PAIN ASSESSMENT METHODS IN LABORATORY RODENTS

FIN3R Annual Symposium

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Helsinki, November 7th 2023

1

WHY IS IT IMPORTANT TO RECOGNIZE AND ALLEVIATE ANIMAL PAIN?

- Minimizing animal pain whenever possible is important both ethically and legally
- The 3R principle of refinement: all procedures involving animals should (1) avoid or minimize discomfort and pain and/or (2) otherwise include the provision of adequate pain relief unless the pain is justified scientifically
- Untreated animal pain and pain-relieving drugs can both affect the animals´s biology in profound ways:

  Example: the early experience of pain in postnatal animals may lead to increased pain sensitivity later in life, while effective pain management may improve healing rates and decrease mortality

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2

2

RODENTS IN PAIN

- Aggressive when handled
- Avoiding food, may eat bedding or their offspring
- Reductions in body weight and growth rate are commonly used as indicators of pain and distress and as humane endpoints in research rodent studies
- Group housed animals change their normal group behaviour or grooming
- Increased insolation from their cage mates and attempts to hide, or no longer exhibiting the nest-building behaviour

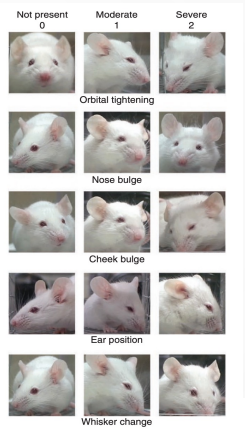
3

FACIAL EXPRESSIONS OF PAIN IN THE LABORATORY MOUSE

Mouse grimace scale (**MGS, Langford et al, 2010**):

Consists of **five facial features** perceived by human facial pain expression experts as potentially reliable indications of pain

1) Orbital tightening is narrowing of the orbital area, with a tightly closed eyelid or an eye squeeze
2) Nose bulge is a rounded extension of skin visible on the bridge of the nose.
3) Cheek bulge refers to convex appearance of the cheek muscle (between eye and whiskers) from its baseline position.
4) Ear position refers to ears pulled apart and back from their baseline
5) Whisker change is movement of whiskers from their baseline position either backward, against the face or forward



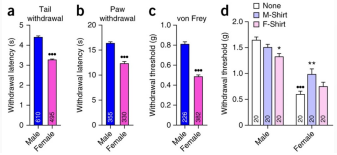
Langford DJ et al. *Nat Methods*. 2010

4

CHALLENGES OF PAIN ASSESSMENT IN LABORATORY RODENTS

- Laboratory rodents are prey species that may show subtle signs of pain
- Studies have shown that prey animals in pain act more normally and less painful when humans ("predators") are present while "non-prey species" do not
- **Sorge et al. (2014):** Mice showed less evidence of pain in the presence of human male observers than female observers, or even in the presence male scientists' worn T-shirts

➢ Male-smelling humans perceived as more of a predatory threat than females? Such reactions affect the rodents' behaviour and potentially confound the results of animal studies

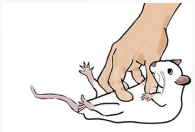


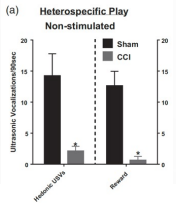
Sorge, R *et al. Nat Methods* 11, 629–632 (2014)

5

CHALLENGES OF PAIN ASSESSMENT IN LABORATORY RODENTS

- Because animals are nonverbal, pain cannot be directly measured in rodents
- Rats and mice in acute pain may vocalize and become unusually aggressive when handled.
- Because rodents also vocalize at ultrasonic frequencies inaudible to humans, the absence of audible vocalization does not necessarily signify the absence of acute pain.





Burgdorf JS et al. *Neuroreport*. 2019

Burgdorf et al (2019):

Chronic constriction injury (CCI) model of neuropathic pain

Ultrasonic vocalizations (50-kHz) were measured in heterospecific play (i.e. tickling) test

Ultrasonic vocalizations during the non-stimulus periods of the tickling test, as well as the rewarding value of tickling, were reduced in CCI rats compared to sham controls

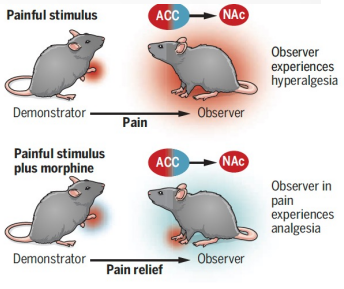
6

MICE EMPATHY: SOCIAL TRANSFER OF PAIN

- **Social transfer of pain**: a brief exposure to an animal of the same species who is experiencing pain will lead to a transfer of the same emotion state to the observer.
- **Smith et al., 2021:** social transfer of pain is mediated by neural projections from the ACC to the nucleus accumbens (NAc) in the observer mouse.

  1) Transfer of hyperalgesia: Observer mice, who has not experienced any pain itself, is more sensitive to painful stimuli and experiences pain more easily

  2) Transfer of analgesia: Observer mice, who were in pain themselves, exhibited less pain responses when observing other mice that had undergone pain-relief treatment with morphine



Smith ML et al. *Science*. 2021

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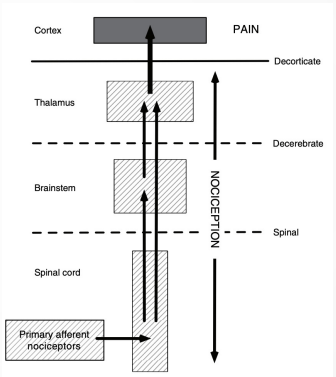
7

7

NOCICEPTION OR PAIN?

- **Pain ≠ Nociception**
- **Nociception**: information about peripheral stimuli is transmitted by primary afferent nociceptors to the spinal cord, brainstem, thalamus, and cortex
- **Pain:** only when there is activity of thalamocortical networks that process the information conveyed by pathways of nociception
- **Nociception can occur in the absence of pain.**

Example: **complete spinal cord transection**; information transmitted by nociceptors is still processed, but because the information cannot be transmitted beyond the transection stimulus-evoked pain is unlikely



Recognition and Alleviation of Pain in Laboratory Animals. Washington (DC): National Academies Press (US); 2009.

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8

8

METHODS FOR ASSESSMENT OF PAIN

- The classical pain evaluation devices aim to assess the **sensory component of pain**, usually by **measurement of withdrawal reflex**.
- New methods make it possible to also assess **pain perception** and to evaluate the **emotional component of pain**
- Methods for pain assessment in laboratory rodents can be divided into 2 groups:

  1) **stimulus-dependent tests** (believed to process **nociception** because stimulus duration is limited by the animal's response, e.g., a nociceptive withdrawal reflex)

  2) **stimulus independent tests** (believed to test the involvement of supraspinal areas of the brain, tests of **both nociception and pain**)

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9

9

1. STIMULUS-DEPENDENT TESTS

- **the latency and the frequency of paw withdrawal** is measured after mechanical or thermal stimulation
- Mechanical stimulation:

  dynamic (triggered by brushing); light stroking (velocity is ~ 2 cm/s) of the external lateral side of the injured hind paw with a paintbrush = very fast lifting of the stimulated paw aside

  static (triggered by pressure); measured by pressure algometer

  punctate (triggered by touch); applying an increasing pressure

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10

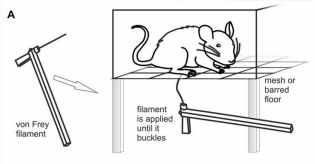
10

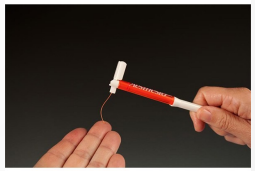
VON-FREY TEST

- Commonly used test used to assess **mechanical hypersensitivity** in rodents
- Series of tests by applying the **pressure of different forces using calibrated von Frey filaments** (ranged from 0.008 to 300 g) on the plantar surface of the hind paw
- Animal is resting on a penetrable grid on its 4 legs, the filament is placed at the angle and pressed against the paw until it bends
- **Paw withdrawal is counted as a positive response**; the weight of stimulus that elicits a response 50% of the times is registered to estimate mechanical threshold
- Limitations:

  the repetitive stimulation is time-consuming and can cause sensitization

  it is assumed that the von Frey test triggers nociception and can be used to assess unpleasantness of the stimulation rather than pain





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11

11

RANDALL-SELITTO TEST

- **Paw pressure test** used to assess **mechanical hypersensitivity in rats** (Randall and Selitto, 1957)
- Animals are restrained and an **increasing pressure is exerted on the hind paw or tail** using a dome-shaped plastic tip.
- When the rat withdraws its paw or vocalizes, threshold is attained and the pressure is stopped
- Limitations:

  Waiting for the animal to vocalize is not recommended since it is a synonym to great pain which may lead to injuring the animal

  Rats should be habituated to being restrained to minimize the stress bias

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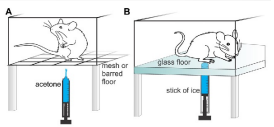
12

12

COLD STIMULATION TESTS

Acetone evaporation test: repetitive application of acetone on the hind paw and a measurement of the latency to withdrawal, leads to a cold hypersensitivity in neuropathic and inflammatory pain models

Limitations: (1) it doesn´t induce nociceptive response in naive animals; (2) the smell of acetone can trigger an olfactory stimulus that can overlap the cold stimulation



Cold plate test: measures the latency to paw withdrawal after applying a stick of ice on the floor of a cage just underneath the paw of a freely moving animal OR placing the rodent on a plate that is cooled down to 5°C

Limitation: animal should stay in place until the temperature transfer

Advantages: measures both cold allodynia and cold anesthesia with low variability

13

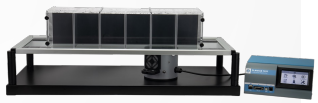
HOT STIMULATION TESTS

Hot plate test: freely moving animal is placed on a hot metallic surface with a constant temperature at 50-55°C OR temperature of the metallic surface increases gradually starting at 42°C until pain-like behaviour is observed (***dynamic hot plate test***)

Limitations: (1) it is recommended to stop the test as soon as the animal withdraws the paw; (2) the latency should not be measured on the forepaws since they are more often used to explore and groom = more reliable to observe hind paw withdrawal

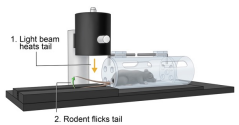
Hargreaves or plantar test: infrared heat source is applied on the hind paw of an unrestrained animal in a clear box which rests on a glass floor, the latency to withdrawal is automatically measured and displayed

Advantage: Compared to the hot plate test, the Hargreaves test offers the possibility to address each hind paw and thus using one as control



Tail Flick test: tail is either dipped in a hot water bath with a constant water temperature between 46° and 52°C or the tail is exposed to a light beam.

Advantages: easy and quick to perform if the animal is habituated to being loosely restrained; **Limitations:** risk of jeopardizing animal's thermoregulation since the tail plays an important role in rodents' thermoregulation



14

2. STIMULUS-INDEPENDENT TESTS

- When in pain, animals are capable of developing a **spontaneous behaviour** that is not a consequence of thermal nor mechanical stimulation.
- Spontaneous pain-like behaviour is believed to be **more relevant clinically**
- **Stimulus-independent tests:**
  - **Face grimace scale**
  - **Weight bearing**
  - **Conditioned place preference**
  - **Burrowing**

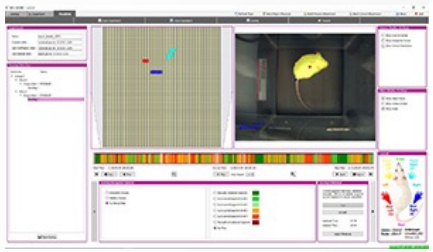
Face grimace scale test: 5 facial features (orbital tightening, nose bulge, cheek bulge, ear position, and whisker position) are observed and scored from 0 (normal) to 2 (severe pain)

Used to access the acute pain since animal are capable of adapting to chronic pain and therefore no longer express facial changes; facial expression is being used to assess emotions associated with pain

15

WEIGHT BEARING TEST

- Measurement of weigh redistribution on the rodent's hind limbs
- Weight bearing is assessed either statically (the animal is standing in inclined cage with the 2 paws relying on 2 separate pressure detectors) or dynamically (the animal is freely moving on pressure sensitive floor)
- In normal conditions: the weight is equally distributed on both hind limbs
- In inflammatory or neuropathic pain models: the weight is shifted towards the non-painful paw



16

CONDITIONED PLACE PREFERENCE TEST

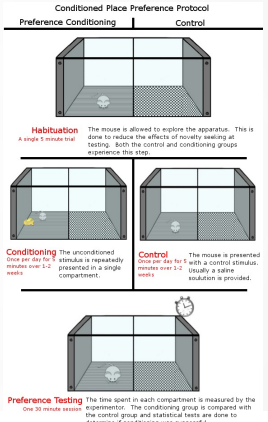
Animals are placed to a chamber with **2 compartments** of identical dimensions but with different floors and walls: **one compartment is associated with pain relief and both compartments communicate through a middle chamber**

Prior to the test, animals are preconditioned for 3 days where they are left moving freely between both compartments, the ones developing a preference to one compartment are excluded

Afterwards animals are conditioned for 4 days by pairing each of the two compartments with either drug or vehicle administration

On the test day, animals are left uninjected and moving freely and the time spent in each compartment is recorded

The animals tend to spend more time in the pain-relief compartment



Cunningham, C., Gremel, C. & Groblewski, A (2006). Drug-induced conditioned place preference and aversion in mice. Nature Protocols. 1(4). 1662-1670.

17

BURROWING

A burrow, filled with appropriate substrate, is placed in the home cage and the amount of material displaced by the animal within a predefined time interval is measured by the experimenter

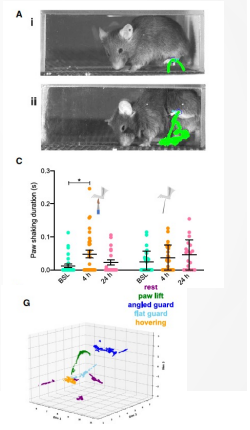
Advantage: (1) very simple, objective test, showing a high level of reproducibility (2) burrowing is reduced in a wide range of inflammatory and neuropathic pain models in rats and mice

18

PAINSPOTTING USING THE COMPUTATIONAL APPROACHES

- Parallel advances in high-speed videography, machine learning, 3D pose analytics, and natural language processing have recently merged together to advance the assessment of pain in mice.
- **Bohic et al, 2023:** Injection of inflammatory substance (carrageenan) to mice leads to guarding of the paw in the air for extended time.

  Unsupervised machine-learning method to analyse patterns in paw position over time: discovery of more fine-grained features of the paw withdrawal behaviour, including angled and flat paw guards, respectively associated with brush and pinprick stimulation following inflammation.



Bohic M et al. *Neuron*. 2023

19

LIMITATIONS OF STUDYING PAIN IN RODENTS

- Animal models of pain have been crucial for the discovery and development of analgesic agents. However, translation of findings from pre-clinical studies to the clinics was so far rather poor.
- There are pronounced genetic and molecular neurochemical differences between humans and animals = difficult to predict the pharmacological effect of a molecule on the clinical level.
- One concern is the reliance of studies on reflexive measures, and it has been suggested that additional measures that test non-reflexive pain-like behaviorus should be included, such as spontaneous pain-like behaviours, quality of life or physical activity measures.
- Heterogeneous nature of human chronic pain can have a multitude of causes while vast majority of animal studies utilize inbred.
- The overuse of male rodents in pre-clinical studies is a clear contrast to the high prevalence of chronic pain in women.

20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21

21