



**HAL**  
open science

## **Animal models of pain: Diversity and benefits**

Cynthia Abboud, Alexia Duveau, Rabia Bouali-Benazzouz, Karine Massé, Joseph Mattar, Louison Brochoire, Pascal Fossat, Eric Boué-Grabot, Walid Hleihel, Marc Landry

► **To cite this version:**

Cynthia Abboud, Alexia Duveau, Rabia Bouali-Benazzouz, Karine Massé, Joseph Mattar, et al.. Animal models of pain: Diversity and benefits. *Journal of Neuroscience Methods*, 2021, 348, pp.108997. 10.1016/j.jneumeth.2020.108997 . hal-03829365

**HAL Id: hal-03829365**

**<https://hal.science/hal-03829365>**

Submitted on 3 Feb 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

**Review article**

**Animal models of pain: diversity and benefits**

Cynthia Abboud<sup>1,2,3,4</sup>, Alexia Duveau<sup>2</sup>, Rabia Bouali-Benazzouz<sup>2</sup>, Karine Massé<sup>2</sup>, Joseph Mattar<sup>3</sup>, Louison Brochoire<sup>2</sup>, Pascal Fossat<sup>2</sup>, Eric Boué-Grabot<sup>2</sup>, Walid Hleihel<sup>3,4\*</sup>, Marc Landry<sup>2\*§</sup>

1: Univ. Bordeaux, CNRS, Interdisciplinary Institute for Neuroscience, IINS, UMR 5297, F-33000 Bordeaux, France

2: Univ. Bordeaux, CNRS, Institute for Neurodegenerative Diseases, IMN, UMR 5293, F-33000 Bordeaux, France

3: School of Medicine and Medical sciences - Holy Spirit University of Kaslik (USEK)

4: Faculty of Arts and Sciences - Holy Spirit University of Kaslik (USEK)

\*: Share seniority

§: Corresponding author, [marc.landry@u-bordeaux.fr](mailto:marc.landry@u-bordeaux.fr)

**Acknowledgements:** This work is supported by an ANR grant (Relax, n° 193992). CA is the recipient of a scholarship from the CNRSL (Lebanon). We thank Dr. Najib NAJJAR, Adjunct Instructor at Holy Spirit University of Kaslik – USEK, Chairperson at American University of Technology – AUT, for English language editing.

**Competing interest statement:** The authors declare they have no competing interests.

**Author contribution:** **Marc Landry** and **Walid Hleihel:** funding acquisition and supervision; **Marc Landry** and **Cynthia Abboud:** conceptualization; **Cynthia Abboud, Alexia Duveau, Rabia Bouali-Benazzouz, Karine Massé, Joseph Mattar, Louison Brochoire, Pascal Fossat, Eric Boué-Grabot, Walid Hleihel** and **Marc Landry:** writing – original draft; **Cynthia Abboud** and **Karine Massé:** Writing – tables; **Marc Landry** and **Louison Brochoire:** Writing – review and editing.

## **Abstract**

Chronic pain is a maladaptive neurological disease that remains a major health problem. A deepening of our knowledge on mechanisms that cause pain is a prerequisite to developing novel treatments. A large variety of animal models of pain has been developed that recapitulate the diverse symptoms of different pain pathologies. These models reproduce different pain phenotypes and remain necessary to examine the multidimensional aspects of pain and understand the cellular and molecular basis underlying pain conditions.

In this review, we propose an overview of animal models, from simple organisms to rodents and non-human primates and the specific traits of pain pathologies they model. We present the main behavioral tests for assessing pain and investigating the underpinning mechanisms of chronic pathological pain. The validity of animal models is analysed based on their ability to mimic human clinical diseases and to predict treatment outcomes. Refine characterization of pathological phenotypes also requires to consider pain globally using specific procedures dedicated to study emotional comorbidities of pain. We discuss the limitations of pain models when research findings fail to be translated from animal models to human clinics. But we also point to some recent successes in analgesic drug development that highlight strategies for improving the predictive validity of animal models of pain. Finally, we emphasize the importance of using assortments of preclinical pain models to identify pain subtype mechanisms, and to foster the development of better analgesics.

## **Highlights:**

- Evaluation tools to assess chronic pain and its comorbidities
- Inventory of a large diversity of pain models including rodents, non-human primates pain models and simple organisms
- Focus on inflammatory and neuropathic pain models
- Limitations of the predictive validity of animal models of pain
- Translational successes from animal model studies to develop new analgesics

## **Introduction**

Pain is a vital physiological function that protects organisms against potential damage. Acute nociceptive pain is a normal function of the nervous system that provides important sensory information about the environment and reacts to harmful stimuli such as noxious heat, extreme cold, chemical irritants, and mechanical tissue damage. These noxious stimuli activate peripheral nociceptors, triggering action potentials that propagate along sensory axons to the dorsal horn of the spinal cord where nociceptive inputs are processed and relayed to the brain. In turn, the activation of specific brain areas produces a broad array of sensory, emotional, autonomic, and motor responses that shape our experience and perception of pain (Basbaum et al., 2009; Burma et al., 2017).

In contrast to acute pain, chronic pain is a maladaptive disease that heightens the sensitivity to sensory stimulation (Woolf and Salter, 2000; Costigan et al., 2009). Chronic pain results from abnormal functioning of the nervous system, with pain persisting far beyond the resolution of the primary injury. Pain hypersensitivity manifests as spontaneous pain (pain in the absence of an external stimulus), allodynia (pain resulting from an innocuous stimulus), and/or hyperalgesia (an exaggerated pain response to a noxious stimulus). Chronic pain is a major health problem that negatively impacts the quality-of-life of sufferers and exacts enormous socio-economic costs with a prevalence of around 8% of the general population (Bouhassira et al., 2008). In the European Union, it is estimated that 20% of the population would suffer from chronic pain during lifespan (Breivik et al., 2006; Alshami, 2014), and it is among the most significant risk factors for suicide.

Chronic pain drastically diminishes quality of life and causes enormous socio-economic costs. Besides high costs for disease management, chronic pain is associated with major impacts on daily activities and quality of life (Groenewald and Palermo, 2015) and high productivity losses due to work absences (Mayer et al., 2019) partly due to common co-morbidities such as depression (Phillips, 2006). The estimated direct and indirect healthcare costs for chronic pain disorders in European Member States vary between two and three percent of GDP across the EU (Breivik et al., 2013). For 2016, this estimate would result in up to €441 billion. In the USA, ~100 million people suffer from pain costing ~\$600 billion/year in health care and lost productivity (Walker et al., 2014). These costs are reported to exceed those estimated for heart disease, cancer and diabetes (Breivik et al., 2013). However, chronic pain is poorly managed with treatment success rates around 30% (Ossipov et al., 2014), mainly because chronic pain mechanisms remain poorly understood (Basbaum et al., 2009; Dolique et al., 2010; Cordero-Erausquin et al., 2016; Kuner and Flor, 2016), and patients often suffer from comorbid disorders such as anxiety and depression (Attal et al., 2011).

It is important to recognize that there is not one overarching, singular condition called chronic pain but rather, there are multiple aetiologies of pain, each resulting from different pathologies and differing in the clinical presentation of signs and symptoms (Burma et al., 2017). Pain is usually subdivided in different categories as a function of the mechanism of injury. Nociceptive pain represents the normal response to noxious insult or injury of tissues such as skin, muscles, visceral organs, joints, tendons, or bones. Inflammatory pain results of activation and sensitization (peripheral and/or central) of the nociceptive pain pathway by a variety of mediators released at a site of tissue inflammation. Neuropathic pain arises from damage to the nervous system itself, central or peripheral, either from disease, injury, or pinching. Other types of pain were characterized, e.g. cancer pain or dysfunctional pain when no biological cause is identified. To address differences in the presentation of pain symptoms across chronic pain conditions, an array of preclinical animal models has been developed to recapitulate the underlying pathology, duration,

and comorbidities of pain phenotypes (Mogil, 2009). This variety of preclinical pain models is essential for understanding the molecular and cellular mechanisms that underlie distinct pain conditions. The development of novel and more efficacious therapies requires a thorough understanding of the mechanistic underpinnings of chronic pain and the design and testing of new drugs.

Rodents are employed in a large majority of preclinical pain studies (Mogil, 2009). However, the use of alternate vertebrates and invertebrates, such as zebrafish, fruit flies (*Drosophila* sp.) and nematodes (*Caenorhabditis elegans*) can also be advantageous for screening assays and for studying the genetic and molecular mechanisms of acute and chronic pain (Way and Chalfie, 1989; Gonzalez-Nunez and Rodríguez, 2009; Milinkeviciute et al., 2012). Each organism confers a distinct advantage for studying pain; the behavioral complexity of the rodents allows the analysis of the affective components of pain (Johansen et al., 2001; Panksepp and Lahvis, 2011), whereas simpler organisms, such as *Drosophila*, can facilitate the discovery of novel molecular players involved in the detection of noxious stimuli (Caldwell and Tracey, 2010; Mogil et al., 2010).

Another important aspect for designing animal models is to recognize that pain is a multi-dimensional experience. Indeed, pain is processed not only at the peripheral and spinal levels, but also in higher brain structures including cortical areas underlying the affective component of pain (Liu and Chen, 2014). As pain transitions from acute to chronic and becomes pathological, the associated negative emotional state not only exacerbates sensory modalities, but also worsens the comorbidities. Indeed, anxiety is a highly common comorbidity of pain where the interactions between pain and anxiety have been demonstrated in human. In addition, chronic pain and depression are complex disorders that often coexist and increase the risk of one another (Radat et al., 2013; Steel et al., 2014; Zhu et al., 2018a). Investigations of pain and psychiatric disorders are mostly conducted separately, but more knowledge of the overlap and interactions between affective and pain circuits is key to better treatments. Refined analysis of animal models of pain and depression has become mandatory to understand interactions between pain and emotional comorbidities (Kremer et al., 2020).

The present review proposes an overview of rodent and alternate models of pain. A large panel of evaluation devices used to characterize **pain-like behavior** in animal models has been developed over the years, and a summary description of these tools is presented here. The review focuses mainly on the broadly studied neuropathic and inflammatory types of pain. It also highlights the necessity of specific procedures dedicated to studying emotional comorbidities to pain. Finally, it summarizes the main limitations of using animal models to mimic clinical pain in humans, but also provides examples of successful translational applications of using animal models.

## **1. Assessment of pain**

The classical pain evaluation devices aim to assess the sensory component of pain, usually by measuring a withdrawal reflex. However, more recently, specific efforts have been made to assess pain perception and to evaluate the emotional component of pain and comorbid affections. Unlike humans, animals are incapable of verbally describing pain, therefore a battery of behavioral tests has been developed in order to assess **pain-like behavior** in animals. These assays can be divided into stimulus-dependent and stimulus independent tests.

### ***Stimulus-dependent tests***

In the evoked tests the latency to or the frequency of paw withdrawal is usually measured after mechanical or thermal stimulation.

#### **Mechanical stimulation.**

Mechanical hypersensitivity can be of various types, namely dynamic (triggered by brushing), static (triggered by pressure), and punctate (triggered by touch) (Deuis et al., 2017a). Dynamic mechanical hypersensitivity is measured by air puffing on the face (Miraucourt et al., 2009) or light stroking (velocity is  $\sim 2$  cm/s) of the external lateral side of the injured hind paw with a paintbrush (Cheng et al., 2017). The typical response of naive rodents to the dynamic mechanical stimulation is a very fast lifting of the stimulated paw aside. In experimental pain models, the response changes into sustained lifting, flinching or even licking behavior. Static mechanical sensitivity can be assessed with a pressure algometer (Deuis et al., 2017a). Testing punctate mechanical hypersensitivity generally consists of applying an increasing pressure and measuring nociception in animals. Some tests are more specific to rats (Randall-Selitto Test) and some are common to rats and mice (von Frey). These tests are described more in detail below.

*von Frey test.* The von Frey test, originally developed by Maximilian von Frey, is used to assess mechanical allodynia in rodents (Piel et al., 2014; Deuis et al., 2017b). The manual von Frey test consists of applying pressure of different force using calibrated von Frey filaments (ranged from 0.008 to 300 g) on the plantar surface of the hind paw. The animal is freely moving in a cage, and can rest on a penetrable grid. Once the animal is standing on its 4 legs, the filament is placed perpendicularly and pressed against the paw until it bends.

(i) Three main methods of using von Frey filaments are commonly used. The “up and down” method determines the weight of stimulus that elicits a response 50% of the times it is applied. It derives of the statistical formula used to determine LD50s (lethal dose: dose that is lethal for 50% of the animals in the group) (Dixon, 1980; Chaplan et al., 1994). The method is based on a series of testing with filament of different forces. The calculation takes into account six responses around the threshold and assess the 50% threshold using the formula: 50% threshold ( $g$ ) =  $10^{(X+kd)}/10^4$ , where  $X$  = the value (in log units) of the final von Frey filament,  $k$  = tabular value for the response pattern (see Appendix 1 in (Chaplan et al., 1994)) and  $d$  = the average increment (in log units) between von Frey filaments (Deuis et al., 2017a). Recent refinements of this methods have been published and proposed easier procedures (Bonin et al., 2014; Christensen et al., 2020). A limitation of this method is the repetitive stimulations that is time-consuming and can cause sensitization.

(ii) In the “percent” method, identical series of stimulations with von Frey filaments of increasing force are applied to all animals and the percentage of withdrawal responses is assessed (Chaplan et al., 1994). Here also the limitation relates to the high number of stimulations applied to one animal.

(iii) In the “ascending” method, filaments with increasing force are used, and nociceptive behavior is considered when the animal retracts the paw, licks it or even shakes it (D’Souza et al., 2011; Papon et al., 2020). The effects of stress are very limited in this test since the animal is unrestrained and is habituated to the environment prior to the test. However, it relies on the experimenter eye and judgment thus a subjective bias has to be taken into consideration. In order to limit this subjectivity, one can use electronic

von Frey where a single filament is pressed against the paw and the force is increased automatically until paw withdrawal.

Overall, despite the popularity of the von Frey test, there is no consensus on the sensation that is triggered in rodents, mainly is because the distinction between noxious and innocuous stimuli relies on subjective human judgment and can hardly be applied to an animal. Moreover, pain is an experience that can be different for human and rodents. Therefore, it is assumed that the von Frey test triggers nociception and can be used to assess unpleasantness of the stimulation, rather than pain (Abdus-Saboor et al., 2019).

*Randall-Selitto Test or paw pressure test.* This test is used to assess mechanical hypersensitivity in rats (Randall and Selitto, 1957). The animal is 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 (Barrot, 2012). Waiting for the animal to vocalize is not recommended since it is a synonym to great pain which may lead to injuring the animal (Muley et al., 2016). Another limitation is that rats should be habituated to being restrained in order to minimize the stress bias (Santos-Nogueira et al., 2012).

Behavioral tests and readouts must be carefully chosen according to the mechanisms under investigation. Withdrawal reflex is usually seen as a measure of spinal reflex. In contrast, vocalizations are considered as a measure of integrated pain-like behavior (Kayser and Christensen, 2000). Since 22 kHz vocalizations are emitted in response to aversive stimuli, the use of ultrasound has been considered. However, ultrasound vocalization do not correlate to pain-like behavior and cannot be considered as a reliable readout of pain sensation in rodents (Wallace et al., 2005).

#### Cold stimulation.

*Acetone evaporation test.* Acetone evaporation test is used to assess cold allodynia in rodents (Choi et al., 1994; Vissers and Meert, 2005; Colburn et al., 2007). The evaporation of acetone on the hind paw leads to a cold stimulation that is not considered nociceptive in naive animals. Therefore, it is used to evaluate allodynia in neuropathic and inflammatory pain models (Deuis et al., 2017). The test consists of a repetitive application of acetone on the hind paw and a measurement of the latency to withdrawal (Xing et al., 2007). Alternatively, cold allodynia assessment may be achieved by monitoring the duration or the number of nocifensive responses, or scoring the severity of the response. The test is done on one paw, leaving the other as control. However, the smell of acetone can trigger an olfactory stimulus that can overlap the cold stimulation (D'Souza et al., 2011a).

*Cold plate.* The cold plate test is used to assess cold allodynia and hyperalgesia. It consists of measuring the latency to paw withdrawal after applying a stick of ice (wet or dry ice) on the floor of a cage just underneath the paw of a freely moving animal (Allchorne et al., 2005). Alternatively, the rodent can be standing on a plate that is cooled down to 5°C by Pelletier effect. One main disadvantage for this test is that the animal should stay in place until the temperature transfer (Barrot, 2012).

The cold plantar assay has been developed to apply a focal ramping cold stimulus to unrestrained, acclimated mice (Brenner et al., 2012). This assay has the ability to establish a detail evaluation of the nocifensive response as a function of the temperature and to measure both cold allodynia and cold anesthesia with low variability in mice. It is also highly sensitive to experimentally altered pain conditions including CFA-induced inflammation, and SNL-induced nerve injury, or morphine treatment.

#### Hot stimulation.

*Hot plate.* Hot plate test is used to assess thermal **nociception** in mice and rats (Woolfe and Macdonald, 1944). The test consists of placing a freely moving animal on a hot metallic surface (with a constant temperature at 50-55°C) and measuring the latency for the animal to exhibit **nocifensive** behavior (paw withdrawal or jumping). For ethical reasons, it is recommended to stop the test as soon as the animal withdraws the paw. The latency should not be measured on the forepaws since they are more often used to explore and groom. Consequently, it is more reliable to observe hind paw withdrawal. In order to avoid tissue damage, explorers should determine a cut-off time if the animal doesn't respond. Another variant of the hot plate is the dynamic hot plate test where the temperature of the metallic surface increases gradually until **pain-like behavior** (D'Souza et al., 2011; Barrot, 2012; Burma et al., 2017; Deuis et al., 2017).

The dynamic hot plate (Ogren and Berge, 1984) differs of the classical hot plate test in the sense that the animal is submitted to a ramp of increasing temperatures, usually starting at 42°C until a nocifensive response is observed. By assessing the response temperature, the dynamic hot plate test gives an estimate of thermal allodynia.

*Hargreaves or plantar test:* Hargreaves test is used to assess heat pain threshold in mice and rats (Hargreaves et al., 1988). The test consists of applying an infrared heat source 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. The animal should be acclimatized to the box prior to the experiment to minimize movement not related to nociception. Compared to the hot plate test, the Hargreaves test offers the possibility to address each hind paw and thus using one as control (Hargreaves et al., 1988; Harvey and Dickenson, 2009). In 2013, Banik and Kabadi have developed a modified Hargreaves for rats where they fix the time and measure the temperature at which the paw is withdrawn (Banik and Kabadi, 2013). The authors apply a constant temperature for 10s, then they increase the temperature by 2.5 °C and apply it for another 10 seconds until paw withdrawal (with temperature starting at 35°C up to a maximum of 70°C).

*Tail Flick test :* The tail flick test is used to assess heat pain threshold in mice and rats (D'amour and Smith, 1941). The test consists of measuring the latency of the tail to flick after a heat stimulation. The 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. This test is easy and quick to perform if the animal is habituated to being loosely restrained, but there is a risk of jeopardizing animal's thermoregulation since the tail plays an important role in rodents' thermoregulation (Barrot, 2012; Deuis et al., 2017b).

### ***Stimulus-independent tests***

When in pain, animals are capable of developing a spontaneous behavior that is not a consequence of thermal nor mechanical stimulation. This type of behavior is believed to be more relevant clinically **although it must be kept in mind that rodents being prey animals, they tend to hide states of discomfort.** Throughout the years a series of methods have been developed to assess these non-evoked behaviors. In this review the grimace scale, weight bearing, **conditioned place preference and burrowing** will be discussed (for a more thorough review on spontaneous behavior please refer to Tappe-Theodor and Küner (Tappe-Theodor and Küner, 2014).

#### **Grimace scale and unbiased behavioral analysis.**

The grimace scale test in rodents consists of observing 5 facial features: orbital tightening, nose bulge, cheek bulge, ear position, and whisker position (Langford et al., 2010; Sotocinal et al., 2011). These features

are scored from 0 (normal) to 2 (severe). It is classically used to assess acute pain since animals are capable of adapting to long-lasting pain, and therefore no longer express facial changes (Langford et al., 2010). Moreover, facial expression is being used to assess emotions associated with pain (Dolensek et al., 2020).

Beyond the grimace scale test, high-throughput, unbiased approaches that accurately measure non-stimulus-evoked pain are currently under development. Increasing the dimensionality of pain assays has the potential to increase the predictive validity of translational pain therapeutics and the transfer from bench to bedside. The speed of rodent responses to sensory stimulations is in the range of the millisecond and cannot be captured only by the eye experimenter. It requires the development of automated systems to monitor pain-like behavior, possibly coupled with statistical modelling and machine-learning (Abdus-Saboor et al., 2019). Automated behavioral analysis can be performed in a dedicated apparatus (Behavioral Spectrometer, Behavioral Instruments) or in a home cage (HomeCageScan, CleverSys; PhenoTyper, Noldus) and captured a broad array of various motor behaviors. It also computes distance traveled and velocity (Brodkin et al., 2014; Roughan et al., 2016). The challenge is to ensure that the combination of measured behaviors is representative of pain states since each individual behavior is not specific to pain. Comparisons with well-established assays, or series of individual assays, are necessary prior to generalize the use of automated, unbiased paradigms to assess pain conditions in rodents.

#### Weight bearing.

The test consists of measuring weight repartition on the rodent's hind limbs. In normal conditions the weight is equally distributed on both hind limbs, whereas in inflammatory or neuropathic pain models, the weight is shifted towards the non-painful paw. 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) (Muley et al., 2016; Burma et al., 2017; Deuis et al., 2017).

#### Conditioned place-preference (CPP).

CPP test is used to assess ongoing pain in rodents (Leite-Almeida et al., 2015). The original protocol was developed in rats by King et al. (King et al., 2009). The test consists of 2 compartments with identical dimensions but with different floors and walls. One compartment is associated with pain relief and both compartments communicate through a middle chamber (King et al., 2009; Blanco-Gandía et al., 2018). 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. The purpose is to associate one compartment with pain and the other one with analgesia. On the test day, animals are left uninjected and moving freely. The time spent in each compartment is recorded (King et al., 2009; Sellmeijer et al., 2018). The animals tend to spend more time in the pain-relief compartment (Vuralli et al., 2019). The test assesses spontaneous pain in an indirect way. To avoid biases in the compartment choice, it is important to maintain the same environment during the whole procedure (Narita et al., 2006).

#### Burrowing

Burrowing is a spontaneous behavior that has been used in rodents to assess non-evoked pain conditions. 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 (Deacon, 2006).

It is a very simple, objective, test, showing a high level of reproducibility. It has been validated for various models of inflammatory and neuropathic pain (Deuis et al., 2017a).

### ***Assessment of pain comorbidities***

When chronic pain lasts for more than 3 months and is considered as an illness, it is often associated with emotional or psychiatric comorbidities. In spite of its prevalence and impact on patients quality of life, chronic pain and emotional disorders are still poorly managed and current therapies are often inadequate (Finnerup et al., 2010). Although many reports suggest that typical anti-depressants are successful in treating pain, their success rate remains lower than 50% (Kroenke et al., 2009). Moreover, anti-depressants have anti-nociceptive effects but are less convincing in preventing the affective component of pain, i.e., pain perception (Boyce-Rustay et al., 2010). Therefore, simply targeting either pain or emotional disorders is not effective and there is a need to identify new common mechanisms. An increased understanding of mechanisms that underlie the overlap of pain with comorbid emotional states are key factors in the development of new therapies (Navratilova et al., 2016). For this purpose, the identification of agents that are active against both pain and emotional pathologies is a priority since these agents are likely to target common mechanisms, engaging overlapping neuronal circuits and underlying both disorders. Such research at the frontier between pain studies and psychiatry requires elaborate animal models and complex analysis to discriminate between the various components of the global pathology. Some highlights on comorbidities between chronic pain and psychiatric disorders are proposed below. A thorough evaluation of pain and anxiety/depression models was recently proposed (Kremer et al., 2020).

#### Anxiety-like behavior.

In the following tests the time and frequency of staying in one compartment or another is measured.

*Elevated plus maze test (EPM).* The EPM test is considered as the standard test for anxiety-like behavior in rodents (Leite-Almeida et al., 2015). It was first developed in rats by Pellow et al. in 1985 (Pellow et al., 1985) and by Lister et al. in 1987 for mice use (Lister, 1987). The test consists of a cross-shaped maze that is elevated from the floor with 2 open arms that are perpendicular to 2 closed arms (Vuralli et al., 2019). The animal is placed in the centre and left to explore the premises for 5 minutes (Narita et al., 2006). The test relies on the quandary between the animal innate tendency to explore and its fear of open, lit and elevated places (Campos et al., 2013). Anxious animals spend less time in open arm. Indeed, inflamed animals exhibit anxious-like behavior just hours after pain induction and lasts for 3-4 weeks, whereas animal models of neuropathic pain develop this kind of behavior 3-4 weeks after the surgery and it lasts for 4 more weeks (Leite-Almeida et al., 2015). It is important to notice that the animal gets familiar with the cross shaped maze, thus introducing some bias (Tucker and McCabe, 2017). Therefore, the elevated zero maze was developed as it consists of a circular-shaped maze (Shepherd et al., 1994). One more pitfall is that the presence of the experimenter interferes with the results. Consequently, animals are videotaped for unbiased data (Vuralli et al., 2019).

*Open field test (OF).* The OF test represents the oldest test to assess anxiety-like behavior in rodents (Leite-Almeida et al., 2015). The test consists of placing the animal in the center of a square with high walls and let the animal explore the area freely for 5 minutes. The concept of this test is similar to the EPM, as it relies on the dilemma between exploratory behavior and fear of open spaces. Anxious animals tend to stay next to the wall and avoid the center, in fact mouse models of inflammatory pain exhibit anxiety-like

behavior from 2 to 28 days after induction of the inflammation whereas neuropathic pain models may develop this kind of behavior 2 to 8 weeks after surgery or may not. This great variability could be linked to the type of neuropathy or to the test protocol; this is why it is not recommended to use this test as the sole determinant of anxiety-like behavior in neuropathic pain models (Kremer et al., 2020).

*Light and dark box test (LDB):* The LDB test was first developed by Crawley and Goodwin in the 1980's (Crawley and Goodwin, 1980). The box is divided into two compartments, one small and dark and the other big and brightly lit (twice as big as the small dark compartment) (Bourin and Hascoët, 2003). The animal is placed in the light compartment and left to explore the box freely for 5 minutes (Vuralli et al., 2019). The LDB concept is the same as the EPM and OF, as it is based on the conflict between the animal exploratory behavior and its aversion to light places. Anxious animal tend to avoid the light compartment. In fact mouse models of inflammatory pain exhibit anxiety-like behavior from 1 to 28 days after induction of the inflammation whereas this kind of behavior may develop 4 to 8 weeks after surgery in neuropathic pain models (Kremer et al., 2020).

#### Depression-like behavior.

*Forced swim test (FST).* The FST is the most commonly used test to assess depression-like behavior in rodents. It was first developed by Porsolt in 1977 (Porsolt et al., 1977a, 1977b). The test consists of dropping the animal in a water filled cylinder for 5 minutes and measure the total duration of immobility (Leite-Almeida et al., 2015). When faced with the inability to escape (closed water cylinder), **the animal stays immobile as a possible manifestation of depression-like behavior**. Increased immobility is observed in mouse models of inflammatory pain between 4 to 35 days post induction and between 4 to 8 weeks post-surgery in neuropathic pain models (Kremer et al., 2020).

*Tail suspension test (TST):* TST is a depression-like behavior test applicable only in mice and is considered as a more sensitive variant of FST (Steru et al., 1985). It consists of suspending the mice by their tail for 6 minutes and measuring their duration of immobility. Increased immobility is observed in inflamed mice between 7 days (complete Freund's adjuvant model in mice, see §2) to 41 weeks (osteoarthritis model in mice, see §2) post induction and between 2 weeks to 2 months post-surgery in animal models of neuropathic pain (Kremer et al., 2020).

*Sucrose preference test (SPT):* The SPT developed by Katz (Katz, 1982), is a depression-like behavior test that assesses anhedonia which refers to the inability to feel pleasure. The animal has the freedom to drink either water or a sweet solution. **Animal models of depression** have a decrease ratio of usage of sweet solution over water. In fact inflammatory pain mouse models have their ratio decreased between 2 days to 4 weeks post induction and between 1 week to 10 weeks post-surgery in neuropathic pain models (Kremer et al., 2020).

## **2. Models of inflammatory pain**

**Two** important parameters to be considered in animal models of pain are the method of injury and the endpoint measurement. The most appropriate models, whether an injury, application of chemical agents, or other manipulations, should be based on 1) understanding the clinical disease presentation and pathology (i.e. face validity); 2) producing nociception by recapitulating the mechanisms of specific clinical conditions (i.e. construct validity). Measures of nociceptive behavior must not only detect pain-like responses but also do so in a manner consistent with the clinical experience of pain (Gregory et al., 2013).

Measures of reflexive behaviors such as withdrawal thresholds to noxious stimuli have been used for decades to examine mechanisms of pain. These have clearly proven useful in advancing our understanding of the physiological basis of nociception, and the identification of neurotransmitters, receptors, intracellular messengers, genes, and circuits implicated in pain-specific mechanisms. They led to better understanding of existing pharmacologic and non-pharmacologic pain treatments (Woolf, 1983, 2011; Basbaum et al., 2009). In addition, past studies in rodent models of acute nociception and chronic pain indicated that the pharmacologic action (i.e. efficacy, potency, duration of action) of a broad spectrum of analgesics to reduce reflexive sensory responses have demonstrated relevance to human analgesia (Yaksh, 2002; Gregory et al., 2013).

Chronic pain can be the consequence of persistent inflammation (Burma et al., 2017). Injured peripheral tissue releases an inflammatory soup made of pro-inflammatory molecules comprising bradykinin, prostaglandin, cytokines and chemokines. This peripheral inflammation stimulates primary afferent neurons and causes peripheral sensitization. This can be accompanied by central sensitization due to the release in the spinal cord of neurotransmitters and neuromodulators such as glutamate, substance P or BD. This sensitization is transient in case of acute inflammation, whereas it becomes long lasting in chronic pain diseases (Ji et al., 2014). In the following, classical animal models of inflammatory pain will be listed first (**Table 1**) and followed by specific models for specific pathologies. As a matter of fact some animal models are specifically developed for well-defined pathologies. In this review, animal models of pain commonly used for osteoarthritis (which is the most common form of arthritis) and rheumatoid arthritis will be detailed.

### ***Pain model induced by capsaicin***

Capsaicin is a proto-alkaloid present in chili peppers and is considered to be the main irritant responsible for hot sensation (Ilie et al., 2019). Capsaicin induces a neurogenic inflammation. In fact capsaicin binds to transient receptor potential vanilloid 1 (TRPV-1) present on free neuronal endings of primary sensory neurons which will leads to the peripheral release of several inflammatory mediators: calcitonin gene-related peptide (CGRP), neurokinin A/B, somatostatin, vasoactive intestinal peptide (VIP), and substance P (Muley et al., 2016; Ilie et al., 2019). These neuropeptides will thus induce the release of pro-inflammatory cytokines by nearby mast cells, endothelial cells, epithelial cells and immune cells. The local inflammation occurs with hyperalgesia. Administration of capsaicin is accompanied by 2 types of hyperalgesia, a primary one that is believed to be a consequence of peripheral sensitization and responds to thermal and mechanical stimuli, and a secondary one which is a result of central sensitization and responds to mechanical stimulation (Frias and Merighi, 2016; Muley et al., 2016; Ilie et al., 2019). Capsaicin effects are dose dependent. In fact, a high concentration or a continuous administration can lead to desensitization of TRPV1 resulting in an analgesic effect.

This inflammatory induced pain model, obtained after intra-plantar injection of capsaicin, is used in mice and rats (Carter and Francis, 1991; Lynn et al., 1992; Caterina et al., 2000; Laird et al., 2001a; Drewes et al., 2003) to mimic skin inflammation and inflammatory bowel disease. It has also been used to assess the efficiency of non-steroid anti-inflammatory drugs (diclofenac) (NSAID), or anti-epileptic drugs (gabapentin) (Muley et al., 2016).

### ***Pain model induced by formalin***

The formalin test is commonly used since it was created in 1977 (Dubuisson and Dennis, 1977). It consists of an injection (sub-cutaneous or intra-plantar) of a diluted solution (0,05-5%) of formaldehyde (Tjølsen et al., 1992). This pain model is characterized by a biphasic response. A short (0-5 minutes) first phase, where pain is believed to be a consequence of direct activation of primary sensory neurons, is followed by a second prolonged phase (10-40 minutes), where pain is the result of spinal cord inflammation within the dorsal horn, leading to central sensitization (Hunnskaar and Hole, 1987; Tjølsen et al., 1992; Raboisson and Dallel, 2004). Among the mechanisms involved in this model, it has been shown that formalin activates transient receptor potential ankyrin 1 (TRPA1) (McNamara et al., 2007) more particularly in phase 2. Interleukin-33 and its receptor ST2 are also believed to be involved in inducing pain in both phases. In fact, IL-33/ST2 pathway activation leads to activating mitogen-activated protein kinase (MAPK) signaling pathway cascades that are implicated in central sensitization (McNamara et al., 2007; Han et al., 2015). In phase 1, **pain-like behavior** (paw licking and lifting, vocalization) can be observed. It is inhibited by lidocaine. In contrast, in phase 2, pain is alleviated by NSAID, morphine and gabapentin (McNamara et al., 2007). The formalin induced model is used in rats (Dubuisson and Dennis, 1977; McNamara et al., 2007) and mice (Hunnskaar and Hole, 1987; Han et al., 2013) has largely contributed to better understanding of pain mechanisms (central and peripheral) (Muley et al., 2016).

### ***Pain model induced by complete Freund's adjuvant (CFA)***

CFA is a suspension of heat-killed *Mycobacterium tuberculosis* in paraffin oil (Barrot, 2012) that is frequently used for inducing inflammatory pain. CFA is injected subcutaneously in the hind paw or intra-articular. When injected in the hind paw, the inflammation occurs 24 hours after the injection and lasts for one to two weeks (Ren and Dubner, 1999; Bas et al., 2016). However, after intra-articular injection, the inflammation takes 7 days to occur (Muley et al., 2016). The mechanisms leading to CFA-induced inflammatory pain are still to be deepened but it is known that the injection of CFA leads to the release of pro-inflammatory mediators (PGE2, TNF alpha, IL-1) that induces synovitis, bone resorption and eventual degeneration. Inflammatory mediators are responsible of neuronal sensitization that is responsible for joint pain (Muley et al., 2016). CFA-induced pain models are frequently used to assess inflammatory pain in mice and rats (Knight et al., 1992; McDougall et al., 1995; Keeble et al., 2005; Simjee et al., 2007; Fernandes et al., 2011; Uematsu et al., 2011; Nisar et al., 2015). For example, the CFA model is considered a robust model to study arthritis, and particularly rheumatic arthritis (RA) since it mimics the synovitis and bone resorption observed in human RA but does not mimic cartilage alteration (Bas et al., 2016).

### ***Pain model induced by carrageenan***

This model is mainly used to study inflammatory pain, more specifically joint inflammation (Mert et al., 2018). Carrageenan is used to induce transient joint inflammation, where hyperalgesia and allodynia are observed (Winter et al., 1962; Fehrenbacher et al., 2012). A carrageenan solution with a concentration centered between 0.5-2 percent is injected subcutaneously, the swelling is observed 3-5 hours after the injection and it lasts for 24 hours (Winter et al., 1962; Otterness and Moore, 1988).

### ***Pain model induced by zymosan***

This self-resolving model is used to study acute inflammation (Cash et al., 2009). Indeed, 30 minutes after injecting zymosan, which is an insoluble polysaccharide component of the cell walls of *Saccharomyces cerevisiae*, animals develop edema and inflammation as well as thermal and mechanical hyperalgesia (Doherty et al., 1985; Meller and Gebhart, 1997). Thermal and mechanical hyperalgesia are dose dependent, and spontaneous pain is observed with greater doses (Doherty et al., 1985; Meller and Gebhart, 1997).

### ***Osteoarthritis pain models***

Arthritis is a medical condition in which the inflammation of the joint leads to chronic pain and movement limitation. In 2012, 52.5 million USA adults suffered from arthritis. This number is thought to increase and reach 78.4 million by year 2040 (Hootman et al., 2016). Osteoarthritis (OA) is a progressive musculoskeletal disease that leads to the failure of the entire joint (Teeple et al., 2013; Alshami, 2014). Novel treatment that tackles pain and helps increase activity is of necessity and therefore animal models of pain are needed to deepen the understanding of the underlying pathophysiology of OA.

#### Chemically induced models

A wide range of chemicals are used to induce OA-like models, including papain, trypsin, carrageenan, kaolin, hyaluronidase, collagenase, sodium urate and mono-iodoacetate (MIA) (D'Souza et al., 2011a; Fang and Beier, 2014). **Notably, MIA is the most commonly used model to assess OA pain and one of the best models to study symptom-modifying OA drugs** (D'Souza et al., 2011b). MIA injection was first used by Dieter Kalbhen (Kalbhen, 1987). The mechanism of action of MIA consists of inhibiting the glyceraldehyde-3-phosphate dehydrogenase in chondrocytes; thus inhibiting the glycolytic pathway and eventually leading to chondrocyte apoptosis, subchondral bone necrosis and cartilage degeneration, consequently mimicking the morphology of end stage OA (D'Souza et al., 2011a; Fang and Beier, 2014). Subsequent to MIA injection, there are 2 phases. The first one consists of an early (few days after) inflammation, where pain is reversed by non-steroidal anti-inflammatory drugs (NSAIDs). In the second phase, the joint is destructed and the expression of the nerve injury marker activating transcription factor-3 (ATF-3) is increased in the L5 dorsal root ganglion. The pain during this phase may be lowered by the administration of morphine/gabapentin. MIA injection is used mainly in rats and also in mice and guinea pigs. In rats and mice the clinical outcomes are characterized by mechanical hypersensitivity in hind paw, alteration of sleep, and locomotive deficit, whereas in guinea pig mechanical allodynia is observed with altered weight bearing (D'Souza et al., 2011a; Malfait et al., 2013; Fang and Beier, 2014). The intra-articular (knee) injection of the above-listed chemicals is a low-cost procedure that leads to rapid severe joint degeneration. Unfortunately the clinical relevance of this model to model OA is not as accurate as with other procedures.

#### Surgically induced models.

Surgically induced models are obtained after joint surgery that is consequently unstable and altered. The cartilage degeneration, which is proportional to the degree of joint instability, depends on the joint structure. The most common procedures are performed on mice knees and leads to a fast and reproducible disease progression.

*Anterior cruciate ligament transection (ACLT)*. It has been used for decades on large animals and nowadays this procedure is performed on rodents (Stoop et al., 2001; Gomis et al., 2007). The post-surgery physiopathology evolves over time with changes in chondrocyte physiology at 4 weeks post-surgery, mild cartilage destruction at 8 weeks, and osteophytes formed at 12 weeks post-surgery. When ACLT is combined to the removal of medial meniscus, the joint destruction and osteophytes formation are observed only at 4 weeks post-surgery. It is important to mention that in human OA, support structures are not all ruptured (D'Souza et al., 2011; Teeple et al., 2013).

*Meniscectomy*. The meniscectomy in rodents could be partial or total, lateral or medial, unilateral or bilateral (Bendele, 1987; Bove et al., 2006; Knights et al., 2012). At 4 weeks post-surgery, the cartilage starts to get damage and OA lesions progressively develop from 8 to 12 weeks post-surgery (Knights et al., 2012). This procedure is questionable since there is no consistency in the amount of meniscus that is being removed (Teeple et al., 2013).

*Destabilisation of medial meniscus (DMM)*. The DMM is very common in mice (Glasson et al., 2007). Transection of the medial meniscotibial ligament leads to mild instability with cartilage destruction, subchondrial bone sclerosis and osteophyte formation. Cartilage lesions appear at 2 weeks post-surgery and progress for 16 weeks after this period. This procedure is the most common since it is reliable, reproducible and structurally similar to human OA. Furthermore, pain is reversed by standard analgesics. Finally, the disease progression is slower when compared to others (D'Souza et al., 2011a).

#### Osteoarthritis in companion animals

OA is a natural occurring disease in cats, dogs and horses and appears to be very similar to human disease. Studies in these large animals, although less used than small animal models, are often required in translational research (McCoy, 2015; Lascelles et al., 2018). Dogs are considered as an excellent model to study human OA and remain the most used, especially in preclinical trials (Aragon et al., 2007; Pelletier et al., 2010; McCoy, 2015). However, natural racehorse OA is the best-suited model to investigate the pathophysiology of post-traumatic osteoarthritis in humans, especially in athletes (McIlwraith et al., 2012; McCoy, 2015). In particular, the stifle joints articular cartilage, the most frequent altered tissue in OA suffering horses, is highly similar to the human knee cartilage. Although some pain research studies have been carried out in induced-OA companion animals, OA-natural companion models are, along with cancer-natural models (see section 4), valid models for modelling human pain (Klinck et al., 2017).

Thermal and mechanical nociception and pain tests commonly used in rodents have been adapted for these large animal models, such as facial expression analysis with the Grimace Scales (Holden et al., 2014; de Grauw and van Loon, 2016). For OA large animal models, subjective lameness scoring and kinetic gait analysis are also a widely method to assess OA pain (Moreau et al., 2014). Clinical metrology instruments (CMIs), questionnaires designed for pet owners, have improved the assessment of sensory and affective effects of pain in companion animals. Several OA CMIs exist for dogs, e.g. The "Canine Brief Pain Inventory" (CBPI) and "Liverpool Osteoarthritis" (LOAD), and cats, e.g. 'The Owner Behavior Watch' (OBW) and 'The Feline Musculoskeletal Pain Index' (FMPI). Although these CMIs are subjective methods to assess pain, they are validated by comparative analysis with objective tests for diagnosis and for outcome measurements in clinical research (Walton et al., 2013; Stadig et al., 2019). The positive therapeutic outcome of the anti-NGF antibodies in OA suffering dogs and cats is a good example of the translational positive values of companion animal models (see § 11; (Gruen et al., 2016; Sanga et al., 2017; Lascelles et al., 2018; Kelly et al., 2019)).

### ***Rheumatoid arthritis models***

An average of 0.5-1% of the general population suffer from rheumatoid arthritis (RA) (Bas et al., 2016). RA is a chronic autoimmune disorder characterized by T cells' activation of macrophages and release of pro-inflammatory cytokines, e.g. tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin (IL) 1, 6 and 17. This results in inflammation of the synovial membrane as well as cartilage and bone erosion (Caplazi et al., 2015; Bas et al., 2016). Immunotherapy is the most commonly used treatment for RA but the underlying cause of T cells reactivation is still unknown. Therefore, animal models are still needed and should new lights on pathophysiological mechanisms.

#### Collagen-induced arthritis (CIA)

CIA is the most frequently used models for RA since there is a good resemblance with human RA with the presence of rheumatoid factor and anti-citrullinated peptide antibody (Caplazi et al., 2015; Bas et al., 2016). The experimental procedure consists of an intra-dermal (ID) injection of a combination of CFA and collagen II, which constitutes the major form of articular cartilage, leading to chronic polyarthritis in periarticular joints (Courtenay et al., 1980; Caplazi et al., 2015; Bas et al., 2016; Fischer et al., 2017).

#### Collagen antibody-induced arthritis (CAIA)

CAIA is obtained after intravenous or intraperitoneal injection of a mixture of anti-CII antibodies, followed by lipopolysaccharide (LPS) injection 3 days after (Nandakumar et al., 2003; Caplazi et al., 2015; Bas et al., 2016). Inflammation is at its maximum 8 days after the injection and lasts for a month (Lynn et al., 1992). This model is frequently used because of its histological similarities with human RA. In addition, bone and cartilage degradation as well as synovitis and pannus are observed in CAIA (Fischer et al., 2017). In this model, mechanical and thermal (hot and cold) hypersensitivity are observed. CAIA can be implanted in rodents that are unsuitable for CIA. However the inflammation observed is innate and not related to B cell nor T cell activation (Caplazi et al., 2015; Fischer et al., 2017).

#### Streptococcal cell wall (SCW)-induced arthritis

This model is used to mimic RA in rats. It consists of an intra-articular injection of *Streptococcus pyogenes* cell walls. A single injection leads to an acute inflammation within one day, whereas multiple injections lead to chronic RA (van den Broek et al., 1988; Bessis et al., 2017).

### **3. Models of migraine pain**

Migraine has not classically been considered an inflammatory disease probably because it is not obviously associated with heat, redness, and swelling. Instead, a vascular etiology was proposed and the prevailing theory of migraine for most of the twentieth century, held that pain results from an abnormal dilatation of intracranial blood vessels, leading to mechanical excitation of sensory fibers. However, in recent years, advancements in the neurobiology of migraine headache have shifted the emphasis away from vascular smooth muscle toward mechanisms related to inflammation (Waeber and Moskowitz, 2005). Accumulating data have come, in large part, from basic science research utilizing small animal models of migraine-related pain (Akerman et al., 2017; Harriott et al., 2019; Vuralli et al., 2019c). Although the vascular system plays a crucial role in the headache associated with migraine that distinguishes it from somatic or visceral pain, a neuronal involvement in the aetiology of migraine has increasingly been considered (Olesen et al., 2009).

Several lines of evidence suggest that activation of trigeminal nociceptors innervating meningeal tissues is central to the initiation of migraine pain. Once activated, trigeminovascular afferents release neuropeptides that, in turn, may mediate additional release of mast cell contents and other immune mediators (Harriott et al., 2019). Exposure of perivascular fibers to inflammatory agents released in the vicinity of sensory fibers alters their sensitivity and leads to the sensation of head pain. Animal models ultimately seek to reproduce the etiology of migraine with various experimental paradigms described below.

### ***Dural stimulation***

Peripheral stimulation of meningeal nerve terminals can be achieved electrically or chemically. The direct electrical stimulation of meningeal nerve terminals (Zagami et al., 1990) derived from the demonstration in human that it results in pain often referred to the face. Such stimulation elicits the polysynaptic activation of the central projection sites of these afferents in the trigeminal nucleus caudalis and ascending projections throughout the brain (Benjamin et al., 2004). These studies have been key in identifying major migraine-related pain processing in preclinical research (Akerman et al., 2013). Direct electrical stimulation is however a nonrecoverable procedure and other protocols are necessary to mimic chronic activation of the dura.

Indeed, direct application of inflammatory soup on the exposed dura mater induces cephalic hypersensitivity (Melo-Carrillo and Lopez-Avila, 2013). This chemical stimulation elicits the expression of pronociceptive markers in the trigeminal ganglia (Lukács et al., 2015). This paradigm authorizes repeated applications producing a long-lasting central sensitization that mimics the pathophysiology of migraine (Munro et al., 2017). Less invasive refinements of this paradigm make it compatible with behavioral testing in conscious freely-moving animals.

Electrophysiology is a common readout for the various procedures of dura mater direct stimulation. It is a powerful assay method that allowed identifying effective mechanisms such as the triptans and CGRP receptor antagonists, while predicting failure of neurokinin (NK)1 receptor antagonists (Akerman et al., 2013).

### ***Trigeminal neuron stimulation***

The stereotaxic insertion of electrodes allows to directly stimulate trigeminal ganglion neurons which causes release of CGRP from perivascular afferent terminals to the dura mater. Short (3-5 min) and long (30 min) stimulation paradigms have been used with the latter inducing morphological changes (Knyihár-Csillik et al., 1997). Notably, neurochemical and morphological changes are sensitive to triptan and dihydroergotamine, thus making the inhibition of peripheral neuropeptide release a plausible mechanism of anti-nociceptive action (Buzzi et al., 1991).

### ***In vivo application of algogenic substances***

The most widely used models in preclinical migraine research rely on systemic infusion of nitric oxide donors, e.g. glycerol trinitrate (GTN) (Munro et al., 2017). Intraperitoneal administration is replaced in some studies by intravenous infusion that can directly activate the trigeminovascular system

(Ramachandran et al., 2014). Beside acute administration, other authors have developed repeated intermittent intraperitoneal administration (Pradhan et al., 2014), or intradermal injection of GTN in the hindpaw (Ferrari et al., 2016). Both variants have clinical relevance, resulting in progressive and sustained basal hyperalgesia and showing sumatriptan sensitivity, respectively. Interestingly in these models, the effects of algogenic substances are not restricted to specific peripheral or central nervous system sites but are likely to act more broadly in migraine-related structures (Harriott et al., 2019).

### ***Genetic models***

Among the rare genetic models of migraine, mouse models expressing gain of function missense mutations of the CACNA1A faithfully recapitulate the symptoms of familial hemiplegic migraine (FHM), a rare monogenic migraine subtype associated with aura. Notably, CACNA1A, as well as other genes implicated in FHM, encode ion channel and transporter subunits that play important roles in neurotransmission.

### ***Cortical spread depression model***

The aura is associated with migraine in up to 30% of patients. The aura results from a wave of intense excitation across the visual cortex that has been attributed to cortical spreading depression (CSD) process. CSD is a slowly propagating depolarizing wave that can be induced experimentally in rodents where it is initiated by injection of KCl into the cortex (Munro et al., 2017). CSD triggers neuronal activation within the trigeminal ganglion and in higher brain regions (Cui et al., 2015). Moreover, CSD also increased activation of microglia that is a key mechanism relevant to central sensitization and contributing to pain sensitivity.

## **4. Models of neuropathic pain.**

Neuropathic pain (NP) is a painful syndrome caused by central or peripheral lesion of the nervous system. It is highly disabling, affecting 7-8% of general population (Bouhassira et al., 2008). NP elicits sensory alteration including dysesthesia and paresthesia, spontaneous pain, increase pain sensation for innocuous stimuli (allodynia) and increase pain sensation for noxious stimuli (hyperalgesia). Animal models of NP are developed, mainly in rodents, to recapitulate one or several symptoms of NP aiming at deciphering the underlying mechanisms. **Larger animals, e.g. cat, have also been used to study NP mechanisms** (Koyama et al., 1993). NP models are distinguished according to the localization, central or peripheral, of the nerve injury.

### ***Models of central NP***

Central pain syndrome (CPS) classified as neuropathic pain is defined as “pain initiated or caused by a primary lesion or dysfunction of the central nervous system” (International Association for the Study of the Pain, Merskey and Bogduk, 1994) or more recently as “pain arising as a direct consequence of a lesion or disease affecting the central somatosensory system” (Treede et al., 2008). CPS patients display somatosensory abnormalities (hyposensitivity to thermal and noxious stimuli), which is the universal feature of this syndrome, due to the spinothalamoparietal thermoalgesic pathway dysfunctions (Canavero and Bonicalzi, 2007). CPS patients suffer from dysesthesia and painful burning sensation whose localization in body regions, and intensity and duration, widely differ, depending of the syndrome etiology. CPS onset

can be delayed several months after CNS damage but is a lifetime condition in the majority of cases (Berić, 1998; Canavero and Bonicalzi, 2007) CPS is mostly induced by stroke (referred as central post-stroke pain), multiple sclerosis or brain/spinal cord injury (SCI). Diseases displaying a CPS-like component such as Parkinson's disease are referred as central pain-allied conditions (CPAC). Depending of the origins of the lesions, patients suffer from brain central pain (brain-brainstem lesion) or cord central pain (spinal cord lesion).

CPS has been mostly studied in rodents by performing SCI, either targeting individual fiber tracts, or extensively affecting the cord. Electrolytic lesions (one or two) of the rat spinothalamic tract afferents are performed using tungsten or quartz-insulated platinum or electrodes (Wang and Thompson, 2008; Masri et al., 2009). The electrode is targeted unilaterally to the ventrolateral spinal quadrant in the lower cervical or thoracic vertebral level (Wang and Thompson, 2008; Masri et al., 2009). Demyelinating lesions have also been carried out by the injection of lysophosphatidylcholine solution through a glass pipette inserted into the region of the rat spinothalamic tract (Wang and Thompson, 2008). CCP rodent models allowed to identify the biological bases of this syndrome (Wang and Thompson, 2008; Masri et al., 2009; Quiton et al., 2010; Naseri et al., 2013) and to test therapeutic approaches (Wang and Thompson, 2008; Lucas et al., 2011).

Other models are generated by a more extensive SCI, not only restricted to fiber tracts, e.g. spinal cord compression, partial or complete sections, photo-chemically induced ischemia, spinal cord crushing, contusion, or application of excitatory neurotoxins (**Table 1**). Even if paralysis is the core symptom of this model due to the lesion of descending motor tracts, other features of SCI impair quality of life such as pain (Simpson et al., 2012; Caplazi et al., 2015; Fischer et al., 2017). Extensive lesions of the spinal cord are associated with spontaneous and evoked pain resulting in both hyperalgesia and allodynia (Campbell and Meyer, 2006; Starkey et al., 2009; Schneider et al., 2017).

Contusive SCI is usually obtained by using a weight-drop technique (Siddall et al., 1995). In this model, rats developed allodynia few days after SCI, that lasts up to 30 days after motor recovery (Siddall et al., 1995). Spinal hemi-section mimics chronic central pain after spinal cord lesion (Christensen et al., 1996). This model offers advantages of maintaining the injured site separate from the intact side. It is obtained by performing a hemi section of a thoracic segment, usually cranial to L1 dorsal root entry. Spinal hemi-section induces changes in withdrawal thresholds in response to both mechanical and thermal stimuli that persist for 5-6 months. In this model, NP develops both below and above the SCI on both sides. This model recapitulates the NP in Brown-Sequard Syndrome, a spinal cord hemi-section injury, where chronic pain also develops on both sides (Koehler and Endtz, 1986).

Photochemical injury, developed by Watson and Colleagues (Watson et al., 1985), presents the advantage to reproduce a mechanical trauma of the spinal cord without surgery. It consists of intravenous injection of the photosensitizing dye, Rose Bengal or erythrosin B, followed by an irradiation. This reaction induces thrombosis leading to ischemia and tissue necrosis of the spinal cord (Gaviria et al., 2002). Injury induces a strong allodynia in different areas innervated by the ischemic spinal segments (caudal trunk, hind limbs, and hind paws) and a hypersensitivity to cold but not to heat stimuli.

### ***Models of peripherally-induced NP***

Various models have been proposed to copy consequences of radiculopathies or post-operative nerve

lesions (**Table 1**). Classical models are obtained by lesioning peripheral nerves, e.g. the infra-orbital nerve (face neuropathy) or the sciatic nerve (hindlimb neuropathy). Peripheral NP models elicit peripheral but also central effects, including spontaneous discharges from afferent neurons, nociceptor sensitization and spinal and cortical reorganization.

#### Nerve transection.

The sciatic nerve total transection is achieved by a complete axotomy of the sciatic nerve removing approximately 5mm of the sciatic nerve in rats or mice. This model mimics the clinical symptoms of “phantom limb” that occur in humans after transversal spinal lesion. The limitation of this axotomy model is the motor impairment, which hampers behavioral evaluation by testing withdrawal latencies, and causes frequent autotomy. For ethical considerations, these axotomy models are now replaced by partial nerve injury in which autotomy is strongly reduced or totally suppressed.

### Partial nerve lesion.

Several partial nerve lesion models have been developed over the last 20 years. These models differ according to their procedure, their reproducibility, the inflammatory component and the duration of NP symptoms. Chronic constriction injury (CCI) (Bennett and Xie, 1988) consists of four loose knots around the sciatic nerve or the infra-orbital nerve. This model involves both neuropathic and inflammatory components triggered by suture thread (Costa et al., 2005). CCI elicits spontaneous pain and pain hypersensitivity that occurs 24 hours after surgery and persists for a duration of at least 7 weeks. Variability in responses occurs upon variation in the tightness of ligature and thread used. Cuffing of sciatic nerve ("cuff" model) allows a standardized and reproducible chronic constriction injury; it is obtained by the implantation of a section of polyethylene tubing placed around the common branch of the sciatic nerve of rats or mice (Pitcher et al., 1999; Benbouzid et al., 2008; Yalcin et al., 2014b). Animals develop heat-hyperalgesia lasting for 3 weeks and mechanical allodynia for at least 2 months (Benbouzid et al., 2008). This model is of interest to study anxio-depressive comorbidities associated with NP that lasts for weeks after removal of hypersensitivity to pain (Yalcin et al., 2014a). Partial sciatic nerve injury (PNI), developed in rats by Seltzer and colleagues (Seltzer et al., 1990), consists of tight partial ligation of the dorsal third or half of sciatic nerve, peroneal or tibial branch or both. Pain symptoms occur within 1 week after surgery and continue up to 6 weeks post-injury. PNI has been adapted in mice by Malmberg and Basbaum (Malmberg and Basbaum, 1998) and showed pain symptoms one day after injury. The thermal allodynia resolves by 49 days, but the mechanical allodynia persists for the duration of the study (70 days). Spinal Nerve Ligation (SNL) was developed in rats by Kim and Chung (Kim and Chung, 1992). It consists of a unilateral and tight ligation of lumbar spinal nerves L5 and L6 distal to dorsal root ganglia. Within 24 to 48 hours after surgery, this model develops a mechanical allodynia, thermal hyperalgesia and spontaneous pain that persist for 4 months without any autotomy. Although the surgery is complex, it is a highly reproducible method with little damage to surrounding tissue (Kim and Chung, 1992; Dobremez et al., 2005; LaBuda and Little, 2005; Fossat et al., 2010; Laffray et al., 2012). SNL model is also available in mice (Kiso et al., 2008) where mechanical allodynia started at day 1 and lasted for at least 2 months after surgery. Spared nerve injury (SNI) corresponds to the lesion of two of the three terminal branches of the sciatic nerve that is obtained by the ligation of common peroneal and tibial nerves leaving the remaining sural nerve intact (Decosterd and Woolf, 2000; Bourquin et al., 2006). Mechanical and thermal responsiveness is increased in the territory of spared sural nerve. SNI model displays early and persistent **pain-like behaviors**; it is easy to perform and highly reproducible. Caudal trunk resection is performed by lesioning the caudal trunk in rat or mice (Na et al., 1994; Sung et al., 2000). Allodynia and hyperalgesia appear within a day of nerve injury and last for weeks (Back et al., 2003). In rats and mice, this model offers several advantages by allowing behavioral tests, thermal and mechanical stimuli to be easily performed at the same spot on the tail.

**These models of peripheral neuropathy induce pain-like behavior as a direct consequence of nerve lesion (i.e. sciatic nerve for the hindlimb or infra-orbital nerve for the face). These models have the ability to recapitulate some particular neuropathic syndromes in human. For instance, CCI, SNI and PNI targeting the sciatic nerve are models of complex regional pain syndromes type II (CRPS) that same sensory disorders similar to those described in patients, e.g. touch hypersensitivity, mechanical hyperalgesia, spontaneous pain, and pain irradiatio. Moreover, in the case of CCI, the strong inflammatory component mimics some features of the chronic low back pain syndromes in human. Finally, SNL that targets dorsal roots of the sciatic nerve models sciatica or radiculopathy (defined as pain resulting from injury or disease of the cervical, thoracic, lumbar or sacral nerve roots). Although these models present interesting features, there**

is still today a large gap between the number of molecules effective in preclinical studies and their poor outcome in clinical trials. Several explanations can be evoked such as the genetic homogeneity of the models (inbred mouse/rat), the low diversity of the behavioral tests generally used (i.e. withdrawal reflexes), the similarities between the different preclinical models (nerve injury modelling mononeuropathy) and the almost exclusive use of male rodents. Future challenges will be to determine precisely which mechanisms is targeted by a given drug, and which patients will be relevant for treatment (Rice et al., 2018). For this purpose, the relevance of pain assessment in animal models, and the similarities between animal behavior and human symptoms are critical.

### ***Models of poly-neuropathy (PN)***

Notably, studies on animal models of mononeuropathic pain most largely report sensory gain (Rice et al., 2018). However, traumatic nerve injury is most often characterized by a loss of sensitivity to non-painful stimuli, and a moderate sensory gain to painful stimuli (Gierthmühlen et al., 2012). In addition, most of clinical trials involve patients with polyneuropathy (Rice et al., 2019). In human clinics, polyneuropathies (peripheral neuropathies) are the most common type of disorder of the peripheral nervous system in adults, and specifically in the elderly, with an estimated prevalence of 5–8%, depending on age. A sensory loss profile is reported in the majority of patients with common polyneuropathies for example: chemotherapy-induced (Ventzel et al., 2018), diabetic (Raputova et al., 2017) and HIV-associated associated (Phillips et al., 2014) polyneuropathies, and spinal cord injury (Finnerup et al., 2003) or postherpetic neuralgia (Fields et al., 1998). Recently, relevant disease models have been developed and validated, especially those related to drug-induced neuropathy, diabetic and HIV-associated neuropathy and autoimmune neuropathy. Some examples of these models are briefly discussed in the following.

#### Chemotherapy-induced neuropathy

Peripheral neuropathy is a dose-limiting side effect for a number of chemotherapeutic agents including platinum analogues, microtubule-directed agents (taxol, vincaalkaloids), suramin or thalidomide. Cisplatin neurotoxicity is very common and can be mimicked in mice and rats by repeated injections of platinum for several weeks. Mechanical and thermal hyperalgesia and allodynia appear in the 2–4 weeks following the beginning of treatment (Authier et al., 2003). Oxaliplatin is another chemotherapeutic drug used for the treatment of gastrointestinal tract tumours, especially in colorectal cancer (Ibrahim et al., 2004). Oxaliplatin induces acute side effects in 80% of the patients, and can evolve to a long-term neuropathy in a fraction of the affected patients (around 15-20%). Mouse models of chemotherapy-induced neuropathy are generated by repeated (e.g. twice a week for 4 weeks) intravenous injection of oxaliplatin in the tail vein (Marmioli et al., 2017). Chronic treatment with oxaliplatin has been reported to induce shrinkage of Dorsal Root Ganglia (DRG) neurons and axonopathy of myelinated fibres in animal models (Renn et al., 2011), and impairs sensory neuron arborisation, indicating that oxaliplatin neurotoxicity occurs mainly in sensory neurons (López-González et al., 2018).

#### Diabetic neuropathy

Diabetic neuropathy is a progressive disease that leads to structural changes in the nerve causing hyperalgesia. Various animal models, with possible different pathogenesis underlying mechanisms, exist and highlight the importance of establishing clear criteria for models of diabetic neuropathy in rodents.

The most common metabolic models are the streptozotocin (STZ) diabetic rats and mice (mainly Swiss, C57/Bl6 or CD1) (Biessels et al., 2014). Depending on the severity of anticipated disease, diabetes is induced by a single intraperitoneal or intravenous dose of 40–80 mg STZ/kg (rat), or 150–200 mg STZ/kg (mouse) body weight, or of lower doses given to mouse over consecutive days. STZ neurotoxicity was reported to induce early behavioral and electro-physiological changes followed by distal nerve fiber loss, axonal atrophy and myelin thinning after many months of diabetes. However, STZ toxicity may be indirectly responsible for neuropathy (Davidson et al., 2009).

A large variety of genetic models have been also proposed to recapitulate the pathophysiology of diabetes. The BB/Wor and BBZDR/Wor rats model type 1 and type 2 diabetes respectively. The BB/Wor rats lack T lymphocytes expressing the RT6 alloantigen, and develop auto-immune attack of the pancreas and spontaneous onset of type 1 diabetes in males between the ages of 70–80 days (Yang and Santamaria, 2006). The BBZDR/Wor-rat spontaneously develops insulin resistance that is preceded by obesity and these animals model type 2 diabetes (Tirabassi et al., 2004). In mouse, there are two common genetic models of type 1 diabetes: the non-obese diabetic (NOD) mouse and the Akita mouse, representing spontaneous auto-immune type 1 diabetes (NOD) and a mutation of the insulin-2 gene, respectively. There is currently relatively little consistent information on the neuropathy in these animal models (Biessels et al., 2014). Models of type 2 diabetes, such as db/db (leptin receptor mutation) and ob/ob (leptin mutation) mice, have been available for many years (Wang et al., 2014). Both models develop diabetes at 4–6 weeks of age with hyperinsulinemia and hyperlipidemia. Early electrophysiological changes, and later structural abnormalities have been reported during the progression of the disease. The main limitation of both the db/db and ob/ob models is that death occurs at 24–30 weeks of diabetes in the absence of insulin supplementation.

A major limitation of the STZ and genetic models is that they do not replicate the evolution of the metabolic imbalance and the natural progression from obesity to diabetes (Preguiça et al., 2020). High fat, or high fat and high sugar diets, alone or associated with low or moderate doses of STZ, more closely model the disease progression. These paradigms induce the hallmarks of diabetic neuropathy and are key in the progressive development of symptoms including behavioural alterations, nerve conduction velocity deficits and nerve structural impairments (O'Brien et al., 2014).

In these different models of diabetic peripheral neuropathy, evoked nociception is not correlated to ongoing pain that remains present whatever the hypo- or hyperalgesic status and the possible loss intra-epidermal nerve fibre endings (Agarwal et al., 2018; Gao et al., 2019).

### Autoimmune neuropathy

Autoimmune neuropathies comprise a diverse group of conditions resulting from an immune attack on the peripheral nervous system. The prototypic immune neuropathy is the heterogeneous Guillain-Barré syndrome (GBS), the most common acute paralytic disorder in industrialized countries. It is classically considered as an acute inflammatory demyelinating polyneuropathy (Fricker et al., 2008). The pathological substrate for GBS has been well established with immune cell infiltration, demyelination with or without axonal damage in the peripheral nervous system (PNS), nevertheless molecular and cellular mechanisms require adapted animal models to be fully understood. In the classical models of experimental allergic neuritis (EAN), the auto-immune aetiology of GBS was reinforced using homogenate of peripheral nerve tissue in adjuvant (Waksman and Adams, 1956). Classical EAN is induced in susceptible rats (the Lewis rat strain is the most sensitive to auto-immune conditions) or mice by active immunization (complete Freund's

adjuvant) with peripheral myelin extract, or the whole or partial purified P2 myelin protein (Hahn, 1996). Adoptive transfer EAN is induced by injection into naive animals of antigen-specific T cells obtained from lymph nodes of EAN syngenic animals (Toyka, 1999). Although EAN has provided valuable information, it has been criticized for its artificial manipulation (Yang et al., 2014). Therefore, genetic models have been raised that develop spontaneous autoimmune peripheral polyneuropathy caused by B7-2 (Salomon et al., 2001) or CD4 (Yang et al., 2014) depletion in mouse.

### Infectious neuropathy

Infectious neuropathies include several diseases associated with infectious micro-organisms targeting the central (e.g. neurosyphilis, viral meningitis) or the peripheral (e.g. leprosy, HIV, Lyme disease, hepatitis C) nervous system. Here we provide examples of animal models of the two most common neuropathies induced by an infectious agent, leprosy and HIV-associated neuropathy. Leprosy is the most common sensory multiple mononeuropathy, still largely present in developing countries. Leprosy is caused by the non-toxic non-cytolytic *Mycobacterium leprae*, an obligate intracellular parasite. The proliferation of *M. leprae* in the mouse footpad (MFP) has been widely used to test bactericidal activity (Ji et al., 2006) and to define the role of immunity in leprosy after MFP infection in immune-deficient mice (Rambukkana et al., 2002; Fricker et al., 2008). However, the development of more representative models of human leprosy is still necessary to better account for a real leprosy neuropathy.

In HIV-associated neuropathy, peripheral sensory structures (DRG, peripheral nerves and cutaneous terminals) are affected. Distal sensory polyneuropathy (DSP) is the most common HIV-associated neuropathy and is present in one third of HIV-infected patients. Classical animal models rely on the postulated direct toxicity of secreted viral proteins (gp120 and Tat) on DRG and axons leading to degeneration and the development of DSP. Gp120 can be delivered acutely through an intradermal injection, or chronically, directly to the sciatic nerve, with a carrier matrix, thus inducing allodynia and hyperalgesia (Herzberg and Sagen, 2001). HIV infection may also heightens the vulnerability to antiretrovirus-induced neurotoxicity. Indeed, in transgenic mice continuously releasing gp120 from astrocytes and Schwann cells, food supplementation with the antiretroviral agent didanosine results in small unmyelinated fiber degeneration and hyperalgesia (Keswani et al., 2006). Similarly, the intravenous injection of a common protease inhibitor, indinavir, triggers hindpaw mechanical hypersensitivity in rats (Huang et al., 2017). Notably, DSP in these models is responsive to analgesic compounds, e.g. gabapentin.

## **5. Models of cancer pain**

Several mammalian and non-mammalian animal models have been developed to improve our understanding of cancer biology (Schachtschneider et al., 2017). However, cancer pain, which is experienced by human patients and animals in advanced stages of cancer, has only been addressed in some of these models.

Immunocompromised and immunocompetent rodents (mouse and rat) are commonly used to study cancer pain. Because of its large size, rat model is better suited for manipulations and injections into bones compared to mouse. However, mice are more widely used compared to rats mostly due to the possibility to address cancer pain in transgenic mice either in knock-out or -in overexpressing gene mice (Ghilardi et al., 2005a; Lindsay et al., 2005). Larger animals also used are dogs and less commonly cats as they develop spontaneous tumors such as mammary, prostate, head and neck squamous cell carcinoma (HNSCC),

squamous cell carcinoma (SCC). In dogs, cancer is the number one cause of mortality with osteosarcoma (OSA) being the most common primary bone tumor, and a painful one. Dogs and cats display similar cancer biology when compared with humans and are therefore appropriate models for human cancer (MacEwen, 1990; Simmons et al., 2015). Methods used to quantify cancer pain are various and dependent of the animal species used. In rodents, the von Frey assay remains most widely used although non-evoked pain tests are also applied (**Table 2**). Cancer pain in dogs is addressed mostly using owner-completed questionnaires and activity sensory function measure assays (Brown et al., 2015; Monteiro et al., 2018) (**Table 2**).

Bone cancer leads to the most painful conditions with patients suffering from chronic pain and breakthrough pain. Moreover, as bone is the major site of metastasis in advanced stage cancer, e.g. mammary and prostate cancers, it is expected that bone cancer induced pain (CIBP) models are the first cancer pain models to be developed. Metastatic bone cancer pain models were developed via intravenous, intra-cardiac (left ventricle of the heart), or orthotopic injections of cancer cells (Arguello et al., 1988; Yoneda et al., 1994; Liepe et al., 2005). However, assessment of bone induced pain cancer has been proven difficult in these models due to the differences in size, tissue/organ localization of the metastases. The first primary bone cancer pain model was developed in 1998 by injection of NCTC 2472 fibrosarcoma cells into mouse intramedullary space of long bone, i.e. femur (Schwei et al., 1999). The first rat model was developed a few years later by injection of MRMT1 mammary carcinoma into the intramedullary space of tibia (Medhurst et al., 2002). A model of breakthrough cancer pain defined as an intermittent episode of extreme pain has been recently developed (Tang et al., 2016). Non-bone cancer models were also developed using orthotopic injections (see **Table 2**). The differences between these different rodent models depend on the site of injection and the type of cancer cell injected (**Table 2**). Development of these diverse models allowed pharmacological interventions with preclinical positive outcome (reviewed in (Slosky et al., 2015)). Indeed, analgesic effects of resiniferatoxin, a TRPV1 activator, were demonstrated on intractable osteosarcoma suffering dogs and in phase I clinical trials on patients with intractable cancer (Brown et al., 2015). Moreover, the use of various animal models has validated the anti-NGF therapy for cancer pain management (Sevcik et al., 2005; Jimenez-Andrade et al., 2011). Tanezumab and Fulranumab, two NGF antibodies, safety and efficiency have been tested in phase I and phase II clinical trials (Sopata et al., 2015; Slatkin et al., 2016). A phase III trial using Tanezumab is currently in progress to determine whether Tanezumab is effective in the treatment of cancer pain due to bone metastasis in patients already taking background opioid therapy (see ClinicalTrials.gov).

The use of these different models has provided insight into the mechanisms involved in cancer pain (reviewed in (Falk and Dickenson, 2014)) and brought *in vivo* evidence of the implication of tumor secreting proteins, e.g. endothelin 1, (Wacnik et al., 2001; Tang et al., 2016)), microRNAs (Bali et al., 2013; Elramah et al., 2017), neurotrophins, receptors (e.g. osteoprotegerin, (Honore et al., 2002)), and TRPV1 (Ghilardi et al., 2005a; Sapio et al., 2018). In particular, the implication of the purinergic P2X receptor family and purinergic pathway in cancer pain has been shown using these rodent models (Gilchrist et al., 2005; Wu et al., 2012; Guedon et al., 2016; Falk et al., 2019).

## 6. Models of visceral pain

Visceral pain includes pain emanating from organs localized into the thoracic, pelvic and abdominal regions. This type of pain is poorly localized, often affecting two or more visceral organs. In particular,

gastrointestinal (GI) pain is common in various disorders including irritable bowel syndrome, inflammatory bowel diseases (IBD) (Crohn's disease and ulcerative colitis), pancreatitis, kidney stones, biliary disorders or associated with cancer. Several factors have been identified as contributing to visceral pain, such as stress, early life influence, microbiota, genetic predispositions, epigenetic changes and miRNA regulation ((Larauche et al., 2012; Zhang and Banerjee, 2015; Fuentes and Christianson, 2018; Lomax et al., 2019; Louwies et al., 2019; van Thiel et al., 2020). However, the mechanisms of visceral pain are complex and identifying its cause and the correct treatments for patient remains a challenge (Drewes et al., 2020). Numerous animal models, mostly rodent models, have been developed to mimic GI diseases (reviewed in (Larauche et al., 2012; Goyal et al., 2014; Greenwood-Van Meerveld and Johnson, 2017)). However, visceral pain has only been investigated in a small number of these available models (Schwartz and Gebhart, 2014). Some examples of naturally occurring or induced visceral pain are given in the **Table 3**.

Visceral pain associated diseases naturally occur in dogs, and pancreatitis is the most frequent visceral pain condition (Catanzaro et al., 2016). Pain is addressed by posture, movement, behavioral, physiological changes and reaction to abdominal palpation (Mansfield and Beths, 2015; Catanzaro et al., 2016).

Classically, visceral pain can be induced by hollow organ isobaric distension using latex balloon inserted into the esophagus or colon that is distended over a certain period of (Ness and Gebhart, 1988; Ozaki et al., 2002; O'Mahony et al., 2012). Colonic sensitivity is then measured by behavior analysis, e.g. abdominal withdrawal reflex (AWR) and/or monitoring abdominal muscles contraction (visceromotor responses, VMR). This method is mostly used in rodents but has been adapted to bigger animals, such as dogs or horses (Ness, 1999).

One widely used technique consists of inducing GI irritation, in order to copy IBD, by intra-colorectal injection or oral ingestion of irritant chemicals such as trinitrobenzene sulphonic acid, dextran sulfate sodium, dibutyltin dichloride (Vera-Portocarrero and Westlund, 2004; Lapointe et al., 2015; Zhu et al., 2018b; Hou et al., 2019). This technique derives from the writhing test developed in 1957. In this method, intraperitoneal injection of chemical irritant, such as acetic acid or phenylquinone, induced writhing responses and has been used as pharmaceutical screening tool (reviewed in (Schwartz and Gebhart, 2014)). However, its use needs to be reconsidered due to limitations and ethical issues. GI inflammation can also be induced by parasite or E.coli infection or lipopolysaccharide (LPS) injection (Quick et al., 2013; Nozu et al., 2018, 2019; Zhang et al., 2019).

More disease-specific models, such as experimental autoimmune pancreatitis induced by mice immunization with rat prostate extracts antigen, or kidney stones obtained by the injection of dental cement into rat ureter, have been generated (Giamberardino et al., 1995; Liu et al., 2019).

Animal models have also been developed to evaluate stress-induced visceral pain. They rely on inducing stress in adult rodents by repeated water avoidance stress, partial restraint stress or in early life of mice by neonatal maternal separation (Miquel et al., 2016; Nozu et al., 2018; Zhu et al., 2019).

In all of these disease models, visceral pain is then measured using abdominal withdrawal reflex, visceromotor responses, mustard oil test, von Frey assay or "up and down" method, or animal behavior analysis such as grimace face (see **Table 3**).

The use of different rodent strains is a plus to address the importance of individual's sensitivity to stress into the induction and maintenance of GI pain (O'Malley et al., 2014). Furthermore, these assays to induce visceral nociception/pain can be carried out in transgenic mice, enabling to highlight the implications of

factors, or signaling pathways, e.g. purinergic signaling pathway in the mechanisms of visceral pain and/or to address the effects of analgesics and probiotics/prebiotics on visceral pain (Trimble et al., 2007; Ochoa-Cortes et al., 2014; Pusceddu and Gareau, 2018).

Visceral pain can also be felt in other somatic regions than the affected one, therefore it is called referred visceral pain (Giamberardino et al., 2010). For example left shoulder, jaw and neck pain are typically observed in case of a cardiac ischemia. The physiopathology underlying referred visceral pain lies in the presence of viscerosomatic convergence at spinal cord level. In fact the dorsal horn of the spinal cord receives a visceral input that converges with a somatic input leading to the sensation of pain (Gebhart and Bielefeldt, 2016). Referred visceral pain is first felt within the deep tissue walls but with persistent stimulation and secondary hyperalgesia the pain is extended to superficial layers (Sikandar and Dickenson, 2012). Laird et al developed an animal model for referred visceral pain in mice, it consists of an intracolonic capsaicin instillation (ICI). In fact 50  $\mu$ l of capsaicin were injected in the colon via a cannula inserted in the anus. Von frey filaments were applied to the abdomen before the instillation and 20 minutes after, and the difference in the frequency to withdrawal was tested (Laird et al., 2001b). ICI was also adapted for rats, in this case 200  $\mu$ l of capsaicin were injected (Sanoja et al., 2010).

## 7. Models of musculoskeletal pain

Similarly to cancer pain or visceral pain, muscle pain results in a diffuse, aching pain that contrasts with sharp and localized cutaneous pain. Models specific to studying muscle pain are scarce in the literature although muscle pain is a major clinical problem. Indeed, musculoskeletal pain is a chronic widespread pain manifestation that affect between one quarter and one third of the US population (Gaskin and Richard, 2012). In chronic conditions like lowback pain, myositis, myofascial pain, and fibromyalgia, muscle pain is one of the major clinical manifestations. Therefore, animal models obtained after muscle insult more reliably replicate many clinical pain syndromes when compared to models of cutaneous pain (Lesnak and Sluka, 2019).

Models of chronic muscle pain include inflammatory and non-inflammatory components. Musculoskeletal hyperalgesia is classically generated by intramuscular injection of either carrageenan (inflammatory) or acidic saline (noninflammatory) (Sluka et al., 2001; Radhakrishnan et al., 2003). The inflammatory pain models mimic a wide range of painful muscular disorders with an inflammatory component such as myositis and tendonitis. The pain behaviors are sensitive to anti-inflammatory drugs (Kehl et al., 2000). The inflammatory model of musculoskeletal pain in rodents is obtained by carrageenan injection in the gastrocnemius muscle and produces hyperalgesia to heat stimuli (Chopade et al., 2014). This carrageenan-induced pain model has been suggested to have greater face validity to pain of musculoskeletal origin in humans [4].

The non-inflammatory model mimics painful muscle disorders such as fibromyalgia, where inflammation is not present. This model is not sensitive to anti-inflammatory agents (Radhakrishnan et al., 2003). The most common and well-characterized models of fibromyalgia-like pain involve repeated insults to the muscle. A non-inflammatory pain model is induced by repeated injections of acid saline (pH 4.0) into the same gastrocnemius muscle. It results in widespread hyperalgesia of the skin, muscle and viscera without observable tissue damage or inflammation (Sluka and Clauw, 2016).

In both inflammatory and non-inflammatory animal models, the findings nicely corroborate clinical observations and contributed to demonstrate that chronic widespread pain is multifactorial. Peripheral alterations are partly responsible of the symptoms through the involvement of the immune system or impaired nociceptor sensitivity, in particular in the induction of hyperalgesia. However, emerging evidence suggest that the central nervous system maintains the hyperalgesia. In inflammatory musculoskeletal pain, recent observations indicate the importance of a spinal COX-2 mechanism, and the spinal site of action of systemically delivered drugs that alleviate hyperalgesia (Chopade et al., 2014). Central excitatory mechanisms have also been implicated in the pathophysiology of fibromyalgia (Sluka and Clauw, 2016). In addition, the inhibitory effects of central pain modulation are decreased in human patients with fibromyalgia and animal model studies point to reduced serotonergic transmission in the brain stem that projects to the spinal dorsal horn (Bobinski et al., 2015).

### **8. The particular case of non-human primates**

Although rodents are mammals, their behavior and the organization of their nervous system remains different from humans. In contrast, studying pain in non-human primates offers the opportunity to investigate mechanisms, and response to analgesic treatment, that are close to what is expected in humans. However, using non-human primates undoubtedly poses acute ethical problems and little exploration of pain-related mechanisms has been done in non-human primates as compared to rodent.

Few attempts have been made to develop NP models in non-human primates. The most classical approach was the ligation of the L7 spinal nerve in rhesus monkeys. At 14 days post lesion, this resulted in the alteration in the activity of spinothalamic tract neurons that displayed increased activity in response to mechanical and thermal stimuli (Palecek et al., 1992). Accordingly, the animals exhibited behavioral responses consistent with mechanical and thermal allodynia (Carlton et al., 1994). Recently, *Macaca fascicularis* has been used to establish a model of mild injury of the sciatic nerve. This does not involve nerve transection, so that nerve transduction can still take place. Under these conditions, behavioral and physiological outcomes model the chronic and long-term nature of sciatic nerve neuropathy that is observed in clinics more closely than in classical rodent models (Guo and Gu, 2014). Sciatic nerve retained the ability of nerve signal transduction, and showed a firing rate profile consistent with injury induced-sensitization of sensory fibres. This model may serve as a non-human primate model to study functional changes of traumatic injury to the sciatic nerve. In a macaque model of chemotherapy-induced neuropathic pain, functional magnetic resonance imaging (fMRI) reported abnormal activation of pain-related brain regions including insular and secondary somatosensory cortices, suggesting that fMRI measurement combined with behavioral outcomes in macaque models could be used to test therapeutic treatments for neuropathic pain and document their modes of action (Nagasaka et al., 2020).

Interestingly, a procedure similar to the rodent CCI model failed to induce symptoms of neuropathic pain in non-human primates (Palecek et al., 1992), likely because of the thicker protective sheath of the primate spinal nerve that prevented rodent-like pathology to develop. This example highlights that specific pain-inducing procedures must be adapted for every species studied.

The use of large animal models is more common to study acute or chronic inflammatory pain (Henze and Urban, 2010). In non-human primates, the formalin test has been used as a tonic pain model with good objectivity, validity, reproducibility and quantification. Moreover, this model exhibits good predictive validity by recapitulating the analgesic effects of morphine and pethidine (Alreja et al., 1984). Similarly,

topical application of capsaicin proved to be efficient in inducing transient hyperalgesia and can serve as a model to test analgesic treatments in primates (Kupers et al., 1997). Spontaneous osteoarthritis exists in rhesus monkeys but despite a good face validity, it has never been used to test effectiveness of analgesics, perhaps because it would require preliminary reliable methods to assess pain in these free-range animals (Henze and Urban, 2010). Another painful affection that develops spontaneously in non-human primates is endometriosis (Braundmeier and Fazleabas, 2009). Such spontaneous endometriosis in *cynomolgus* monkeys displays similar clinic-pathological characteristics to the human disease and was useful as an experimental model (Nishimoto-Kakiuchi et al., 2018). *Cynomolgus* monkeys have similar lesions to those in humans as revealed with MRI studies (Nishimoto-Kakiuchi et al., 2018). The implications of these findings are that well established conventional laboratory methods and parameters for assessment can be applied for evaluating disease progress and drug efficacy in this model.

### **9. Pain models in simple organisms**

To reduce the use of rodents in pain studies and carry out high-throughput screening studies, new models have started to appear in pain research, using lower vertebrate species such as zebrafish or *Xenopus* and invertebrates such as *Drosophila* and *Caenorhabditis elegans* (*C. elegans*).

The shared advantages of these models reside in their simplicity of use including low cost and easy maintenance, large brood and egg size and rapid external development. They are genetically similar to humans and genome manipulation (random or directed mutagenesis, transgenesis, Crisp/Cas9...) or gene expression alteration (knock-down or over-expression) can be easily and quickly carried out (St Johnston, 2013; Sassen and Köster, 2015; Chen et al., 2016; Tandon et al., 2017). Genetic, chemical and behavioral screening can also be performed in multiwell-format screening (Jorgensen and Mango, 2002; Wheeler and Brändli, 2009; Giacomotto and Ségalat, 2010; Venken and Bellen, 2014). These diverse and efficient technologies together with online resources make them powerful model to human neuropathologies (Jeibmann and Paulus, 2009; Bessa et al., 2013; Pratt and Khakhalin, 2013; Fontana et al., 2018; de Abreu et al., 2019; Vaz et al., 2019; Markaki and Tavernarakis, 2020). Furthermore, the lower level of ethical constraints for *Drosophila* and *C. elegans* and zebrafish and *Xenopus* embryos and larvae under European and USA legislation places them as a replacement approach in animal experimentation and alternative promising models to study nocifensive reactions, in respect of the 3R principles (Sneddon et al., 2017).

There are numerous debates regarding the capacities of non-mammals to experience pain (Sneddon et al., 2014; Brown, 2015; Key, 2015; Sneddon, 2018, 2019; Williams et al., 2019). Indeed these species are quite distant phylogenetically from humans and their nervous system is indeed not organized as in mammals (White et al., 1986; Rein et al., 2002; Pratt and Khakhalin, 2013; Lee-Liu et al., 2017; Zheng et al., 2018; Vaz et al., 2019). The simplicity of nervous system and the lack of neocortex in zebrafish and somatocortex in *Xenopus* is one of the main arguments for no possible pain experience in these species. However, it has been established that nociceptive pathways in these four animals are similar to mammalian ones (Tobin and Bargmann, 2004; Kahn-Kirby and Bargmann, 2006; Smith and Lewin, 2009; Stevens, 2011, 2015; Khuong and Neely, 2013; Demin et al., 2018b, 2018a; Sneddon, 2018; Walters, 2018). This includes (1) nociceptors with morphological and functional resemblance to vertebrate ones, (2) ion channels which, based on their sequence and structure homology and functional assays, are orthologous to the mammalian TRP channels, (3) opioid system and (4) neurotransmitters of primary afferents (Table 4). These four species

are capable of sensing mechanical, chemical and thermal noxious stimuli although only amphibians can sense cold stimuli (Sneddon, 2018).

Stereotypical behavioral responses to a noxious stimulus have been well described in these **simple organisms** and helped to develop numerous assays to study thermal, mechanical and chemical nociception and also neuropathic and chronic pain models (**Table 5**). In *Drosophila*, the classical response of larvae to heat or mechanical noxious stimuli is the rolling (corkscrew-like) response (Milinkeviciute et al., 2012). *C.elegans* respond to a heat stimulus with the stereotypical escape behavior called Tav (thermal avoidance), which includes a stop movement followed by reversal, reposition and a forward movement to another new direction (Wittenburg and Baumeister, 1999). However, new heat avoidance assays rely on other behavior responses such as crossing a thermal barrier or distance travelled on heated assay plate (Glauser et al., 2011; Nkambeu et al., 2019). *Xenopus* nociceptive response to the application of acetic acid test (AAT) drops of dilute acetic drops (5%) to the hindlimb, a pain model developed by Pezalla in 1983 (Pezalla, 1983), is called “wipping response”. In zebrafish, nociception has been assessed by changes in behavior, such as reduction of activity, swimming distance and space use, physiological changes (opercular beat rate) or more recently by abdominal constriction like response (Costa et al., 2019; Sneddon, 2019). Some of these assays such as the hotplate or von Frey test, successfully implemented in *Drosophila* larvae and adults, are similar to the mammalian ones (Milinkeviciute et al., 2012). These nociception like assays based on behavioral responses coupled to the ease of genomic manipulation allowed the discovery of new molecular mechanisms involved in nociception and pain. As an example, painless, a TRP channel related to the TRPA1 channel subfamily, was identified in the first nociception study through a forward genetic screen for *Drosophila* mutants defective in heat stimulus response (Tracey et al., 2003).

It is now admitted that these four **animals can be used as alternatives** to mammalian models to study nocifensive behaviors. However, it remains unclear if they can be used to improve our understanding of pain integration. Pain models, such as neuropathic pain, have been developed in *Drosophila* and zebrafish (Malafoglia et al., 2014; Khuong et al., 2019) (**Table 5**). The ATT test, performed in *Xenopus* and zebrafish have been considered as a pain model, mimicking the rodent visceral pain protocol when injected intraperitoneally (Costa et al., 2019). These animals have also been used in analgesia and anaesthesia research and it has been proven that negative effects of noxious stimulus are blocked by the use of anaesthetics (Morgan et al., 2007; Stevens, 2011; Lopez-Luna et al., 2017). To access pain events is complicated in these four **organisms**, but it has shown they display cognitive processes such as memory, learning, active avoidance learning, punishment learning, pain-relief learning and if trained, opioids self-administration (Millhr et al., 1974; Ardiel and Rankin, 2010; Gerber et al., 2014; Rothman et al., 2016; Bossé and Peterson, 2017; Yang et al., 2018).

The large number of *Drosophila*, *C.elegans* and zebrafish and *Xenopus* larvae obtained from one mating combined to the absence of ethical issues for these individuals made them very attractive for large genetic and chemical screenings. Teratogenicity assays in zebrafish and *Xenopus* embryos and larvae have been used to address toxicity of pain drugs and herbal remedies and could be powerful tests for future analgesics and anaesthetics development (Chae et al., 2015; Jayasinghe and Jayawardena, 2019). High-throughput screenings have already been implemented with the aim to identify new pain drugs (Ellis et al., 2018). *Xenopus* wild type or overexpressing human proteins related to chronic pain oocytes are frequently used to screen libraries of compounds and test their specificity using electrophysiology or voltage-clamp fluorometry (Zeng et al., 2020). In particular, inhibitors of the P2X7 purinergic receptor or an ion channel involved in chemotherapy induced peripheral neuropathy have been identified using *Xenopus* oocytes

(Zeng et al., 2020). *Straightjacket*, member of the voltage-gated calcium channel family involved in acute and chronic pain in humans, and the thermoreceptor TRPA1 were identified among 580 new pain genes in a 10,000 knockdown adult flies screen (Neely et al., 2010, 2011). Recently, a forward behavioral genetic screen allowed testing more than 600,000 transgenic tgMOR *C.elegans* mutants and identifying a novel and conserved orphan-opioid system (Wang et al., 2019). The recent development of novel behavioral assays with large numbers of animals, automated behavioral tool (such as the Fish Behavior Index (FBI)), tracking software (AnyMaze®) and quantitative models should become very useful tools for assessing nociceptive responses high-throughput screening (Curtright et al., 2015; Leung et al., 2016; Taylor et al., 2017; Deakin et al., 2019). Indeed, the Multi-Worm Tracker software (Swierczek et al., 2011) was used to record the animal movement and identify *C.elegans* mutants displaying behavioral changes to opioids (Wang et al., 2019). Finally, habituation alterations have been successfully tested in these high-throughput models for neurodevelopmental disorders (Kepler et al., 2020). Such assay could be a promising approach to provide insight into nociception and pain.

The easiness of these four **organisms**, the ethical issues and the development of recent automated methodologies have contributed to the increase of studies on nociception and pain research. Although these animals feel nociception and can be used to test behavioral responses to thermal, chemical and mechanical noxious stimuli with a great potential for pharmacological research, for molecular mechanisms understanding and for the 3R respect, there is some limitations to their use. One of these limitations is their phylogenetic distance to humans and therefore to some molecular and cellular mechanisms differences. For example, the P2X3 receptor involved in pain is not found in *Xenopus* genome (Burnstock, 2016; Bernier et al., 2018; Blanchard et al., 2019). The main limitation resides **in the difficulty to assess pain perception, if any**. However, the use of these models, especially in high-throughput screening, could be the first *in vivo* step before any experimentation on rodents or other mammals.

## **10. Limitations of animal models of pain**

The study of pain always relied on preclinical animal models that attempted to explore the complex physiological and sensory implications of the condition. Various animal models of chronic pain aim to emulate different types of pain and they have been instrumental in the discovery and development of analgesic agents. While there is no doubt about the necessity of such models in chronic pain research, there are several limitations to their use. This is especially true when considering their failure to facilitate the translation of basic science data into effective and commercially available therapies. This is supported by the fact that there is a high rate of translational failures associated with preclinical animal models (Le Bars et al., 2001; Negus et al., 2006). One well-known failure is that of the neurokinin-1 receptor antagonists (substance P) (Hill, 2000). Several reasons have been suggested for these failures (Mogil, 2009). This debate focuses partly on the utility of animal models of pain and behavioral measures for screening new potential analgesics. One concern is the reliance of studies on reflexive measures, and it has been suggested that additional measures of supraspinal integration that use non-reflexive **pain-like behaviors** should be included, such as operant learning measures, spontaneous nocifensive behaviors and quality of life or physical activity measures. Another concern is the use of animal models of disease that do not reflect the clinical condition the experimenter is trying to model, such as using inflammatory pain in animals to study chronic low back pain. **Finally, the insufficiency of the knowledge on fundamental neuronal mechanisms must be also emphasized. In particular, recent studies pointed to the importance of NK1 receptor signaling along the endocytotic pathway, and not only at the plasma membrane, in the**

transduction of nociceptive information (Jensen et al., 2017). Therefore, classical NK1 antagonists that target membrane NK1 may not be able to prevent atypical intracellular NK1 signaling.

Most molecules that successfully demonstrated analgesic effects in preclinical studies fail to produce similar effects when tested on a clinical level. One of the reasons this might occur relies on the genetic and molecular neurochemical differences between humans and animals. This makes it difficult to predict the pharmacological effect of a molecule on the clinical level. In contrast, after clinical validation, most approved drugs have proven good efficiency in reverse translational studies in animals. The difficult translatability of pain across species and transfer to clinical settings could also be due to non-optimal translational research conditions and not directly related to the failure of the model to simulate human pain mechanisms (Le Bars et al., 2001; Henze and Urban, 2010). In fact, failure in translational approaches can be due to the presence of severe side effects that appear in clinics but are not detected in preclinical studies (Mogil, 2009).

Another major cause is likely to be the heterogeneous nature of human chronic pain which can have a multitude of aetiologies. This heterogeneity clashes with the controlled environment often used in animal studies that might interfere with the translation of the research to a human population. In particular, a vast majority of animal studies utilize inbred animal. While some injury models are available, most studies only use sensory-evoked thresholds (e.g., von Frey filaments) in order to assess nociceptive sensitivity; whereas the evaluation of ongoing pain based on non-evoked stimulus (such as the grimace scale, weight bearing, CPP...) may be clinically more relevant (Negus et al., 2006; Deuis et al., 2017b). Other challenges researchers are facing include the replication of the transitional process of pain from acute to chronic in animal models. The complexity of pain mechanisms and differences between pain types (i.e. neuropathic vs. inflammatory) indicates a lack of a mechanistic delineation between conditions (Le Bars et al., 2001; Ferrari et al., 2015).

In addition, several demographic factors are implicated in pain mechanisms in humans including age. Pain mechanisms are often considered independent from age in animals, which lead to the predominant use of young mice in preclinical studies. This is questioned by the increasing evidence of the involvement of an age-dependent mechanism (Mogil, 2009). Sexual dimorphism is another demographic factor that might lead to the misrepresentation of chronic pain in animal models. This is apparent through the overuse of male subjects in studies which is a clear contrast to the high prevalence of chronic pain in females. The underutilization of female subjects in preclinical trials might provide an explanation to the lack of effective analgesic therapies for the human global population (Le Bars et al., 2001; Negus et al., 2006; Henze and Urban, 2010). Pain perception differs between female and male; with female perceiving more pain perhaps due to high levels of estrogen and progesterone, and low testosterone levels. In fact, pain perception is modulated by the immune system that is under the control of gonadal hormones (Mogil, 2009). This difference has been highlighted in a study that indicated only male mice developed acute thermal hypersensitivity when tested in an environment linked to a painful memory (Martin et al., 2019). Furthermore it has been showed that T-cell deficient female mice needed 2-3 times the morphine dose administered to male mice (Rosen et al., 2019). Another example pointed that the hypersensitivity induced by nerve injury is different between male and female mice since the spinal mechanisms differ between the two genders (Mapplebeck et al., 2018).

Interstrain animal genotypic variabilities can also account for the failure of human chronic pain modelling in animals (Le Bars et al., 2001; Negus et al., 2006; Henze and Urban, 2010). The example of the gene encoding sodium channel Nav1.7 is illustrative. This gene is present on adrenal and pituitary gland of

rodents, and its mutation is lethal. However, the situation is different in primates where this gene mutation leads to pain insensitivity (Mogil, 2009). **Notably, recent studies pointed that Nav1.7 may not be critical for survival even in rodents, but appears as an key molecular component for transmitting high-threshold noxious stimuli** (Gingras et al., 2014). There is also genetic variability among rodents that is also to blame for clinical failure. In fact, mice show an increase of morphine analgesia when **isolated in a cage**, whereas it decreases with rats. Furthermore chronic pain was shown to modulate 43 genes in rats versus only 10 in mice (Mogil, 2009).

## **11. Translational lessons learned from animal model studies**

Translation is defined by the National center for advancing translation science as “the process of turning observations in the laboratory, clinic, and community into interventions that improve the health of individuals and populations – from diagnostics and therapeutics to medical procedures and behavioral interventions” (Nakao, 2019). It is worth noting that despite the aforementioned limitations, a growing number of examples demonstrate the potential to convert new agents into widely used analgesics through pre-clinical testing in animal models. The following will consider such examples for treating neuropathic or inflammatory pain, as well as low back pain and post-operative pain.

### ***Targeting purinergic signalling***

ATP induces acute pain when applied to human skin (Bleehen and Keele, 1977; Hamilton et al., 2000) where it activates ATP-gated channels (P2X receptors), mainly P2X3 receptors that are almost exclusively expressed in nociceptive sensory neurons. P2X3 and heteromeric P2X2/3 play a key role in pain sensing and in various sensory transduction such as taste and visceral afferent sensation. Several P2X receptors expressed in neurons and/or microglia, including P2X3 and P2X4 or P2X7, are also involved in the chronic nociceptive behavior that follow nerve injury or inflammation indicating that P2X receptors represent therapeutic targets for pain syndrome (Khakh and North, 2006).

Nerve injury mouse models recently allowed the understanding of a spinal mechanism in which the purinergic receptor P2X4 expressed in microglia plays a critical role specifically in neuropathic pain but not in acute or inflammatory pain. Using a spinal nerve ligation rat model of neuropathic pain, a pioneered work showed that the increase of P2X4 expression specifically in reactive microglia within the dorsal horn of the spinal cord is determinant in tactile allodynia (Tsuda et al., 2003). The authors also showed that the pharmacological blockade of P2X4 or the expression knock-down using siRNA dramatically reduced pain-like behavior indicating that P2X4 activation is required to maintain pain hypersensitivity. The key role of P2X4 was confirmed in P2X4 knockout mice (P2X4KO) in which tactile allodynia after peripheral nerve ligation was drastically reduced (Ulmann et al., 2008). Several studies established that activation of microglial P2X4 by ambient ATP increases nociceptive signaling within the spinal cord by reducing inhibitory currents within the spinal cord. This neuronal disinhibition results from a rise in the intracellular concentration of chloride ions that suppresses GABA or Glycine mediated-inhibitory responses. This phenomenon may eventually convert inhibition into excitation in a subset of neurons, sufficient to induce hypersensitivity of spinal neurons and tactile allodynia (Coull et al., 2005; Trang et al., 2009). These changes in chloride concentration are triggered by the potassium-chloride co-transporter (KCC2) downregulation, which is mediated by the extracellular release of BDNF and the activation of Trk-B receptor located at the surface of inhibitory neurons. The neuronal hyper-excitability mediated by microglial P2X4 signaling may account for the main symptoms of neuropathic pain suggesting that the blockade of P2X4 expression or function alleviates this debilitating condition in human. Although no commercially available P2X4-derived pain killer exists, the therapeutic potential of targeting this system is well recognized, and current research efforts are made for P2X4 drug development. TNP-ATP or 5-BDBD are P2X4 antagonists that have been commonly used to show the involvement of P2X4 in rodent nervous tissue but they are not very selective (Suurväli et al., 2017). New antagonists of rodent P2X4 (BAY-1797 or NP-1815-PX) and a highly selective human P2X4 antagonist (BX430) with no effect on rodent P2X4 were recently identified (Ase et al., 2015; Matsumura et al.,

2016). Kyushu University and Nippon Chemiphar Co., Ltd. initiated a phase 1 clinical trial of the NC2600 P2X4 antagonist but no data is available since then (Bhattacharya, 2016).

An alternative to P2X4 blockade could be the enhancement of KCC2. Actually, chloride extrusion enhancers have been proposed as novel therapeutics for pain alleviation (Gagnon et al., 2013). Chloride enhancers could also be used in synergy with other pharmacological agents. Indeed, a recent study pointed to the benefit of rescuing chloride homeostasis with the KCC2-enhancer CLP25728 on the analgesic action of the benzodiazepine site ligand L838,417. This combination produced an efficient analgesia even with high doses of benzodiazepine, whereas they remained otherwise ineffective when administered alone (Lorenzo et al., 2020).

P2X7 is expressed in most of the immune cells and regulate the inflammation, thus representing a valuable therapeutic target for many diseases involving inflammation, including persistent pain (Burnstock and Knight, 2018). P2X7 antagonists such as AZ106006120 or nanobodies that are small single chain antibodies blocking or modulating P2X7 functions have been proposed as drug candidates for inflammatory disorders (Danquah et al., 2016; Mishra et al., 2016). P2X7 knockout mice showed reduced behavioral responses to inflammation or tactile allodynia (Chessell et al., 2005). These effects seem to be mediated by the decrease of the pro-inflammatory interleukin-1 release from microglia. P2X7 is abundantly expressed in reactive microglia after CNS injury, and neuropathic pain is reduced by P2X7 antagonist such as A-740003 and A-438079. These data suggest that targeting microglial P2X7 may be useful for the treatment of chronic pain (Tsuda, 2017).

Antagonist selective of P2X3 or P2X2/3 receptors have also been shown to have anti-inflammatory and anti-nociceptive effects in different models of neuropathy and peripheral inflammation when expressed in sensory neurons (Jarvis et al., 2002; Kuan and Shyu, 2016). Mechanical allodynia in animal models was also reduced in mouse with deletion of P2X3 gene (Cockayne et al., 2000; Souslova et al., 2000). In addition, P2X3 knockout mice exhibited a marked urinary bladder hyporeflexia and it has been showed that ATP released by the distension of the urothelium activates P2X3 expression in afferent nerves. A similar link have been established for the taste buds, the wall intestine and reflexes such as cough, indicating that P2X3 and/or P2X2/3 receptors have a more widespread role in sensory transduction. Therefore, they represent attractive targets for drugs, and not only for pain. Among the numerous selective antagonists of P2X3 discovered, some of them have been studied in clinical trials for pain-related disorders but also for other diseases such as overactive bladder, irritable bowel syndrome, or chronic cough. Gefapixant (MK-7264) significantly reduced cough frequency in patients with refractory chronic cough or unexplained chronic cough (Smith et al., 2020) and is one of the most promising P2X3 antagonists in phase 3 clinical trials for the treatment of chronic cough.

### **Targeting 5-HT receptors**

Lumbar disc herniation is a well known aetiology of chronic lower back pain. A herniated disc induces pain by a mechanical deformation (in addition to a biochemical irritation) to the dorsal root ganglion and the nerve root. Many substances such as proinflammatory cytokines and monoamines play a role in the biochemical irritation of the nerve. Serotonin (5-HT) is one of the monoamine participating in pain modulation mainly *via* 5-HT<sub>2A</sub> receptors. Nowadays, scientists are using animal models to understand the complexity of chronic pain pathways, aiming to project these hypothesis and find a curative treatment for chronic pain, and especially low back pain in humans (Kato et al., 2015).

In their paper, Hashizume H et al. evaluated the effect of a 5-HT<sub>2A</sub> receptor antagonist (sarpogrelate) on lumbar disc herniation pain (Hashizume et al., 2007). They performed a study on rats that underwent surgery to place an autologous nucleus pulposus at the 4<sup>th</sup> and 5<sup>th</sup> left lumbar nerve roots in order to mimic lumbar disc herniation pain. After studying pain related behaviors, researchers found that sarpogrelate treatment considerably reduced mechanical allodynia at 5 and 8 days after drug administration (Hashizume et al., 2007). In a related experiment, the same surgical model of rats was used to study the effect of a similar 5-HT<sub>2A</sub> receptor antagonist (sarpogrelate hydrochloride) on pain related behavior, and on the expression of 5-HT<sub>2A</sub> receptors in dorsal root ganglia. The results confirmed that sarpogrelate used at high doses (10 mg/kg) dramatically reduced mechanical withdrawal threshold evaluated by von Frey tests. In addition to decreasing nociceptive transmission, researchers confirmed that this 5-HT<sub>2A</sub> antagonist down-regulates 5-HT<sub>2A</sub> receptor expression in dorsal root ganglia (Kato et al., 2015). Furthermore, the effect of the serotonin noradrenaline reuptake inhibitor (SNRI) duloxetine was evaluated on radiculopathy in the same rat model of lumbar disc herniation pain (Handa et al., 2016). Rats received duloxetine at different doses (low dose 0.4 mg/kg, high dose 1.2 mg/kg). Results confirmed that duloxetine had a positive impact on neuropathic pain in rats probably by down regulating the expression of tumor necrosis factor alpha and nerve growth factor and microglia activation (Handa et al., 2016).

These preclinical data obtained on animal models drove clinical trials in order to demonstrate SNRI drug efficacy on chronic back pain in humans. A recent meta-analysis reported the results of 3 randomized double blind clinical studies of patients taking duloxetine or placebo for chronic low back pain (Enomoto et al., 2017). Duloxetine-treated patients (n=400) and placebos (n=451) were considered and the statistical analysis revealed that duloxetine considerably alleviated pain. Duloxetine-induced analgesic effect was mainly modulated by interaction with the pain pathway rather than with the anti-depressive effect of the drug, indicating that duloxetine primarily targeted pain-specific mechanisms and not comorbid disorders (Enomoto et al., 2017). The effectiveness and safety of duloxetine monotherapy was assessed in a Japanese population suffering from chronic low back pain. Duloxetine (60 mg daily) was effective in pain management. Moreover, duloxetine was well tolerated with mild side effects that may resolve and improve with the course of the treatment (Konno et al., 2016). These results were confirmed by independent studies with randomized, double-blind trials on chronic low back pain patients (Skljarevski et al., 2010a, 2010b).

Taken together, these studies have led to the recommendation of duloxetine in the management of chronic low back pain as a second line therapy by the American college of physicians in 2017 (Qaseem et al., 2017).

In addition, a novel dual antagonist of the GlyT2 glycine transporter and 5-HT<sub>2A</sub> receptor (VVZ-149) is being tested in healthy volunteers for future applications in the management of post-operative pain (Oh et al., 2018). In case of success, the therapeutic indication for this drug may be extended beyond post-operative pain to other pain pathologies.

### **Targeting TRPV1 receptors**

Capsaicin is a TRPV1 receptor agonist (see §2.) with anaesthetic effects that is approved as a pain killer and proved to be efficient in various types of pain. **Although the analgesic properties of capsaicin were empirically known for long, animal model studies allowed to determine its mechanisms of action.** Early

evidence, back in the 1980s, indicated that capsaicin treatment causes an alteration of the synthesis and storage of substance P in the dorsal horn and primary afferent neurons, thus affecting nociceptive transmission (Gamse et al., 1980; Lembeck and Donnerer, 1981). Subsequently, capsaicin-mediated thermal anti-nociceptive effects were demonstrated, and the suggested mechanisms pointed to a possible suppression of axoplasmic impulses in sensory neurons (Yaksh et al., 1979; Buck et al., 1981; Gamse et al., 1982). More recent studies revealed that capsaicin alleviates pain in various animal models including post-surgical pain (Hamalainen et al., 2009) and bone cancer pain (Ghilardi et al., 2005b).

Based on animal model studies, the use of topical capsaicin for the management of human chronic pain became increasingly popular (Wagner et al., 2013; Blair, 2018). The effect of an 8% capsaicin patch were assessed on peripheral neuropathic pain in comparison with oral Pregabalin (Haanpää et al., 2016). Capsaicin treatment contributed to remarkable pain alleviation at 8 weeks in comparison with optimal Pregabalin doses. Patches are associated with less systemic side effect, rapid action and greater treatment satisfaction (Haanpää et al., 2016). Another study also indicated the superiority of 8% capsaicin patches over Pregabalin on mechanical allodynia in patients suffering from peripheral neuropathic pain (Cruccu et al., 2018). These findings were confirmed by a network meta-analysis of 25 randomized controlled trials (van Nooten et al., 2017). Consequently, Capsaicin patch is indicated in treating neuropathic and skeletomuscular pain (Chang and Quick, 2020). In addition, a recent paper supported the use of capsaicin patches to attenuate chemotherapy-induced peripheral neuropathy that may occur in cancer patients (Anand et al., 2019).

Prolonged application of capsaicin results in the desensitization of TRPV1 channel. As a consequence, the nociceptive afferent becomes functionally silent (Zhang and Li Wan Po, 1994; Lambert, 2009). The efficacy of capsaicin-induced desensitization in treating pain pushed pharmaceutical companies to develop TRPV1 antagonists (Dray, 2008). Moreover, TRPV1 was shown to play a role in neural sensitization (Ramsey et al., 2006), and preclinical studies suggested TRPV1 antagonists may exhibit analgesic effects in inflammatory conditions (García-Martinez et al., 2002). These findings paved the way for clinical research, and at least seven orally active TRPV1 antagonist substances were under development in 2009, with many more in preclinical development (Gunthorpe and Chizh, 2009). In the same period, phase II clinical trials were performed for compounds such as GRC 6211 (Lilly/Glenmark), NDG6243 (Merck/Neurogen) and AZD1386 (AstraZeneca). However, to date, no TRPV1 antagonists are available for the clinician.

The main reasons for this failure relies in the lack of precise and rigorous preclinical evidence for using TRPV1 antagonists in pain treatment. Hence, it is necessary to optimize preclinical pharmacology to define precisely pain profiles sensitive to the compounds tested. Alternatively, refinements in preclinical studies may avoid making overenthusiastic assumptions, and help in a timely manner not to pursue clinical developments, thus saving money and resources for the pharmaceutical industry (M Keppel Hesselink, 2016).

### **Targeting Nerve Growth Factor**

Evidence for a contribution of locally produced nerve growth factor (NGF) to joint pathology, as well as pain in arthritic joints has emerged from animal model studies. A single intraarticular (IA) injection of NGF into normal rat knees produced a direct sensitisation of nociceptors through dose-dependent, long-lasting increases in pain-like behavior, joint swelling and synovial macrophage infiltration (Ashraf et al.,

2014). Other work provided evidence that NGF is released by damaged cells in arthritic joints, and elevated NGF is detected in synovium of murine OA model (Kc et al., 2016). Elevated levels of NGF has been also found in synovial fluid in dogs with naturally occurring OA (Isola et al., 2011). Administration of an anti-NGF/TrkA signalling molecule significantly decreased pain-like behaviors in a murine model of OA (McNamee et al., 2010). In particular, anti-NGF monoclonal antibody (mAb) produced a robust analgesia that is equal to or greater than current analgesics in animal models (Xu et al., 2016; Enomoto et al., 2019).

In view of experimental preclinical findings on the role of NGF in pain, clinical trials have been initiated to demonstrate the effectiveness of the strategy targeting the NGF system (Takasusuki and Yaksh, 2011; Bannwarth and Kostine, 2014, 2017). Anti-NGF mAbs are in development as treatments for several pain conditions and three NGF-blocking drugs have been developed: tanezumab (humanised mAb; Pfizer, in collaboration with Eli Lilly), fulranumab (fully human mAb; Amgen) and fasinumab (fully human mAb; Regeneron Pharmaceuticals, in collaboration with Sanofi). The first attempts to develop clinical trials aimed at evaluating the efficacy of anti NGF antibodies on osteoarthritis pain (Ishikawa et al., 2015; Miyagi et al., 2017). Tanezumab prevents NGF binding to its TrkA receptor resulting in pain alleviation. Although many studies emphasized Tanezumab positive effects against osteoarthritis pain, Tanezumab exhibits also serious adverse effects such as arthralgia, paraesthesias and osteonecrosis that question Tanezumab approval to treat chronic pain conditions in humans (Schnitzer et al., 2015; Walicke et al., 2018; Webb et al., 2018). After being put on a clinical hold between 2010 and 2012, trials for the development of anti-NGF mAbs in human restarted in 2015, and Tanezumab received fast track designation by the Food and Drug Administration in 2017. Tanezumab is currently in phase III studies in patients with OA of the hip and knee, chronic lower back pain, and bone cancer pain trials (Enomoto et al., 2019). Other companies are developing TrkA antagonist (Sanofi, GZ389988; Glenmark Pharmaceuticals Ltd., GBR 900). Comparisons with anti-NGF antibodies in animal models of inflammatory pain demonstrated that GBR 900's efficacy is similar as that of anti-NGF antibodies

Since OA naturally occurs in cats, dogs and horses (see §2), companies are developing canine and feline anti-NGF mAbs (NexVet, Ranevetmab and Frunevetmab, respectively). Overall, significant improvement has been seen in both dogs and cats following administration of anti-NGF mAbs. Assessment was made in dogs with OA through the subjective (owned completed questionnaire) and objective (activity) measures. These results suggested a positive analgesic effect possibly greater than that expected with NSAIDs (Webster et al., 2014). Anti-NGF mAB effectiveness was also demonstrated in dogs with pain artificially induced by kaolin injection (Gearing et al., 2013).

### **Targeting calcium channels**

Conopeptides are interesting candidates to design novel drugs for alleviating chronic neuropathic pain. Conopeptides are extracted from the venoms of marine snails and have an effect on calcium channels in modulating pain (Schroeder and Craik, 2012; Patel et al., 2018). Efficiency of conopeptides were first assessed in animal models of pain and then transferred to human clinic (Nielsen et al., 2005). Among conopeptides, a peptide blocker of CA<sub>v</sub> 2.2 channels (synthetic version of W-conotoxin MVIIA, named Ziconotide) has attracted much attention (Patel et al., 2018). Intrathecal administration of Ziconotide for refractory chronic pain (e.g. neuropathic pain) has proved antiallodynic and antihyperalgesic effects (Wermeling and Berger, 2006; McDowell and Pope, 2016). Ziconotide however show severe drawbacks,

and various alarming side effects have been monitored, e.g. sedation, confusion, ataxia, memory impairment, vertigo, gait imbalance, hallucinations, nausea and vomiting, that limit its indications (Wermeling and Berger, 2006). Actually Ziconotide is approved as a nonopioid analgesic but it must be administered intrathecally in refractory chronic pain cases, allowing patients to benefit from a potent long lasting analgesic effect related to malignant and non-malignant aetiologies (McGivern, 2007; Wie and Derian, 2020). To facilitate the use of Ziconotide, recent studies are evaluating the possibility of administering the drug intranasally (Manda et al., 2016).

## **12. Conclusion**

Animal models of pain were historically viewed as reliable tools that have served to advance research in the field over the past decades. They have been instrumental in constructing a global picture of how key proteins, signalling systems, and neural circuits contribute to **pain-like behaviors**. However, there are limitations and caveats to these models that must be acknowledged when considering the translation of research findings from the bench to the bedside. There is not one “best model” for the study of pain. Instead, the use of different organisms has been instrumental for integrating a comprehensive array of genetic, molecular, cellular, and behavioral data on normal and pathological pain processing in the central and peripheral nervous systems.

Classical rodent models usually display good face validity. However, it is still needed to improve construct validity by choosing refine animal models that mimic faithfully the human diseases. In particular, considering sex-differences, genetic variations, or comorbid interactions will contribute to describe a greater diversity of mechanisms that accurately account for clinical situations. In addition, defining more accurate behavioral measures will assist investigators in developing an appropriate set of experiments to better test mechanisms and potential treatments.

In contrast, using simpler organisms provides an efficient mean to achieve high throughput screening of possible key players in pain processes. These approaches are based on alterations of aversive behaviors that are considered surrogates of aberrant pain processes. Although far from the human clinics, these assays are likely to inform the construct of specific rodent models, or the design of protocols dedicated to test the actual role of these candidates.

The combination of distinct animal models may be the alternative to the lack of good predictive validity of animal models that basic research is facing so far. As long as the interpretation of the results remains constrained by their intrinsic limitations, integrating data obtained from different animal models is a powerful method to build up a comprehensive understanding of pain mechanisms and for the identification of potentially more relevant therapeutic targets.

Developing clinical assessment of pain is another important path to help defining more accurate animal models. Quantitative sensory testing, assessment of evoked vs resting pain, are parameters that are needed to understand how pain develops in humans. Clinical trials may also be designed to test outcomes and mechanisms derived from animal studies. Finally, an open dialogue between basic research results and clinical challenges posed by human studies is necessary to improve the translation of findings from bench to the bedside and to ensure the development of better pain therapies and pain management strategies

## Bibliography

- Abdus-Saboor I, Fried NT, Lay M, Burdge J, Swanson K, Fischer R, Jones J, Dong P, Cai W, Guo X, Tao Y-X, Bethea J, et al. 2019. Development of a Mouse Pain Scale Using Sub-second Behavioral Mapping and Statistical Modeling. *Cell Rep* 28:1623-1634.e4.
- Abreu MS de, Genario R, Giacomini ACVV, Demin KA, Lakstygai AM, Amstislavskaya TG, Fontana BD, Parker MO, Kalueff AV. 2019. Zebrafish as a Model of Neurodevelopmental Disorders. *Neuroscience*.
- Agarwal N, Helmstädter J, Rojas DR, Bali KK, Gangadharan V, Kuner R. 2018. Evoked hypoalgesia is accompanied by tonic pain and immune cell infiltration in the dorsal root ganglia at late stages of diabetic neuropathy in mice. *Mol Pain* 14:1744806918817975.
- Akerman S, Holland PR, Hoffmann J. 2013. Pearls and pitfalls in experimental in vivo models of migraine: dural trigeminovascular nociception. *Cephalalgia Int J Headache* 33:577–592.
- Akerman S, Romero-Reyes M, Holland PR. 2017. Current and novel insights into the neurophysiology of migraine and its implications for therapeutics. *Pharmacol Ther* 172:151–170.
- Allchorne AJ, Broom DC, Woolf CJ. 2005. Detection of cold pain, cold allodynia and cold hyperalgesia in freely behaving rats. *Mol Pain* 1:36.
- Alreja M, Mutalik P, Nayar U, Manchanda SK. 1984. The formalin test: a tonic pain model in the primate. *Pain* 20:97–105.
- Alshami AM. 2014. Knee osteoarthritis related pain: a narrative review of diagnosis and treatment. *Int J Health Sci* 8:85–104.
- Anand P, Elsaifa E, Privitera R, Naidoo K, Yiangou Y, Donatien P, Gabra H, Wasan H, Kenny L, Rahemtulla A, Misra P. 2019. Rational treatment of chemotherapy-induced peripheral neuropathy with capsaicin 8% patch: from pain relief towards disease modification. *J Pain Res* 12:2039–2052.
- Aragon CL, Hofmeister EH, Budsberg SC. 2007. Systematic review of clinical trials of treatments for osteoarthritis in dogs. *J Am Vet Med Assoc* 230:514–521.
- Ardiel EL, Rankin CH. 2010. An elegant mind: Learning and memory in *Caenorhabditis elegans*. *Learn Mem* 17:191–201.
- Arguello F, Baggs RB, Frantz CN. 1988. A murine model of experimental metastasis to bone and bone marrow. *Cancer Res* 48:6876–6881.
- Ase AR, Honson NS, Zaghdane H, Pfeifer TA, Séguéla P. 2015. Identification and characterization of a selective allosteric antagonist of human P2X4 receptor channels. *Mol Pharmacol* 87:606–616.
- Ashraf S, Mapp PI, Burston J, Bennett AJ, Chapman V, Walsh DA. 2014. Augmented pain behavioural responses to intra-articular injection of nerve growth factor in two animal models of osteoarthritis. *Ann Rheum Dis* 73:1710–1718.

- Attal N, Lanteri-Minet M, Laurent B, Fermanian J, Bouhassira D. 2011. The specific disease burden of neuropathic pain: results of a French nationwide survey. *Pain* 152:2836–2843.
- Authier N, Gillet JP, Fialip J, Eschalier A, Coudore F. 2003. An animal model of nociceptive peripheral neuropathy following repeated cisplatin injections. *Exp Neurol* 182:12–20.
- Back SK, Kim JS, Hong SK, Na HS. 2003. Ascending pathways for mechanical allodynia in a rat model of neuropathic pain. *Neuroreport* 14:1623–1626.
- Bali KK, Selvaraj D, Satagopam VP, Lu J, Schneider R, Kuner R. 2013. Genome-wide identification and functional analyses of microRNA signatures associated with cancer pain. *EMBO Mol Med* 5:1740–1758.
- Banik RK, Kabadi RA. 2013. A modified Hargreaves' method for assessing threshold temperatures for heat nociception. *J Neurosci Methods* 219:41–51.
- Bannwarth B, Kostine M. 2014. Targeting nerve growth factor (NGF) for pain management: what does the future hold for NGF antagonists? *Drugs* 74:619–626.
- Bannwarth B, Kostine M. 2017. Nerve Growth Factor Antagonists: Is the Future of Monoclonal Antibodies Becoming Clearer? *Drugs* 77:1377–1387.
- Barrot M. 2012. Tests and models of nociception and pain in rodents. *Neuroscience* 211:39–50.
- Bas DB, Su J, Wigerblad G, Svensson CI. 2016. Pain in rheumatoid arthritis: models and mechanisms. *Pain Manag* 6:265–284.
- Basbaum AI, Bautista DM, Scherrer G, Julius D. 2009. Cellular and molecular mechanisms of pain. *Cell* 139:267–284.
- Benbouzid M, Pallage V, Rajalu M, Waltisperger E, Doridot S, Poisbeau P, Freund-Mercier MJ, Barrot M. 2008. Sciatic nerve cuffing in mice: a model of sustained neuropathic pain. *Eur J Pain Lond Engl* 12:591–599.
- Bendele AM. 1987. Progressive Chronic Osteoarthritis in Femorotibial Joints of Partial Medial Meniscectomized Guinea Pigs. *Vet Pathol* 24:444–448.
- Benjamin L, Levy MJ, Lasalandra MP, Knight YE, Akerman S, Classey JD, Goadsby PJ. 2004. Hypothalamic activation after stimulation of the superior sagittal sinus in the cat: a Fos study. *Neurobiol Dis* 16:500–505.
- Bennett GJ, Xie YK. 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33:87–107.
- Berić A. 1998. Central pain and dysesthesia syndrome. *Neurol Clin* 16:899–918.
- Bernier L-P, Ase AR, Séguéla P. 2018. P2X receptor channels in chronic pain pathways. *Br J Pharmacol* 175:2219–2230.

- Bessa C, Maciel P, Rodrigues AJ. 2013. Using *C. elegans* to decipher the cellular and molecular mechanisms underlying neurodevelopmental disorders. *Mol Neurobiol* 48:465–489.
- Bessis N, Decker P, Assier E, Semerano L, Boissier M-C. 2017. Arthritis models: usefulness and interpretation. *Semin Immunopathol* 39:469–486.
- Bhattacharya A. 2016. Clinical optimism for antagonists targeting certain ion channels. 3:10–13.
- Biessels GJ, Bril V, Calcutt NA, Cameron NE, Cotter MA, Dobrowsky R, Feldman EL, Fernyhough P, Jakobsen J, Malik RA, Mizisin AP, Oates PJ, et al. 2014. Phenotyping animal models of diabetic neuropathy: a consensus statement of the diabetic neuropathy study group of the EASD (Neurodiab). *J Peripher Nerv Syst JPNS* 19:77–87.
- Blair HA. 2018. Capsaicin 8% Dermal Patch: A Review in Peripheral Neuropathic Pain. *Drugs* 78:1489–1500.
- Blanchard C, Boué-Grabot E, Massé K. 2019. Comparative Embryonic Spatio-Temporal Expression Profile Map of the Xenopus P2X Receptor Family. *Front Cell Neurosci* 13:.
- Blanco-Gandía MC, Aguilar MA, Miñarro J, Rodríguez-Arias M. 2018. Reinstatement of Drug-seeking in Mice Using the Conditioned Place Preference Paradigm. *J Vis Exp JoVE*.
- Bleehen T, Keele CA. 1977. Observations on the algogenic actions of adenosine compounds on the human blister base preparation. *Pain* 3:367–377.
- Bobinski F, Ferreira TAA, Córdova MM, Dombrowski PA, Cunha C da, Santo CC do E, Poli A, Pires RGW, Martins-Silva C, Sluka KA, Santos ARS. 2015. Role of brainstem serotonin in analgesia produced by low-intensity exercise on neuropathic pain after sciatic nerve injury in mice. *Pain* 156:2595–2606.
- Bonin RP, Bories C, De Koninck Y. 2014. A simplified up-down method (SUDO) for measuring mechanical nociception in rodents using von Frey filaments. *Mol Pain* 10:26.
- Bossé GD, Peterson RT. 2017. Development of an opioid self-administration assay to study drug seeking in zebrafish. *Behav Brain Res* 335:158–166.
- Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C. 2008. Prevalence of chronic pain with neuropathic characteristics in the general population: *Pain* 136:380–387.
- Bourin M, Hascoët M. 2003. The mouse light/dark box test. *Eur J Pharmacol* 463:55–65.
- Bourquin A-F, Süveges M, Pertin M, Gilliard N, Sardy S, Davison AC, Spahn DR, Decosterd I. 2006. Assessment and analysis of mechanical allodynia-like behavior induced by spared nerve injury (SNI) in the mouse. *Pain* 122:14.e1-14.
- Bove SE, Laemont KD, Brooker RM, Osborn MN, Sanchez BM, Guzman RE, Hook KE, Juneau PL, Connor JR, Kilgore KS. 2006. Surgically induced osteoarthritis in the rat results in the development of both osteoarthritis-like joint pain and secondary hyperalgesia. *Osteoarthritis Cartilage* 14:1041–1048.

Boyce-Rustay JM, Zhong C, Kohnken R, Baker SJ, Simler GH, Wensink EJ, Decker MW, Honore P. 2010. Comparison of mechanical allodynia and the affective component of inflammatory pain in rats. *Neuropharmacology* 58:537–543.

Braundmeier AG, Fazleabas AT. 2009. The non-human primate model of endometriosis: research and implications for fecundity. *Mol Hum Reprod* 15:577–586.

Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. 2006. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *Eur J Pain Lond Engl* 10:287–333.

Breivik H, Eisenberg E, O'Brien T, OPENMinds. 2013. The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. *BMC Public Health* 13:1229.

Brenner DS, Golden JP, Gereau RW. 2012. A novel behavioral assay for measuring cold sensation in mice. *PLoS One* 7:e39765.

Brodkin J, Frank D, Grippo R, Hausfater M, Gulinello M, Achterholt N, Gutzen C. 2014. Validation and implementation of a novel high-throughput behavioral phenotyping instrument for mice. *J Neurosci Methods* 224:48–57.

Broek MF van den, Berg WB van den, Putte LB van de, Severijnen AJ. 1988. Streptococcal cell wall-induced arthritis and flare-up reaction in mice induced by homologous or heterologous cell walls. *Am J Pathol* 133:139–149.

Brown C. 2015. Comparative evolutionary approach to pain perception in fishes. 7.

Brown DC, Agnello K, Iadarola MJ. 2015. Intrathecal resiniferatoxin in a dog model: efficacy in bone cancer pain. *Pain* 156:1018–1024.

Buck SH, Deshmukh PP, Yamamura HI, Burks TF. 1981. Thermal analgesia and substance P depletion induced by capsaicin in guinea-pigs. *Neuroscience* 6:2217–2222.

Burma NE, Leduc-Pessah H, Fan CY, Trang T. 2017a. Animal models of chronic pain: Advances and challenges for clinical translation. *J Neurosci Res* 95:1242–1256.

Burma NE, Leduc-Pessah H, Fan CY, Trang T. 2017b. Animal models of chronic pain: Advances and challenges for clinical translation. *J Neurosci Res* 95:1242–1256.

Burnstock G. 2016. Purinergic Mechanisms and Pain. *Adv Pharmacol San Diego Calif* 75:91–137.

Burnstock G, Knight GE. 2018. The potential of P2X7 receptors as a therapeutic target, including inflammation and tumour progression. *Purinergic Signal* 14:1–18.

Buzzi MG, Carter WB, Shimizu T, Heath H, Moskowitz MA. 1991. Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. *Neuropharmacology* 30:1193–1200.

- Caldwell JC, Tracey WD. 2010. Alternatives to mammalian pain models 2: using *Drosophila* to identify novel genes involved in nociception. *Methods Mol Biol Clifton NJ* 617:19–29.
- Campbell JN, Meyer RA. 2006. Mechanisms of Neuropathic Pain. *Neuron* 52:77–92.
- Campos AC, Fogaça MV, Aguiar DC, Guimarães FS. 2013. Animal models of anxiety disorders and stress. *Rev Bras Psiquiatr Sao Paulo Braz* 1999 35 Suppl 2:S101-111.
- Canavero S, Bonicalzi V. 2007. Central pain syndrome: elucidation of genesis and treatment. *Expert Rev Neurother* 7:1485–1497.
- Caplazi P, Baca M, Barck K, Carano R a. D, DeVoss J, Lee WP, Bolon B, Diehl L. 2015. Mouse Models of Rheumatoid Arthritis. *Vet Pathol* 52:819–826.
- Carlton SM, Lekan HA, Kim SH, Chung JM. 1994. Behavioral manifestations of an experimental model for peripheral neuropathy produced by spinal nerve ligation in the primate. *Pain* 56:155–166.
- Carter RB, Francis WR. 1991. Capsaicin desensitization to plasma extravasation evoked by antidromic C-fiber stimulation is not associated with antinociception in the rat. *Neurosci Lett* 127:43–45.
- Cash JL, White GE, Greaves DR. 2009. Chapter 17. Zymosan-induced peritonitis as a simple experimental system for the study of inflammation. *Methods Enzymol* 461:379–396.
- Catanzaro A, Di Salvo A, Steagall PV, Zampini D, Polisca A, Della Rocca G. 2016. Preliminary study on attitudes, opinions and knowledge of Italian veterinarians with regard to abdominal visceral pain in dogs. *Vet Anaesth Analg* 43:361–370.
- Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitl KR, Koltzenburg M, Basbaum AI, Julius D. 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288:306–313.
- Chae J-P, Park MS, Hwang Y-S, Min B-H, Kim S-H, Lee H-S, Park M-J. 2015. Evaluation of developmental toxicity and teratogenicity of diclofenac using *Xenopus* embryos. *Chemosphere* 120:52–58.
- Chang A, Quick J. 2020. Capsaicin. StatPearls, Treasure Island (FL): StatPearls Publishing,.
- Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. 1994. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 53:55–63.
- Chen X, Feng X, Guang S. 2016. Targeted genome engineering in *Caenorhabditis elegans*. *Cell Biosci* 6:60.
- Cheng L, Duan B, Huang T, Zhang Y, Chen Y, Britz O, Garcia-Campmany L, Ren X, Vong L, Lowell BB, Goulding M, Wang Y, et al. 2017. Identification of spinal circuits involved in touch-evoked dynamic mechanical pain. *Nat Neurosci* 20:804–814.
- Chessell IP, Hatcher JP, Bountra C, Michel AD, Hughes JP, Green P, Egerton J, Murfin M, Richardson J, Peck WL, Grahames CBA, Casula MA, et al. 2005. Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. *Pain* 114:386–396.

- Choi Y, Yoon YW, Na HS, Kim SH, Chung JM. 1994. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. *Pain* 59:369–376.
- Chopade AR, Sayyad FJ, Naikwade NS. 2014. Pharmacological characterization of carrageenan induced heat muscle hyperalgesia in rats using non-selective, preferential and selective COX-2 inhibitors. *Pharmacol Rep PR* 66:353–362.
- Christensen MD, Everhart AW, Pickelman JT, Hulsebosch CE. 1996. Mechanical and thermal allodynia in chronic central pain following spinal cord injury. *Pain* 68:97–107.
- Christensen SL, Hansen RB, Storm MA, Olesen J, Hansen TF, Ossipov M, Izarzugaza JMG, Porreca F, Kristensen DM. 2020. Von Frey testing revisited: Provision of an online algorithm for improved accuracy of 50% thresholds. *Eur J Pain Lond Engl* 24:783–790.
- Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, Malmberg AB, Cain G, Berson A, Kassotakis L, Hedley L, Lachnit WG, et al. 2000. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature* 407:1011–1015.
- Colburn RW, Lubin ML, Stone DJ, Wang Y, Lawrence D, D’Andrea MR, Brandt MR, Liu Y, Flores CM, Qin N. 2007. Attenuated cold sensitivity in TRPM8 null mice. *Neuron* 54:379–386.
- Cordero-Erausquin M, Inquimbert P, Schlichter R, Hugel S. 2016. Neuronal networks and nociceptive processing in the dorsal horn of the spinal cord. *Neuroscience* 338:230–247.
- Costa B, Trovato AE, Colleoni M, Giagnoni G, Zarini E, Croci T. 2005. Effect of the cannabinoid CB1 receptor antagonist, SR141716, on nociceptive response and nerve demyelination in rodents with chronic constriction injury of the sciatic nerve. *Pain* 116:52–61.
- Costa FV, Rosa LV, Quadros VA, Santos ARS, Kalueff AV, Rosemberg DB. 2019. Understanding nociception-related phenotypes in adult zebrafish: Behavioral and pharmacological characterization using a new acetic acid model. *Behav Brain Res* 359:570–578.
- Costigan M, Scholz J, Woolf CJ. 2009. Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu Rev Neurosci* 32:1–32.
- Coull JAM, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y. 2005. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438:1017–1021.
- Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. 1980. Immunisation against heterologous type II collagen induces arthritis in mice. *Nature* 283:666–668.
- Crawley J, Goodwin FK. 1980. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav* 13:167–170.
- Cruccu G, Nurmikko TJ, Ernault E, Riaz FK, McBride WT, Haanpää M. 2018. Superiority of capsaicin 8% patch versus oral pregabalin on dynamic mechanical allodynia in patients with peripheral neuropathic pain. *Eur J Pain Lond Engl* 22:700–706.

Cui Y, Toyoda H, Sako T, Onoe K, Hayashinaka E, Wada Y, Yokoyama C, Onoe H, Kataoka Y, Watanabe Y. 2015. A voxel-based analysis of brain activity in high-order trigeminal pathway in the rat induced by cortical spreading depression. *NeuroImage* 108:17–22.

Curtright A, Rosser M, Goh S, Keown B, Wagner E, Sharifi J, Raible DW, Dhaka A. 2015. Modeling Nociception in Zebrafish: A Way Forward for Unbiased Analgesic Discovery. *PLoS ONE* 10:.

D'amour FE, Smith DL. 1941. A Method for Determining Loss of Pain Sensation. *J Pharmacol Exp Ther* 72:74–79.

Danquah W, Meyer-Schwesinger C, Rissiek B, Pinto C, Serracant-Prat A, Amadi M, Iacenda D, Knop J-H, Hammel A, Bergmann P, Schwarz N, Assunção J, et al. 2016. Nanobodies that block gating of the P2X7 ion channel ameliorate inflammation. *Sci Transl Med* 8:366ra162.

Davidson E, Coppey L, Lu B, Arballo V, Calcutt NA, Gerard C, Yorek M. 2009. The roles of streptozotocin neurotoxicity and neutral endopeptidase in murine experimental diabetic neuropathy. *Exp Diabetes Res* 2009:431980.

Deacon RMJ. 2006. Burrowing in rodents: a sensitive method for detecting behavioral dysfunction. *Nat Protoc* 1:118–121.

Deakin AG, Buckley J, AlZu'bi HS, Cossins AR, Spencer JW, Al'Nuaimy W, Young IS, Thomson JS, Sneddon LU. 2019. Automated monitoring of behaviour in zebrafish after invasive procedures. *Sci Rep* 9:1–13.

Decosterd I, Woolf CJ. 2000. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* 87:149–158.

Demin K, Lakstygal A, Kalueff A. 2018a. Time to (finally) acknowledge that fish have emotionality and pain. *Anim Sentience* 3.

Demin KA, Meshalkina DA, Kysil EV, Antonova KA, Volgin AD, Yakovlev OA, Alekseeva PA, Firuleva MM, Lakstygal AM, Abreu MS de, Barcellos LJG, Bao W, et al. 2018b. Zebrafish models relevant to studying central opioid and endocannabinoid systems. *Prog Neuropsychopharmacol Biol Psychiatry* 86:301–312.

Deuis JR, Dvorakova LS, Vetter I. 2017a. Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci* 10:284.

Deuis JR, Dvorakova LS, Vetter I. 2017b. Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci* 10:284.

Dixon WJ. 1980. Efficient analysis of experimental observations. *Annu Rev Pharmacol Toxicol* 20:441–462.

Dobremez E, Bouali-Benazzouz R, Fossat P, Monteils L, Dulluc J, Nagy F, Landry M. 2005. Distribution and regulation of L-type calcium channels in deep dorsal horn neurons after sciatic nerve injury in rats. *Eur J Neurosci* 21:3321–3333.

- Doherty NS, Poubelle P, Borgeat P, Beaver TH, Westrich GL, Schrader NL. 1985. Intraperitoneal injection of zymosan in mice induces pain, inflammation and the synthesis of peptidoleukotrienes and prostaglandin E2. *Prostaglandins* 30:769–789.
- Dolensek N, Gehrlach DA, Klein AS, Gogolla N. 2020. Facial expressions of emotion states and their neuronal correlates in mice. *Science* 368:89–94.
- Dolique T, Landry M, Nagy F. 2010. Spinal cord: dorsal horn. *Handbook of Brain Microcircuits*, Oxford University Press, p 237–248.
- Dray A. 2008. Neuropathic pain: emerging treatments. *Br J Anaesth* 101:48–58.
- Drewes AM, Olesen AE, Farmer AD, Szigethy E, Rebours V, Olesen SS. 2020. Gastrointestinal pain. *Nat Rev Dis Primer* 6:1.
- Drewes AM, Schipper KP, Dimcevski G, Petersen P, Gregersen H, Funch-Jensen P, Arendt-Nielsen L. 2003. Gut pain and hyperalgesia induced by capsaicin: a human experimental model. *Pain* 104:333–341.
- D’Souza WN, Ng GY, Youngblood BD, Tsuji W, Lehto SG. 2011a. A review of current animal models of osteoarthritis pain. *Curr Pharm Biotechnol* 12:1596–1612.
- D’Souza WN, Ng GY, Youngblood BD, Tsuji W, Lehto SG. 2011b. A review of current animal models of osteoarthritis pain. *Curr Pharm Biotechnol* 12:1596–1612.
- Dubuisson D, Dennis SG. 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain* 4:161–174.
- Ellis LD, Berrue F, Morash M, Achenbach JC, Hill J, McDougall JJ. 2018. Comparison of cannabinoids with known analgesics using a novel high throughput zebrafish larval model of nociception. *Behav Brain Res* 337:151–159.
- Elramah S, López-González MJ, Bastide M, Dixmérias F, Roca-Lapirot O, Wielanek-Bachelet A-C, Vital A, Leste-Lasserre T, Brochard A, Landry M, Favereaux A. 2017. Spinal miRNA-124 regulates synaptopodin and nociception in an animal model of bone cancer pain. *Sci Rep* 7:10949.
- Enomoto H, Fujikoshi S, Funai J, Sasaki N, Ossipov MH, Tsuji T, Alev L, Ushida T. 2017. Assessment of direct analgesic effect of duloxetine for chronic low back pain: post hoc path analysis of double-blind, placebo-controlled studies. *J Pain Res* 10:1357–1368.
- Enomoto M, Mantyh PW, Murrell J, Innes JF, Lascelles BDX. 2019. Anti-nerve growth factor monoclonal antibodies for the control of pain in dogs and cats. *Vet Rec* 184:23.
- Falk S, Appel CK, Bennedbæk HB, Al-Dihaissy T, Unger A, Dinkel K, Heegaard A-M. 2019. Chronic high dose P2X7 receptor inhibition exacerbates cancer-induced bone pain. *Eur J Pharmacol* 845:48–55.
- Falk S, Dickenson AH. 2014. Pain and nociception: mechanisms of cancer-induced bone pain. *J Clin Oncol Off J Am Soc Clin Oncol* 32:1647–1654.

- Fang H, Beier F. 2014. Mouse models of osteoarthritis: modelling risk factors and assessing outcomes. *Nat Rev Rheumatol* 10:413–421.
- Fehrenbacher JC, Vasko MR, Duarte DB. 2012. Models of Inflammation: Carrageenan- or Complete Freund's Adjuvant-Induced Edema and Hypersensitivity in the Rat. *Curr Protoc Pharmacol Editor Board SJ Enna Ed--Chief AI 0 5:Unit5.4*.
- Fernandes ES, Russell FA, Spina D, McDougall JJ, Graepel R, Gentry C, Staniland AA, Mountford DM, Keeble JE, Malcangio M, Bevan S, Brain SD. 2011. A distinct role for transient receptor potential ankyrin 1, in addition to transient receptor potential vanilloid 1, in tumor necrosis factor  $\alpha$ -induced inflammatory hyperalgesia and Freund's complete adjuvant-induced monoarthritis. *Arthritis Rheum* 63:819–829.
- Ferrari LF, Bogen O, Reichling DB, Levine JD. 2015. Accounting for the delay in the transition from acute to chronic pain: axonal and nuclear mechanisms. *J Neurosci Off J Soc Neurosci* 35:495–507.
- Ferrari LF, Levine JD, Green PG. 2016. Mechanisms mediating nitroglycerin-induced delayed-onset hyperalgesia in the rat. *Neuroscience* 317:121–129.
- Fields HL, Rowbotham M, Baron R. 1998. Postherpetic neuralgia: irritable nociceptors and deafferentation. *Neurobiol Dis* 5:209–227.
- Finnerup NB, Johannesen IL, Fuglsang-Frederiksen A, Bach FW, Jensen TS. 2003. Sensory function in spinal cord injury patients with and without central pain. *Brain J Neurol* 126:57–70.
- Finnerup NB, Sindrup SH, Jensen TS. 2010. The evidence for pharmacological treatment of neuropathic pain. *Pain* 150:573–581.
- Fischer BD, Adeyemo A, O'Leary ME, Bottaro A. 2017. Animal models of rheumatoid pain: experimental systems and insights. *Arthritis Res Ther* 19:146.
- Fontana BD, Mezzomo NJ, Kalueff AV, Rosemberg DB. 2018. The developing utility of zebrafish models of neurological and neuropsychiatric disorders: A critical review. *Exp Neurol* 299:157–171.
- Fossat P, Dobremez E, Bouali-Benazzouz R, Favereaux A, Bertrand SS, Kilk K, Léger C, Cazalets J-R, Langel U, Landry M, Nagy F. 2010. Knockdown of L calcium channel subtypes: differential effects in neuropathic pain. *J Neurosci Off J Soc Neurosci* 30:1073–1085.
- Frias B, Merighi A. 2016. Capsaicin, Nociception and Pain. *Mol Basel Switz* 21:.
- Fricker B, Muller A, René F. 2008. Evaluation tools and animal models of peripheral neuropathies. *Neurodegener Dis* 5:72–108.
- Fuentes IM, Christianson JA. 2018. The Influence of Early Life Experience on Visceral Pain. *Front Syst Neurosci* 12:2.
- Gao Z, Wei H, Chen Z, Jalava N,, A. Koivisto A, Pertovaara A. 2019. Ongoing pain in streptozotocin model of diabetes in the rat: correlation with cutaneous chemnociception. *J. Physiol. Pharm.* 70, 6, 969-978.

- Gagnon M, Bergeron MJ, Lavertu G, Castonguay A, Tripathy S, Bonin RP, Perez-Sanchez J, Boudreau D, Wang B, Dumas L, Valade I, Bachand K, et al. 2013. Chloride extrusion enhancers as novel therapeutics for neurological diseases. *Nat Med* 19:1524–1528.
- Gamse R, Holzer P, Lembeck F. 1980. Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. *Br J Pharmacol* 68:207–213.
- Gamse R, Petsche U, Lembeck F, Jancsó G. 1982. Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. *Brain Res* 239:447–462.
- García-Martínez C, Humet M, Planells-Cases R, Gomis A, Caprini M, Viana F, De La Pena E, Sanchez-Baeza F, Carbonell T, De Felipe C, Pérez-Paya E, Belmonte C, et al. 2002. Attenuation of thermal nociception and hyperalgesia by VR1 blockers. *Proc Natl Acad Sci U S A* 99:2374–2379.
- Gaskin DJ, Richard P. 2012. The economic costs of pain in the United States. *J Pain Off J Am Pain Soc* 13:715–724.
- Gaviria M, Haton H, Sandillon F, Privat A. 2002. A mouse model of acute ischemic spinal cord injury. *J Neurotrauma* 19:205–221.
- Gearing DP, Virtue ER, Gearing RP, Drew AC. 2013. A fully caninised anti-NGF monoclonal antibody for pain relief in dogs. *BMC Vet Res* 9:226.
- Gebhart GF, Bielefeldt K. 2016. Physiology of Visceral Pain. *Compr Physiol* 6:1609–1633.
- Gerber B, Yarali A, Diegelmann S, Wotjak CT, Pauli P, Fendt M. 2014. Pain-relief learning in flies, rats, and man: basic research and applied perspectives. *Learn Mem Cold Spring Harb N* 21:232–252.
- Ghilardi JR, Röhrich H, Lindsay TH, Sevcik MA, Schwei MJ, Kubota K, Halvorson KG, Poblete J, Chaplan SR, Dubin AE, Carruthers NI, Swanson D, et al. 2005a. Selective blockade of the capsaicin receptor TRPV1 attenuates bone cancer pain. *J Neurosci Off J Soc Neurosci* 25:3126–3131.
- Ghilardi JR, Röhrich H, Lindsay TH, Sevcik MA, Schwei MJ, Kubota K, Halvorson KG, Poblete J, Chaplan SR, Dubin AE, Carruthers NI, Swanson D, et al. 2005b. Selective blockade of the capsaicin receptor TRPV1 attenuates bone cancer pain. *J Neurosci Off J Soc Neurosci* 25:3126–3131.
- Giacomotto J, Ségalat L. 2010. High-throughput screening and small animal models, where are we? *Br J Pharmacol* 160:204–216.
- Giamberardino MA, Affaitati G, Costantini R. 2010. Visceral Referred Pain. *J Musculoskelet Pain* 18:403–410.
- Giamberardino MA, Valente R, Bigontina P de, Vecchiet L. 1995. Artificial ureteral calculosis in rats: behavioural characterization of visceral pain episodes and their relationship with referred lumbar muscle hyperalgesia. *Pain* 61:459–469.
- Gierthmühlen J, Maier C, Baron R, Tölle T, Treede R-D, Birbaumer N, Hüge V, Koroschetz J, Krumova EK, Lauchart M, Maihöfner C, Richter H, et al. 2012. Sensory signs in complex regional pain syndrome and peripheral nerve injury. *Pain* 153:765–774.

- Gilchrist LS, Cain DM, Harding-Rose C, Kov AN, Wendelschafer-Crabb G, Kennedy WR, Simone DA. 2005. Re-organization of P2X3 receptor localization on epidermal nerve fibers in a murine model of cancer pain. *Brain Res* 1044:197–205.
- Gingras J, Smith S, Matson DJ, Johnson D, Nye K, Couture L, Feric E, Yin R, Moyer BD, Peterson ML, Rottman JB, Beiler RJ, et al. 2014. Global Nav1.7 knockout mice recapitulate the phenotype of human congenital indifference to pain. *PLoS One* 9:e105895.
- Glasson SS, Blanchet TJ, Morris EA. 2007. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. *Osteoarthritis Cartilage* 15:1061–1069.
- Glauser DA, Chen WC, Agin R, Macinnis BL, Hellman AB, Garrity PA, Tan M-W, Goodman MB. 2011. Heat avoidance is regulated by transient receptor potential (TRP) channels and a neuropeptide signaling pathway in *Caenorhabditis elegans*. *Genetics* 188:91–103.
- Gomis A, Miralles A, Schmidt RF, Belmonte C. 2007. Nociceptive nerve activity in an experimental model of knee joint osteoarthritis of the guinea pig: effect of intra-articular hyaluronan application. *Pain* 130:126–136.
- Gonzalez-Nunez V, Rodríguez RE. 2009. The zebrafish: a model to study the endogenous mechanisms of pain. *ILAR J* 50:373–386.
- Goyal N, Rana A, Ahlawat A, Bijjem KRV, Kumar P. 2014. Animal models of inflammatory bowel disease: a review. *Inflammopharmacology* 22:219–233.
- Grauw JC de, Loon JP a. M van. 2016. Systematic pain assessment in horses. *Vet J Lond Engl* 1997 209:14–22.
- Greenwood-Van Meerveld B, Johnson AC. 2017. Stress-Induced Chronic Visceral Pain of Gastrointestinal Origin. *Front Syst Neurosci* 11:86.
- Gregory NS, Harris AL, Robinson CR, Dougherty PM, Fuchs PN, Sluka KA. 2013. An overview of animal models of pain: disease models and outcome measures. *J Pain Off J Am Pain Soc* 14:1255–1269.
- Groenewald CB, Palermo TM. 2015. The price of pain: the economics of chronic adolescent pain. *Pain Manag* 5:61–64.
- Gruen ME, Thomson AE, Griffith EH, Paradise H, Gearing DP, Lascelles BDX. 2016. A Feline-Specific Anti-Nerve Growth Factor Antibody Improves Mobility in Cats with Degenerative Joint Disease-Associated Pain: A Pilot Proof of Concept Study. *J Vet Intern Med* 30:1138–1148.
- Guedon J-MG, Longo G, Majuta LA, Thompsen ML, Fealk MN, Mantyh PW. 2016. Dissociation between the relief of skeletal pain behaviors and skin hypersensitivity in a model of bone cancer pain. *Pain* 157:1239–1247.
- Gunthorpe MJ, Chizh BA. 2009. Clinical development of TRPV1 antagonists: targeting a pivotal point in the pain pathway. *Drug Discov Today* 14:56–67.

Guo N, Gu X. 2014. Sciatic Nerve Neuropathy in Cynomolgus Monkey *Macaca Fascicularis*: Altered Leg Usage and Peripheral Nerve Firing. *J Neurol Neurophysiol* 05:

Haanpää M, Cruccu G, Nurmikko TJ, McBride WT, Docu Axelarad A, Bosilkov A, Chambers C, Ernault E, Abdulahad AK. 2016. Capsaicin 8% patch versus oral pregabalin in patients with peripheral neuropathic pain. *Eur J Pain Lond Engl* 20:316–328.

Hahn AF. 1996. Experimental allergic neuritis (EAN) as a model for the immune-mediated demyelinating neuropathies. *Rev Neurol (Paris)* 152:328–332.

Hamalainen MM, Subieta A, Arpey C, Brennan TJ. 2009. Differential effect of capsaicin treatment on pain related behaviors after plantar incision. *J Pain Off J Am Pain Soc* 10:637–645.

Hamilton SG, Warburton J, Bhattacharjee A, Ward J, McMahon SB. 2000. ATP in human skin elicits a dose-related pain response which is potentiated under conditions of hyperalgesia. *Brain J Neurol* 123 (Pt 6):1238–1246.

Han P, Liu S, Zhang M, Zhao J, Wang Y, Wu G, Mi W. 2015. Inhibition of Spinal Interleukin-33/ST2 Signaling and Downstream ERK and JNK Pathways in Electroacupuncture Analgesia in Formalin Mice. *PLoS One* 10:e0129576.

Han P, Zhao J, Liu S-B, Yang C-J, Wang Y-Q, Wu G-C, Xu D-M, Mi W-L. 2013. Interleukin-33 mediates formalin-induced inflammatory pain in mice. *Neuroscience* 241:59–66.

Handa J, Sekiguchi M, Krupkova O, Konno S-I. 2016. The effect of serotonin-noradrenaline reuptake inhibitor duloxetine on the intervertebral disk-related radiculopathy in rats. *Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc* 25:877–887.

Hargreaves K, Dubner R, Brown F, Flores C, Joris J. 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 32:77–88.

Harriott AM, Strother LC, Vila-Pueyo M, Holland PR. 2019. Animal models of migraine and experimental techniques used to examine trigeminal sensory processing. *J Headache Pain* 20:91.

Harvey VL, Dickenson AH. 2009. Behavioural and electrophysiological characterisation of experimentally induced osteoarthritis and neuropathy in C57Bl/6 mice. *Mol Pain* 5:18.

Hashizume H, Kawakami M, Yoshida M, Okada M, Enyo Y, Inomata Y. 2007. Sarpogrelate hydrochloride, a 5-HT<sub>2A</sub> receptor antagonist, attenuates neurogenic pain induced by nucleus pulposus in rats. *Spine* 32:315–320.

Henze DA, Urban MO. 2010. Large Animal Models for Pain Therapeutic Development. In: Kruger L, Light AR, editors. *Translational Pain Research: From Mouse to Man*, Boca Raton (FL): CRC Press/Taylor & Francis,.

Herzberg U, Sagen J. 2001. Peripheral nerve exposure to HIV viral envelope protein gp120 induces neuropathic pain and spinal gliosis. *J Neuroimmunol* 116:29–39.

Hill R. 2000. NK1 (substance P) receptor antagonists--why are they not analgesic in humans? *Trends Pharmacol Sci* 21:244–246.

Holden E, Calvo G, Collins M, Bell A, Reid J, Scott EM, Nolan AM. 2014. Evaluation of facial expression in acute pain in cats. *J Small Anim Pract* 55:615–621.

Honore P, Kage K, Mikusa J, Watt AT, Johnston JF, Wyatt JR, Faltynek CR, Jarvis MF, Lynch K. 2002. Analgesic profile of intrathecal P2X(3) antisense oligonucleotide treatment in chronic inflammatory and neuropathic pain states in rats. *Pain* 99:11–19.

Hootman JM, Helmick CG, Barbour KE, Theis KA, Boring MA. 2016. Updated Projected Prevalence of Self-Reported Doctor-Diagnosed Arthritis and Arthritis-Attributable Activity Limitation Among US Adults, 2015–2040. *Arthritis Rheumatol Hoboken NJ* 68:1582–1587.

Hou T, Xiang H, Yu L, Su W, Shu Y, Li H, Zhu H, Lin L, Hu X, Liang S, Zhang H, Li M. 2019. Electroacupuncture inhibits visceral pain via adenosine receptors in mice with inflammatory bowel disease. *Purinergic Signal* 15:193–204.

Huang W, Calvo M, Pheby T, Bennett DLH, Rice ASC. 2017. A rodent model of HIV protease inhibitor indinavir induced peripheral neuropathy. *Pain* 158:75–85.

Hunskar S, Hole K. 1987a. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* 30:103–114.

Hunskar S, Hole K. 1987b. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* 30:103–114.

Ibrahim A, Hirschfeld S, Cohen MH, Griebel DJ, Williams GA, Pazdur R. 2004. FDA drug approval summaries: oxaliplatin. *The Oncologist* 9:8–12.

Ilie MA, Caruntu C, Tampa M, Georgescu S-R, Matei C, Negrei C, Ion R-M, Constantin C, Neagu M, Boda D. 2019. Capsaicin: Physicochemical properties, cutaneous reactions and potential applications in painful and inflammatory conditions. *Exp Ther Med* 18:916–925.

Ishikawa G, Koya Y, Tanaka H, Nagakura Y. 2015. Long-term analgesic effect of a single dose of anti-NGF antibody on pain during motion without notable suppression of joint edema and lesion in a rat model of osteoarthritis. *Osteoarthritis Cartilage* 23:925–932.

Isola M, Ferrari V, Miolo A, Stabile F, Bernardini D, Carnier P, Busetto R. 2011. Nerve growth factor concentrations in the synovial fluid from healthy dogs and dogs with secondary osteoarthritis. *Vet Comp Orthop Traumatol VCOT* 24:279–284.

Jarvis MF, Burgard EC, McGaraughty S, Honore P, Lynch K, Brennan TJ, Subieta A, Van Biesen T, Cartmell J, Bianchi B, Niforatos W, Kage K, et al. 2002. A-317491, a novel potent and selective non-nucleotide antagonist of P2X3 and P2X2/3 receptors, reduces chronic inflammatory and neuropathic pain in the rat. *Proc Natl Acad Sci U S A* 99:17179–17184.

Jayasinghe CD, Jayawardena UA. 2019. Evidence-Based Complementary and Alternative Medicine. <https://www.hindawi.com/journals/ecam/2019/7272808/>. Accessed April 2, 2020.

Jeibmann A, Paulus W. 2009. *Drosophila melanogaster* as a model organism of brain diseases. *Int J Mol Sci* 10:407–440.

Jensen DD, Lieu T, Halls ML, Veldhuis NA, Imlach WL, Mai QN, Poole DP, Quach T, Aurelio L, Conner J, Herenbrink CK, Barlow N, et al. 2017. Neurokinin 1 receptor signaling in endosomes mediates sustained nociception and is a viable therapeutic target for prolonged pain relief. *Sci Transl Med* 9:.

Ji B, Chauffour A, Andries K, Jarlier V. 2006. Bactericidal activities of R207910 and other newer antimicrobial agents against *Mycobacterium leprae* in mice. *Antimicrob Agents Chemother* 50:1558–1560.

Ji R-R, Xu Z-Z, Gao Y-J. 2014. Emerging targets in neuroinflammation-driven chronic pain. *Nat Rev Drug Discov* 13:533–548.

Jimenez-Andrade JM, Ghilardi JR, Castañeda-Corral G, Kuskowski MA, Mantyh PW. 2011. Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. *Pain* 152:2564–2574.

Johansen JP, Fields HL, Manning BH. 2001. The affective component of pain in rodents: Direct evidence for a contribution of the anterior cingulate cortex. *Proc Natl Acad Sci* 98:8077–8082.

Jorgensen EM, Mango SE. 2002. The art and design of genetic screens: *caenorhabditis elegans*. *Nat Rev Genet* 3:356–369.

Kahn-Kirby AH, Bargmann CI. 2006. TRP channels in *C. elegans*. *Annu Rev Physiol* 68:719–736.

Kalbhenn DA. 1987. Chemical model of osteoarthritis—a pharmacological evaluation. *J Rheumatol* 14 Spec No:130–131.

Kato K, Sekiguchi M, Kikuchi S, Konno S. 2015. The effect of a 5-HT<sub>2A</sub> receptor antagonist on pain-related behavior, endogenous 5-hydroxytryptamine production, and the expression 5-HT<sub>2A</sub> receptors in dorsal root ganglia in a rat lumbar disc herniation model. *Spine* 40:357–362.

Katz RJ. 1982. Animal model of depression: pharmacological sensitivity of a hedonic deficit. *Pharmacol Biochem Behav* 16:965–968.

Kayser V, Christensen D. 2000. Antinociceptive effect of systemic gabapentin in mononeuropathic rats, depends on stimulus characteristics and level of test integration. *Pain* 88:53–60.

Kc R, Li X, Kroin JS, Liu Z, Chen D, Xiao G, Levine B, Li J, Hamilton JL, Wijnen AJ van, Piel M, Shelly DA, et al. 2016. PKC $\delta$  null mutations in a mouse model of osteoarthritis alter osteoarthritic pain independently of joint pathology by augmenting NGF/TrkA-induced axonal outgrowth. *Ann Rheum Dis* 75:2133–2141.

Keeble J, Russell F, Curtis B, Starr A, Pinter E, Brain SD. 2005. Involvement of transient receptor potential vanilloid 1 in the vascular and hyperalgesic components of joint inflammation. *Arthritis Rheum* 52:3248–3256.

Kehl LJ, Trempe TM, Hargreaves KM. 2000. A new animal model for assessing mechanisms and management of muscle hyperalgesia. *Pain* 85:333–343.

- Kelly KM, Sanga P, Zaki N, Wang S, Haeussler J, Louie J, Thippawong J. 2019. Safety and efficacy of fulranumab in osteoarthritis of the hip and knee: results from four early terminated phase III randomized studies. *Curr Med Res Opin* 35:2117–2127.
- Kepler LD, McDiarmid TA, Rankin CH. 2020. Habituation in high-throughput genetic model organisms as a tool to investigate the mechanisms of neurodevelopmental disorders. *Neurobiol Learn Mem* 171:107208.
- Keswani SC, Jack C, Zhou C, Höke A. 2006. Establishment of a rodent model of HIV-associated sensory neuropathy. *J Neurosci Off J Soc Neurosci* 26:10299–10304.
- Key B. 2015. Fish do not feel pain and its implications for understanding phenomenal consciousness. *Biol Philos* 30:149–165.
- Khakh BS, North RA. 2006. P2X receptors as cell-surface ATP sensors in health and disease. *Nature* 442:527–532.
- Khuong TM, Neely GG. 2013. Conserved systems and functional genomic assessment of nociception. *FEBS J* 280:5298–5306.
- Khuong TM, Wang Q-P, Manion J, Oyston LJ, Lau M-T, Towler H, Lin YQ, Neely GG. 2019. Nerve injury drives a heightened state of vigilance and neuropathic sensitization in *Drosophila*. *Sci Adv* 5:eaaw4099.
- Kim SH, Chung JM. 1992. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain* 50:355–363.
- King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. 2009. Unmasking the tonic-aversive state in neuropathic pain. *Nat Neurosci* 12:1364–1366.
- Kiso T, Watabiki T, Tsukamoto M, Okabe M, Kagami M, Nishimura K, Aoki T, Matsuoka N. 2008. Pharmacological characterization and gene expression profiling of an L5/L6 spinal nerve ligation model for neuropathic pain in mice. *Neuroscience* 153:492–500.
- Klinck MP, Mogil JS, Moreau M, Lascelles BDX, Flecknell PA, Poitte T, Troncy E. 2017. Translational pain assessment: could natural animal models be the missing link? *Pain* 158:1633–1646.
- Knight B, Katz DR, Isenberg DA, Ibrahim MA, Le Page S, Hutchings P, Schwartz RS, Cooke A. 1992. Induction of adjuvant arthritis in mice. *Clin Exp Immunol* 90:459–465.
- Knights CB, Gentry C, Bevan S. 2012. Partial medial meniscectomy produces osteoarthritis pain-related behaviour in female C57BL/6 mice. *Pain* 153:281–292.
- Knyihár-Csillik E, Tajti J, Samsam M, Sárosi G, Slezák S, Vécsei L. 1997. Effect of a serotonin agonist (sumatriptan) on the peptidergic innervation of the rat cerebral dura mater and on the expression of c-fos in the caudal trigeminal nucleus in an experimental migraine model. *J Neurosci Res* 48:449–464.
- Koehler PJ, Endtz LJ. 1986. The Brown-Séquard syndrome. True or false? *Arch Neurol* 43:921–924.

Konno S, Oda N, Ochiai T, Alev L. 2016. Randomized, Double-blind, Placebo-controlled Phase III Trial of Duloxetine Monotherapy in Japanese Patients With Chronic Low Back Pain. *Spine* 41:1709–1717.

Koyama S, Katayama Y, Maejima S, Hirayama T, Fujii M, Tsubokawa T. 1993. Thalamic neuronal hyperactivity following transection of the spinothalamic tract in the cat: involvement of N-methyl-D-aspartate receptor. *Brain Res* 612:345–350.

Kremer M, Becker LJ, Barrot M, Yalcin I. 2020a. How to study anxiety and depression in rodent models of chronic pain? *Eur J Neurosci* n/a:

Kremer M, Becker LJ, Barrot M, Yalcin I. 2020b. How to study anxiety and depression in rodent models of chronic pain? *Eur J Neurosci*.

Kroenke K, Bair MJ, Damush TM, Wu J, Hoke S, Sutherland J, Tu W. 2009. Optimized antidepressant therapy and pain self-management in primary care patients with depression and musculoskeletal pain: a randomized controlled trial. *JAMA* 301:2099–2110.

Kuan Y-H, Shyu B-C. 2016. Nociceptive transmission and modulation via P2X receptors in central pain syndrome. *Mol Brain* 9:58.

Kuner R, Flor H. 2016. Structural plasticity and reorganisation in chronic pain. *Nat Rev Neurosci* 18:20–30.

Kupers RC, Chen CC, Bushnell MC. 1997. A model of transient hyperalgesia in the behaving monkey induced by topical application of capsaicin. *Pain* 72:269–275.

LaBuda CJ, Little PJ. 2005. Pharmacological evaluation of the selective spinal nerve ligation model of neuropathic pain in the rat. *J Neurosci Methods* 144:175–181.

Laffray S, Bouali-Benazzouz R, Papon M-A, Favereaux A, Jiang Y, Holm T, Spriet C, Desbarats P, Fossat P, Le Feuvre Y, Decossas M, Héliot L, et al. 2012. Impairment of GABAB receptor dimer by endogenous 14-3-3 $\zeta$  in chronic pain conditions. *EMBO J* 31:3239–3251.

Laird J, Martinez-Caro L, Garcia-Nicas E, Cervero F. 2001a. A new model of visceral pain and referred hyperalgesia in the mouse. *Pain* 92:335–342.

Laird JM, Martinez-Caro L, Garcia-Nicas E, Cervero F. 2001b. A new model of visceral pain and referred hyperalgesia in the mouse. *Pain* 92:335–342.

Lambert DG. 2009. Capsaicin receptor antagonists: a promising new addition to the pain clinic. *Br J Anaesth* 102:153–155.

Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, et al. 2010. Coding of facial expressions of pain in the laboratory mouse. *Nat Methods* 7:447–449.

Lapointe TK, Basso L, Iftinca MC, Flynn R, Chapman K, Dietrich G, Vergnolle N, Altier C. 2015. TRPV1 sensitization mediates postinflammatory visceral pain following acute colitis. *Am J Physiol Gastrointest Liver Physiol* 309:G87-99.

Larauche M, Mulak A, Taché Y. 2012. Stress and visceral pain: from animal models to clinical therapies. *Exp Neurol* 233:49–67.

Lascelles BDX, Brown DC, Maixner W, Mogil JS. 2018. Spontaneous painful disease in companion animals can facilitate the development of chronic pain therapies for humans. *Osteoarthritis Cartilage* 26:175–183.

Le Bars D, Gozariu M, Cadden SW. 2001. Animal models of nociception. *Pharmacol Rev* 53:597–652.

Lee-Liu D, Méndez-Olivos EE, Muñoz R, Larraín J. 2017. The African clawed frog *Xenopus laevis*: A model organism to study regeneration of the central nervous system. *Neurosci Lett* 652:82–93.

Leite-Almeida H, Pinto-Ribeiro F, Almeida A. 2015. Animal Models for the Study of Comorbid Pain and Psychiatric Disorders. *Mod Trends Pharmacopsychiatry* 30:1–21.

Lembeck F, Donnerer J. 1981. Time course of capsaicin-induced functional impairments in comparison with changes in neuronal substance P content. *Naunyn Schmiedebergs Arch Pharmacol* 316:240–243.

Lesnak J, Sluka KA. 2019. Chronic non-inflammatory muscle pain: central and peripheral mediators. *Curr Opin Physiol* 11:67–74.

Leung K, Mohammadi A, Ryu WS, Nemenman I. 2016. Stereotypical Escape Behavior in *Caenorhabditis elegans* Allows Quantification of Effective Heat Stimulus Level. *PLoS Comput Biol* 12:.

Liepe K, Geidel H, Haase M, Hakenberg OW, Runge R, Kotzerke J. 2005. New model for the induction of osteoblastic bone metastases in rat. *Anticancer Res* 25:1067–1073.

Lindsay TH, Jonas BM, Sevcik MA, Kubota K, Halvorson KG, Ghilardi JR, Kuskowski MA, Stelow EB, Mukherjee P, Gendler SJ, Wong GY, Mantyh PW. 2005. Pancreatic cancer pain and its correlation with changes in tumor vasculature, macrophage infiltration, neuronal innervation, body weight and disease progression. *Pain* 119:233–246.

Lister RG. 1987. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl)* 92:180–185.

Liu M-G, Chen J. 2014. Preclinical research on pain comorbidity with affective disorders and cognitive deficits: Challenges and perspectives. *Prog Neurobiol* 116:13–32.

Liu Y, Li Y, Liu Q, Wu Z, Cui J, Zhu K, Zhao H, Zhou C, Shi B. 2019. Role of GM-CSF in a mouse model of experimental autoimmune prostatitis. *Am J Physiol Renal Physiol* 317:F23–F29.

Lomax AE, Pradhananga S, Sessenwein JL, O'Malley D. 2019. Bacterial modulation of visceral sensation: mediators and mechanisms. *Am J Physiol Gastrointest Liver Physiol* 317:G363–G372.

López-González MJ, Soula A, Landry M, Favereaux A. 2018. Oxaliplatin treatment impairs extension of sensory neuron neurites in vitro through miR-204 overexpression. *Neurotoxicology* 68:91–100.

Lopez-Luna J, Al-Jubouri Q, Al-Nuaimy W, Sneddon LU. 2017. Reduction in activity by noxious chemical stimulation is ameliorated by immersion in analgesic drugs in zebrafish. *J Exp Biol* 220:1451–1458.

Lorenzo L-E, Godin AG, Ferrini F, Bachand K, Plasencia-Fernandez I, Labrecque S, Girard AA, Boudreau D, Kianicka I, Gagnon M, Doyon N, Ribeiro-da-Silva A, et al. 2020. Enhancing neuronal chloride extrusion rescues  $\alpha 2/\alpha 3$  GABAA-mediated analgesia in neuropathic pain. *Nat Commun* 11:869.

Louwies T, Ligon CO, Johnson AC, Greenwood-Van Meerveld B. 2019. Targeting epigenetic mechanisms for chronic visceral pain: A valid approach for the development of novel therapeutics. *Neurogastroenterol Motil Off J Eur Gastrointest Motil Soc* 31:e13500.

Lucas JM, Ji Y, Masri R. 2011. Motor cortex stimulation reduces hyperalgesia in an animal model of central pain. *Pain* 152:1398–1407.

Lukács M, Haanes KA, Majláth Z, Tajti J, Vécsei L, Warfvinge K, Edvinsson L. 2015. Dural administration of inflammatory soup or Complete Freund's Adjuvant induces activation and inflammatory response in the rat trigeminal ganglion. *J Headache Pain* 16:564.

Lynn B, Ye W, Cotsell B. 1992. The actions of capsaicin applied topically to the skin of the rat on C-fibre afferents, antidromic vasodilatation and substance P levels. *Br J Pharmacol* 107:400–406.

M Keppel Hesselink J. 2016. Arguments to develop TRPV1 antagonist in neuropathic pain. Lessons for drug development. *Clin Res Trials* 3:.

MacEwen EG. 1990. Spontaneous tumors in dogs and cats: models for the study of cancer biology and treatment. *Cancer Metastasis Rev* 9:125–136.

Malafoglia V, Colasanti M, Raffaelli W, Balciunas D, Giordano A, Bellipanni G. 2014. Extreme Thermal Noxious Stimuli Induce Pain Responses in Zebrafish Larvae. *J Cell Physiol* 229:300–308.

Malfait AM, Little CB, McDougall JJ. 2013. A commentary on modelling osteoarthritis pain in small animals. *Osteoarthritis Cartilage* 21:1316–1326.

Malmberg AB, Basbaum AI. 1998. Partial sciatic nerve injury in the mouse as a model of neuropathic pain: behavioral and neuroanatomical correlates. *Pain* 76:215–222.

Manda P, Kushwaha AS, Kundu S, Shivakumar HN, Jo SB, Murthy SN. 2016. Delivery of ziconotide to cerebrospinal fluid via intranasal pathway for the treatment of chronic pain. *J Control Release Off J Control Release Soc* 224:69–76.

Mansfield C, Beths T. 2015. Management of acute pancreatitis in dogs: a critical appraisal with focus on feeding and analgesia. *J Small Anim Pract* 56:27–39.

Mapplebeck JCS, Dalgarno R, Tu Y, Moriarty O, Beggs S, Kwok CHT, Halievski K, Assi S, Mogil JS, Trang T, Salter MW. 2018. Microglial P2X4R-evoked pain hypersensitivity is sexually dimorphic in rats. *Pain* 159:1752–1763.

Markaki M, Tavernarakis N. 2020. *Caenorhabditis elegans* as a model system for human diseases. *Curr Opin Biotechnol* 63:118–125.

- Marmiroli P, Riva B, Pozzi E, Ballarini E, Lim D, Chiorazzi A, Meregalli C, Distasi C, Renn CL, Semperboni S, Morosi L, Ruffinatti FA, et al. 2017. Susceptibility of different mouse strains to oxaliplatin peripheral neurotoxicity: Phenotypic and genotypic insights. *PLoS One* 12:e0186250.
- Martin LJ, Acland EL, Cho C, Gandhi W, Chen D, Corley E, Kadoura B, Levy T, Mirali S, Tohyama S, Khan S, MacIntyre LC, et al. 2019. Male-Specific Conditioned Pain Hypersensitivity in Mice and Humans. *Curr Biol* CB 29:192-201.e4.
- Masri R, Quiton RL, Lucas JM, Murray PD, Thompson SM, Keller A. 2009. Zona incerta: a role in central pain. *J Neurophysiol* 102:181–191.
- Matsumura Y, Yamashita T, Sasaki A, Nakata E, Kohno K, Masuda T, Tozaki-Saitoh H, Imai T, Kuraishi Y, Tsuda M, Inoue K. 2016. A novel P2X4 receptor-selective antagonist produces anti-allodynic effect in a mouse model of herpetic pain. *Sci Rep* 6:32461.
- Mayer S, Spickschen J, Stein KV, Crevenna R, Dorner TE, Simon J. 2019. The societal costs of chronic pain and its determinants: The case of Austria. *PLoS One* 14:e0213889.
- McCoy AM. 2015. Animal Models of Osteoarthritis: Comparisons and Key Considerations. *Vet Pathol* 52:803–818.
- McDougall JJ, Karimian SM, Ferrell WR. 1995. Prolonged alteration of vasoconstrictor and vasodilator responses in rat knee joints by adjuvant monoarthritis. *Exp Physiol* 80:349–357.
- McDowell GC, Pope JE. 2016. Intrathecal Ziconotide: Dosing and Administration Strategies in Patients With Refractory Chronic Pain. *Neuromodulation J Int Neuromodulation Soc* 19:522–532.
- McGivern JG. 2007. Ziconotide: a review of its pharmacology and use in the treatment of pain. *Neuropsychiatr Dis Treat* 3:69–85.
- McIlwraith CW, Frisbie DD, Kawcak CE. 2012. The horse as a model of naturally occurring osteoarthritis. *Bone Jt Res* 1:297–309.
- McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, Hayward NJ, Chong JA, Julius D, Moran MM, Fanger CM. 2007. TRPA1 mediates formalin-induced pain. *Proc Natl Acad Sci U S A* 104:13525–13530.
- McNamee KE, Burleigh A, Gompels LL, Feldmann M, Allen SJ, Williams RO, Dawbarn D, Vincent TL, Inglis JJ. 2010. Treatment of murine osteoarthritis with TrkAd5 reveals a pivotal role for nerve growth factor in non-inflammatory joint pain. *Pain* 149:386–392.
- Medhurst SJ, Walker K, Bowes M, Kidd BL, Glatt M, Muller M, Hattenberger M, Vaxelaire J, O'Reilly T, Wotherspoon G, Winter J, Green J, et al. 2002. A rat model of bone cancer pain. *Pain* 96:129–140.
- Meller ST, Gebhart GF. 1997. Intraplantar zymosan as a reliable, quantifiable model of thermal and mechanical hyperalgesia in the rat. *Eur J Pain* 1:43–52.

- Melo-Carrillo A, Lopez-Avila A. 2013. A chronic animal model of migraine, induced by repeated meningeal nociception, characterized by a behavioral and pharmacological approach. *Cephalalgia Int J Headache* 33:1096–1105.
- Mert T, Sahin E, Yaman S, Sahin M. 2018. Pain-Relieving Effectiveness of Co-Treatment with Local Tramadol and Systemic Minocycline in Carrageenan-Induced Inflammatory Pain Model. *Inflammation* 41:1238–1249.
- Milinkeviciute G, Gentile C, Neely GG. 2012. Drosophila as a tool for studying the conserved genetics of pain. *Clin Genet* 82:359–366.
- Millhr RR, Berk AM, Springer AD. 1974. Acquisition and retention of active avoidance in *Xenopus laevis*. *Bull Psychon Soc* 3:139–141.
- Miquel S, Martín R, Lashermes A, Gillet M, Meleine M, Gelot A, Eschalier A, Ardid D, Bermúdez-Humarán LG, Sokol H, Thomas M, Theodorou V, et al. 2016. Anti-nociceptive effect of *Faecalibacterium prausnitzii* in non-inflammatory IBS-like models. *Sci Rep* 6:19399.
- Miracourt LS, Moisset X, Dallel R, Voisin DL. 2009. Glycine inhibitory dysfunction induces a selectively dynamic, morphine-resistant, and neurokinin 1 receptor- independent mechanical allodynia. *J Neurosci Off J Soc Neurosci* 29:2519–2527.
- Mishra A, Guo Y, Zhang L, More S, Weng T, Chintagari NR, Huang C, Liang Y, Pushparaj S, Gou D, Breshears M, Liu L. 2016. A Critical Role for P2X7 Receptor-Induced VCAM-1 Shedding and Neutrophil Infiltration during Acute Lung Injury. *J Immunol Baltim Md* 195:2828–2837.
- Miyagi M, Ishikawa T, Kamoda H, Suzuki M, Inoue G, Sakuma Y, Oikawa Y, Orita S, Uchida K, Takahashi K, Takaso M, Ohtori S. 2017. Efficacy of nerve growth factor antibody in a knee osteoarthritis pain model in mice. *BMC Musculoskelet Disord* 18:428.
- Mogil JS. 2009. Animal models of pain: progress and challenges. *Nat Rev Neurosci* 10:283–294.
- Mogil JS, Davis KD, Derbyshire SW. 2010. The necessity of animal models in pain research. *Pain* 151:12–17.
- Monteiro BP, Lorimier L-P de, Moreau M, Beauchamp G, Blair J, Lussier B, Pelletier J-P, Troncy E. 2018. Pain characterization and response to palliative care in dogs with naturally-occurring appendicular osteosarcoma: An open label clinical trial. *PLoS One* 13:e0207200.
- Moreau M, Lussier B, Ballaz L, Troncy E. 2014. Kinetic measurements of gait for osteoarthritis research in dogs and cats. *Can Vet J Rev Veterinaire Can* 55:1057–1065.
- Morgan PG, Kayser E-B, Sedensky MM. 2007. C. *Elegans* and Volatile Anesthetics. *WormBook*.
- Muley MM, Krustev E, McDougall JJ. 2016. Preclinical Assessment of Inflammatory Pain. *CNS Neurosci Ther* 22:88–101.
- Munro G, Jansen-Olesen I, Olesen J. 2017. Animal models of pain and migraine in drug discovery. *Drug Discov Today* 22:1103–1111.

Na HS, Han JS, Ko KH, Hong SK. 1994. A behavioral model for peripheral neuropathy produced in rat's tail by inferior caudal trunk injury. *Neurosci Lett* 177:50–52.

Nagasaka K, Takashima I, Matsuda K, Higo N. 2020. Brain activity changes in a monkey model of central post-stroke pain. *Exp Neurol* 323:113096.

Nakao K. 2019. Translational science: Newly emerging science in biology and medicine - Lessons from translational research on the natriuretic peptide family and leptin. *Proc Jpn Acad Ser B Phys Biol Sci* 95:538–567.

Nandakumar KS, Svensson L, Holmdahl R. 2003. Collagen Type II-Specific Monoclonal Antibody-Induced Arthritis in Mice. *Am J Pathol* 163:1827–1837.

Narita M, Kaneko C, Miyoshi K, Nagumo Y, Kuzumaki N, Nakajima M, Nanjo K, Matsuzawa K, Yamazaki M, Suzuki T. 2006a. Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* 31:739–750.

Narita M, Kaneko C, Miyoshi K, Nagumo Y, Kuzumaki N, Nakajima M, Nanjo K, Matsuzawa K, Yamazaki M, Suzuki T. 2006b. Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* 31:739–750.

Naseri K, Saghaei E, Abbaszadeh F, Afhami M, Haeri A, Rahimi F, Jorjani M. 2013. Role of microglia and astrocyte in central pain syndrome following electrolytic lesion at the spinothalamic tract in rats. *J Mol Neurosci* 49:470–479.

Navratilova E, Morimura K, Xie JY, Atcherley CW, Ossipov MH, Porreca F. 2016. Positive emotions and brain reward circuits in chronic pain. *J Comp Neurol* 524:1646–1652.

Neely GG, Hess A, Costigan M, Keene AC, Goulas S, Langeslag M, Griffin RS, Belfer I, Dai F, Smith S, Diatchenko L, Gupta V, et al. 2010. A genome-wide *Drosophila* screen for heat nociception identifies  $\alpha 2\delta 3$  as an evolutionary-conserved pain gene. *Cell* 143:628–638.

Neely GG, Keene AC, Duchek P, Chang EC, Wang Q-P, Aksoy YA, Rosenzweig M, Costigan M, Woolf CJ, Garrity PA, Penninger JM. 2011. TrpA1 regulates thermal nociception in *Drosophila*. *PLoS One* 6:e24343.

Negus SS, Vanderah TW, Brandt MR, Bilsky EJ, Becerra L, Borsook D. 2006. Preclinical assessment of candidate analgesic drugs: recent advances and future challenges. *J Pharmacol Exp Ther* 319:507–514.

Ness TJ. 1999. Models of Visceral Nociception. *ILAR J* 40:119–128.

Ness TJ, Gebhart GF. 1988. Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudoaffective reflexes in the rat. *Brain Res* 450:153–169.

Nielsen CK, Lewis RJ, Alewood D, Drinkwater R, Palant E, Patterson M, Yaksh TL, McCumber D, Smith MT. 2005. Anti-allodynic efficacy of the  $\chi$ -conopeptide, Xen2174, in rats with neuropathic pain. *Pain* 118:112–124.

- Nisar A, Akhter N, Singh G, Masood A, Malik A, Banday B, Zargar MA. 2015. Modulation of T-helper cytokines and inflammatory mediators by *Atropa accuminata*. Royle in adjuvant induced arthritic tissues. *J Ethnopharmacol* 162:215–224.
- Nishimoto-Kakiuchi A, Netsu S, Okabayashi S, Taniguchi K, Tanimura H, Kato A, Suzuki M, Sankai T, Konno R. 2018. Spontaneous endometriosis in cynomolgus monkeys as a clinically relevant experimental model. *Hum Reprod Oxf Engl* 33:1228–1236.
- Nkambeu B, Salem JB, Leonelli S, Marashi FA, Beaudry F. 2019. EGL-3 and EGL-21 are required to trigger nocifensive response of *Caenorhabditis elegans* to noxious heat. *Neuropeptides* 73:41–48.
- Nooten F van, Treur M, Pantiri K, Stoker M, Charokopou M. 2017. Capsaicin 8% Patch Versus Oral Neuropathic Pain Medications for the Treatment of Painful Diabetic Peripheral Neuropathy: A Systematic Literature Review and Network Meta-analysis. *Clin Ther* 39:787-803.e18.
- Nozu T, Miyagishi S, Kumei S, Nozu R, Takakusaki K, Okumura T. 2018. Lovastatin inhibits visceral allodynia and increased colonic permeability induced by lipopolysaccharide or repeated water avoidance stress in rats. *Eur J Pharmacol* 818:228–234.
- Nozu T, Miyagishi S, Kumei S, Nozu R, Takakusaki K, Okumura T. 2019. Metformin inhibits visceral allodynia and increased gut permeability induced by stress in rats. *J Gastroenterol Hepatol* 34:186–193.
- O'Brien, P.D.; Sakowski, S.A.; Feldman, E.L. Mouse models of diabetic neuropathy. *ILAR J.* 2014, 54, 259–272.
- Ochoa-Cortes F, Liñán-Rico A, Jacobson KA, Christofi FL. 2014. Potential for developing purinergic drugs for gastrointestinal diseases. *Inflamm Bowel Dis* 20:1259–1287.
- Ogren SO, Berge OG. 1984. Test-dependent variations in the antinociceptive effect of p-chloroamphetamine-induced release of 5-hydroxytryptamine. *Neuropharmacology* 23:915–924.
- Oh J, Lee S, Kim A, Yoon J, Jang K, Lee DH, Cho S, Lee SR, Yu K-S, Chung J-Y. 2018. Safety, Tolerability, and Pharmacokinetic Characteristics of a Novel Nonopioid Analgesic, VVZ-149 Injections in Healthy Volunteers: A First-in-Class, First-in-Human Study. *J Clin Pharmacol* 58:64–73.
- Olesen J, Burstein R, Ashina M, Tfelt-Hansen P. 2009. Origin of pain in migraine: evidence for peripheral sensitisation. *Lancet Neurol* 8:679–690.
- O'Mahony SM, Tramullas M, Fitzgerald P, Cryan JF. 2012. Rodent models of colorectal distension. *Curr Protoc Neurosci* Chapter 9:Unit 9.40.
- O'Malley D, Julio-Pieper M, O'Mahony SM, Dinan TG, Cryan JF. 2014. Differential visceral pain sensitivity and colonic morphology in four common laboratory rat strains. *Exp Physiol* 99:359–367.
- Ossipov MH, Morimura K, Porreca F. 2014. Descending pain modulation and chronification of pain. *Curr Opin Support Palliat Care* 8:143–151.
- Otterness IG, Moore PF. 1988. Carrageenan foot edema test. *Methods Enzymol* 162:320–327.

- Ozaki N, Bielefeldt K, Sengupta JN, Gebhart GF. 2002. Models of gastric hyperalgesia in the rat. *Am J Physiol Gastrointest Liver Physiol* 283:G666-676.
- Palecek J, Dougherty PM, Kim SH, Palecková V, Lekan H, Chung JM, Carlton SM, Willis WD. 1992. Responses of spinothalamic tract neurons to mechanical and thermal stimuli in an experimental model of peripheral neuropathy in primates. *J Neurophysiol* 68:1951–1966.
- Panksepp JB, Lahvis GP. 2011. Rodent empathy and affective neuroscience. *Neurosci Biobehav Rev* 35:1864–1875.
- Patel R, Montagut-Bordas C, Dickenson AH. 2018. Calcium channel modulation as a target in chronic pain control. *Br J Pharmacol* 175:2173–2184.
- Pelletier JP, Boileau C, Altman RD, Martel-Pelletier J. 2010. Experimental models of osteoarthritis: Usefulness in the development of disease-modifying osteoarthritis drugs/agents. *Therapy* 7:621–634.
- Pellow S, Chopin P, File SE, Briley M. 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* 14:149–167.
- Pezalla PD. 1983. Morphine-induced analgesia and explosive motor behavior in an amphibian. *Brain Res* 273:297–305.
- Phillips CJ. 2006. Economic burden of chronic pain. *Expert Rev Pharmacoecon Outcomes Res* 6:591–601.
- Phillips TJC, Brown M, Ramirez JD, Perkins J, Woldeamanuel YW, Williams AC de C, Orengo C, Bennett DLH, Bodi I, Cox S, Maier C, Krumova EK, et al. 2014. Sensory, psychological, and metabolic dysfunction in HIV-associated peripheral neuropathy: A cross-sectional deep profiling study. *Pain* 155:1846–1860.
- Piel MJ, Kroin JS, Wijnen AJ van, Kc R, Im H-J. 2014. Pain assessment in animal models of osteoarthritis. *Gene* 537:184–188.
- Pitcher GM, Ritchie J, Henry JL. 1999. Paw withdrawal threshold in the von Frey hair test is influenced by the surface on which the rat stands. *J Neurosci Methods* 87:185–193.
- Porsolt RD, Bertin A, Jalfre M. 1977a. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 229:327–336.
- Porsolt RD, Le Pichon M, Jalfre M. 1977b. Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266:730–732.
- Pradhan AA, Smith ML, McGuire B, Tarash I, Evans CJ, Charles A. 2014. Characterization of a novel model of chronic migraine. *Pain* 155:269–274.
- Pratt KG, Khakhalin AS. 2013. Modeling human neurodevelopmental disorders in the *Xenopus* tadpole: from mechanisms to therapeutic targets. *Dis Model Mech* 6:1057–1065.
- Preguiça I, Alves A, Nunes S, Gomes P, Fernandes R, Viana SD, Reis F. Diet-Induced Rodent Models of Diabetic Peripheral Neuropathy, Retinopathy and Nephropathy. *Nutrients*. 2020 Jan 18;12(1):250.

- Pusceddu MM, Gareau MG. 2018. Visceral pain: gut microbiota, a new hope? *J Biomed Sci* 25:73.
- Qaseem A, Wilt TJ, McLean RM, Forciea MA, Clinical Guidelines Committee of the American College of Physicians. 2017. Noninvasive Treatments for Acute, Subacute, and Chronic Low Back Pain: A Clinical Practice Guideline From the American College of Physicians. *Ann Intern Med* 166:514–530.
- Quick ML, Wong L, Mukherjee S, Done JD, Schaeffer AJ, Thumbikat P. 2013. Th1-Th17 cells contribute to the development of uropathogenic *Escherichia coli*-induced chronic pelvic pain. *PLoS One* 8:e60987.
- Quiton RL, Masri R, Thompson SM, Keller A. 2010. Abnormal activity of primary somatosensory cortex in central pain syndrome. *J Neurophysiol* 104:1717–1725.
- Raboisson P, Dallel R. 2004. The orofacial formalin test. *Neurosci Biobehav Rev* 28:219–226.
- Radat F, Margot-Duclot A, Attal N. 2013. Psychiatric co-morbidities in patients with chronic peripheral neuropathic pain: a multicentre cohort study. *Eur J Pain Lond Engl* 17:1547–1557.
- Radhakrishnan R, Moore SA, Sluka KA. 2003. Unilateral carrageenan injection into muscle or joint induces chronic bilateral hyperalgesia in rats. *Pain* 104:567–577.
- Ramachandran R, Bhatt DK, Ploug KB, Hay-Schmidt A, Jansen-Olesen I, Gupta S, Olesen J. 2014. Nitric oxide synthase, calcitonin gene-related peptide and NK-1 receptor mechanisms are involved in GTN-induced neuronal activation. *Cephalalgia Int J Headache* 34:136–147.
- Rambukkana A, Zanazzi G, Tapinos N, Salzer JL. 2002. Contact-dependent demyelination by *Mycobacterium leprae* in the absence of immune cells. *Science* 296:927–931.
- Ramsey IS, Delling M, Clapham DE. 2006. An introduction to TRP channels. *Annu Rev Physiol* 68:619–647.
- Randall LO, Selitto JJ. 1957. A method for measurement of analgesic activity on inflamed tissue. *Arch Int Pharmacodyn Ther* 111:409–419.
- Raputova J, Srotova I, Vlckova E, Sommer C, Üçeyler N, Birklein F, Rittner HL, Reborn C, Adamova B, Kovalova I, Kralickova N, Kvakpilova E, Forer L, et al. 2017. Sensory phenotype and risk factors for painful diabetic neuropathy: a cross-sectional observational study. *Pain* 158:2340–2353.
- Rein K, Zöckler M, Mader MT, Grübel C, Heisenberg M. 2002. The *Drosophila* standard brain. *Curr Biol CB* 12:227–231.
- Ren K, Dubner R. 1999. Inflammatory Models of Pain and Hyperalgesia. *ILAR J* 40:111–118.
- Renn CL, Carozzi VA, Rhee P, Gallop D, Dorsey SG, Cavaletti G. 2011. Multimodal assessment of painful peripheral neuropathy induced by chronic oxaliplatin-based chemotherapy in mice. *Mol Pain* 7:29.
- Rice ASC, Finnerup NB, Kemp HI, Currie GL, Baron R. 2018. Sensory profiling in animal models of neuropathic pain: a call for back-translation. *Pain* 159:819–824.

- Rosen SF, Ham B, Haichin M, Walters IC, Tohyama S, Sotocinal SG, Mogil JS. 2019. Increased pain sensitivity and decreased opioid analgesia in T-cell-deficient mice and implications for sex differences. *Pain* 160:358–366.
- Rothman GR, Blackiston DJ, Levin M. 2016. Color and intensity discrimination in *Xenopus laevis* tadpoles. *Anim Cogn* 19:911–919.
- Roughan JV, Bertrand HGMJ, Isles HM. 2016. Meloxicam prevents COX-2-mediated post-surgical inflammation but not pain following laparotomy in mice. *Eur J Pain Lond Engl* 20:231–240.
- Salomon B, Rhee L, Bour-Jordan H, Hsin H, Montag A, Soliven B, Arcella J, Girvin AM, Padilla J, Miller SD, Bluestone JA. 2001. Development of spontaneous autoimmune peripheral polyneuropathy in B7-2-deficient NOD mice. *J Exp Med* 194:677–684.
- Sanga P, Katz N, Polverejan E, Wang S, Kelly KM, Haeussler J, Thippawong J. 2017. Long-Term Safety and Efficacy of Fulranumab in Patients With Moderate-to-Severe Osteoarthritis Pain: A Phase II Randomized, Double-Blind, Placebo-Controlled Extension Study. *Arthritis Rheumatol Hoboken NJ* 69:763–773.
- Sanoja R, Tortorici V, Fernandez C, Price TJ, Cervero F. 2010. Role of RVM neurons in capsaicin-evoked visceral nociception and referred hyperalgesia. *Eur J Pain Lond Engl* 14:120.e1-120.e9.
- Santos-Nogueira E, Redondo Castro E, Mancuso R, Navarro X. 2012. Randall-Selitto Test: A New Approach for the Detection of Neuropathic Pain after Spinal Cord Injury. *J Neurotrauma* 29:898–904.
- Sapio MR, Neubert JK, LaPaglia DM, Maric D, Keller JM, Raithe SJ, Rohrs EL, Anderson EM, Butman JA, Caudle RM, Brown DC, Heiss JD, et al. 2018. Pain control through selective chemo-axotomy of centrally projecting TRPV1+ sensory neurons. *J Clin Invest* 128:1657–1670.
- Sassen WA, Köster RW. 2015. Advances in Genomics and Genetics. <https://www.dovepress.com/a-molecular-toolbox-for-genetic-manipulation-of-zebrafish-peer-reviewed-article-AGG>. Accessed April 2, 2020.
- Schachtschneider KM, Schwind RM, Newson J, Kinachtchouk N, Rizko M, Mendoza-Elias N, Grippo P, Principe DR, Park A, Overgaard NH, Jungersen G, Garcia KD, et al. 2017. The Oncopig Cancer Model: An Innovative Large Animal Translational Oncology Platform. *Front Oncol* 7:190.
- Schneider LE, Henley KY, Turner OA, Pat B, Niedzielko TL, Floyd CL. 2017. Application of the Rat Grimace Scale as a Marker of Supraspinal Pain Sensation after Cervical Spinal Cord Injury. *J Neurotrauma* 34:2982–2993.
- Schnitzer TJ, Ekman EF, Spierings ELH, Greenberg HS, Smith MD, Brown MT, West CR, Verburg KM. 2015. Efficacy and safety of tanezumab monotherapy or combined with non-steroidal anti-inflammatory drugs in the treatment of knee or hip osteoarthritis pain. *Ann Rheum Dis* 74:1202–1211.
- Schroeder CI, Craik DJ. 2012. Therapeutic potential of conopeptides. *Future Med Chem* 4:1243–1255.
- Schwartz ES, Gebhart GF. 2014. Visceral pain. *Curr Top Behav Neurosci* 20:171–197.

Schwei MJ, Honore P, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, Clohisy DR, Mantyh PW. 1999. Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. *J Neurosci Off J Soc Neurosci* 19:10886–10897.

Sellmeijer J, Mathis V, Hugel S, Li X-H, Song Q, Chen Q-Y, Barthas F, Lutz P-E, Karatas M, Luthi A, Veinante P, Aertsen A, et al. 2018. Hyperactivity of Anterior Cingulate Cortex Areas 24a/24b Drives Chronic Pain-Induced Anxiodepressive-like Consequences. *J Neurosci Off J Soc Neurosci* 38:3102–3115.

Seltzer Z, Dubner R, Shir Y. 1990. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain* 43:205–218.

Sevcik MA, Ghilardi JR, Peters CM, Lindsay TH, Halvorson KG, Jonas BM, Kubota K, Kuskowski MA, Boustany L, Shelton DL, Mantyh PW. 2005. Anti-NGF therapy profoundly reduces bone cancer pain and the accompanying increase in markers of peripheral and central sensitization. *Pain* 115:128–141.

Shepherd JK, Grewal SS, Fletcher A, Bill DJ, Dourish CT. 1994. Behavioural and pharmacological characterisation of the elevated “zero-maze” as an animal model of anxiety. *Psychopharmacology (Berl)* 116:56–64.

Siddall P, Xu CL, Cousins M. 1995. Allodynia following traumatic spinal cord injury in the rat. *Neuroreport* 6:1241–1244.

Sikandar S, Dickenson AH. 2012. Visceral Pain – the Ins and Outs, the Ups and Downs. *Curr Opin Support Palliat Care* 6:17–26.

Simjee SU, Jawed H, Quadri J, Saeed SA. 2007. Quantitative gait analysis as a method to assess mechanical hyperalgesia modulated by disease-modifying antirheumatoid drugs in the adjuvant-induced arthritic rat. *Arthritis Res Ther* 9:R91.

Simmons JK, Hildreth BE, Supsavhad W, Elshafae SM, Hassan BB, Dirksen WP, Toribio RE, Rosol TJ. 2015. Animal Models of Bone Metastasis. *Vet Pathol* 52:827–841.

Simpson LA, Eng JJ, Hsieh JTC, Wolfe DL, Spinal Cord Injury Rehabilitation Evidence Scire Research Team. 2012. The health and life priorities of individuals with spinal cord injury: a systematic review. *J Neurotrauma* 29:1548–1555.

Skljarevski V, Desai D, Liu-Seifert H, Zhang Q, Chappell AS, Detke MJ, Iyengar S, Atkinson JH, Backonja M. 2010a. Efficacy and safety of duloxetine in patients with chronic low back pain. *Spine* 35:E578-585.

Skljarevski V, Zhang S, Desai D, Alaka KJ, Palacios S, Miazgowski T, Patrick K. 2010b. Duloxetine versus placebo in patients with chronic low back pain: a 12-week, fixed-dose, randomized, double-blind trial. *J Pain Off J Am Pain Soc* 11:1282–1290.

Slatkin N, Zaki N, Sanga P, Wang S, Louie J, Kelly K, Thippawong J. 2016. (382) Efficacy, safety, and tolerability of Fulranumab as adjunctive therapy for cancer-related pain: a randomized, double-blind, placebo-controlled, multicenter study. *J Pain* 17:S70–S71.

Slosky LM, Largent-Milnes TM, Vanderah TW. 2015. Use of Animal Models in Understanding Cancer-induced Bone Pain. *Cancer Growth Metastasis* 8:47–62.

- Sluka KA, Clauw DJ. 2016. Neurobiology of fibromyalgia and chronic widespread pain. *Neuroscience* 338:114–129.
- Sluka KA, Kalra A, Moore SA. 2001. Unilateral intramuscular injections of acidic saline produce a bilateral, long-lasting hyperalgesia. *Muscle Nerve* 24:37–46.
- Smith ESJ, Lewin GR. 2009. Nociceptors: a phylogenetic view. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 195:1089–1106.
- Smith JA, Kitt MM, Morice AH, Birring SS, McGarvey LP, Sher MR, Li Y-P, Wu W-C, Xu ZJ, Muccino DR, Ford AP, Protocol 012 Investigators. 2020. Gefapixant, a P2X3 receptor antagonist, for the treatment of refractory or unexplained chronic cough: a randomised, double-blind, controlled, parallel-group, phase 2b trial. *Lancet Respir Med* 8:775–785.
- Sneddon LU. 2018. Comparative Physiology of Nociception and Pain. *Physiol Bethesda Md* 33:63–73.
- Sneddon LU. 2019. Evolution of nociception and pain: evidence from fish models. *Philos Trans R Soc Lond B Biol Sci* 374:20190290.
- Sneddon LU, Elwood RW, Adamo SA, Leach MC. 2014. Defining and assessing animal pain. *Anim Behav* 97:201–212.
- Sneddon LU, Halsey LG, Bury NR. 2017. Considering aspects of the 3Rs principles within experimental animal biology. *J Exp Biol* 220:3007–3016.
- Sopata M, Katz N, Carey W, Smith MD, Keller D, Verburg KM, West CR, Wolfram G, Brown MT. 2015. Efficacy and safety of tanezumab in the treatment of pain from bone metastases. *Pain* 156:1703–1713.
- Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JCS, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, et al. 2011. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain* 7:55.
- Souslova V, Cesare P, Ding Y, Akopian AN, Stanfa L, Suzuki R, Carpenter K, Dickenson A, Boyce S, Hill R, Nebunius-Oosthuizen D, Smith AJ, et al. 2000. Warm-coding deficits and aberrant inflammatory pain in mice lacking P2X3 receptors. *Nature* 407:1015–1017.
- St Johnston D. 2013. Using mutants, knockdowns, and transgenesis to investigate gene function in *Drosophila*. *Wiley Interdiscip Rev Dev Biol* 2:587–613.
- Stadig S, Lascelles BDX, Nyman G, Bergh A. 2019. Evaluation and comparison of pain questionnaires for clinical screening of osteoarthritis in cats. *Vet Rec* 185:757.
- Starkey ML, Davies M, Yip PK, Carter LM, Wong DJN, McMahon SB, Bradbury EJ. 2009. Expression of the regeneration-associated protein SPRR1A in primary sensory neurons and spinal cord of the adult mouse following peripheral and central injury. *J Comp Neurol* 513:51–68.
- Steel Z, Marnane C, Iranpour C, Chey T, Jackson JW, Patel V, Silove D. 2014. The global prevalence of common mental disorders: a systematic review and meta-analysis 1980–2013. *Int J Epidemiol* 43:476–493.

- Steru L, Chermat R, Thierry B, Simon P. 1985. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* 85:367–370.
- Stevens CW. 2011. Analgesia in amphibians: preclinical studies and clinical applications. *Veterinary Clin North Am Exot Anim Pract* 14:33–44.
- Stevens CW. 2015. Bioinformatics and evolution of vertebrate nociceptin and opioid receptors. *Vitam Horm* 97:57–94.
- Stoop R, Buma P, Kraan PM van der, Hollander AP, Billingham RC, Meijers TH, Poole AR, Berg WB van den. 2001. Type II collagen degradation in articular cartilage fibrillation after anterior cruciate ligament transection in rats. *Osteoarthritis Cartilage* 9:308–315.
- Sung B, Loh HH, Wei L. 2000. Association of kappa opioid receptor mRNA upregulation in dorsal root ganglia with mechanical allodynia in mice following nerve injury. *Neurosci Lett* 291:163–166.
- Suurväli J, Boudinot P, Kanellopoulos J, Rüütel Boudinot S. 2017. P2X4: A fast and sensitive purinergic receptor. *Biomed J* 40:245–256.
- Swierczek NA, Giles AC, Rankin CH, Kerr RA. 2011. High-throughput behavioral analysis in *C. elegans*. *Nat Methods* 8:592–598.
- Takasusuki T, Yaksh TL. 2011. Regulation of Spinal Substance P Release by Intrathecal Calcium Channel Blockade. *Anesthesiology* 115:153–164.
- Tandon P, Conlon F, Furlow JD, Horb ME. 2017. Expanding the genetic toolkit in *Xenopus*: Approaches and opportunities for human disease modeling. *Dev Biol* 426:325–335.
- Tang Y, Peng H, Liao Q, Gan L, Zhang R, Huang L, Ding Z, Yang H, Yan X, Gu Y, Zang X, Huang D, et al. 2016. Study of breakthrough cancer pain in an animal model induced by endothelin-1. *Neurosci Lett* 617:108–115.
- Tappe-Theodor A, Kuner R. 2014. Studying ongoing and spontaneous pain in rodents--challenges and opportunities. *Eur J Neurosci* 39:1881–1890.
- Taylor JC, Dewberry LS, Totsch SK, Yessick LR, DeBerry JJ, Watts SA, Sorge RE. 2017. A novel zebrafish-based model of nociception. *Physiol Behav* 174:83–88.
- Teeple E, Jay GD, Elsaid KA, Fleming BC. 2013a. Animal models of osteoarthritis: challenges of model selection and analysis. *AAPS J* 15:438–446.
- Teeple E, Jay GD, Elsaid KA, Fleming BC. 2013b. Animal models of osteoarthritis: challenges of model selection and analysis. *AAPS J* 15:438–446.
- Thiel I a. M van, Botschuijver S, Jonge WJ de, Seppen J. 2020. Painful interactions: Microbial compounds and visceral pain. *Biochim Biophys Acta Mol Basis Dis* 1866:165534.
- Tirabassi RS, Flanagan JF, Wu T, Kislauskis EH, Birckbichler PJ, Guberski DL. 2004. The BBZDR/Wor rat model for investigating the complications of type 2 diabetes mellitus. *ILAR J* 45:292–302.

- Tjølsen A, Berge O-G, Hunskaar S, Rosland JH, Hole K. 1992. The formalin test: an evaluation of the method. *Pain* 51:5–17.
- Tobin DM, Bargmann CI. 2004. Invertebrate nociception: behaviors, neurons and molecules. *J Neurobiol* 61:161–174.
- Toyka KV. 1999. Eighty three years of the Guillain-Barré syndrome: clinical and immunopathologic aspects, current and future treatments. *Rev Neurol (Paris)* 155:849–856.
- Tracey WD, Wilson RI, Laurent G, Benzer S. 2003. painless, a *Drosophila* gene essential for nociception. *Cell* 113:261–273.
- Trang T, Beggs S, Wan X, Salter MW. 2009. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. *J Neurosci Off J Soc Neurosci* 29:3518–3528.
- Treede R-D, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, Hansson P, Hughes R, Nurmikko T, Serra J. 2008. Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology* 70:1630–1635.
- Trimble N, Johnson AC, Foster A, Greenwood-van Meerveld B. 2007. Corticotropin-releasing factor receptor 1-deficient mice show decreased anxiety and colonic sensitivity. *Neurogastroenterol Motil Off J Eur Gastrointest Motil Soc* 19:754–760.
- Tsuda M. 2017. P2 receptors, microglial cytokines and chemokines, and neuropathic pain. *J Neurosci Res* 95:1319–1329.
- Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K. 2003. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 424:778–783.
- Tucker LB, McCabe JT. 2017. Behavior of Male and Female C57BL/6J Mice Is More Consistent with Repeated Trials in the Elevated Zero Maze than in the Elevated Plus Maze. *Front Behav Neurosci* 11:13.
- Uematsu T, Sakai A, Ito H, Suzuki H. 2011. Intra-articular administration of tachykinin NK<sub>1</sub> receptor antagonists reduces hyperalgesia and cartilage destruction in the inflammatory joint in rats with adjuvant-induced arthritis. *Eur J Pharmacol* 668:163–168.
- Ulmann L, Hatcher JP, Hughes JP, Chaumont S, Green PJ, Conquet F, Buell GN, Reeve AJ, Chessell IP, Rassendren F. 2008. Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. *J Neurosci Off J Soc Neurosci* 28:11263–11268.
- Vaz R, Hofmeister W, Lindstrand A. 2019. Zebrafish Models of Neurodevelopmental Disorders: Limitations and Benefits of Current Tools and Techniques. *Int J Mol Sci* 20:.
- Venken KJT, Bellen HJ. 2014. Chemical mutagens, transposons, and transgenes to interrogate gene function in *Drosophila melanogaster*. *Methods San Diego Calif* 68:15–28.

- Ventzel L, Madsen CS, Karlsson P, Tankisi H, Isak B, Fuglsang-Frederiksen A, Jensen AB, Jensen AR, Jensen TS, Finnerup NB. 2018. Chronic Pain and Neuropathy Following Adjuvant Chemotherapy. *Pain Med* 19:1813–1824.
- Vera-Portocarrero LP, Westlund KN. 2004. Attenuation of nociception in a model of acute pancreatitis by an NK-1 antagonist. *Pharmacol Biochem Behav* 77:631–640.
- Vissers K, Meert T. 2005. A behavioral and pharmacological validation of the acetone spray test in gerbils with a chronic constriction injury. *Anesth Analg* 101:457–464, table of contents.
- Vuralli D, Wattiez A-S, Russo AF, Bolay H. 2019a. Behavioral and cognitive animal models in headache research. *J Headache Pain* 20:11.
- Vuralli D, Wattiez A-S, Russo AF, Bolay H. 2019b. Behavioral and cognitive animal models in headache research. *J Headache Pain* 20:11.
- Vuralli D, Wattiez A-S, Russo AF, Bolay H. 2019c. Behavioral and cognitive animal models in headache research. *J Headache Pain* 20:11.
- Wacnik PW, Eikmeier LJ, Ruggles TR, Ramnaraine ML, Walcheck BK, Beitz AJ, Wilcox GL. 2001. Functional interactions between tumor and peripheral nerve: morphology, algogen identification, and behavioral characterization of a new murine model of cancer pain. *J Neurosci Off J Soc Neurosci* 21:9355–9366.
- Waeber C, Moskowitz MA. 2005. Migraine as an inflammatory disorder. *Neurology* 64:S9-15.
- Wagner T, Poole C, Roth-Daniek A. 2013. The Capsaicin 8% Patch for Neuropathic Pain in Clinical Practice: A Retrospective Analysis. *Pain Med Malden Mass* 14:1202–1211.
- Waksman BH, Adams RD. 1956. A comparative study of experimental allergic neuritis in the rabbit, guinea pig, and mouse. *J Neuropathol Exp Neurol* 15:293–334.
- Walicke PA, Hefti F, Bales R, Lu S-P, Ruckle JL, Brown MT, West CR, Shelton DL. 2018. First-in-human randomized clinical trials of the safety and efficacy of tanezumab for treatment of chronic knee osteoarthritis pain or acute bunionectomy pain. *Pain Rep* 3:e653.
- Walker AK, Kavelaars A, Heijnen CJ, Dantzer R. 2014. Neuroinflammation and Comorbidity of Pain and Depression. *Pharmacol Rev* 66:80–101.
- Wallace VCJ, Norbury TA, Rice ASC. 2005. Ultrasound vocalisation by rodents does not correlate with behavioural measures of persistent pain. *Eur J Pain Lond Engl* 9:445–452.
- Walters ET. 2018. Nociceptive Biology of Molluscs and Arthropods: Evolutionary Clues About Functions and Mechanisms Potentially Related to Pain. *Front Physiol* 9:.
- Walton MB, Cowderoy E, Lascelles D, Innes JF. 2013. Evaluation of construct and criterion validity for the “Liverpool Osteoarthritis in Dogs” (LOAD) clinical metrology instrument and comparison to two other instruments. *PLoS One* 8:e58125.

- Wang B, Chandrasekera PC, Pippin JJ. 2014. Leptin- and leptin receptor-deficient rodent models: relevance for human type 2 diabetes. *Curr Diabetes Rev* 10:131–145.
- Wang D, Stoveken HM, Zucca S, Dao M, Orlandi C, Song C, Masuho I, Johnston C, Opperman KJ, Giles AC, Gill MS, Lundquist EA, et al. 2019. Genetic behavioral screen identifies an orphan anti-opioid system. *Science* 365:1267–1273.
- Wang G, Thompson SM. 2008. Maladaptive homeostatic plasticity in a rodent model of central pain syndrome: thalamic hyperexcitability after spinothalamic tract lesions. *J Neurosci Off J Soc Neurosci* 28:11959–11969.
- Watson BD, Dietrich WD, Busto R, Wachtel MS, Ginsberg MD. 1985. Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann Neurol* 17:497–504.
- Way JC, Chalfie M. 1989. The *mec-3* gene of *Caenorhabditis elegans* requires its own product for maintained expression and is expressed in three neuronal cell types. *Genes Dev* 3:1823–1833.
- Webb MP, Helander EM, Menard BL, Urman RD, Kaye AD. 2018. Tanezumab: a selective humanized mAb for chronic lower back pain. *Ther Clin Risk Manag* 14:361–367.
- Webster RP, Anderson GI, Gearing DP. 2014. Canine Brief Pain Inventory scores for dogs with osteoarthritis before and after administration of a monoclonal antibody against nerve growth factor. *Am J Vet Res* 75:532–535.
- Wermeling DP, Berger JR. 2006. Ziconotide infusion for severe chronic pain: case series of patients with neuropathic pain. *Pharmacotherapy* 26:395–402.
- Wheeler GN, Brändli AW. 2009. Simple vertebrate models for chemical genetics and drug discovery screens: lessons from zebrafish and *Xenopus*. *Dev Dyn Off Publ Am Assoc Anat* 238:1287–1308.
- White JG, Southgate E, Thomson JN, Brenner S. 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 314:1–340.
- Wie CS, Derian A. 2020. Ziconotide. StatPearls, Treasure Island (FL): StatPearls Publishing,.
- Williams CJ, James LE, Bertelsen MF, Wang T. 2019. Analgesia for non-mammalian vertebrates. *Curr Opin Physiol* 11:75–84.
- Winter CA, Risley EA, Nuss GW. 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc Soc Exp Biol Med Soc Exp Biol Med N Y N* 111:544–547.
- Wittenburg N, Baumeister R. 1999. Thermal avoidance in *Caenorhabditis elegans*: an approach to the study of nociception. *Proc Natl Acad Sci U S A* 96:10477–10482.
- Woolf CJ. 1983. Evidence for a central component of post-injury pain hypersensitivity. *Nature* 306:686–688.
- Woolf CJ. 2011. Central sensitization: implications for the diagnosis and treatment of pain. *Pain* 152:S2–15.

- Woolf CJ, Salter MW. 2000. Neuronal plasticity: increasing the gain in pain. *Science* 288:1765–1769.
- Woolfe G, Macdonald AD. 1944. The Evaluation of the Analgesic Action of Pethidine Hydrochloride (demerol). *J Pharmacol Exp Ther* 80:300–307.
- Wu JX, Xu MY, Miao XR, Lu ZJ, Yuan XM, Li XQ, Yu WF. 2012. Functional up-regulation of P2X3 receptors in dorsal root ganglion in a rat model of bone cancer pain. *Eur J Pain Lond Engl* 16:1378–1388.
- Xing H, Chen M, Ling J, Tan W, Gu JG. 2007. TRPM8 mechanism of cold allodynia after chronic nerve injury. *J Neurosci Off J Soc Neurosci* 27:13680–13690.
- Xu L, Nwosu LN, Burston JJ, Millns PJ, Sagar DR, Mapp PI, Meesawatsom P, Li L, Bennett AJ, Walsh DA, Chapman V. 2016. The anti-NGF antibody muMab 911 both prevents and reverses pain behaviour and subchondral osteoclast numbers in a rat model of osteoarthritis pain. *Osteoarthritis Cartilage* 24:1587–1595.
- Yaksh TL. 2002. Future advances in pain pharmacology: what does the present say about the future? *Proc West Pharmacol Soc* 45:211–218.
- Yaksh TL, Farb DH, Leeman SE, Jessell TM. 1979. Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. *Science* 206:481–483.
- Yalcin I, Barthas F, Barrot M. 2014a. Emotional consequences of neuropathic pain: insight from preclinical studies. *Neurosci Biobehav Rev* 47:154–164.
- Yalcin I, Barthas F, Barrot M. 2014b. Emotional consequences of neuropathic pain: insight from preclinical studies. *Neurosci Biobehav Rev* 47:154–164.
- Yalcin I, Megat S, Barthas F, Waltisperger E, Kremer M, Salvat E, Barrot M. 2014c. The sciatic nerve cuffing model of neuropathic pain in mice. *J Vis Exp JoVE*.
- Yang M, Rainone A, Shi XQ, Fournier S, Zhang J. 2014. A new animal model of spontaneous autoimmune peripheral polyneuropathy: implications for Guillain-Barré syndrome. *Acta Neuropathol Commun* 2:5.
- Yang P, Kajiwara R, Tonoki A, Itoh M. 2018. Successive and discrete spaced conditioning in active avoidance learning in young and aged zebrafish. *Neurosci Res* 130:1–7.
- Yang Y, Santamaria P. 2006. Lessons on autoimmune diabetes from animal models. *Clin Sci Lond Engl* 1979 110:627–639.
- Yoneda T, Sasaki A, Mundy GR. 1994. Osteolytic bone metastasis in breast cancer. *Breast Cancer Res Treat* 32:73–84.
- Zagami AS, Goadsby PJ, Edvinsson L. 1990. Stimulation of the superior sagittal sinus in the cat causes release of vasoactive peptides. *Neuropeptides* 16:69–75.
- Zeng SL, Sudlow LC, Berezin MY. 2020. Using *Xenopus* oocytes in neurological disease drug discovery. *Expert Opin Drug Discov* 15:39–52.

Zhang J, Banerjee B. 2015. Role of MicroRNA in Visceral Pain. *J Neurogastroenterol Motil* 21:159–171.

Zhang L, Wang R, Bai T, Xiang X, Qian W, Song J, Hou X. 2019. EphrinB2/ephB2-mediated myenteric synaptic plasticity: mechanisms underlying the persistent muscle hypercontractility and pain in postinfectious IBS. *FASEB J Off Publ Fed Am Soc Exp Biol* 33:13644–13659.

Zhang WY, Li Wan Po A. 1994. The effectiveness of topically applied capsaicin. A meta-analysis. *Eur J Clin Pharmacol* 46:517–522.

Zheng Z, Lauritzen JS, Perlman E, Robinson CG, Nichols M, Milkie D, Torrens O, Price J, Fisher CB, Sharifi N, Calle-Schuler SA, Kmecova L, et al. 2018. A Complete Electron Microscopy Volume of the Brain of Adult *Drosophila melanogaster*. *Cell* 174:730-743.e22.

Zhu C, Xu J, Lin Y, Ju P, Duan D, Luo Y, Ding W, Huang S, Chen J, Cui D. 2018a. Loss of Microglia and Impaired Brain-Neurotrophic Factor Signaling Pathway in a Comorbid Model of Chronic Pain and Depression. *Front Psychiatry* 9:442.

Zhu H, Xiao X, Chai Y, Li D, Yan X, Tang H. 2019. MiRNA-29a modulates visceral hyperalgesia in irritable bowel syndrome by targeting HTR7. *Biochem Biophys Res Commun* 511:671–678.

Zhu X, Liu Z, Qin Y, Niu W, Wang Q, Li L, Zhou J. 2018b. Analgesic effects of electroacupuncture at ST25 and CV12 in a rat model of postinflammatory irritable bowel syndrome visceral pain. *Acupunct Med J Br Med Acupunct Soc* 36:240–246.

|   | Animal species                              | Procedure   | Clinical relevance  | References  |
|---|---|---|---|---|
| <b>Inflammatory pain models</b>                               |   |   |   |   |
| <i>Pain model induced by capsaicin</i>                        | Mice<br>Rats                                | Intra-plantar injection of capsaicin  | Mimic skin inflammation and inflammatory bowel disease                          | Kenins P., 1982<br>Muley et al., 2016   |
| <i>Pain model induced by formalin</i>                         | Mice<br>Rats                                | Injection (sub-cutaneous, intra-plantar...) of diluted formaldehyde solution (0.5-5%)   | Not specific to a clinical pathology.   | Dubuisson et al., 1977<br>Muley et al., 2016  |
| <i>Pain model induced by complete freund's adjuvant (CFA)</i> | Mice<br>Rats                                | Subcutaneous injection of CFA in the hind paw or intra-articular  | Assess inflammatory pain in mice and rats<br>Robust pain model for the study RA | Stein et al., 1988<br>Muley et al., 2016  |
| <i>Pain model induced by carrageenan</i>                      | Mice<br>Rats                                | Subcutaneously injection of carrageenan   | Joint pain inflammation   | Winter et al., 1962   |
| <i>Pain model induced by zymosan</i>                          | Mice  | Injection of cell walls of <i>Saccharomyces cerevisiae</i>  | Acute inflammation  | Doherty et al., 1985  |
| <b><i>Osteoarthritis pain models</i></b>                      | Mice<br>Rats<br>Guinea-Pigs<br>Dogs<br>Cats | <ul style="list-style-type: none"> <li>• Chemical injections</li> <li>• Joint surgery and ligament manipulation</li> <li>• Streptococcus injection</li> </ul> | Musculoskeletal disease and joint inflammation                                  | Kalbhen, 1987<br>Stoop et al., 2001<br>Gomis et al., 2007<br>Knights et al., 2012<br>van den Broek et al., 1988<br>Mc Coy, 2015 |
| <b><i>Rheumatoid arthritis models</i></b>                     | Mice  | <ul style="list-style-type: none"> <li>• Collagen injection</li> <li>• Collagen antibody injection</li> </ul>   | chronic autoimmune disorder   | Courtenay et al., 1980<br>Nandakumar et al., 2003   |
| <i>Streptococcal cell wall induced arthritis (SCW)</i>        | Rats  | Intra-articular injection of <i>Streptococcus pyogenes</i>  | Mimic RA  | van den Broek et al., 1988<br>Bessis et al., 2017   |
| <b>Centrally induced neuropathic pain models</b>              |   |   |   |   |
| <i>Contusive Spinal cord injury</i>                           | Mice<br>Rats                                | Weight drop on spinal cord  | Clinically relevant model, but not specific to a pathology                      | Siddall et al., 1995  |
| <i>Spinal hemi-section</i>                                    | Rats  | Hemi section of a thoracic segment usually cranial to L1 dorsal root entry  | Mimic chronic central pain after spinal cord trauma                             | Koehler and Endtz, 1986   |
| <i>Photochemical injury</i>                                   | Rats  | Ischemia & tissue necrosis of spinal cord   | Reproduce a mechanical trauma   | Watson et al., 1986   |

|  |              |  |  |  |
|--|--------------|--|--|--|
|  |              |  | of the spinal cord without surgery   |  |
| <b><i>Peripherally induced neuropathic pain models</i></b> |              |  |  |  |
| <i>Sciatic nerve total transection</i>                     | Mice<br>Rats | Complete sciatic nerve transection   | Mimic the clinical symptoms of “phantom limb”  | Wall et al., 1979                                  |
| <i>Chronic constriction injury</i>                         | Mice<br>Rats | Four loose knots around sciatic nerve or the infra-orbital nerve                             | Mimic causalgia or complex regional pain syndrome  | Bennett and Xie, 1988                              |
| <i>Cuffing of sciatic nerve</i>                            | Mice<br>Rats | Implantation of a polyethylene tubing (cuff) around the common branch of the sciatic nerve   | Study anxiety-depressive comorbidities associated with NP that lasts for weeks after <b>loss</b> of hypersensitivity to pain | Mosconi et al., 1996<br>Benbouzid et al., 2008     |
| <i>Partial sciatic nerve injury</i>                        | Mice<br>Rats | Partial ligation of dorsal third or half of sciatic nerve, peroneal or tibial branch or both | Mimic human causalgia symptoms (injury to the peripheral nerve)  | Seltzer et al., 1990<br>Malmberg and Basbaum, 1998 |
| <i>Spinal Nerve Ligation</i>                               | Mice<br>Rats | Unilateral ligation of lumbar spinal nerves L5 and L6 distal to dorsal root ganglia          | Mimic human causalgia symptoms (injury to the peripheral nerve)  | Kim and Chung, 1992.<br>Kiso et al., 2008          |
| <i>Spared nerve injury</i>                                 | Mice<br>Rats | Lesion of two of the three terminal branches of the sciatic nerve                            | Not specific to a clinical pathology.  | Decosterd and Woolf, 2000                          |

**Table 1:** Classical inflammatory and neuropathic pain models in rodents with notes on companion animals.

|  | <b>Animal Species</b> | <b>Procedure</b>  | <b>Cancer cell line</b>   | <b>Pain Tests</b>  | <b>References</b>  |
|--|-----------------------|---|---|--|--|
| <i>Bone Cancer Induced Pain (CIBP)</i> | Mice                  | Intraosseous Injection Cancer Cells into Femur<br>Humerus<br>Calcaneus bone of hindpaw  | 66.1 or 4T1-Luc2 mammary adenocarcinoma<br>NTCT2472<br>Fibrosarcoma<br>B16 melanoma<br>C26 colon adenocarcinoma | Flinching<br>Guarding<br>von Frey Test<br>Limb use<br>Tail Flick Assay<br>Dynamic weight bearing<br>Palpation (light touching) | Schwei et al., 1999; Wacnik et al., 2001; Sabino et al., 2003; Vermeirsch et al., 2004; Grenald et al., 2017; Elmarah et al., 2017; Appel et al., 2019 |
|  |                       | Transgenic mice   | n.a.  | Flinching<br>Guarding  | Ghilardi et al., 2005  |
|  | Rat                   | Intraosseous Injection Cancer Cells into Tibia<br>Femur                                 | MRMT-1 murine mammary carcinoma<br>Walker 256 rat mammary carcinoma<br>MLL rat prostate adenocarcinoma          | von Frey Test<br>Limb use<br>Weight-bearing test   | Medhurst et al., 2002; Wu et al., 2012; Zhu et al., 2018; Wang et al., 2018; Falk et al., 2018   |
|  | Dog                   | Naturally-Occurring Bone Cancer   | n.a.  | Lameness<br>Canine BPI<br>QST<br>VAL<br>QoL  | Brown et al., 2015; Sapio et al., 2018; Monteiro et al., 2018  |
| <i>Breakthrough Cancer pain (BTcP)</i> | Mice                  | Injection of Cancer Cells into Femur +ET-1 injection into tumor cells                   | Lewis Lung carcinoma  | von Frey Test<br>Limb use  | Tang et al., 2016;   |
| <i>Metastasis Bone Cancer Pain</i>     | Mice/rat              | Injection of Cancer Cells into: Tail vein<br>Intracardiac<br>Intraosseous<br>Orthotopic | ACE-1 canine prostate carcinoma<br>R3327 Mat LyLu cells rat prostatic adenocarcinoma                            | Flinching<br>Guarding<br>Thermal Paw stimulator<br>Posture changes, movement limitations                                       | Liepe et al., 2005; Halvorson et al., 2005   |
| <i>Breast Cancer Pain</i>              | Mice                  | Injection of cancer cells into mammary fat pad  | 4T1 murine breast cancer  | von Frey Test<br>Acetone drop<br>grimace scale   | De Almeida et al., 2019  |
| <i>Pancreatic Cancer Pain</i>          | Mice                  | Injection of cancer cells into pancreas   | SW1990 human pancreatic cancer  | Abdominal withdrawal threshold<br>Hunching<br>VMR  | Wang et al., 2017  |
|  |                       | Transgenic mice   | n.a.  | Hunching<br>Vocalization   | Lindsay et al., 2005   |
| <i>Peritoneal carcinomatosis</i>       | Mice                  | Injection of cancer cells into abdominal cavity   | 60As6Luc derived from HSC60 human gastric scirrrous carcinoma   | von Frey test<br>licking<br>Abdominal withdrawal threshold<br>Hunching   | Suzuki et al., 2012  |

**Table 2:** Animal Models of Cancer Pain. Canine BPI: Canine Brief Pain Inventory; ET-1: Endothelin-1 ; n.a: not applicable; QoL: Quality of Life; QST: quantitative sensory testing; VAL; Visual analog scale; VMR: Visceromotor responses

|                          | Animal Species | Procedure  | Clinical relevance               | Pain/Nociceptive tests   | References  |
|--------------------------|----------------|--|----------------------------------|--|---|
| <i>Abdominal Viscera</i> | Mice           | Intraperitoneal injection of chemicals<br>Colorectal distension (CRD)  | Visceral Pain                    | Writhing Test<br>Responses<br><br>VMR  | Alonso-Castro et al., 2017<br>Kamp et al., 2002             |
|                          | Rat            | Colorectal distension  |                                  | Passive avoidance behavioral paradigm<br>AWR   | Ness and Gebhart, 1988;<br>Ness and Gebhart, 1991;          |
|                          | Dog            | Colorectal distension  |                                  | VMR  | Lyubashina et al., 2016                                     |
| <i>Digestive Tract</i>   | Mice           | Intracolorectal injection of chemicals   | IBD                              | “up and down” method<br>von Frey Test  | Hou et al., 2019  |
|                          |                | Oral ingestion of chemicals  | IBD<br>Ulcerative colitis        | Intracolonical administration of allyl isithiocyanate (mustard oil) (0,5%)<br>VMR    | Lapointe et al., 2015                                       |
|                          | Rat            | Intracolorectal injection of chemicals   | IBD                              | VMR<br>AWR   | Huang et al., 2019; Parisio et al., 2020                    |
| <i>Colon-Bowel</i>       | Mice           | Castor-oil induced diarrhea  | <i>IBS-D</i>                     | Intracolonical administration of allyl isithiocyanate (mustard oil)<br>Writhing Test | Sobczak et al., 2014  |
|                          |                | Repeated WAS<br>NMS<br>PRS   | <i>IBS</i>                       | AWR<br><br>VMR   | Zhu et al., 2019<br>Miquel et al., 2016                     |
|                          | Rat            | Colorectal injection of inflamogens or irritants<br>Intragastric<br>Parasite infection<br>Subcutaneous LPS injection | PI-IBS<br>Ulcerative colitis     | AWR<br>VMR   | Zhu et al., 2018<br>Zhang et al., 2019<br>Nozu et al., 2019 |
|                          |                | Repeated WAS   | <i>IBS</i>                       |  | Nozu et al., 2019   |
| <i>Stomach</i>           |                | Hollow stomach distention<br>Gastric injection/Oral injection of irritant chemical                                   | Gastric hyperalgesia             | VMR<br>Passive Avoidance Behavior paradigm   | Ozaki et al., 2002  |
| <i>Ureter</i>            | Rat            | Intraureteral injection of dental cement   | Ureteric calculi (Kidney stones) | vocalization to electrical stimulation   | Giamberardino et al., 1995                                  |

|                               |              |   |  |  |  |
|-------------------------------|--------------|---|--|--|--|
|                               | Mice         | oral injection of DBTC  | Chronic pancreatitis   | von Frey Test<br>animal posture<br>gait disturbances                           | Oz, 2016   |
| <i>Pancreas</i>               | Rat          | High fat (65%)/ethanol (6%) diet (10 weeks)   | Chronic pancreatitis   | von Frey Test<br>up-down method<br>Hot plate                                   | McIlwrath and Westlund, 2015                               |
|                               |              | Intraductal infusion of a bile salt + Intraperitoneal injection of aCCK analogue<br>Intravenous injection of chemical | Acute pancreatitis   | Exploratory activity<br>von Frey Test<br>abdominal withdrawal to heat stimulus | Zhang et al., 2004<br>Vera-Portocarrero and Westlund, 2004 |
|                               | Dog          | Naturally-Occurring   | Pancreatitis   | Glasgow Composite Pain Scale   | Mansfield and Beths, 2015<br>Catanzaro et al., 2016        |
| <i>Prostate</i>               | Mice         | Infection with <i>E.coli</i> bacteria isolate from patient<br>EAP induction   | Chronic prostatitis/<br>chronic pelvic pain syndrome (CP/CPPS) | von Frey Test  | Quick et al., 2013<br>Liu et al., 2019                     |
| <i>Biliary system</i>         | Mice         | Injection of solvents into bile duct  | Cholangiopathy   | Grimace face<br>Parameters<br>activity   | Berntsen et al., 2017                                      |
|                               | Rat          | Bile duct resection   | Cholestasis  | Tail-flick latency   | Hasanein, 2010   |
| <i>Referred visceral pain</i> | Mice<br>Rats | Intracolonic capsaicin instillation   | Prolonged referred hyperalgesia                                | Von frey test  | Laird et al., 2001<br>Sanoja et al., 2010                  |

**Table 3:** Visceral pain models. AWR: Abdominal Withdrawal Reflex; CRD : Colorectal distension; EAP: Experimental autoimmune prostatitis; IBD: Inflammatory Bowel Disease; IBS: Irritable Bowel syndrome; IBS-D: Irritable Bowel syndrome - diarrhea-predominant; NMS : Neonatal Maternal Separation; PRS: Partial restraint stress; VMR: Visceromotor responses, WAR: Water avoidance stress

|                   | Nociception                      | Nociceptors   | TRP-like channels   | Neuromodulators                                      | Opioid system                               | References   |
|-------------------|----------------------------------|---|---|--|---|--|
| <i>C.elegans</i>  | Thermal, chemical and mechanical | ASH pair of neurons DRG polymodal nociceptors                               | Osm-9 and ocr-2 (=TRPV)   | SubstanceP, calcitonin, Glutamate FLP18/FLP-21/NRP-1 | μ-opioid like receptor                      | Bargmann and Kaplan, 1998; Kahn-Kirby and Bargmann, 2006; Venkatachalam et al., 2014; Wittenburg and Baumesiter, 1999; Nkambeu et al., 2018; Glauser et al., 2011; Hills et al., 2016              |
| <i>Drosophila</i> | Thermal, chemical and mechanical | Multidendritic (Md) class IV and class III neurons                          | Painless, TRPA1, pyrexia (≈ TRPA) Pkd2, NompC, and Trpm Inactive (≈ TRPV) | NRP1 Neuropeptide F Amnesiac                         | μ-opioid like receptor                      | Hwang et al., 2007; Walters, 2019; Tracey et al., 2003; Neely et al., 2011; Lee et al., 2005; Turner et al., 2016; Kwon et al., 2010; Hu et al., 2017; Aldrich et al., 2010; Kuong and Neely, 2013 |
| <i>Zebrafish</i>  | Thermal, chemical and mechanical | Polymodal nociceptors<br><br>Un-myelinated C-fibre and Myelinated Aδ-fibres | 10 TRP channels (TRPV, TRPM, TRPA)  | Substance P, Glutamate,                              | 14 opioid (μ,δ,κ and opioid-like) receptors | Williams et al., 2019; Sneddon, 2019; Saito and Shangai, 2006; Demin et al., 2018; Stevens, 2015; <a href="#">Demin et al., 2018</a>   |
| <i>Xenopus</i>    | Thermal, chemical and mechanical | Polymodal nociceptors<br><br>Un-myelinated C-fibre and myelinated Aδ-fibres | 14 TRP channels (TRPV, TRPM, TRPA)  | Substance P, CGRP, Glutamate*                        | 4 opioid (μ,δ,κ and opioid-like) receptors  | Williams et al., 2019; Stevens, 2011; Saito and Shangai, 2006; Stevens et al., 2009  |

**Table 4:** The nociceptive pathways in invertebrates (*C.elegans* and *Drosophila*) and lower vertebrates (*Xenopus* and *Zebrafish*). Some examples of neurotransmitters or TRP channels are given; only TRPM, TRPA and TRPV channels are indicated for *Xenopus* and *Zebrafish*, \*demonstrated in amphibian class (*Rana*).

|                   | Age           | Thermal assay   | Mechanical assay  | Chemical assay   | Chronic pain-like assay                               | Neuropathic pain-like assay          | References  |
|-------------------|---------------|---|---|--|---|--------------------------------------|---|
| <i>C. elegans</i> |               | Heated metal pen tip or laser diode<br>Thermal barrier assay<br>Noxious heat thermogradient assay and four quadrants assay  | Harsh touch assay   | Octanol avoidance assay<br>Thrashing assay                                     | N.T.  | N.T.                                 | Mills et al., 2016;<br>Cohen et al., 2014;<br>Tobin and Bargmann, 2004;<br>Wittenburg and Baumeister, 1999;<br>Glauser et al., 2011;<br>Nkambeu et al., 2019; Wang et al., 2019 |
| <i>Drosophila</i> | Larvae        | Soldering iron heated to 46°C<br>Heated Water Droplet<br>Cold probe contact   | von Frey fibres assay   | HCl (quick exposure or incubation)   | N.T.  | N.T.                                 | Khuong et al., 2019;<br>Calvo et al., 2019;<br>Im and Galko, 2012;<br>Milinkeviciute et al., 2012; Lopez-Bellido et al., 2019; Heather et al., 2017                             |
|                   | Adult         | Water-tight chamber surrounded by hot water<br>'Jump' reflex assay<br>'Hotplate' assay<br>The light-driven heat avoidance test<br>Thermal challenge (warm water bath) | N.T.  | Feeding session<br>Direct contact with food/liquid laced with noxious compound | 'Sunburn' UV radiation                                | Nerve injury (Middle leg amputation) |   |
| <i>Zebrafish</i>  | Embryo/Larvae | Temperature aversion assay<br>Cold and warm water tank  | N.T.  | Acetic Acid exposure   | Acetic acid test (exposure)                           | Extreme Thermal exposure             | Malafoglia et al., 2014; Curtright et al., 2015; Taylor et al., 2017; Deakin et al., 2019; Ellis et al., 2018; Yang et al., 2018; Costa et al., 2019; Sneddon, 2018             |
|                   | Adult         | N.T.  | Electric shock  | Acetic acid test (exposure or subcutaneous injection)<br>Algogen injections    | Acetic acid test (exposure or subcutaneous injection) | N.T.                                 |   |
| <i>Xenopus</i>    | Adult         | Hargreaves test   | Pinch by a bulldog clamp in the dactyls of the forelimbs<br>Pull of the ovaries<br>Electric shock | Acetic acid test (exposure)  | Acetic acid test (exposure)                           | N.T.                                 | Stevens, 2011;<br>Miller et al., 1974   |

**Table 5:** Examples of assays to study nociception performed in *C. elegans*, *Drosophila*, Zebrafish and *Xenopus*