Update of project: **Ex**ploring irreversible pulp**iti**s and its t**r**eatment's effects on **p**ripher**a**l and cen**t**ral m**e**chanisms: **ExtirPate** study

## Objectives

Develop a new translatable model of symptomatic irreversible pulpitis (SIP) and explore its nociceptive peripheral and central mechanisms.

Identify the effect of current approaches to managing SIP on peripheral and central nociceptive mechanisms.

## Methods (to date)

A small access cavity was drilled into the first maxillary molar of Naïve adult male Sprague Dawley rats (180-250g, n=33), after which a sterilised paper point was introduced into the pulp (Figure 1). A glass-ionomer restoration was placed with the paper point protruding through it. At days 1-5 nociceptive mechanisms were assessed using behavioural, biochemical, and immuno-histological analysis. A further experiment (n=3) involved the placement of fluorogold directly onto the exposed pulp to undertake neuronal tracing.

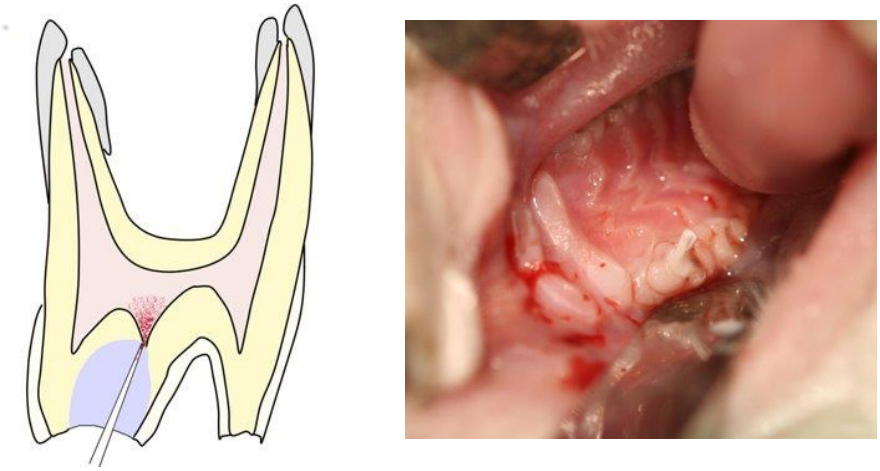

*Figure 1: Initiation of symptomatic irreversible pulpitis through the insertion of a paper point into the pulp followed by temporary restoration.*

## Results (to date)

Behavioural assessment demonstrated:

- Increased rat grimace scale score in SIP *vs* sham at day 1 (change from baseline: 0.234 *vs* -0.049, p=0.003, n=25).
- Mechanical allodynia (Von Frey test) demonstrated in SIP *vs* sham at days 1 and 2: 11.75g *vs* 25.00g (p=0.012, n=25); 9.36g *vs* 26.58g, (p=0.006, n=18) respectively.

- Thermal allodynia caused less attempts to access reward in SIP *vs* sham at days 1, 2 and 5: 5.86 *vs* 21.58 (p=0.008, n=33); 3.34 *vs* 20.83 (p=0.037, n=25); 10.42 *vs* 25.38 (p=0.033, n=10) respectively.
- Soft food preference in SIP *vs* sham at day 5 (change from baseline: 4.36 *vs* -6.55, p=0.021, n=10).

Biochemical analyses demonstrated:

- Serum IL-1β increased in SIP *vs* sham at day 2 (948.5pg/ml vs 616.58pg/ml, p=0.039, n=18).
- Serum TNFα increased in SIP *vs* sham at day 5 (238.32pg/ml vs 50.09pg/ml, p=0.039, n=10).

Histological (Hematoxylin and Eosin) analyses demonstrated: progressive coronal pulp inflammation from day 2 to day 5, with the formation of micro-abscesses and areas of necrosis at day 5 (Figure 2).

Immunohistochemical analyses of sub-nucleus caudalis demonstrated: increased levels of GFAP, AMPA and phosphorylated α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid ipsilateral to SIP compared to sham animals.

Examination of rats with fluorogold applied showed autofluorescence of the tooth and TG at 5-days, but no autofluorescence was identified in the brainstem.

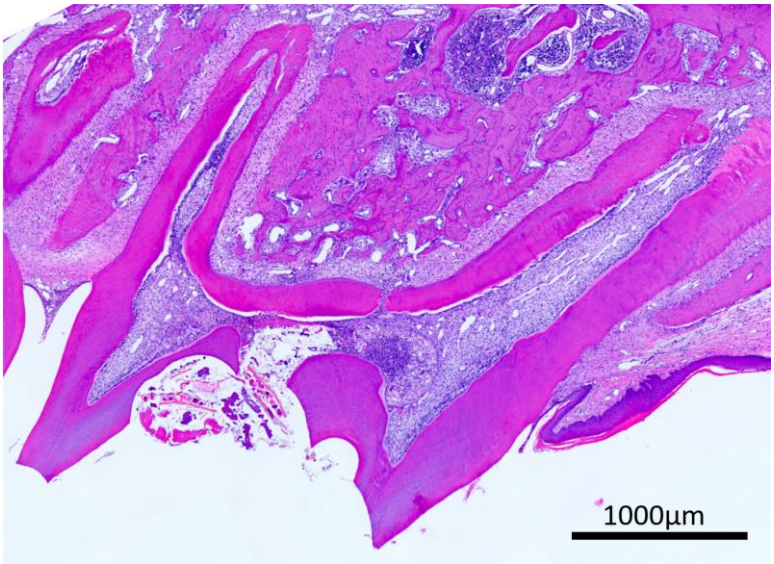


*Figure 2: H+E stained section showing inflammatory cell infiltrate and formation micro abscess at 5-days*

## Conclusion

This novel murine model of SIP is moving towards validation and presents previously unreported behavioural and histological changes in rats. It offers a more translatable biological model to SIP pathogenesis in humans compared to previously reported models. Further work will be undertaken to validate the model under the BES grant. This includes RNA-Seq of the brainstem, further immunohistochemistry of the TG and brainstem to explore NMDAR1 receptor upregulation, ATF-3 protein upregulation and IDO enzyme. Following this I intend to explore the impact of current approaches to managing SIP (corticosteroids, antimicrobials) in addition to novel approaches (manipulation of kynurenine pathway).

## Outputs

The lead applicant (DE) has been successful in the award of a Wellcome 4Ward North doctoral research fellowship and an FDS RCS(Eng) research fellowship to further the work.

The lead applicant is now out of programme undertaking a PhD (full time).

The lead applicant has had an abstract accepted at the upcoming 2022 IADR General session and will present the findings of the research so far.