 <p>Standard Operating Procedure</p>	Title: Hot plate test	
	Doc. Number: Doc. Number: ESLIM_012_001 Rev No. 0	Date Issued: 22/02/2007

1.0 Purpose:

- 1.1 The hot plate test is used to assess acute pain sensitivity to a thermal stimulus.

2.0 Scope:

- 2.1 Individuals who have been trained, and are competent in performing the procedures described herein must follow this procedure.
- 2.2 Any queries, comments or suggestions, either relating to this SOP in general or to a specific problem encountered during a procedure, should be addressed to the Behavioural Neuroscience Project Leader.
- 2.3 Any deviances from this protocol must be reported to the Behavioural Neuroscience Project Leader.


3.0 Safety Requirements:

- 3.1 General laboratory procedures should be followed, which include: no eating, no chewing gum, no drinking, and no applying of cosmetics in the work area. Laboratory coats and gloves must be worn at all times in the work area, unless the protocol specifically describes the appropriate attire for the procedure.

4.0 Associated Documents:

5.0 Notes:

- 5.1 The validity of results obtained from behavioural phenotyping is largely dependent on methods of animal husbandry. It is of vital importance that individuals following this procedure are experienced and aware of the animal's welfare.

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- 5.2 The majority of mouse behavioural studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.
- 5.3 Environmental factors may contribute to the levels of anxiety within the mouse. The temperature, humidity, ventilation, noise intensity and lighting intensity must be maintained at levels appropriate for mice. It is essential that the mice be kept in a uniform environment before and after testing to avoid anomalous results being obtained.
- 5.4 It is recommended that all phenotyping experimentation is conducted at approximately the same time of day because physiological and biochemical parameters change throughout the day.

6.0 Quality Control

7.0 Equipment:


- 71 Different Hot plate variants from different suppliers can be used (e.g. Panlab, Spain; TSE GmbH, Germany)
- 7.2 A viewing jar made of PVC (Suitable diameter and height to observe correctly the mouse behaviour; generally about 20cm diameter and 25cm height.)

8.0 Supplies:

- 8.1 Tap Water, 70% Ethanol
- 8.2 Tissue paper
- 8.3 Stopwatch if the Hot plate is not equipped with one.

9.0 Procedure:

9.1 General design

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9.1.1 The mice are submitted to a single trial during which they are placed into a glass cylinder on a hot plate adjusted to 52°C and the latency of the first reaction (lick, shake, jump) is recorded (cut off for the first trial 30 seconds).

9.2 Transportation and acclimation:

9.2.1 Animals are transported to the testing room in their rack (whenever possible) and left undisturbed for at least 15 minutes before the test (assuming the conditions in the testing room are the same as in the rest of the facility – if they are not, allow 1 hour for acclimatisation).

9.2.2 Illumination in the testing room is 100-130 Lux at the level of the bench.

It should be noted that mice have previously been labelled. If animals are submitted subsequently to other behavioural evaluations, labelling is also checked at the end of the testing session.

9.3 Testing:


9.3.1 A work sheet is previously prepared and contains particularly the day of experiment, the title of the study, the list of the animals and the parameters of the heat stimulus.

9.3.2 Switch on the hot plate apparatus and wait until the plate reaches the defined temperature (52°C, adjust if necessary). Put the cylindrical jar on the plate.

9.3.3 Clean the metal surface and the plastic cover with disinfectant (e.g. 70% ethanol). Wait 1 minute to re-establish the surface temperature before commencing with the test.

9.3.4 Put the first mouse on the plate and simultaneously start the stopwatch to measure the withdrawal latency.

9.3.5 Stop the stopwatch after the mouse displays any reaction to heat (paw shaking, licking or jumping). If the mouse does not react to heat after 30 seconds, it is removed from the hot plate and 30 seconds is considered as latency by default.

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9.3.6 Note the performance on the worksheet for subsequent data analysis.

9.3.7 The hot plate and the jar are cleaned with water and disinfectant (e.g. 70% ethanol) before testing another animal, wait 1 minute to re-establish the surface temperature.

9.3.8 At the end of testing, return the mice to the housing rooms and thoroughly clean the equipment.

10.0 Parameters and measures:

Time of first response (seconds)
Type of response (shake/lick/jump)
Comment on other behaviours

11.0 Supporting information

- 11.1 Kew J. C., Koester A., Bourson A., Moreau J.L., Jenck F., Ouagazzal A.M., Mutel V., Richards G., Trube G., Fischer G., Montkowski A., Hundt W., Reinscheid R.K., Pauly-Evers M., Kemp J.A. & Bluethmann H. (2000). Functional consequences of reduction in NMDA receptor glycine affinity in mice carrying targeted point mutations in amino acids contributing to the glycine binding site. *J. Neurosci.*, 20 : 4037-4049.
- 11.2 Wilson SG & Mogil JS (2001). Measuring pain in the knockout mouse: big challenges in small mammals. *Behav Brain Res* 125: 65-73.

12.0 History Review:

13.0 Emergency Procedures: