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Review

Drosophila as a tool for studying the conserved genetics of pain

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Survival of all animals depends on an accurate representation of the world, and an organism must be capable of prioritizing and responding to potentially hazardous conditions. This ability is dependent on nociception, the sensory process allowing animals to detect and avoid potentially harmful stimuli. Nociception is the sensory process that results in the subjective experience of 'pain' in humans. Because of its vital and broad role in animal biology, pain/nociception is a complex, whole-body physiological process that is under stringent evolutionary pressure. Here, we discuss the utility of *Drosophila melanogaster* as an emerging model organism for studying the conserved genetics of nociception, particularly with respect to recently developed high-throughput *Drosophila* 'pain' paradigms.

Conflict of interest

The authors declare no conflict of interest.

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Animals have developed complex conserved mechanisms to survive in an often hostile environment. Acute detection of harmful stimuli and the ability to react and avoid them (pain/nociception) became of paramount importance. The International Association for the Study of Pain (http://www.iasp-pain.org) describes pain as an 'unpleasant sensory and emotional experience associated with actual or potential tissue damage' (1). Pain is a subjective experience, whereas nociception, the objective neural processes of encoding and processing noxious stimuli, is an evolutionary-conserved mechanism that alerts an organism to potential tissue damage and is crucial for the survival (2). Pain/nociception is a protective sensation that is a natural part of life; however, sensitization through inflammation or nerve injury can lead to allodynia (nociceptive response to normally non-noxious stimuli) or hyperalgesia (exaggerated response to noxious stimuli) (3). Excessive acute pain can be treated effectively with opiates (4) or non-steroidal anti-inflammatories (5), whereas persistent or chronic pain represents an unmet clinical challenge. Chronic or persistent pain is defined as the type of pain that lasts beyond the term of an injury or painful stimulus. Persistent pain can protect an organism from further damage while tissue is healing; however, in various chronic pathological conditions, allodynia or hyperalgesia can remain present long after the tissue has healed (6, 7). The prevalence of chronic pain in the population has been estimated to be 34–53% (8) and can reach close to 90% in older subjects (65–74 years old) (9). Chronic pain causes an enormous financial burden and more importantly involves physical discomfort that can have a negative impact on social life and relationships (1), and can also lead to cognitive issues and the development of psychological disorders, such as depression (10). Although known to involve sensitization of second- and higher-order neurons, the molecular pathogenesis of chronic widespread pain conditions remains unclear (11).

Elucidation of the genetics of pain diseases in human populations can lead to identifying genes that predispose to severe chronic pain and/or genes that can be considered as novel drug targets for the treatment of chronic pain disease. Unfortunately, the genetics of chronic pain has been difficult to unravel. Population approaches have established a clear genetic constituent to pain perception, nociceptive thresholds, and development of chronic pain diseases in humans. These findings suggest a complex genetic component responsible for the process of pain sensing and pain

diseases in humans that would go far beyond the few genes already implicated in pain-associated diseases (12). Preliminary human population efforts have identified some genetic alterations that predispose an individual to severe chronic pain (13–19), and these results have initiated the generation of novel classes of chronic pain therapies, for example, with BH4 (tetrahydrobiopterin) interventions (20, 21). Although in many circumstances mammal model organisms are the best choice for nociception studies, when considering large-scale *in vivo* screening strategies, scientists are confronted with great obstacles including cost, timeframe of research, and ethical concerns. Over the last decade, the fruit fly in particular has become a powerful model organism for studying the genetics of nociception, in part due to the short generation time, powerful genetic tools, and robust nociceptive responses to a range of noxious stimuli.

The simplest animal behavior that could be considered nociceptive is found in Protozoa and involves changes of movement as well as body shape. For example, a unicellular protozoan *Paramecium* is able to detect hazardous chemical environment around it and swim to the safer area. *Paramecium* was suggested to be used as a biosensor to detect water pollutants (22). The oldest metazoan phylum Porifera (sponges) (23) does not have a nervous system; however, this animal uses chemical messengers such as glutamate and GABA (γ-aminobutyric acid), which are also used in nociception systems of higher metazoans (24), and in sponges an intracellular calcium influx and subsequent contraction is elicited in response to thermal stress (25). Glass sponges show coordinated inflation and deflation of their canal system within 20 s of mechanical stimulation (25), which can be considered an aversive response. The Cnidaria, phylum that includes jellyfish, hydra-like animals, sea anemones and others, has one of the simplest nervous systems – a diffuse nerve net (26). In *Aurelia* sp., (moon jellies), neurons are organized in nets and bundles and serve as central nervous system that receives the input from chemosensors and mechanoreceptors (21). They increase their swimming speed in response to touch (27) and react to chemicals released by injured prey (28). Moon jellies can sense water salinity and change position when salinity is too low (29). Turbellaria class flatworms possess a cephalic ganglion, which serves as a center for signal integration and coordination of peripheral systems (30). Flatworm contracts longitudinally and moves away from the stimulus when prodded with a needle (31). More advanced invertebrates exhibit diverse nociceptive behaviors, for example, leeches (Annelida, Hirudinea) display rapid withdrawal or pronounced writhing in response to noxious mechanical stimuli like pinching (32) and *Cepaea* snails (Gastropoda) lift the anterior portion of their extended foot in response to noxious temperatures (40°C) (33). The more complex *Aplysia* sea slug (Gastropoda) shows withdrawal and escape locomotion in response to noxious cutaneous stimulation as well as discharges ink and opaline at the source of noxious stimulation (34). Opiates, which have been used as analgesics for centuries in humans, also have anti-nociceptive effects on invertebrates, highlighting the basic conservation of this process. For example, terrestrial snail *Megalobulimus abbreviatus* exhibits increased response latency to noxious heat (50°C) in the hot-plate assay when pre-treated with morphine (Gastropoda) (35). In addition, invertebrate systems exhibit desensitization to morphine-induced thermal analgesia treatment. For example, the snail *Cepaeanemoralis* (Gastropoda) shows increased latency of the foot-lifting response when injected with morphine. Effect is reduced and abolished upon administration of naloxone (antagonist of morphine) along with morphine (33). A 50% increase in escape reaction time is observed in the cricket *Pteronemobius* sp. 90 min after morphine administration (36). Thus, nociception is an ancient conserved strategy to promote survival in the animal kingdom.

Drosophila models of nociception/pain

It has been estimated that 75% of human disease genes have conserved homologs in *Drosophila melanogaster*, making this fly a model organism of great potential (37). *Drosophila* has been used extensively as a model for human disease already, for example, to study cancer (38), Alzheimer disease (39), cardiac diseases (40), innate immunity (41), obesity and diabetes (40) and others. Learning and memory studies in *D. melanogaster* (42, 43) proved that despite being evolutionary far apart from humans, fruit flies show enough complexity to mirror some elaborated human behavior and disorders. *Drosophila* nociceptors were shown to be preserved throughout the metamorphosis and persist in the adult fly (44), but most importantly, they show morphological and functional resemblance to vertebrate nociceptors with the characteristic naked-nerve endings. In both cases, dendrite endings tile the entire epidermis with hardly any overlap, which enables them to sense and respond quickly to tissue damage and potential injury and empowers to utilize this extraordinary invertebrate for nociception studies (45–49).

The first *Drosophila* experimental model of nociception described used a thermal noxious stimulus (heat) to induce a nociceptive response in the fly larvae (45). In this elegant system, fly larvae were collected and placed in a 35-mm Petri dish and then touched with a soldering iron heated to 46°C. In this paradigm, wild-type larvae respond to heat insult within a few seconds with a stereotypical rolling response (Fig. 1a). This simple system was a powerful advance allowing a merger of nociception research with the many genetics options already available to *Drosophila* researchers. For example, using this system, it was found that the fruit fly larvae use naked multidendritic peripheral sensory nerves across the larval body, and this was confirmed by genetic expression of the tetanus toxin light chain within these cells, which silences neuronal output and completely blocked this nociceptive response. Most importantly, this assay system allowed the isolation of the first *Drosophila* 'pain' gene, *painless*,

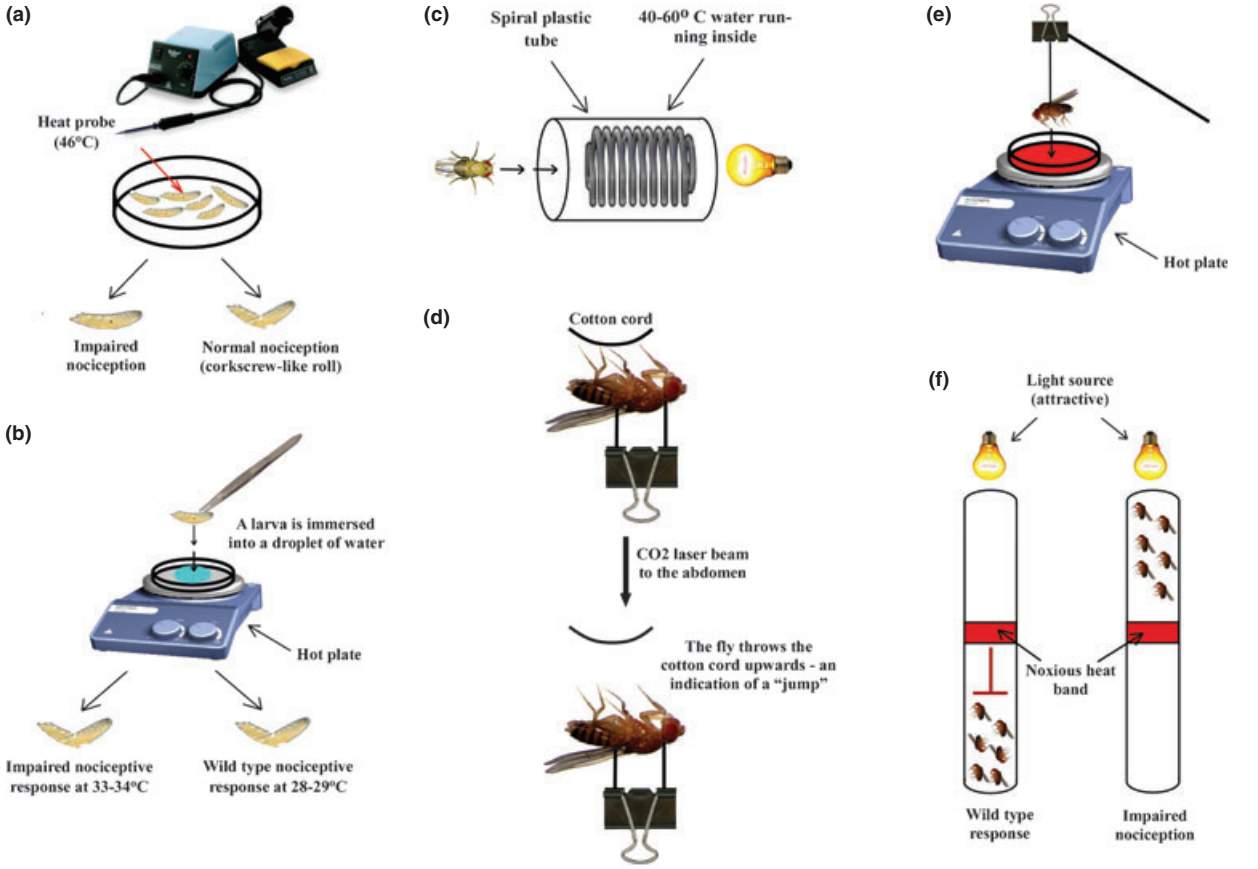

Fig. 1. Fly models of acute nociception. (**a**) *Drosophila* larvae elicit a stereotypical rolling response when touched with a tip of soldering iron heated to 46°C (45). (**b**) Wild-type *Drosophila* larvae and larvae with impaired nociception elicit a characteristic nociceptive response when immersed into a droplet of water heated to 28–29°C and 33–34°C, respectively (54). (**c**) The water-tight chamber surrounded by hot water is used as a noxious barrier between flies and attractive light source. Flies with intact thermal nociception do not cross the barrier (52). (**d**) Adult *Drosophila* throws the cotton cord upward when laser beam is directed to the fly's abdomen (55). (**e**) After dropping an adult fly on the 47°C hot plate, latency to jump is recorded (55). (**f**) The light-driven heat avoidance test with a heated aluminum ring used as a noxious barrier between flies and the attractive light source (46°C) (50, 56).

a transient receptor potential (TRP) family member (temperature-responsive voltage-gated cation channels) of the TRPA1 subfamily (45). Unfortunately, *painless* is not conserved in vertebrates. While the larval thermal nociception paradigm described above really opened the door to the use of *Drosophila* in nociception or pain research, there are now many models of *Drosophila* nociception reported (2, 45, 50–53).

An alternative method to study larval heat nociception has also been reported (54). In this paradigm, a larva is immersed into a droplet of water on the Petri dish lid and then placed on the hot plate (Fig. 1b). The temperature is gradually raised and measured with a thermocouple, and the temperature point at which larvae exhibit the stereotypic rolling response is then recorded (54). Wild-type and *painless* mutant larvae showed nociceptive behavior at 28–29°C and 33–34°C, respectively (54), which is ~10°C lower than the heat probe results showed (45). This temperature difference may be due to the activation of multiple thermosensitive neurons across the larval body wall, while a touch with the heat probe activates just a subset of neurons at the point of stimulus (54).

The first reported technique to use adult fruit flies to study nociception focused on the pharmacology of nociception and the utility of *Drosophila* for pharmacological analgesic research (52). In this system, adult *Drosophila*, which prefer light, are placed in a horizontal water-tight chamber, and this chamber is then surrounded by hot water (24–60°C) and a light is placed at the far end of the chamber to attract flies (Fig. 1c). Flies with intact thermal nociception will not pass through the tube when the water is heated to a noxious temperature (i.e. above 42°C) despite the attractive light at the opposite end, whereas flies with a defective heat nociception system would be expected to ignore the noxious heat and pass through the tube to reach the light source. While this system has not yet exploited the power of *Drosophila* genetics, it has been used to establish that the GABA agonist 3-aminopropyl(methyl)phosphinic acid (3-APMPA) can act as a thermal analgesic when injected into the adult fruit fly (52).

Two other models of heat nociception developed for adult fruit flies attempted to mimic the mammalian hot-plate assay (55). In the first of these systems, a fly is

immobilized with glue, held upside down, and a cotton thread is balanced on the fly's legs (Fig. 1d). The fly is then heated with a laser, and the latency to response (time until the fly throws the cotton cord upward) is recorded. This assay is thought to model the 'jump' reflex a fly (as well as mammals) can exhibit when exposed to noxious heat. The second model for a 'hot-plate' assay involves gluing a nylon thread to the back of a fly and then suspending this fly over a 47°C hot plate. The fly is then dropped on the hot plate, and the latency to jump is recorded (Fig. 1e). Using both of these models, adult *painless* mutant flies exhibit a delay in heat nociception responses. These assays were also used to establish the first anatomical characterization of the adult *Drosophila* nociception response, showing that the central complex, but not the mushroom body, is required for the fly heat pain response (55).

Another recent model for studying heat nociception in the adult *Drosophila* also combines the adult *Drosophila* light preference response with the noxious heat avoidance (50). In this paradigm, a modified countercurrent phototaxis chamber, originally described in 1967 (56), is used with a heated aluminum ring (40–50°C) used as a noxious barrier between flies and the attractive light source. Wild-type flies avoid this heated ring, whereas *painless* mutant flies exhibit less avoidance of this noxious heat barrier (Fig. 1f). Importantly, use of this system highlighted a role for the neuropeptide amnesiac as an essential component of the adult *Drosophila* nociception apparatus. Moreover, *amnesiac* appears to be a *bona fide Drosophila* pain gene, as *amnesiac* mutants, like *painless* mutants, also exhibit defects in larval heat nociception, as well as the laser 'jump' response. Unfortunately, the neuropeptide amnesiac is also not conserved in mammals (50).

In addition to heat pain, *Drosophila* has also been used to study mechanical nociception, which was also reported in the initial *Drosophila* pain study (45). In this system, *Drosophila* larvae are again isolated and placed in a tissue culture dish, but in this case are subjected to noxious mechanical insult and assayed for the characteristic nociceptive rolling response (Fig. 2). The stimulus is delivered by calibrated von Frey fibers, the same ones used in standard mammalian mechanical pain studies (57). Larvae are less mobile and nociceptive response can be easily evaluated; therefore, this method cannot be easily applied for the mechanical nociception studies in the adult fly. Therefore, at present it is unclear if the genes and cells required for larval mechanical nociception play a similar role in adult mechanical nociception responses. Using this technique, larvae were observed to pause their normal feeding behavior upon light touch; however, a force of 45 mN induced the stereotypic rolling reaction. The *Drosophila* pain gene *painless* was also shown to be a polymodal nociceptor, responding to both noxious thermal and mechanical input. The corkscrew-like roll was seen only when *painless* mutant larvae were stimulated with 100 mN von Frey fiber (45). Another class of ion channels involved in mechanosensation is the DEG/ENaC channels, which have been shown to play a role in the sensing of gentle

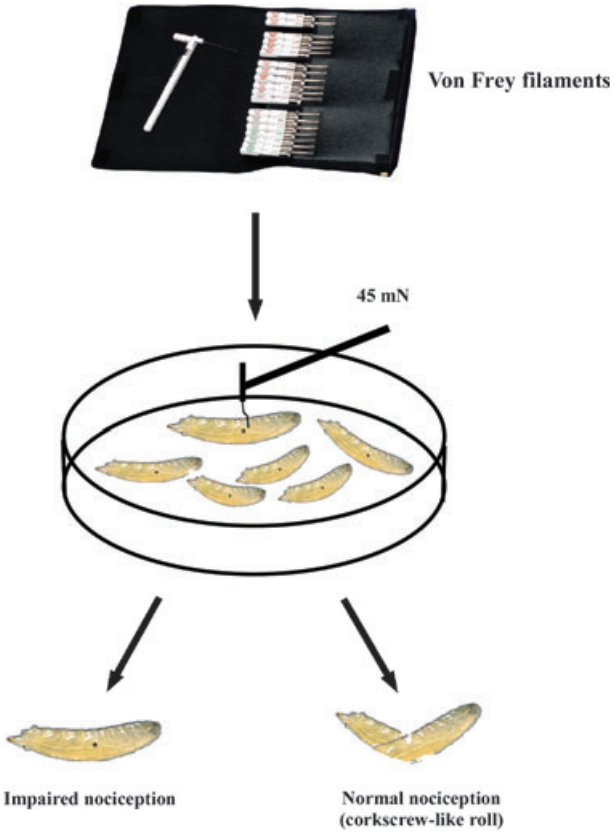

Fig. 2. Mechanical nociception assay with calibrated von Frey filaments. *Drosophila* larvae elicit stereotypical rolling behavior in response to forces stronger than ~45 mN (45).

touch in *C. elegans* and aversive mechanosensation in *Drosophila* (58, 59). In *Drosophila*, *pickpocket* (*ppk*) codes a DEG/ENaC subunit and is expressed in Class III and IV multidendritic neurons. *ppk* mutant larvae lack the characteristic rolling response to harsh mechanical stimulation with 50 mN von Frey filaments but show a wild-type response to gentle touch (58). In contrast to *painless*, *ppk* seems to be implicated only in mechanical but not in thermal nociception. Importantly, *ppk* shows some homology with multiple human channels, including the amiloride-sensitive cation channels like Accn3 known to play a role in mammalian mechanical pain perception (60, 61).

An exciting development in the conserved genetics of mechanical nociception occurred this year. An assessment of *DmPiezo*, a *Drosophila* version of the recently described mechanosensing channel *piezo* (51), revealed that *DmPiezo* can also form mechanosensing pores (62) and is essential for mechanical nociception in the *Drosophila* larvae (59). Although *DmPiezo* expression was found to be localized in multidendritic *ppk*-positive neurons, signaling pathways were found to function in parallel (59). While floxed conditional and reporter embryonic stem (ES) cells exist for the two mammalian counterparts of *DmPiezo* (*FAM38A* and *FAM38B*), the phenotype for these mice in mechanical nociception has not yet been published (51).

In addition to models of acute nociception, *Drosophila* researchers have also reported a model of pain 'sensitization' using UV radiation, giving *Drosophila* larvae a 'sunburn'. This assay is of particular importance as there are no other *Drosophila* chronic pain models available. UV treatment results in thermal hyperalgesia (altered pain intensity) as well as allodynia (pain after subnoxious stimulus). Non-noxious temperatures (up to 39°C) do not provoke nociceptive behavior in *D. melanogaster* larvae when tested in the hot-probe assay described above (Fig. 1a). However, starting 4 h after exposure to UV radiation, third-instar larvae exhibit thermal allodynia, showing the stereotypic nociceptive response (corkscrew-like roll) to previously innocuous temperature of 38°C (Fig. 3). The peak response latency is observed 24 h after UV exposure, which correlates with the time required for epidermal cells to undergo apoptosis (53). Tumor necrosis factor (TNF) and its receptor modulates the UV-induced sensitization in vertebrates (63), and *Drosophila* encodes a TNF-like factor Eiger and its receptor Wengen, which show similar signaling mechanisms to their vertebrate counterparts. After UV radiation, Eiger is released from epidermal cells and binds to Wengen expressed on *Drosophila* nociceptive neurons. *eiger* and *wengen* knockdown larvae show inhibition of thermal allodynia induced by UV radiation (53).

hedgehog (*hh*) signaling was found to operate in parallel to *eiger* in mediation of UV-induced allodynia. Activation of both, *hh* and TNF, signaling pathways had a cumulative effect and resulted in austere allodynia. Furthermore, *hh* pathway was shown to mediate thermal hyperalgesia (7). *painless* and *dTrpA1* role in sensitization was analyzed because of their involvement in detection of noxious stimuli (*dTrpA1* role in acute pain perception is discussed in more detail below) (45, 64). *painless* was shown to be activated in both signaling pathways and modulate thermal allodynia, whereas *dTRPA1* was found to take part only in *hh*-induced thermal hyperalgesia (7). Discovery of *hh* involvement in UV-induced allodynia and hyperalgesia may uncover novel potential therapeutic targets, and impressively in this study, in addition to genetics in flies, the authors pharmacologically blocked smoothened, part of the *hh* pathway, in rats and found synergy with morphine to promote analgesia in CFA and neuropathic pain models (7). Importantly, *hh* signaling had never been linked to nociception or sensitization before work in *Drosophila*, highlighting that, in addition to mimicking vertebrate nociception, fruit fly research also allows us to rapidly learn new things about mammalian nociception and pain diseases.

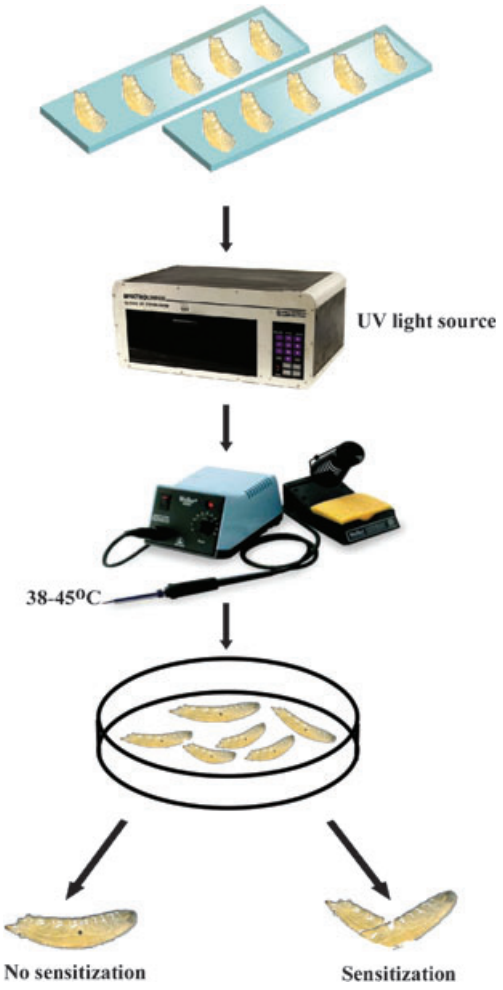

Fig. 3. Chronic 'pain' assay. *Drosophila* larvae exhibit heat sensitization (hyperalgesia and allodynia) starting 4 h after UV exposure (53).

A high-throughput screening model of nociception

While all the above assays are useful and accurate measures of various acute and chronic nociception modalities in the fruit fly, none of these assays is intended for high-throughput screening. Thus, to this end, we designed a nociception behavioral assay system that could be used for high-throughput genetic and pharmacological screening. We based our system on the observation that heat above ~39°C is acutely noxious to adult fruit flies, rapidly incapacitating them. We designed a system in which about 20 fruit flies are placed in a 35-mm tissue culture plate, the plate is taped closed, flies are tested for basic coordination, then acclimated to the test chamber, and finally floated on a water bath heated to 46°C in the dark. This results in the bottom of the chamber heating to 46°C within ~20 s and the top of the chamber reaches a subnoxious temperature of 31°C after a 4-min assay. The logic behind this assay was that flies that could sense noxious heat would rapidly avoid it, and flies with defects in noxious heat sensation (nociception by definition) would fail to avoid the hot plate and become rapidly incapacitated. Indeed, with this system we found that all wild flies avoid the hot side of the plate and stay on the cooler top side of the plate (Fig. 4). Importantly, when we tested *painless* mutant flies, many of them failed to avoid the noxious side of the chamber (64). We then used this system combined with *in vivo* neural-specific RNAi to screen the entire fruit fly genome to find genes required for heat nociception *in vivo*. This was a large,

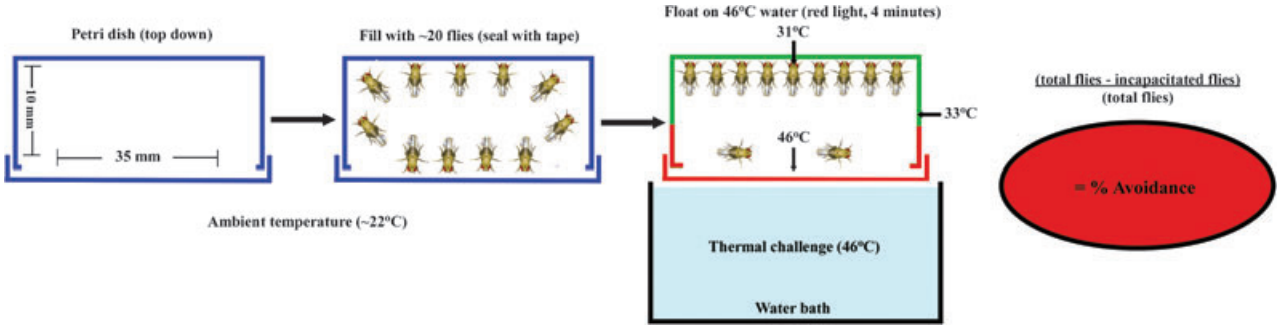

Fig. 4. Schematic for high-throughput heat nociception using adult *Drosophila*. Chamber with flies is floated on a 46°C water bath for 4 min and immobilized flies are counted as 'incapacitated'. Total fly numbers are recorded to calculate the values for percent avoidance (64).

~5-year study involving the generation and screening of 11,664 individual neural specific knockdown flies, which represents about 82% of the *Drosophila* genome. This screening allowed us to identify 580 new fruit fly 'pain' genes, 399 of which are conserved through to humans. In addition, we describe about 1400 genes that were required for development of the *Drosophila* nervous system, about 1000 of which are conserved through to humans and many of which had never previously been annotated or functionally characterized in any way (2).

A global functional assessment of nociception

Of the 580 nociception genes identified through our screening efforts, we identified fly orthologs for multiple known mammalian pain genes. For example, our screen identified two additional fly orthologs of the amiloride-sensitive cation channel 3 (Accn3), an acid-sensing channel that sets tonic pain thresholds (60), GDNF family receptor alpha2 responsible for maintaining the size and terminal innervation of cutaneous nociceptors (65) also implicated in neuropathic pain, and Protein Kinase G, implicated in promoting thermal sensitization in response to inflammation (66). We also identified the fly orthologs of arrestin 1 and 2, which are involved in morphine desensitization (67), the adenosine receptor Adora important for both acute and chronic pain in mice (68, 69), a potential ortholog of the mammalian galanin receptor contributing to neuropathic pain behavior in mice (70), NF-kappaB signaling important for acute and inflammatory thermal pain in mice (71), a fly GPCR similar to mouse cannabinoid and lysophosphatidic acid receptors (72), a fly ortholog of DREAM, a component of the spinal gate (73), and a fly ortholog of the cholecystokinin receptor that influences thermal pain thresholds (74).

We used Gene Ontology (GO) enrichment and gene set enrichment analysis to annotate our candidate pain hits into biological, molecular, and cellular categories. Statistical analysis of GO terms revealed a significant enrichment of 45 GO terms, including genes known to be involved in ATP synthesis, mitochondrial function, neurotransmission (33 genes), ISWI and NURF nucleosome remodeling, vesicle trafficking including synaptic vesicle transport, and secretion (46 genes), in addition to genes involved in basic housekeeping functions. From our analysis, we identified 189 putative nociception genes with previously unknown GO annotations. We also performed Kegg analysis on our screening data to identify fly pathways statistically enriched in our data set. Amazingly, we saw an enrichment for *hedgehog* signaling in fly nociception as confirmed later by others using the larval sensitization paradigm (7). We also found a significant enrichment for ubiquitin-mediated proteolysis pathways, signaling pathways such as Wnt, ErbB, JAK-Stat, Notch, mTOR, TGFβ, and Ca^{2+} signaling (64).

Ca^{2+} signaling is a conserved core component of the nociception apparatus

Because Ca^{2+} signaling was one of the pathways we found significantly enriched in our functional fly pain screen, and because Ca^{2+} signaling is already associated with pain perception in mammals, we focused our initial efforts on confirming some of these novel Ca^{2+} signaling components implicated in nociception. One of the first genes we characterized was a relative of the original fly pain gene *painless*, a warmth activated TRP channel called *dTrpA1*. While *painless* is not conserved in mammals, *dTrpA1* is (75), and *TRPA1* is well characterized as a heat pain gene in mammals, at least during conditions of inflammation (76). We were able to confirm that *dTrpA1* is indeed a heat pain gene in adult fruit flies as well as fruit fly larvae. In addition, we could detect a role for *dTrpA1* in heat nociception using both RNAi transgenic animals and conventional *dTrpA1* mutant flies, and these data were specific because we could add back the *dTrpA1* gene on the mutant background and rescue the observed heat nociception defect (64). Finally, using tissue-specific knockdown of *dTrpA1* specifically in *Drosophila* sensory neurons (MD-Gal4), we could show that *dTrpA1* is at least in part acting directly in the sensory nerves to detect and mediate the nociceptive response to noxious heat. While *TRPA1* has been known as a pain gene in mice for some time (76), recent evidence also implicates *TRPA1* in human pain, specifically in generating spontaneous pain in patients with familial episodic pain syndrome

(77). Thus, a role for *TRPA1* in nociception is conserved from flies through mice to humans, although the specific role in the process of nociception seems to have a degree of flexibility through evolution.

Another Ca^{2+} signaling gene we found by screening the fruit fly genome for nociception behavior was *straightjacket* (*stj*, α2δ*3*, *CACNA2D3*). α2δ*3* is a peripheral component of multiple Ca^{2+} channels, and mammalian α2δ*3* is closely related to α2δ*1*, the molecular target of gabapentin and pregabalin (2, 78). Using multiple RNAi hairpins and somatic mutant flies, we were able to confirm a role for α2δ*3* in *Drosophila* larval and adult heat nociception. Further, we could also rescue this defect by adding back the α2δ*3* gene; thus, α2δ*3* is another *bona fide Drosophila* pain gene. To evaluate if α2δ*3* is a conserved pain gene in mammals, we tested α2δ*3* knockout mice. Indeed, mice with mutations in the *stj* ortholog gene α2δ*3* displayed an impaired response to noxious temperatures in the hot-plate assay at 50–56°C and also a delayed kinetics of inflammatory pain sensitization. Interestingly, α2δ*3* site of action was traced up to the thalamus, where α2δ*3* is required for containing heat pain processing to the pain centers of the brain, and without α2δ*3* pain impulses spread to other sensory processing centers including the visual and auditory cortex. Thus, α2δ*3* represents the first gene ever shown to participate in sensory cross activation, termed synesthesia in humans. Importantly, we identified single-nucleotide polymorphisms in the human α2δ*3* gene that associate with altered acute and chronic pain perception in human patients. Thus, we have again showed the value and medical relevance of nociception research using *Drosophila*, and without approach we have identified α2δ*3* as a novel acute pain gene in flies, and acute and chronic pain gene in mice and humans. Again, the particular mechanistic details may not necessarily be conserved from flies to humans (for example, α2δ*3* is expressed in the peripheral nervous system (PNS) and central nervous system (CNS) of fly, but only in the CNS of mice); however, nociception research in *Drosophila* can clearly identify novel conserved nociception and chronic pain pathways (2).

Summary

Mammalian animal models are the primary models of choice for investigation of various human diseases including nociception and chronic pain. Nevertheless, introduction of *Drosophila* into the field of nociception has facilitated discovery into the conserved genetics of nociception and has become a powerful new addition to the field. In this review, we describe the techniques available to study the conserved genetics of nociception in the fruit fly. Recent work by our group and others has highlighted known and novel conserved regulators of nociception and has proven that *Drosophila* is an influential new model for studying nociception, particularly with respect to powerful genetic tools available for fruit fly researchers, and the feasibility of *in vivo*, tissue-specific high-throughput screening for nociception behavior.

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