AMAZING PAPERS IN NEUROSCIENCE
The Grasshopper Mouse and Bark Scorpion: Evolutionary Biology Meets Pain Modulation and Selective Receptor Inactivation

Benjamin M. Thompson
School of Psychology and Neuroscience, University of St. Andrews, St. Andrews, UK KY16 9JP.

Pain, however unpleasant, is a vital part of survival, providing a motivating response to noxious stimuli that helps move us away from danger. In medicine, adequate pain control can be maintained using analgesics, many of which produce unwanted and complicating side effects, most notably opioid analgesics. Here, I review a study which explored the unique predator/prey relationship between the Southern grasshopper mouse (*Onychomys torridus*) and its natural prey, the Arizona bark scorpion (C*entruroides sculpturatus*). *O. torridus* has developed an analgesic response to the scorpion's usually highly painful sting and, in doing so, provides a wonderful display of ion channel function and evolutionary biology.

O. torridus' unique adaptation serves as a strong example of Krugg's Principle, which states there exists a best animal specimen for any scientific question. This principle is utilized to great effect by the authors to better understand receptor activation in pain. The study gradually progresses from an animal behavior model to isolating the amino acid residue in the ion channel responsible for the pain-relieving effect of scorpion venom on *O. torridus.* This provides a convincing argument for the potential of highly selective analgesics and the prospective sites of action for these future drugs.

Key words: Analgesia; Voltage-gated Sodium Channels; Neuropharmacology; Pain; Evolutionary Biology

The safe and effective management of pain is a challenge that medicine has wrestled with for thousands of years. Our modern efforts still raise a variety of complicating issues, often due to the unsavory systemic effects of analgesic drugs such as opioids. The development of highly-specific analgesics which produce pain relief without side effects is a pharmacological holy grail, but what neural sites could be targeted, and what effects would they have?

Acute pain transmission is mediated by small diameter dorsal root ganglion (DRG) neurons in the central nervous system (Purves et al., 2001). These neurons express the main nociceptive receptor channels, the voltage-gated sodium (Na^+) channels Nav1.7 and Nav1.8, which are responsible for the initiation and propagation, respectively, of the action potentials that relay noxious stimuli, and subsequently produce a painful sensation. Many creatures have developed evolutionary adaptations to deter predators which exploit voltage-gated sodium channels to cause pain by administering a venom that hijacks the system, causing hyperactivation of sodium channels and a lasting painful response (Jami et al., 2018). It is uncommon to see a higher pain threshold as a counter-adaptation in predators as this would greatly increase the risk of injury or death. However, Rowe et al. (2013) explored one example of this in the unique and fascinating evolutionary adaptation of the southern grasshopper mouse (*Onychomys torridus*) to the venom of its prey, the Arizona bark scorpion (C*entruroides sculpturatus*).

The authors present an exceptional example of a well-structured study which investigates *O. torridus*' immunity to *C. sculpturatus* venom. They begin by eliciting pain response behavior in house mice (*Mus musculus*) and *O. torridus* through the injection of bark scorpion venom, formalin, which induces a prolonged pain stimulus, or saline into the hind paw. House mice licked their paws furiously on injection of the venom, whereas *O. torridus* licked their paws only briefly, and for less time than they did with the saline control. To assess whether this reduced sensitivity to toxins was generalized or specific to the scorpion venom, formalin was also injected. *O. torridus* exhibited an increased pain response to formalin compared to saline and venom, but this was still reduced in comparison to the *M. musculus* pain response to formalin. The striking difference in behavioral response to venom is illustrated well in Figure 1A (figure references to Rowe et al., 2018) and serves as a good example of an animal behavioral paradigm with readily interpretable results. As such, it appeared that *O. torridus* showed a partially selective reduction in pain response in the presence of *C. sculpturatus* venom. The modest reduction in pain response seen after formalin injection may also indicate a higher general threshold for pain than *M. musculus*.

By injecting slow ramping currents into dissociated small-diameter DRG cells, the authors determined the baseline membrane excitability of DRG cells in both species of mouse (Fig. 3). The action of bark scorpion venom significantly increased the membrane excitability of *M. musculus* neurons and conversely, decreased excitability in *O. torridus* neurons. Venom blocked action potentials from firing, thereby appearing to induce a pain-relieving effect, contrasting with the highly painful response in *M. musculus* caused by venom-induced DRG hyperactivity. This electrophysiological representation of the pain-relieving effect of venom is shown beautifully in Figure 3A, with membrane action potentials absent in *O. torridus* after venom application. Returning to the paw-licking behavior, venom-induced analgesia was further investigated in both mice by injecting formalin into the hind paw after a previous injection of *C. sculpturatus* venom. *M. musculus* experienced an increased paw licking response

to formalin, expressing a hypersensitivity to further painful stimulus after pre-treatment. As might be expected by this point, the response in *O. torridus* was the opposite, with formalin-induced paw licking significantly reduced after venom pre-treatment, showing that bark scorpion venom produces lasting analgesia in the grasshopper mouse.

The next step was to determine the physiological change in *O. torridus* that reduces the painful symptoms of the venom. It was established that *C. sculpturatus* venom selectively binds to the Nav1.7 channel in *M. musculus*, causing a hypersensitivity of the DRG neuron, with no effect on the sodium current of the Nav1.8 channels in the same cell. This was discovered by selectively blocking Nav1.7 in *M. musculus* DRG neurons with tetrodotoxin (TTX). In the absence of TTX, *C. sculpturatus* venom increases Nav1.7 current. After TTX application, venom did not affect the channel currents of either Nav1.7 or Nav1.8. Interestingly, the current recorded from Nav1.8 was reduced by venom in *O. torridus* DRG neurons in a dose-dependent manner, suggesting a difference in venom binding and action between mouse species.

Having highlighted NaV1.8 as a point of interest, the Rowe team sequenced this sodium channel and searched for changes in its structure between *O. torridus* and *M. musculus*. Using expression vectors to produce multiple chimaeras of the *O. torridus* channel (otNav1.8), the domain where venom binding takes place could be isolated (Fig. 4). Each of the 4 otNav1.8 transmembrane domains were replaced with the corresponding domain from *M. musculus* (mNav1.8) and the channel was then assessed for changes in activity on venom introduction. In the chimaera with domain II replaced, the inhibitory effect of venom was essentially abolished. This clear change in channel activity after replacing domain II is seen in Figure 4D. Next, domain II in mNav1.8 was exchanged with domain II from otNav1.8, resulting in the development of sensitivity of that channel to *C. sculpturatus* venom, and thereby development of the pain-relieving response.

The final aim of the paper was to identify the specific amino acids in domain II which permitted venom binding to Nav1.8. Using site-directed mutagenesis of amino acid variants at the SS2-S6 linker structure at the channel pore (it is known that *C. sculpturatus* venom binds to these areas in Nav1.7), it was shown that a glutamic acid residue at position 862 (E^{862}) was responsible for the sensitivity of otNav1.8 to venom. By replacing this residue with a hydrophilic glutamine residue, the sensitivity of the channel to *C. sculpturatus* venom was almost abolished, highlighting its role as a major, but likely not exclusive, site of venom action.

The presence of glutamic acid or glutamine at positions 862 and 859 of domain II of the Nav1.7 channel is conserved among a variety of rodent and primate species, suggesting the importance of these amino acids in the structure and function of this channel. As such, it appears that, due to a novel interaction between these residues and the binding properties of bark scorpion venom, *O. torridus* has benefited from a unique opportunity. By developing an adaptation that not only negates the usually painful venom of its main prey, but produces a pain-relieving effect, the grasshopper mouse uses the scorpion's venom to its own advantage in a fascinating counter-attack in the evolutionary arms race.

VALUE

Through the study of *O. torridus'* response to its prey venom, Rowe and colleagues highlight the value of interdisciplinary approaches in research, creating a set of experiments that relate animal behavior, electrophysiology and molecular neuroscience with clarity. They also provide a robust example of Krugg's principle, where there is thought to be an ideal animal for any given experiment; a useful theory in the teaching of good practice in experimental design.

The structure of the paper is such that the reader can readily follow the researchers' journey of discovery from the whole animal model, to the isolation of the Nav1.8 channel, channel domains, and finally the specific amino acid residues responsible for *O. torridus'* pain-relieving response. The findings also suggest the potential role of both Nav1.7 and Nav1.8 channels as targets for analgesics in the future. The adaptation of the grasshopper mouse provides a tantalizing window into how selective activation or inactivation of the right channel could produce highly specific analgesia, possibly abolishing the negative side effects of present clinical analgesic drugs.

It is worth noting that the paper makes use of established experiments to elicit novel findings from a unique animal model rather than attempt to develop an entirely new experimental method for this research. Not only does this emphasize the importance of finding inspiration in novel attributes of animals and continued exploration of conventional and successful experimental paradigms, but it also ensures that the paper is easy to follow for the student, who has likely already encountered the methods used in this work. The study therefore provides a valuable context for the student to better understand the often challenging systems of pain modulation and voltage-gated sodium channel function.

AUDIENCE

This paper provides an elegant consolidation of animal behavior, neuropharmacology, neurophysiology and molecular biology teaching for students in advanced undergraduate classes, allowing them to apply their understanding of channel structure and function to a real life, experimental model of pain. There is a strong emphasis here on the movement in modern neuroscience research to be more interdisciplinary. The complimentary use of approaches from animal behavior, electrophysiology, and genetics may allow a better contextualization of these disciplines than would be possible separately. This may help students understand multiple complex concepts with an easily visualized animal model as a more digestible point of reference. This could be particularly useful in courses from as wide a range as advanced undergraduate pharmacology to neurobiology.

The paper can spark lively debate on the future of analgesic pharmacology and the clinical applications of

highly-specific drugs. The clear example of Krugg's Principle in this study can also spur the imagination to consider what other unique and fascinating adaptations creatures have evolved, and what they might teach us.

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Address correspondence to: Ben Thompson, School of Psychology and Neuroscience, University of St. Andrews, St. Andrews, UK KY16 9JP.
Email: bmt2@st-andrews.ac.uk

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