

# Measuring Pain-Related Behaviour in Four Inbred Rat Strains. Differences in Hot Plate Behaviour

M.W.H. Schaap<sup>1</sup>, S.S. Arndt<sup>2</sup>, L.J. Hellebrekers<sup>1</sup>

<sup>1</sup>*Faculty of Veterinary Medicine, Department of Clinical Sciences of Companion Animals, Utrecht University, Utrecht, The Netherlands. [m.schaap@uu.nl](mailto:m.schaap@uu.nl)*

<sup>2</sup>*Faculty of Veterinary Medicine, Department of Animals in Science & Society, Utrecht University, Utrecht, The Netherlands.*

Very little is known about differences in behaviour of different strains during the hot plate test, and the strain-related effects on reported nociceptive thresholds. The hot plate is a commonly used test to study thermal (anti)nociception in rats and mice [1]. In this test, the animal is placed on a hot plate until a predetermined behavioural endpoint is observed, typically a hind-paw lick or jump response [2, 3]. Differences in latencies between animals are considered to reflect differences in nociceptive threshold. It is known that when studying drug-induced changes in nociceptive threshold, latencies of various behavioural endpoints are affected differently depending on the drugs used [2]. However, strain-related differences have to be taken into consideration as well.

The experimental protocol was approved by the Animal Experiments Committee of the Academic Biomedical Centre, Utrecht, The Netherlands. The Animal Experiments Committee based its decision on 'De Wet op de Dierproeven' (the Dutch 'Experiments on Animals Act', 1996) and on the 'Dierproevenbesluit' (the Dutch 'Experiments on Animals Decision', 1996). Both documents are available online at <http://wetten.overheid.nl>. Further, all animal experiments followed the 'Principles of Laboratory Animal Care' and refer to the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioural Research (National Research Council 2003).

Four phylogenetically distant inbred rat strains (Elevage Janvier, Le Genet St. Isle, France; n = 14 for each strain), were used, including Wistar Kyoto (WKY), Fawn Hooded (FH), Brown Norway (BN) and Lewis (LE). Body weights ( $\pm$  SEM) were 225.07 ( $\pm$  2.02), 202.43 ( $\pm$  2.92), 163.64 ( $\pm$  1.13) and 233.57 ( $\pm$  2.04) gram at the time of arrival, respectively, and all rats were 8 weeks old at the time of arrival. All rats were housed individually in a clear 1500U Eurostandard Type IV S cage of 48 x 37.5 x 21 cm (Techniplast, Buguggiate, Italy). Rats were provided with bedding material (Aspen chips), *ad lib* access to food (CRM, Expanded, Special Diets Services Witham, United Kingdom), water and carton houses (Rat Corner House, Bio Services B.V., Uden, The Netherlands) and plastic tubes (Rat retreat Amber, Plexx, Elst, The Netherlands) as cage enrichment. The environment was temperature ( $21 \pm 2$  °C) and humidity ( $47 \pm 3\%$ ) controlled with an inversed 12:12 h light-dark cycle (lights off from 7.00 – 19.00 hrs) and a radio played constantly as background noise. Animals were handled at least three times a week by the experimenters. All testing procedures occurred in a room different than the animal housing room.

After an acclimatization period of three weeks, all animals were subjected to the hot plate test. The hot plate apparatus (Model 35100, Ugo Basile, Varese, Italy), was maintained at  $50.0 \pm 0.1$  °C. Animals were placed in a glass cylinder of 24 cm diameter on the heated surface of the hot plate and removed immediately when either a hind paw lick or jumping response was observed [4]. The latency until one of these responses was scored. A cut-off time of 120 seconds was used to prevent tissue damage.

Two types of behavioural endpoints were measured, i.e. the hind-paw lick and the jumping response. None of the rats reached the 120 seconds cut-off time. Thermal nociceptive thresholds differed per strain ( $F_{3,51} = 15.83$ ,  $p < 0.000$ ; see Figure 1a). Post hoc test (Sidak) revealed that the latency time of the FH was significantly increased compared to WKY and LE, the BN showed a significantly increased latency time compared to the WKY. The type of behavioural endpoint observed differed per strain,  $\chi^2(3, N = 56) = 12.36$ ,  $p < 0.01$ , see Figure 1b, but no effect of type of response was found on latency time (response x strain:  $F_{3,48} = 1.23$ ,  $p = 0.35$ ; response:  $F_{1,51} =$

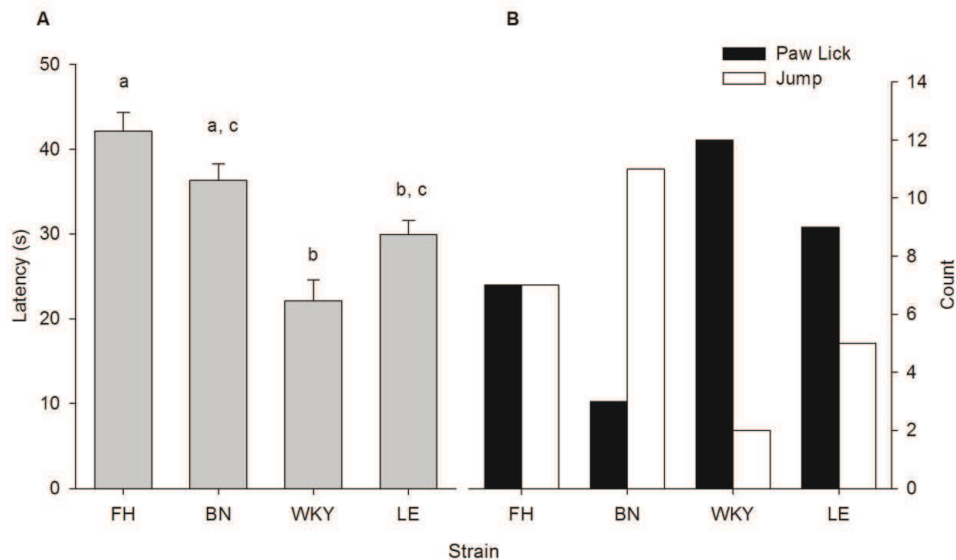


Figure 1. (A) The type of response did not significantly affect the latency time. Latency times differed between strains. Data are represented as mean  $\pm$  SEM. Values which are different between strains are marked with different characters, i.e. FH = BN, FH > WKY = LE, BN = LE, BN > WKY. (Sidak;  $p < 0.05$ ). (B) Strains differed in their behavioural endpoint. Data are represented as number of responses per strain. FH = Fawn Hooded, BN = Brown Norway, WKY = Wistar Kyoto and LE = Lewis.

0.56,  $p = 0.46$ ). The BN showed mainly the jumping response, while within the WKY strain paw licks were most prevalent. The FH and LE showed both behavioural endpoints with equal prevalence.

As the strains differed in body weight, one concern is the potential influence of body weight on latency. However, a recent study showed that this effect is weak and therefore unlikely to fully explain differences in latency up to 50% in this study [5]. In conclusion, when selecting behavioural endpoints the animal strain should be considered. Since the type of observed behavioural endpoint did not influence the baseline latency (i.e. paw licks and jumping responses occurred at the same latency) it is recommended to take both types of behaviours as endpoint for the hot plate test as indicator for the baseline (i.e. non-drug related) thermal nociceptive threshold.

## References

1. Le Bars, D., Gozariu, M. & Cadden, S.W. (2001). Animal Models of Nociception. *Pharmacological reviews* **53**, 597-652.
2. Carter, R.B. (1991). Differentiating analgesic and non-analgesic drug activities on rat hot plate: effect of behavioral endpoint. *Pain* **47**, 211-220.
3. Espejo, E.F. & Mir, D. (1993). Structure of the rat's behaviour in the hot plate test. *Behavioural brain research* **56**, 171-176.
4. Allen, J.W. & Yaksh, T.L. (2004). Assessment of acute thermal nociception in laboratory animals. In J.M. Walker (ed), *Pain Research: Methods and Protocols*. Humana Press Inc., Totowa, NJ, 111-123.
5. Gunn, A., Bobeck, E.N., Weber, C. & Morgan, M.M. (2011). The Influence of Non-Nociceptive Factors on Hot-Plate Latency in Rats. *The Journal of Pain* **12**, 222-227.