

The streptozocin model of diabetes induces neuropathic pain, anhedonia and impaired burrowing in rats

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Introduction

- ❖ 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, polydipsia and frequently neuropathic pain.
- ❖ In this study 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 can be improved by the analgesic pregabalin (PGB).

Experimental Procedures

- ❖ This work was conducted in accordance with guidelines established by the Animals (Scientific Procedures) Act 1986 / ASPA Amendment Regulations 2012.
- ❖ Male Wistar rats (325-425g, Charles River) were administered a single i.p. injection of 65mg/kg STZ or 20mM citrate buffer (pH4.5).
- ❖ Animals; Control (CTRL, N=16) or STZ (N=18) were pair housed into 5 CTRL/CTRL, 5 mixed STZ/CTRL and 6 STZ/STZ.
- ❖ Evoked mechanical allodynia was evaluated using von Frey hairs and the Dixon's up-down method (1), 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 (2) 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.

Results

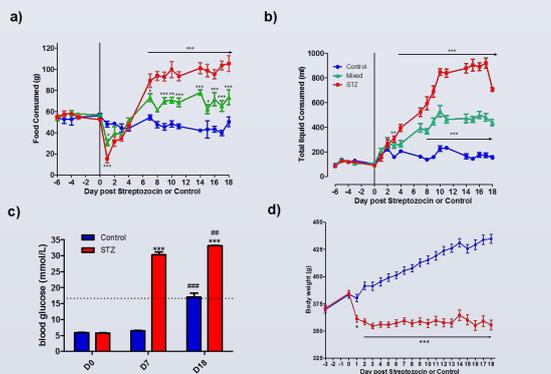


Figure 1: Development of STZ induced diabetes polyphagia (a), polydipsia (b), hyperglycaemia (c) and body weight change (d) over 18 days. For (a) and (b) Control and STZ pairs N=5, mixed pair N=7; for (c) and (d) control N=16, STZ N=17. *P<0.05, **P<0.01, ***P<0.001 time matched versus control, ###P<0.01, ####P<0.001 versus day 7.

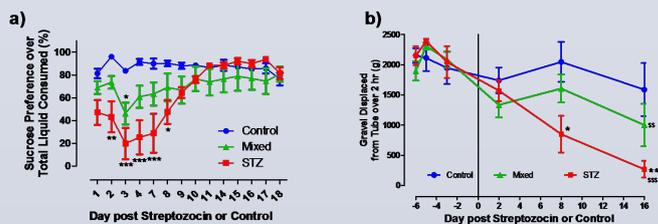


Figure 2: Development of sucrose preference and naturalistic paired burrowing over 18 days (a) % Sucrose preference and (b) naturalistic paired burrowing. Control and STZ pairs N=5, mixed pair N=7; *P<0.05, **P<0.01, ***P<0.001 time matched versus control water or control group, #P<0.05, ###P<0.001 vs control sucrose, \$P<0.01, \$\$\$P<0.001 vs baseline (day-6).

Results cont.....

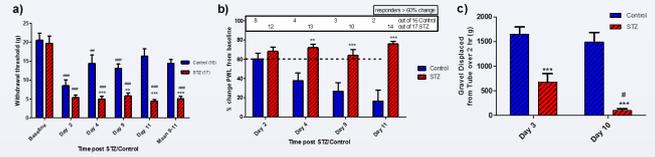


Figure 3: Development of mechanical static allodynia (a & b) and naturalistic individual 2 hr burrowing (c) over 11 days in the STZ model. ***P<0.001, **P<0.01, vs control ###P<0.01 vs baseline, #P<0.05 vs Day 3.

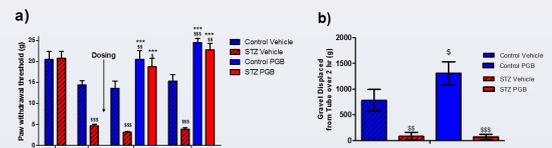


Figure 4: Effect of PGB (30mg/kg po) on (a) mechanical static allodynia and (b) individual burrowing between day 14-18. N=16 controls ± PGB, N=17 STZ ±PGB (cross over study) on day 14-18. Mechanical static allodynia was measured on day 14 and day 17, burrowing was observed on day 15 and day 18 and wash out non testing was day 16. \$\$\$P<0.001, \$\$P<0.01, \$P<0.05 vs control vehicle animals. ***P<0.001, **P<0.01, *P<0.05 vs STZ treated animals.

- ❖ 94% (17/18) STZ developed diabetes (blood glucose level 30.2 ± 0.9 mmol/L) by day 7.
- ❖ 5.5% (1/18) STZ developed hypoglycaemia and coma on day 1.
- ❖ STZ pairs showed significant polydipsia and polyphagia within 7 days which stabilized during the second week **Figure 1 (a) & (b)**.
- ❖ STZ pairs showed elevated blood glucose levels from day 7, which continued until day 18 **Figure 1 (c)**.
- ❖ STZ pairs showed attenuated body weight gain over 18 days following STZ **Figure 1 (d)**.
- ❖ CTRL pairs showed increased blood glucose at day 18 compared with day 7 following 2% sucrose in the drinking water **Figure 1 (c)**.
- ❖ STZ pairs demonstrated a significant decrease in sucrose preference transiently 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 **Figure 2 (a)**.
- ❖ In CTRL pairs >80% sucrose preference was not altered over 18 days **Figure 2 (a)**.
- ❖ STZ paired animals demonstrated impaired naturalistic burrowing by day8 **Figure 2 (b)**.
- ❖ STZ animals developed sensory static allodynia by day 3 post STZ injection (>70% had 68.2 ± 4.2% change from baseline) and impaired individual burrowing **Figure 3 (a-c)**.
- ❖ PGB (30mg/kg po at 1 and 2 hours post treatment) completely reversed static allodynia but not impaired burrowing as compared with vehicle treated rats between day 14 and 18 post STZ **Figure 4 (a) and (b)**.

Summary

- ❖ We have extensively explored the behavioural phenotype of the STZ rat model of diabetes induced neuropathic pain.
- ❖ As demonstrated previously, rats injected with STZ develop a reproducible polydipsia, polyphagia, hyperglycaemia and a painful neuropathy which is sensitive to PGB.
- ❖ We have established that acute anhedonia and impaired burrowing in STZ diabetic rats may offer an affective sensitive and objective measure of pain and/or diabetes in this model.
- ❖ In STZ diabetic rats, the anhedonia and impaired burrowing become apparent at the same time as the mechanical allodynia. However, the anhedonia disappears but the impaired burrowing deteriorates further after 9 days, whilst the static allodynia remains consistent over 18 days.
- ❖ Social pairing of STZ diabetic rats can have a positive impact on burrowing in the early stage of disease.

References

- 1) Dixon WJ. (1980) Ann Rev Pharmacol Toxicol. 20, 441-62.
- 2) Deacon RMJ (2006) Nat Protoc;1:1117-9.