The effect of a reduced streptozocin dose and social housing on well-being, mechanical hypersensitivity and burrowing behaviour in type 1 diabetes rodents.

M. Lanigan, M. Burnett, P. Kennard, N. Upton, S. Prichard, A. Fisher, L. Lione

Introduction

The Streptozocin (STZ) induced type-1 rodent model of diabetes is widely used to preclinically evaluate the efficacy of novel analgesics against diabetic polyneuropathy (Islam, 2013).

Burrowing is reduced in mononeuropathic pain states and reversed by clinical analgesics (Andrews, 2011, Lau, 2013, Rutten, 2018) suggesting its usefulness as a measure of pain with improved face validity over evoked measures of hypersensitivity.

However, burrowing deficits following a high dose of STZ (75mg/kg) reflects diabetes-associated alteration of the animals' well-being; and not spontaneous pain, due to a lack of reversal with the clinical analgesic, pregabalin (Rutten, 2018).

Previously our group have reported a similar decline in burrowing following a high dose (65mg/kg) of STZ that is resistant to pregabalin, but can be reversed by social pairing, indicating pair housing of diabetic rats can improve their well-being and consequently burrowing behaviour (Fisher 2016).

In this study we compared a low and high dose of STZ on the development of type-1 diabetes, burrowing performance, mechanical hypersensitivity and reversal with pregabalin.

Methods

This work was conducted in accordance with guidelines established by the Animals (Scientific Procedures) Act 1986 / ASPA Amendment Regulations 2012.

Study data was combined from two studies using either a high dose (65 mg/kg I.P.) and or low dose (55 mg/kg I.P.) of STZ. All control animals received 20mM Citrate buffer (pH 4.5).

Male Wistar rats (300-450g at time of STZ dosing) (Charles River UK) Pair housed as either STZ/STZ (5 per study), Control/Control (5 per study) or STZ/Control (Mixed) (7 per study).

Mechanical hypersensitivity was evaluated using von Frey threshold via Dixon's up-down method, burrowing was assessed by displacement of 2.5kg pea shingle after 120 min.

Water or 2% sucrose were provided as options from day 0 to 2 post STZ. Between day 14 and 18 post-STZ pregabalin (30mg/kg) was administered orally for 2 days and mechanical hypersensitivity and burrowing were evaluated up to 2 hours post-treatment.

Data is displayed as mean +/- SEM and compared by one-way ANOVA (Bodyweight), two-way ANOVA (Blood glucose level & Sucrose preference) or two-way repeated measures ANOVA (Von Frey & Burrowing).

Results: Blood Glucose Level and Bodyweight

Figure 1: Blood glucose level 7 days post STZ shows no difference between low and high dose of STZ.

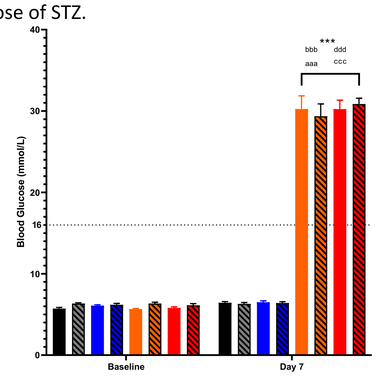

Figure 2: Lower dose STZ animals show significantly less weight loss compared with higher dose STZ animals.

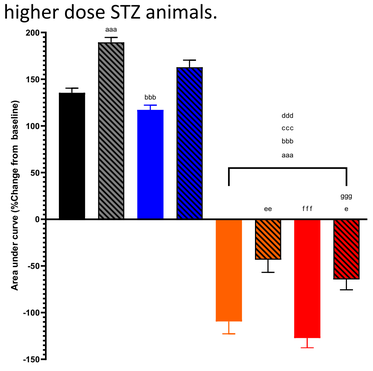

Figure 1 – Blood glucose level at baseline and 7 days post STZ. Two-way ANOVA with Bonferroni's and Tukey's post hoc tests for within and between groups. Cut off for development of hyperglycaemia set at 16 mmol/L.

Figure 2 – Bodyweight displayed as total area under the curve for percentage change from baseline after STZ injection. One-way ANOVA with Tukey's post hoc test between groups.

Stats: * = p<0.05, ** = p<0.01, *** = p< 0.001. * = to baseline, **a** = to Control/Control High Dose, **b** = to Control/Control Low Dose, **c** = to Control in Mixed High Dose, **d** = to Control in Mixed Low Dose, **e** = to STZ in Mixed High Dose, **f** = to STZ in Mixed Low Dose, **g** = to STZ/STZ High Dose.

Results: Von Frey

Figure 3: Von Frey data shows that mechanical allodynia can be induced at both STZ dose levels and can be reversed by 30 mg/kg pregabalin with no difference between levels of allodynia induced.

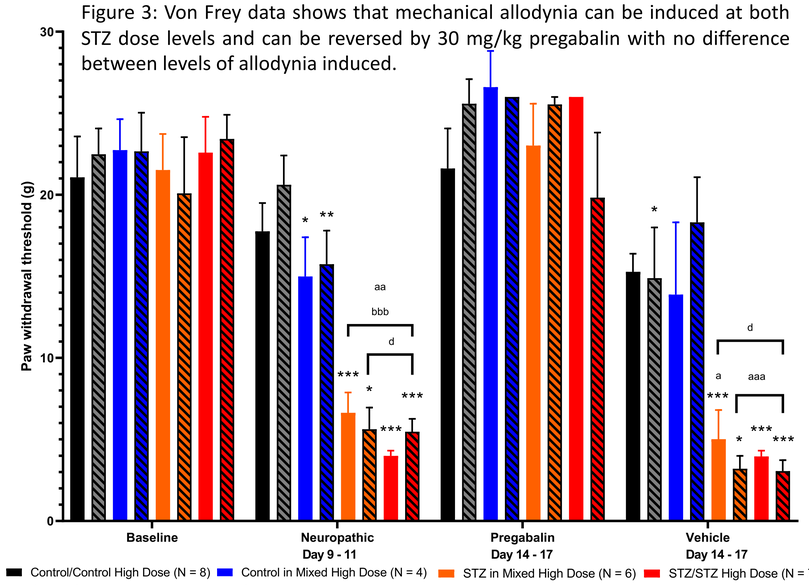

Figure 3 – Von Frey data at baseline, average of day 9-11 post STZ and cross-over testing with 30 mg/kg pregabalin or Vehicle. Two-way repeated measures ANOVA with Geisser-Greenhouse's correction for sphericity. Compared within subject to baseline with Dunnett's post hoc and between groups with Tukey's post hoc test.

Stats: * = p<0.05, ** = p<0.01, *** = p< 0.001. * = to baseline, **a** = to Control/Control High Dose, **b** = to Control/Control Low Dose, **d** = to Control in Mixed Low Dose.

Results: Individual Burrowing

Figure 4: High dose STZ diabetic animals show a temporal reduction in burrowing to negligible levels that is not reversed by pregabalin or home caging with a control partner. Low dose STZ animals show an initial reduction in burrowing at day 3 however by day 10 burrowing increases compared with high dose and can be further increased by home caging with control partner.

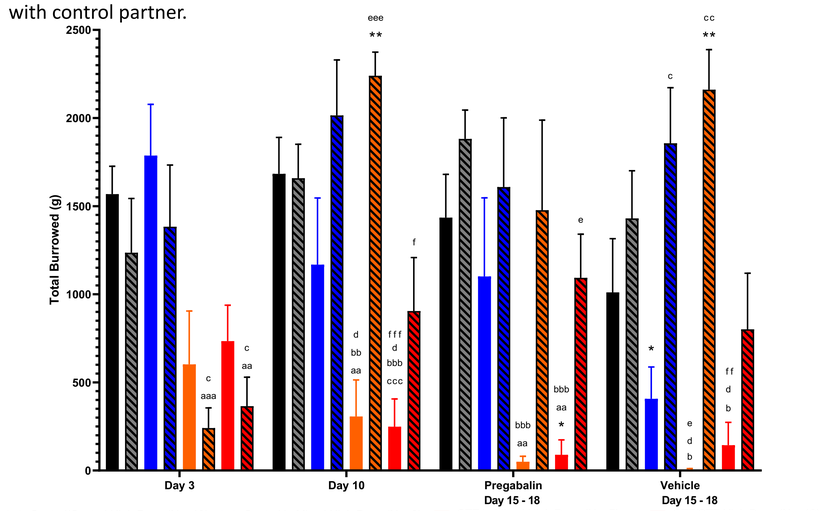

Figure 4 – Individual burrowing of 2.5kg pea shingle at 3 days and 10 days post STZ along with cross-over testing with 30 mg/kg pregabalin or Vehicle. Two-way repeated measures ANOVA with Geisser-Greenhouse's correction for sphericity. Compared within subject to all timepoints with Dunnett's post hoc and between groups with Tukey's post hoc test.

Stats: * = p<0.05, ** = p<0.01, *** = p< 0.001. * = to baseline, **a** = to Control/Control High Dose, **b** = to Control/Control Low Dose, **c** = to Control in Mixed High Dose, **d** = to Control in Mixed Low Dose, **e** = to STZ in Mixed High Dose, **f** = to STZ in Mixed Low Dose.

Results: Sucrose Preference

Figure 5: A deficit in sucrose preference is seen in all STZ animals on day 1 and 2 post STZ dosing

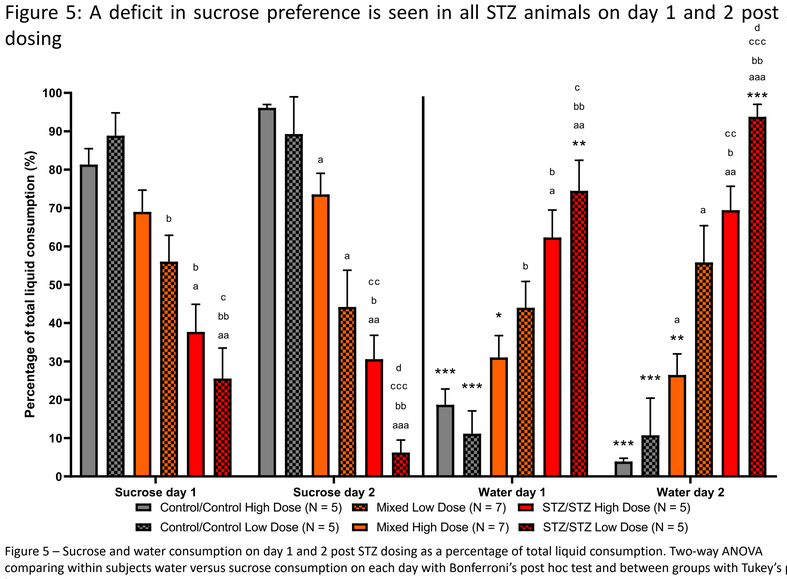

Figure 5 – Sucrose and water consumption on day 1 and 2 post STZ dosing as a percentage of total liquid consumption. Two-way ANOVA comparing within subjects water versus sucrose consumption on each day with Bonferroni's post hoc test and between groups with Tukey's post hoc test.

Stats: * = p<0.05, ** = p<0.01, *** = p< 0.001. * = to baseline, **a** = to Control/Control High Dose, **b** = to Control/Control Low Dose, **c** = to Mixed High Dose, **d** = to Mixed Low Dose.

Conclusions

A low 55mg/kg dose of STZ induces an equivalent level of hyperglycaemia and mechanical allodynia (reversed by pregabalin) to a high 65mg/kg dose whilst improving wellbeing demonstrated by increased burrowing.

Presented here is the first evidence that home caging a low dose STZ diabetic animal with a control animal improves their individual burrowing to levels equivalent to control animals.

Pregabalin reverses mechanical allodynia in low and high dose STZ diabetic animals but lacks efficacy in reversing reduced burrowing, indicating that it does not improve QOL or well being in the STZ type-1 diabetic model.

Together this study demonstrates that at a low STZ dose burrowing deficits can be reversed and as such may provide a sensitive marker for analgesics that improve allodynia and wellbeing whilst excluding sedative compounds.

STZ (low and high dose) animals drink less sucrose than the controls and more water which is likely a result of the emerging diabetic phenotype (polydipsia) rather than anhedonia. Further, sucrose preference is greater in high-dose STZ animals than low-dose STZ animals who may be more reliant on sucrose to overcome the initial hypoglycaemia cause by STZ injection.

Both the dose of STZ used as well as the housing conditions should be considered when designing pain and analgesic studies using this diabetic model of polyneuropathy.

References

Andrews N, Harper S, Issop Y, Rice ASC, (2011). *Ann N Y Acad Sci 1245*:11–13.

Dixon WJ, (1965). *J Am Stat Assoc 60*:967–978.

Fisher A, *et al.* (2016). Poster at 2016 IASP at the 16th World Congress on Pain, Japan.

Islam MS, (2013). *Journal of diabetes research, 2013*:149452.

Lau W, *et al.* (2013). *Neuropharmacology 73*:204–215.

Rutten K, *et al.* (2018). *Behav Brain Res, 350*:129–138.