transpharmation A global leader in translational biology

Poster # 2332

The streptozocin model of diabetes, induces neuropathic pain and changes in quality of life measures which can be modulated by social interaction/welfare

A. Fisher[1], M. Burnett[2], P. Kennard[2], L. Lione [2], N. Upton[1]

1 Transpharmation Ltd, London, UK | **2** School of Life and Medical Sciences, University of Hertfordshire, Hatfield, UK.

Introduction:

- Diabetes is one of the leading causes of neuropathic pain[1].
- In rodents insulin-deficient diabetes and neuropathic pain is often mimicked by systemic injection of streptozotocin[2a&b,3].
- The predictive validity of animal models for analgesia may be improved by looking to reinstate specific innate rodent well-being behaviours suppressed by pain (e.g. burrowing and sucrose preference).
- Streptozocin (STZ) given systemically to rats induces rapid and sustained changes that are seen in diabetic patients i.e. hyperglycaemia, polydypsia and frequently neuropathic pain.

Aim:

- We investigated whether development of STZ induced diabetes in rats over 18 days reduces burrowing and sucrose preference along with inducing mechanical allodynia and if these wellbeing behaviours could be improved by the analgesic pregabalin (PGB) and/or social paired housing.

Methods:

- Studies were conducted in accordance with guidelines established by the Animals (Scientific Procedures) Act 1986 / ASPA Amendment Regulations 2012. ed
- Male Wistar rats (325-425g, Charles River) were administered a single i.p. injection of 65mgkg STZ or 20mM citrate buffer (pH4.5).
- Animals; Control (CTRL, N=16) or STZ (N=18) were pair housed into 5 CTRL/CTRL, 7 mixed STZ/CTRL and 5 STZ/STZ.
- Evoked mechanical allodynia was evaluated using von Frey hairs and the Dixon's updown method, burrowing behaviour in the home cage measured the amount of pea shingle (2.5kg) displaced from hollow plastic tubes (320 mm long x 100 mm diameter), as previously described[4] and preference for 2% sucrose was expressed as a percentage of the total amount of liquid consumed each day ((volume (sucrose solution)/ volume (sucrose solution + water)).
- 2-Way repeated measures ANOVA was used with effect of 'time' (day) and effect of 'treatment' factors for food intake, water intake, sucrose preference, blood glucose, burrowing and mechanical allodynia. All data are presented as mean ± sem. For all the data control from control pair N=10, STZ from STZ pair N=10, STZ from mixed pair N=7, Control from mixed pair N=6.

Figure 1

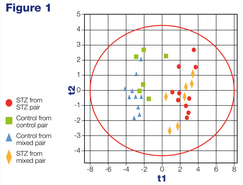

Figure 2

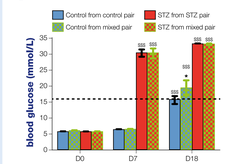

Figure 3a

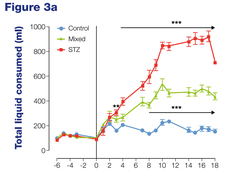

Figure 3b

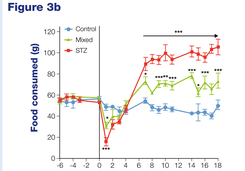

Figure 4

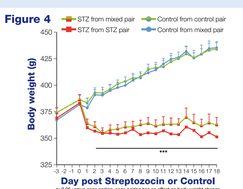

Figure 5

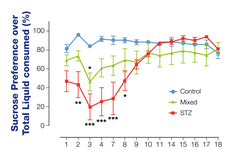

Figure 6a

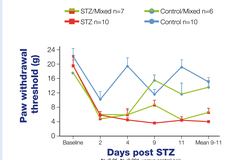

Figure 6b

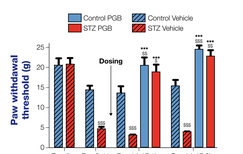

Figure 7a

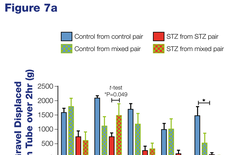

Figure 7b

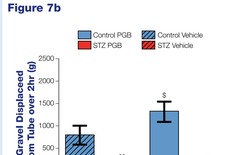

Figure 8

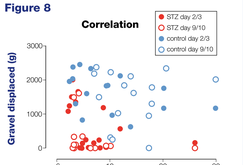

Figure 1: Principal component analysis (PCA) clearly grouped animals according to treatment.

Figure 2: CTRL rats maintained a blood glucose level within the expected/normal range until day 18. By day 18 blood glucose had increased to just above the threshold (16mmol/L) for hyperglycaemia in CTRL rats from the mixed pair only. Animals injected with STZ developed marked hyperglycaemia irrespective of social housing. *P<0.05 c.f. time matched CTRL/CTRL pair. $^{\$\$\$}$P<0.001 c.f. day 0.

Figure 3 a & b: STZ injection resulted in the development of a clear polydipsia and polyphagia and hyperglycaemia. *P<0.05, **P<0.01 ***P<0.001 c.f. time matched CTRL.

Figure 4: CTRL rats gained weight irrespective of social housing. STZ treated rats lost weight and maintained this reduced weight throughout the study irrespective of cage partner. *P<0.05, **P<0.01 and ***P<0.001 c.f. time matched control/control pair.

Figure 5: Anhedonic emotional behaviour was monitored with a 2% sucrose test. In CTRL pairs >80% sucrose preference was not altered over 18 days. STZ pairs demonstrated a significant decrease in sucrose preference from day1 to day8 and a similar trend was noted in the mixed pairs before both pairs recovered after day8 to control levels of >80% until day18. This decline in sucrose preference correlates with the early development of STZ evoked static allodynia. *P<0.05, **P<0.01 and ***P<0.001 time matched c.f. CTRL/CTRL water.

Figure 6: a) STZ injection evoked static mechanical allodynia as early as day 2. This allodynia was stable over the duration of the study from day 2 until day 18. There were no clear beneficial effects of social housing on the development of static mechanical allodynia. b) PGB (30mg/kg p.o.) reversed the mechanical allodynia induced by STZ. *P<0.05, **P<0.01 and ***P<0.001 c.f. time matched control/control pair.

Figure 7: a) STZ injection induced a progressive impairment in burrowing that was apparent as early as day 3. Housing an STZ animal with a CTRL animal had a clearly beneficial effect on burrowing at day 7 but this was not maintained. Whereas housing a CTRL animal with an STZ animal had a clearly detrimental effect at day 18. b) PGB did not improve the burrowing deficit in STZ treated animals $^{\$}$P<0.05, $^{\$\$}$P<0.01 $^{\$\$\$}$P<0.001 c.f. control vehicle.

Conclusion:

There was no correlation between burrowing scores and mechanical static allodynia for both control and STZ animals suggesting different underlying mechanisms for the two behaviours (Figure 8). This was further exemplified by the clear efficacy of Pregabalin in reducing mechanical allodynia whilst leaving the burrowing deficit un-touched. There was a clear temporal impact of social housing, both positive and negative in the development of burrowing deficits.

References: 1) Davies *et al.*, Diabetes Care. 2006, 29(7):1518-22. **2)** Courteix *et al.*, [a]1993, Pain 53:81-88. [b]1994, Pain 57:153-160. **3)** Calcutt *et al.*, 1996, Pain 68:293-299. **4)** Andrews *et al.*, 2012, Eur J Pain. 16(4): 485-95. The authors acknowledge the assistance of Simon Bate for PCA analysis.