Characterisation of the High Fat Diet/Streptozotocin Model of Diabetes and the Effects of the PPARγ Ligand Pioglitazone

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Introduction
Diabetes is a widespread problem with 285 million people worldwide currently living with the disease1. Diabetic neuropathy is one of the most common complications of diabetes, with painful symptoms including hyperalgesia and allodynia. Our model involves feeding a high fat diet (HFD) to induce a degree of insulin resistance, followed by a single low dose of streptozotocin (STZ), which selectively destroys the pancreatic β-cells. This model exhibits changes indicative of mechanical allodynia, as well as the expected metabolic changes associated with diabetes such as hyperglycaemia and hyperinsulinaemia.

Pioglitazone (pio) is a PPARγ agonist with anti-inflammatory effects2 and is an insulin sensitizer3 which is used clinically to treat diabetes. Recently it has been shown to alleviate mechanical hyperalgesia in a model of neuropathic pain4. The aim of this study was to investigate whether the HFD/STZ model alters neuronal responses in the spinal cord, and whether pioglitazone has any effect on parameters such as neuronal activity, hyperglycaemia and mechanical allodynia.

Methods
Male Sprague-Dawley rats (200-250g) were divided into four groups (lean controls, HFD controls, HFD/STZ and pioglitazone). They had free access to water and chow, and were kept on a 12-hour light/dark cycle.

Three weeks after arrival rats received either a single i.p. injection of STZ (45mg/kg, at a volume of 3ml/kg) or citric acid buffer. Mechanical withdrawal thresholds on the plantar hindpaw following stimulation with von Frey filaments (1-15g) were measured twice weekly for the duration of the study as an index of mechanical allodynia. Body weight, and food and water intake were monitored, in addition to blood sampling to determine plasma glucose and insulin levels.

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Results
FIG. 1 HFD/STZ decreases weight gain and increases water intake
The weight intakes in the HFD/STZ group is increased threefold compared to the control groups, and their body weight gain is significantly reduced.

FIG. 2 HFD/STZ increases plasma glucose and decreases plasma insulin
The plasma glucose concentration is significantly increased, and the plasma insulin is significantly decreased in the HFD/STZ group in comparison to HFD controls at all points throughout the study. This increase in glucose could be seen from 3 days after injection with STZ (data not shown).

FIG. 3 HFD/STZ decreases mechanical withdrawal thresholds
The mean mechanical withdrawal threshold of lean controls (13.1±0.5g) and HFD controls (13.4±0.6g) was consistent with that seen in naive rats. The mechanical withdrawal threshold of the HFD/STZ group was significantly reduced compared to HFD controls from day 14 post-STZ, and this threshold decreased further throughout the duration of the study, reaching a peak of 7.0±3.2g at day 78.

FIG. 4 HFD/STZ causes a decrease in mechanically-evoked neuronal responses
Electrophysiological experiments demonstrated that low weight mechanically-evoked responses of spinal neurones were decreased in HFD/STZ rats at day 49 (HFD: 15.4±3.1Hz, HFD/STZ: 8.9±2.0Hz). These responses were still decreased in the HFD/STZ rats at day 78, but the mean responses were higher in both groups.

Conclusion
We have demonstrated that the HFD/STZ model of diabetes causes mechanical allodynia as well as decreased mechanically-evoked responses of spinal neurones. Despite pioglitazone not altering blood glucose, it did attenuate the changes in neuronal responses in the spinal cord. However these effects were not sustained once treatment had ceased. Pioglitazone did not have an effect on the development of pain behaviour in awake responding animals. This indicates that although pioglitazone returns the firing of spinal neurones to a higher level, perhaps through ameliorating peripheral nerve function, there must be factors aside from the altered spinal neuronal responses that are important in the continuing mechanical allodynia.

This study helps us to dissociate the different causes of allodynia, and how they each contribute to neuropathic pain. Further work will involve using Fluorolude-B to stain for neuronal degeneration in DRGs and the spinal cord to assess how these populations are affected in the HFD/STZ diabetic model.

References