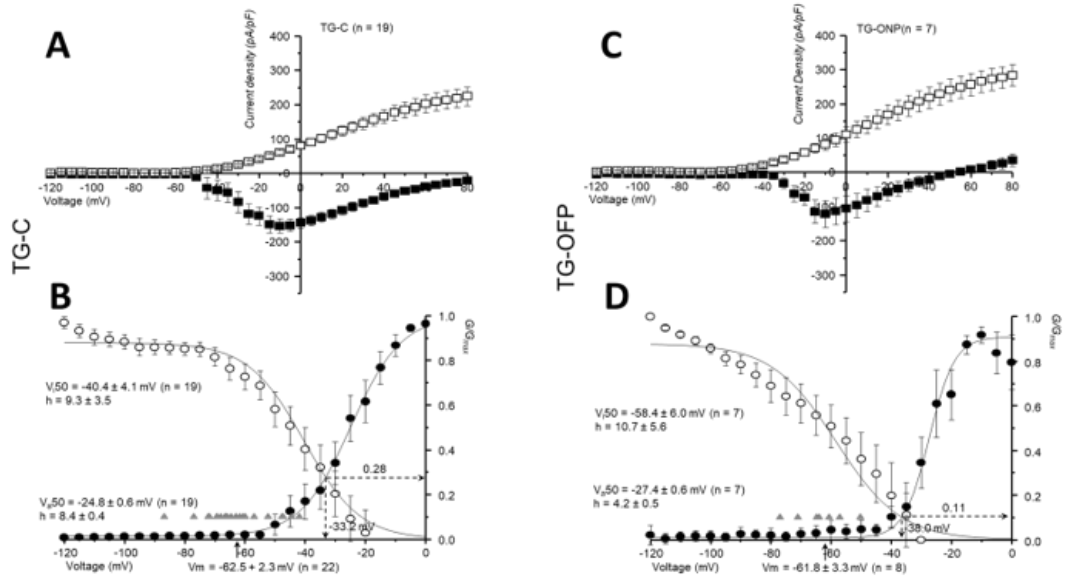
i



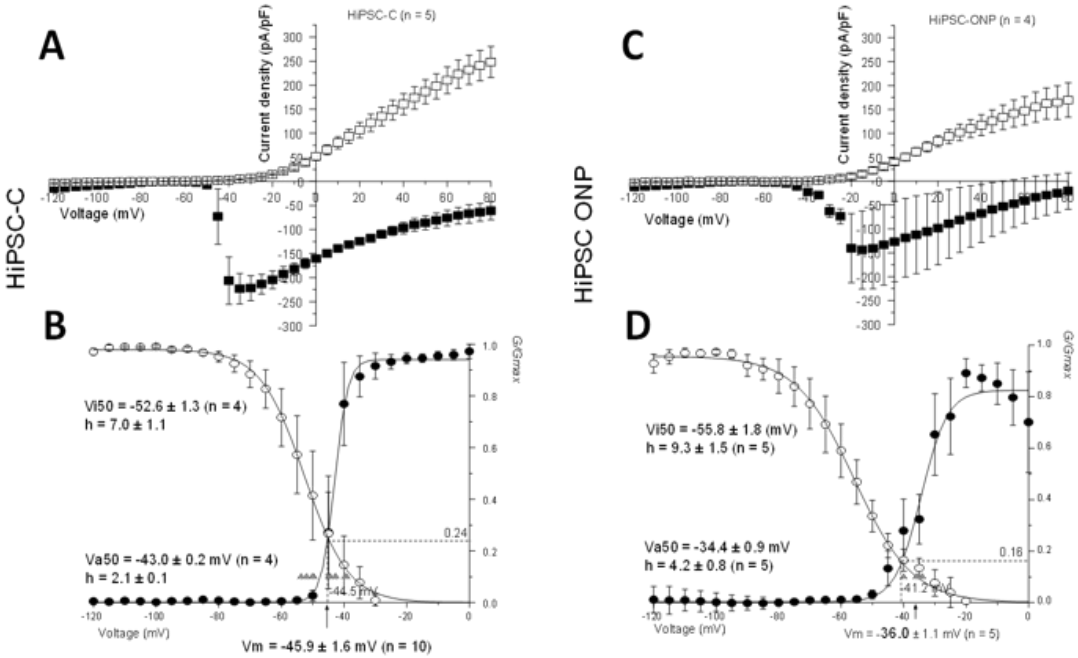
ii

hiPSC-neurons	Mean peak Na^+ current density (pA/pF)	SEM	N	Voltage at peak Na^+ current (mV)
Control	**-147.8**	**35.4**	**20**	**-30**
PC1	-187.3	85.2	7	-15
PC2	-141.6	56.5	11	-5
PC3	-116.5	29.3	17	-35
PC4	-119	31.5	7	-30

4. **The developed pain-sensing nerve cells when compared to real sensory nerves from rats demonstrated comparable functional properties. This suggests the laboratory model of a cell which responds like a human nerve with orofacial neuropathic pain is achievable.**



Mean current-voltage characteristics for [A] Healthy rat orofacial sensory neurons (TG-C)[C] Healthy rat orofacial sensory neurons treated with allogenic substances (TG-ONP). Mean activation and inactivation curves of whole-cell voltage-gated Na^+ currents for [B] Heathy rat (TG-C) and {D] Healthy rat orofacial sensory neurons treated with allogenic substances (TG-ONP)



Mean current-voltage characteristics for the developed pain-sensing nerves (hiPSC-C) [A] and developed pain-sensing nerves treated with allogenic substances (hiPSC-ONP) [C]. Mean activation and inactivation curves of whole-cell voltage-gated Na^+ currents for [B] developed pain sensing nerves (hiPSC-C) and [D] developed pain-sensing nerves treated with allogenic substances (hiPSC-ONP)

Secondary outcomes

1. This project has supported the training of the next generation of clinical scientists

Primary applicant Emma Beecroft is a dentist who manages orofacial pain in the patients she treats. The limited available treatment options for the individuals in her care triggered her interest in research to improve patient outcome. Funding provided by the Pain Relief Foundation has provided Emma the opportunity to learn the laboratory techniques required to grow and test pain-sensing nerve cells, allowing her to complete further research in this field supporting her development and long-term aim of becoming an independent clinical scientist.

2. This project has springboarded further research into orofacial neuropathic pain

As a direct result of preliminary data generated from this research project primary applicant, Emma Beecroft was successfully appointed as a full-time Clinical Doctoral Fellow and Honorary Specialty trainee in oral surgery (March 2022). This appointment includes registration of a staff-funded PhD (January 2023). The PhD project will build on this Pain Relief Foundation funded project by:

a. Further characterising the orofacial neuropathic pain model
b. Using the model to look at the cells in detail to try and work out what changes they display with pain compared to health
c. Using drugs to target and try to correct pain related changes

Involvement of the public in this research project

To design this laboratory-based project in a manner which will generate the most impactful results, patient and public opinion was sought through several avenues. This project was directly discussed with individual living with orofacial neuropathic pain. Patients identified that the long lasting presence (persistency) of pain was the most significant driver in their suffering even when compared to the severity of pain. This highlighted the importance to develop a model which accurately reflects long-lasting nerve-based pain, which became the projects focus. Another consistent concern was that in trying to better understand their condition patients expressed difficulty with understanding how laboratory-based research was working to help their condition. To try to improve understanding a formal presentation of this study with a patient focus group was completed on the 4th February 2021 and a lay representative was recruited and continues to support the research team with fellowship applications and future funding applications, ensuring the project meets the needs and is understandable to patients.

Development of tissue culturing and neuronal differentiation techniques through the course of this project (see methodological adaptations over the course of BRC project section) has improved the viability and quality of the hiPSC derived sensory like neurons outputted by the Pain Research Group at the Dental School, Newcastle University supporting the generation of data which is more reliable and robust.

Data gathered through this project has crucially confirmed feasibility of an *in vitro* disease relevant hiPSC sensory like neuronal model for orofacial neuropathic pain. Peer review publication of the results with international dissemination and clinically meaningful change for individuals with neurodegenerative conditions remain primary aims.

Intellectual property is expected to develop through further research by primary applicant spring boarded as a result of this BRC funded project. BRC will be acknowledged as appropriate.

As a direct result of preliminary data generated from this research project PI, Emma Beecroft was successfully appointed as a full-time Clinical Doctoral Fellow and Honorary Specialty trainee in oral surgery (March 2022). This appointment includes registration of a staff-funded PhD (January 2023) and initiates the clinical academic career of PI, Emma Beecroft, supporting her development and long-term aim of becoming an independent clinical scientist.