EVALUATION OF THE ZUCKER FATTY RAT AS A MODEL OF DIABETIC NEUROPATHY

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Introduction

Diabetic neuropathy (DBN) is the most common clinical form of neuropathic pain and is associated with numbness, tingling sensation and pain (Callahan et al, 2012). One of the most common approaches to model DBN in rodents is the streptozotocin (STZ) model. STZ is an antibiotic that is diabetogenic due to a direct cytotoxic effect on pancreatic beta cells. STZ treated rats rapidly develop signs of diabetes such as elevated blood glucose levels and a reduced threshold to a tactile, but not necessarily a thermal stimulus. However STZ rats also develop marked signs of general ill health which raises concerns about the validity of the model (Fox et al, 1999).

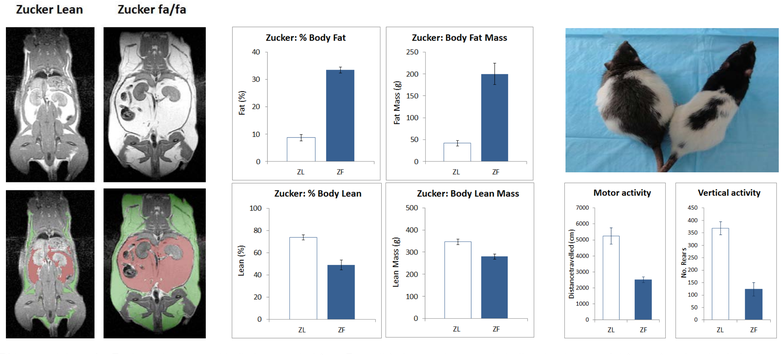
In the search for alternative models of DBN, we have examined the Zucker fatty rat, a genetic model of type 2 diabetes. Although the Zucker rat is widely used as a model of obesity and type 2 diabetes, relatively few reports have described this model in the context of pain (Brussee et al, 2008; Otto et al, 2011; Lupachyk et al, 2012; Vera et al, 2012). These studies represent an investigation of two cohorts of Zucker rats (Charles River, strain code 185, Crl: ZUC-Lepr fa) and their lean controls (strain code 186) in which we specifically assess their value as a model of diabetic neuropathy. We also compare to the Spared Nerve Injury (SNI) model of neuropathic pain (Decostered and Woolf, 2000).

References:
Brussee et al (2008) Diabetes 57: 1664-1673.
Callahan et al (2012) Lancet Neurol. 11: 521-534.
Decostered and Woolf (2000) Pain 87: 149-158.
Fox et al (1999) Pain 81: 307-316
Lupachyk et al (2012) Free Radical Biology & Med. 52: 1255-1263.
Otto et al (2011) Pain Medicine 12: 437-450
Vera et al (2012) Pharmacol. Biochem. Behav. 102: 335-343.

General characterisation of the Zucker rat

Male, Zucker rats (Charles River, strain code 185, Crl: ZUC-Lepr fa) and their lean controls (strain code 186) were acquired at 10 weeks age. Animals were fed regular diet (Lab Diet 5001: 4.07 kcal/g) throughout lifespan. All animals were singly housed in a temperature and humidity controlled environment under a 12h light:dark cycle (lights on 05:00 – 17:00h) throughout the study. A variety of measures including QMR and MRI imaging, behaviour and clinical biomarkers (blood cholesterol, triglycerides, glucose) were made to characterise the Zucker rat relative to its lean control.

Body composition: QMR and MRI imaging



MRI images taken of a Zucker rat compared to its lean control (age 7 month) show an increase in both subcutaneous fat (green) and particularly visceral fat (red). Quantification of fat content was made using QMR imaging which measures total fat and lean mass (this technology is not possible to distinguish between the visceral or subcutaneous fat compartments). MRI images courtesy of Dr. Howard Dobson.

Blood biomarkers

		Blood glucose (fasted) (mmol/l)	OGTT (AUC)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Insulin (pmol/l)
Zucker lean	3 months	5.48±0.18	1688±42	1.89±0.10	1.48±0.16	662±274
	6 months	6.17±0.06	1841±70	2.80±0.16	1.95±0.18	ND
	9 months	7.53±0.39	2063±110	3.96±0.54	2.27±0.37	ND
	12 months	7.55±0.52	2031±103	4.56±0.65	2.30±0.33	105±31
Zucker obese	3 months	7.66±0.28*	2723±125*	3.50±0.23*	10.53±0.99*	4538±1947*
	6 months	9.99±0.50*	3248±160*	9.16±1.11*	21.47±4.52*	ND
	9 months	10.60±0.45*	3019±227*	13.12±1.35*	9.93±2.18*	ND
	12 months	12.77±1.34*	3332±207*	11.45±1.15*	27.57±3.48*	318±67

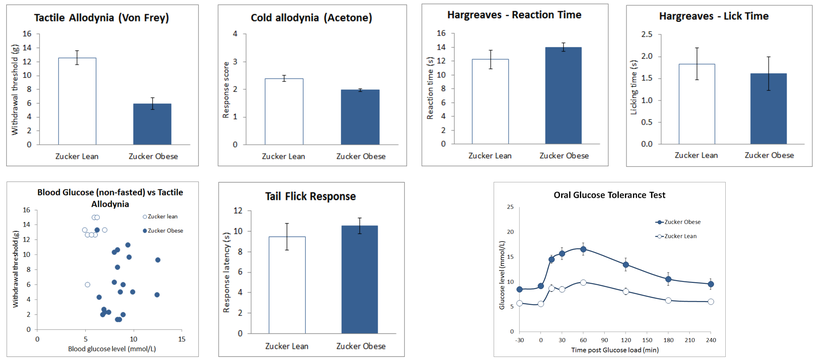
In Zucker study cohort 2, various blood biomarkers were measured at 3, 6, 9, and 12 months. Over 3-12 months age the Zucker rats have considerably higher levels (3-10 fold) of cholesterol and triglyceride content compared to lean controls. Blood [insulin] and [glucose] are also significantly higher, consistent with the Zucker being a model of type 2 diabetes. The magnitude of change in these biomarkers generally appears to increase with age.

Cohort 1

A group of 20 Zucker rats (ZR) aged 10 months, and 9 age matched, Zucker lean (ZL) counterparts were study subjects. The ZR rats were confirmed diabetic as measured by elevated blood glucose, increased water intake and heighted response to an oral glucose load.

The rats were then evaluated in a battery of tests to measure their response to a variety of nociceptive stimuli. Specifically:-

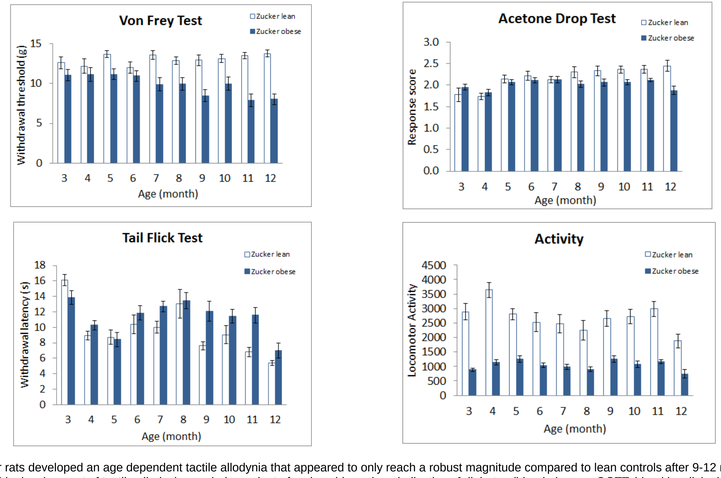
Mechanical static allodynia: Animals were singly placed in clear elevated chambers on a Perspex grid floor and allowed 10-15 minutes to settle. The lateral plantar surface of the paw was stimulated with a series of ascending force Von Frey hair filaments (0.4, 1, 2, 4, 6, 8, 10, and 15g). The threshold was taken as the lowest force that evokes a brisk withdrawal response. The average score from three separate assessments was taken as the final measure for that animal.
Cold allodynia: The acetone drop test was conducted in the same chamber immediately after the Von Frey test. A drop of acetone solution was carefully placed onto the lateral plantar surface of the paw, using a blunt needle connected to a syringe, without touching the skin. The magnitude of the withdrawal response was scored according to a 4 point rating scale where 0 = no visible response, 1 = response but without paw withdrawal, 2 = clear withdrawal of the paw, 3 = prolonged withdrawal (5-30sec) combined with flinching and licking of the paw. Average score from three separate assessments was taken as the final measure for that animal.
Thermal hyperalgesia: Animals were placed in an enclosed observation arena on a glass pane, beneath which an infrared light source is located. Following a 15min acclimatization, a light source (Ugo Basile plantar test, model 37370) was positioned beneath the planter surface of the hindpaw. The time from stimulus onset to the withdrawal of the paw was automatically recorded using a reaction time counter. The average score from 3 separate assessments was taken.
Tail flick: Method as for Thermal hyperalgesia except the light source was positioned approx. 3-4cm from the base of the tail. The time from onset to the withdrawal of the tail was recorded and the average score from 3 separate assessments taken.



At 10 months of age, the Zucker rats demonstrated a significant tactile allodynia relative to Zucker lean controls (P<0.01; T-test). The magnitude of the tactile allodynia was not correlated to blood glucose levels measured within a week of testing. The animals showed equivalent response to a thermal stimulus measured using Hargreaves appuratus, and a normal tail flick response. Interestingly the animals were not hypersensitive to an acetone drop test of cold allodynia, rather they were significantly less responsive compared to lean controls (P<0.05; T-test). All Zucker rats showed evidence of type 2 diabetes as indicated by an elevated OGTT response, hyperglycaemia and hyperdipsia compared to lean controls. However not all rats were necessarily neuropathic as approximately 15% Zucker rats had Von Frey scores of ≥10g.

Cohort 2

A group of 16 Zucker rats aged 3 months, and 12 age matched, Zucker lean (ZL) counterparts were study subjects. The objective of this study was to assess each cohort monthly in a battery of tests to characterise the development of diabetes, general locomotor activity and response to tactile Von Frey, acetone drop and tail flick to a thermal stimulus. Blood biomarkers of diabetes (glucose, OGTT, insulin) were assessed at 3 monthly intervals. The rats received no drug treatments during the course of these studies.



Zucker rats developed an age dependent tactile allodynia that appeared to only reach a robust magnitude compared to lean controls after 9-12 months age. This development of tactile allodynia was independent of various biomarkers indicative of diabetes (blood glucose, OGTT, blood insulin) which was evident from earliest testing (3 months). Also similar to cohort 1, within animal comparisons failed to identify any correlation between blood glucose level and degree of tactile allodynia. A significant correlation (Pearson coefficient 0.811) between readings taken from the same animals at 11 and 12 months age indicated a reliability to this measure. Again, a small subgroup of Zucker rats (3/16; 19%) did not develop a tactile allodynia by 12 months age.

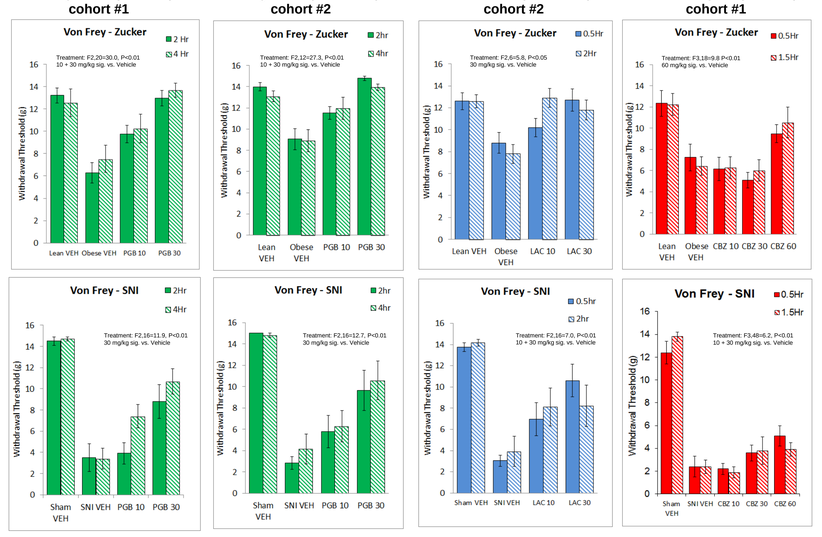
Similar to cohort 1, Zucker rats did not show a hypersensitivity to an acetone drop test or tail flick to a thermal stimulus, indeed, the Zucker rats became less responsive to both these stimuli relative to lean controls (see also Brussee et al, 2008; Vara et al, 2012). Throughout the 3-12 month test period, the Zucker rats showed considerably lower spontaneous locomotor activity compared to lean controls.

Pharmacological characterisation: Zucker vs. SNI model of neuropathic pain

Male Zucker rats from cohorts 1 and 2 (approximate age 10 -12 months) were evaluated for their response to pregabalin, lacosamide and carbamazepine. In parallel, these same drugs were tested in a group of age matched Sprague-Dawley rats with Spared nerve injury (SNI: Decostered and Woolf, 2000). Briefly, male SD rats were surgically prepared by sectioning the tibial and peroneal branches of the sciatic nerve, leaving the sural branch intact. Sham animals underwent similar surgical procedures except the sciatic nerve was not exposed and all descending branches of the nerve remained intact. Following a recovery period of at least 20 days, the animals were familiarised to the test procedures which included measurement of tactile allodynia (Von Frey).

All experiments were conducted according to a repeated measures design with 2-4 days between each treatment cycle. Blood was collected from the saphenous vein to determine plasma [drug]. Zucker rats with Von Frey scores >10g were excluded from any drug study.

Pregabalin (IP route) cohort #1 | **Pregabalin (oral route) cohort #2** | **Lacosamide (oral route) cohort #2** | **Carbamazepine (IP route) cohort #1**



In all studies, SNI surgery produced a more profound tactile allodynia compared to the Zucker, i.e Von Frey threshold: SNI = 2-4g; Zucker = 6-9g.

In separate experiments conducted in two Zucker rat cohorts, Pregabalin effectively reversed the tactile allodynia, with significant effects at both 10 and 30 mg/kg (IP and oral routes). Pregabalin was similarly effective in the SNI rat model, although only partially reversed the tactile allodynia. Comparison of plasma [PGB] levels suggest exposure may be slightly higher in the Zucker compared to SNI rat at the equivalent dose of 10 mg/kg (PGB 10 mg/kg IP 4h timepoint: Zucker: 5.9±0.3µg/ml; SNI model: 4.0±0.3µg/ml).

Lacosamide (30mg/kg oral) also reversed the tactile allodynia in Zucker rats, and produced a partial reversal in the SNI rats. In this regard its profile was similar to pregabalin. Although significant, carbamazepine was virtually ineffective against tactile allodynia in the SNI model, yet some reversal was evident in the Zucker rat at a high dose of 60 mg/kg.

Summary

1. **Two cohorts of Zucker rats were profiled and each found to develop an age related tactile allodynia that was sensitive to pregabalin treatment.**
2. **The diabetic condition clearly preceded the tactile allodynia which only reliably emerged at 9-12 months age. Similar to the STZ rat model, no thermal allodynia was evident.**
3. **The Zucker rat may represent a useful alternative to surgical models of neuropathic pain, such as the SNI model. A comparison between each model is summarised below.**

	Zucker model	SNI (surgical) model
Behavioural profile of model	Restricted. Tactile allodynia is evident, however no hypersensitivity to acetone drop or tail flick - infact less responsive compared to lean controls (see also Brussee et al, 2008; Vera et al, 2012 with respect to Zucker; Fox et al, 1999 with respect to STZ model). Sedentary nature of Zucker rat may influence responses to certain sensory stimulii and complicate their interpretation.	Rats develop robust tactile and thermal allodynia, i.e VF scores typically 1-4. Thermal and mechanical hyperalgesia also evident. Despite these effects, rats do not develop obvious signs of ill health/discomfort, e.g motor activity, neurological function, weight gain, similar to sham controls.
Pharmacological profile	Pregabalin effective at a dose range and plasma exposure consistent with clinical exposure. Lacosamide partially effective (plasma concentration under evaluation). Carbamazepine active but only at a relatively high dose.	Pregabalin effective at a dose range and plasma exposure consistent with clinical exposure. Lacosamide effective (plasma concentration under evaluation). Carbamazepine only weakly active at a relatively high dose.
Reliability of model	Not all Zucker rats necessarily develop robust tactile allodynia, i.e VF scores of <10g. However once developed there appears to be a good reliability over 1-3 months.	Assuming surgeries are accurate, all rats develop robust tactile and thermal allodynia, i.e VF scores of <10. Once developed there is good reliability over 3-6 months.
Window of model	Onset approx. 9-12 months age, Lifespan approx. 15 months with declining health evident in some animals from ~12 months. Therefore Zucker model probably limited to 3-4 months usage.	Onset approx. 5-20 days post surgery. Effects may persist for >6 months. Therefore opportunity to test for several months. Because surgery can be conducted at any age, such studies may be conducted in young, middle-aged or aged rats.
Advantages of model	High construct validity as (1) Non-sugical, (2) tactile allodynia likely related to diabetic condition of Zucker rat. (3) Chronicity allows for repeated measures or longitudinal study designs. (4) Construct validity suggests model might provide insight into disease pathogenesis and/or detect broader NCE classes (see Brussee et al, 2008; Lupachyk et al, 2012).	(1) Extremely robust tactile and thermal allodynia. (2) Opportunity to measure side effects of NCE. (3) Chronicity allows for repeated measures or longitudinal study designs, and also assessment of alternative endpoints. (4) Timed surgery and predictable development of neuropathy allows for prophylactic study designs. (5) Animals appear in good health.
Disadvantages of model	(1) Sensitivity to nociceptive stimulii restricted to tactile allodynia, (2) Sedentary nature of Zucker rat makes evaluation of NCE side effects difficult. (3) Time and resource challenges to establish a suitable cohort of study animals. (4) Complications of declining health in Zucker rats after 12 months age. (5) Lack of biomarker to predict which Zucker rats will develop allodynia and when.	(1) Limited construct validity as surgically induced model, typically conducted in young rats. (2) Significant surgical expertise necessary. (3) Magnitude of allodynia is severe, i.e VF scores of 1-4 typical. Risk of insensitivity to NCE?