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Do “Prey Species” Hide Their Pain? Implications for Ethical Care and Use of Laboratory Animals

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Abstract

Accurate pain evaluation is essential for ethical review of laboratory animal use. Warnings that “prey species hide their pain,” encourage careful accurate pain assessment. In this article, I review relevant literature on prey species’ pain manifestation through the lens of the applied ethics of animal welfare oversight. If dogs are the species whose pain is most reliably diagnosed, I argue that it is not their diet as predator or prey but rather because dogs and humans can develop trusting relationships and because people invest time and effort in canine pain diagnosis. Pain diagnosis for all animals may improve when humans foster a trusting relationship with animals and invest time into multimodal pain evaluations. Where this is not practical, as with large cohorts of laboratory mice, committees must regard with skepticism assurances that animals “appear” pain-free on experiments, requiring thorough literature searches and sophisticated pain assessments during pilot work.

Keywords

laboratory animal – pain – animal welfare – ethics – animal behavior

1 Introduction

As a veterinarian with an interest in laboratory animal pain management, I have read articles and reviewed manuscripts on how to diagnose a mouse in pain. The challenge, some authors warn, is that mice and other “prey species”

may hide or mask their pain (Dwyer, 2004; Malik & Leach, 2017; Allweller, 2019; McLennan et al., 2019; Mogil, 2019; Turner, Pang & Lofgren, 2019; WIRES Northern Rivers, 2020). You approach the cage, the mouse does her best to look fit and strong, and you wrongly assume she's not in pain. When this happens, pain scientists record incorrect data, or scientists withhold the analgesic medicines that might help the mouse feel better, or the veterinarian tells the researcher to continue with their animal experiments, all the while assuring the ethics committee, incorrectly, that the experiments are going well and need no further refinement.

In this article, I ask if this claim about prey species' pain manifestation is true, and what ethical implications for practice should follow from deciding on its truth. It is essential to have the most accurate facts about the animals to aim for the most ethical (animal) cost / (human) benefit balance. It is essential to conduct an ethical reckoning of what to do when the facts about animal suffering are limited and uncertain (Carbone, 2019; Institute for Laboratory Animal Research, 2011).

If mice, rabbits, sheep or others truly hide signs of pain from humans they perceive as dangerous predators, there are important implications for practice. Pain biologists must factor this into their measurements of animal pain or shift their studies to "non prey" species, if such exist, that are less deceptive. Veterinarians and animal ethics committees must factor in the likelihood of under-diagnosed animal pain in their protocol reviews and pain management plans. All scientists who work with animals must receive training on pain assessment strategies that are not confounded by pain-masking behaviors, if there are any. On the other hand, if prey species are easily diagnosed when painful, or by contrast, if *all* animals challenge easy pain diagnosis, different ethical norms will follow.

Three overlapping communities have a stake in accurate mouse pain diagnosis in the laboratory. Pain biologists using mice to model human pain need accurate, reproducible analgesiometric methods to quantify pain—often pain that they have induced—and the relief that experimental analgesics they are testing provide. Scientists in other fields may also induce pain, but as an unwanted contingency, a side-effect of the experiments they are performing (Russell & Burch, 1959). They must work with their ethics committee and veterinarian to assess the likely severity for the animals of experiments they are planning, to plan for preemptive pain management, and to monitor animal welfare during an experiment. Veterinarians and animal caregivers must also monitor for spontaneous pain separate from what the experiments causes, such as fight wounds, cage injuries, and a variety of illnesses animals may

develop as they live and age in the vivarium. All of this is extra challenging if human presence suppresses the pain behaviors they are trying to monitor.

Pain biologists have refined methods for performing analgesimetry, i.e., *measuring* pain in animals, especially rodents (Mogil, 2019). Their tools are efficient, quantifiable, reproducible. The data they report may generalize to other species, especially humans. Their goal is to generate data, even at the cost of causing pain in their animals, not to manage their research animals as clinical patients. On the other hand, veterinarians in companion animal practice collaborate with animal guardians or owners, devoting time and effort to *diagnose* the individual household animal. Their labor is patient-centered and aimed at developing a successful therapeutic plan to treat current pain and reduce situations that exacerbate it. Both approaches to pain assessment can inform best practices for pain management for animals of all species in laboratory experiments and both can be thwarted if some species consciously or reflexively somehow mask their pain.

Pain biologists' practices and needs frequently differ from the others. In many model systems, they will choose one or two pain measurement assays and at most a handful of time points, and conduct their studies when they expect peak pain, whether that is hours after injecting an inflammatory agent or weeks after surgically injuring a nerve. If studying analgesic medications, they will run the test when the drug should be at peak levels. After the tests, most such experiments end with the animals' immediate euthanasia, obviating concerns for ongoing diagnostics and pain management (Mogil, 2019). For the sake of quality data, they must minimize the confounding effects of animals somehow hiding or masking the pain the scientists need to measure. Mice are thus better objects of study when the scientist's presence does not affect the readout of the pain measurement.

Contrast this with the needs of mice as the feeling subjects of clinical concern. By "clinical" in the laboratory context I mean all of the actions scientists and animal care professionals do to maximize animal health and welfare for the sake of the animal. Pain may be acute, during a painful event like a surgery, fight or cage injury; subacute, in the convalescent days following a surgery; or chronic as illnesses progress. Whereas pain scientists may succeed with a handful of time point measurements to graph the trajectory of pain or analgesia, welfare is a continuous concern for the animal, even if the humans will only evaluate them periodically. Ironically, scientists with the least specialized training in pain recognition may be the ones conducting experiments with the highest severity. Their animals are the most in need of accurate and sensitive pain diagnostics. If scientists, veterinarians or caregivers scare

the animals into hiding their pain, the animals and sometimes the data will suffer (Peterson, Nunamaker & Turner, 2017; Carbone, 2019). My concern here is with a particular subset of painful states, those that are severe enough to affect the animals' subjective well-being in ways that might benefit from analgesics, but not so overwhelming that even a casual observer would know that something is wrong.

I appreciate the warning that mouse pain can be hard to detect with an observer present. I've known scientists and even veterinarians who assume they can quickly look at a cage of five identical white mice, asleep or awake, and know if one of them is painful. When they miss seeing that their mice are in pain during the course of an experiment, they then propagate a cycle, asking their ethics committee to approve further experiments and writing up their scientific reports without ever knowing, and therefore, their audience ever knowing, that the animals on a particular experiment are in fact quite painful and could benefit from analgesics, from refined procedures, or from not doing the procedure at all (Carbone & Austin, 2016). They also miss seeing under-treated pain as a potential source of data confound (Peterson, Nunamaker & Turner, 2017).

The idea that rodents as prey animals actively hide their pain is widespread in veterinary, animal welfare, and pain biology literature. But is it true, and are there animals who differ in this regard? Writing with a dual concern for animal welfare and for the predictive value of rodent pain models for human pain biology, Mogil has noted that the idea of prey animals masking their pain seemed a reasonable hypothesis, but could find "no actual data demonstrating differential willingness to display overt pain-related behaviors in prey versus predator species" (Mogil, 2019). I too have challenged authors making this claim to provide a reference to relevant primary data; invariably manuscripts come back with the prey species statement removed rather than supported by citation of data.

Scientists, veterinarians and ethics committees need solid information on mouse pain, and on pain in all the animals in laboratories. Does this prey-species-hide-their-pain trope help? Is it true? Might it actually cloud our thinking about animals' pain, with resulting failures to meet their needs?

In this article, I review:

- What signs of pain an animal might hide in the presence of a predator
- Which species are "prey species" in a world in which human predation touches even the largest of animals
- What empirical data support the claim that prey species hide their pain and that non-prey species do not

- The possibility that prior acclimation to humans may be a more important factor than species-membership and dietary practices in determining how visible an animal's pain can be to a human observer
- How diagnosis of pain in household dogs differs from pain diagnosis in laboratory animals, with implications for clearer thinking about pain in the animal laboratory

I conclude that while species membership is fixed, acclimation to humans is a learned and trainable behavior. I propose that pain diagnostics will be most robust if animals learn to trust human handlers and observers following a gold standard of clinical pain management for household companion animals. I encourage ethics committees to set a high evidentiary bar for any claim that a procedure or condition that would be painful to a person is not in fact similarly painful to a nonhuman.

2 What It Means to Hide Pain from a Predator

First, what does it mean to hide pain in the presence of a perceived predator? Animal pain diagnosis has several components, including:

1. Evaluate decreases in normal behaviors
2. Watch for increases in pain-associated behaviors
3. Physical examination, including palpation of suspected painful body parts
4. Dynamic evaluation of behaviors when animals are forced or encouraged to move or work

Animals in pain may decrease normal behaviors, such as eating, grooming, nest-building, exploring, interacting socially, and more. Decreases in normal behavior may be visible during real-time observation of animals' behavior, as well as looking for what I call the "footprints of pain," such as weight loss (an indicator of reduced eating), untouched nesting material, or unkempt fur. Animals, in pain or in health, may also "freeze," stopping feeding or grooming or other normal behaviors in the presence of humans they find scary (Boiles, 1970; Roelofs, 2017).

Animals in pain may increase abnormal behaviors. Pain-associated signs that may emerge in a painful animal include vocalizing and emitting pheromones, along with the more visible signs of grimacing, twitching, panting or attending to an injured body part. Pain diagnosis also includes physical examination of pain through palpation of body parts: press on the sore area and the human or nonhuman patient may vocalize, tense up, or attempt to move

away. Finally, pain diagnosis includes dynamic evaluation, forcing or encouraging the animal to walk, to reach up for treats, to incorporate new materials into a nest.

How does this suite of signs change in the presence of a perceived predator? An animal in pain is unlikely to suddenly mask a decrease in normal behaviors. She will not suddenly start eating, grooming or building a nest when a human "predator" approaches, trying to hide that she has not been performing those normal behaviors, the murine equivalent of saying, "I'm fit and strong; don't try to eat me." Normal behaviors such as climbing to the cage top for a treat could be observed in real time; perhaps an animal in pain would do this with an intimidating human predator observing, to somehow hide that he hurts? Physical examination is a component of pain assessment; an animal in pain may tense or squirm or vocalize when the sore spot is touched. Perhaps a scared prey animal will submit to physical examination and palpation but will suppress any tensing or vocalization that might betray to the veterinarian that she has touched a sore spot or manipulated a painful joint?

Rather, those who say animals hide their pain are presumably talking mostly about emergent pain-associated behaviors, such as twitching and writhing or assuming postures and facial expressions that animals may do when in pain and presumably stop doing when a human approaches. This is testable, both that prey animals in pain act more normally and less painful when humans are present, and that "non-prey species" do not. But testing this is not easy as it requires scoring painfulness in animals believed to be deceptive about their pain. One analogy could be studying animals' behavior in the dark by entering the room and turning on the lights so the scientist can see the behaviors; the very act of measurement distorts the behaviors being measured.

To evaluate predator effects on prey species' pain behaviors, Young studied ewes subjected to a device that delivered measured mechanical pressure on the lower leg, in the presence or absence of a dog, whom the sheep might perceive as a potential predator (Young, 2006). The ewes showed a higher tolerance of the pressure (greater latency to move the leg) when a dog in a nearby pen than when a non-predatory goat was present. The assay required that humans directly handle the sheep, and Young acknowledged that she found no way to control for human presence, a concern if sheep are one of those prey species that may perceive humans as threatening potential predators. Thus, it appears in Young's work that dogs may indeed alter the ewes' leg-moving behavioral response to pain, or in other words, that ewes may consciously or not hide their pain from dogs. As for the crucial question of whether human presence affects signs of pain in sheep, the data do not say. Perhaps the response to

dogs is additive to the already-fearful response to human presence, or perhaps human presence elicited no aversion from the sheep whatsoever. Nor is it clear in her description of the project how well the individual people and sheep knew each other, a concern I will return to below. But also, consider the possibility that in the presence of scary dogs, the ewes' pain *levels*, not just their pain behaviors, may shift.

Sorge et al. (2014) used different pain assays to measure pain in a different "prey species," injecting mice with zymosan (an inflammatory chemical) and then monitoring their facial grimaces as evidence of pain, as well as scoring licking and self-biting in response to formalin injections. They found that mice showed less evidence of pain in the presence of human male observers than female observers, or even in the presence male scientists' worn T-shirts, perhaps somehow categorizing male-smelling humans as more of a predatory threat than females.

Young (2006) and Sorge et al. (2014) presented evidence corroborating a prey behavior of pain-masking, but pain really is quite complicated, and so Sorge's group explored the phenomenon in greater depth. They measured stress hormones along with pain-associated gene-expression in spinal cords, and they manipulated the pain the mice would feel by using the opioid-antagonist drug, naloxone. They concluded that fear of male "predators" decreases signs of pain not because the painful animals are actively suppressing the grimaces that signal pain, but because of stress-induced analgesia (SIA). In SIA, humans and other animals, including perhaps Young's ewes, actually suppress pain itself in moments of crisis; they appear less painful because they actually feel less pain (Butler & Finn, 2009). Opioids and opioid antagonists (naloxone) can manipulate animals' pain perception with resultant effects on freezing behavior (Hammer & Kapp, 1986). Perhaps animals in pain are at least somewhat more inclined to freeze when scared by unfamiliar humans even as their fear acts via SIA to reduce their painfulness.

The mechanism does not need to involve conscious deliberation for it to be successful, though words like "hiding" and "masking" do seem to imply that. That said, even if not a conscious deliberation, these responses of painful and pain-free animals in response to perceived threats may be modified with acclimation.

The clinical situation however is rarely about survival and more often about veterinary decisions to treat pain and ethics committee decisions to allow painful experimental procedures. Whether fear of some humans leads to momentary suppression of pain or to momentary suppression of *signs* of pain may at first seem like hair-splitting, especially if both result in enhanced

survival when predators are lurking, or both equally result in skewed data in pain biologists' assays. For a clinical/welfare focus in animal laboratories, fear-induced analgesia may make painful mice harder to diagnose, but if the effect wanes when the observer leaves, the mouse is once again in pain, while the person with the analgesic medicines has left the room, and the mouse has had a temporary, stressful, scary interaction layered onto the pain. In Sorge's mice, the analgesic effect of stress did not last more than a few minutes, so mice not only suffered the fear, but did not even get a lasting analgesic effect from it. Thus, it is in fact worth knowing both if and why mice or sheep in pain act differently when perceived predators are too close for comfort. What practical applications might this information result in? Should all animal work be performed only by female scientists and prepubertal boys? Should all scientists switch from using "prey species" like mice, rabbits and sheep to other species for experiments that might cause pain?

3 Are Prey Animals the Only Ones Whose Pain Is Hard to Diagnose?

The "prey species hide their pain" claim implies there are other species whose pain is easy to read. So, what species are prey who hide their pain and what species are non-prey who do not? What animals are in fact easy to diagnose when in pain?

Reports of different species being more or less readable in their pain than others are more anecdotal than scientifically documented. In fact, few who make this claim even offer anecdotes as evidence, so pervasive is this belief. Malik and Leach (2017) say that pain is more evident in rats and mice than in "other prey species" such as rabbits. Allweller contrasts "predatory" dogs to horses and rabbits and other prey animals that may hide their pain (Allweller, 2019). Dalla Costa et al. (2014) posit horses as a species that may suppress obvious signs of pain in the presence of humans, their perceived predators. Pierce writes that cats and rabbits exhibit "stoicism" and "mask their pain quite effectively," while also noting that dogs in pain can be tricky to evaluate too, behaving differently when humans are present (Pierce, 2013). Even injured echinids are said to hide pain from potential predators (WIREs Northern Rivers, 2020).

The ideal experiment to test the claim that prey species differ from others in how they hide their pain would be a within-taxon comparison, comparing two near-identical species that differ only in their tendencies to be prey to other animals (Kavaliers & Choleris, 2001; Mellor, Kinkaid & Mason, 2018). Instead, we compare species with multiple potentially significant differences. If mice

somehow hide pain from humans but dogs, for example, do not, is it because dogs are more predatory, more genetically domestic, larger than mice, more social, more vocal in ranges that humans can hear, or that dogs tend to receive more time and attention from humans? Why single out predatory behavior as the cause for a difference in pain behaviors? Better to compare a mouse to a similar, related species that is not hunted, if there were one. But there is not.

Mice, horses, Guinea pigs, and sheep are paradigmatic prey species. They are hunted. But while sheep and horses may not hunt, mice will, in fact, hunt various tasty, nutritious bugs (Hoy et al., 2016). The category of “prey species” is problematic. Cats are consummate predators, at least as much as the omnivorous dogs who sometimes kill cats. Where I live, in northern California, both are also prey to the occasional coyote or mountain lion. They are thus mesopredators, hunter and hunted, as are so many species, including mice, rats and some other rodents. Even large predators may fall prey to other species, such as orcas killing large sharks, or to cannibalism from their own species-mates (Amstrup et al., 2006; Engelbrecht, Kock & O’Riain, 2019; Jorgensen et al., 2019).

Predator-prey dynamics differ widely, and we should expect different avoidance behaviors among prey animals, some learned and some innate (Boiles, 1970). An owl will likely pounce on a mouse no matter how strong and healthy the mouse looks, so the mouse’s task is to hide her entire self, not just a sore leg or a painful grimace from the owl. On the other hand, a zebra on the savannah cannot hide well from a lion who is watching the herd and plotting her strategy. That zebra may indeed gain value in not being visible as the most vulnerable member of the group, passing himself off as capable of giving a lion a serious kick. Some sharks, likely whether in pain or not, avoid waters frequented by shark-killing orcas (Engelbrecht, Kock & O’Riain, 2019).

Beyond fear of perceived predators, animals have reasons to modulate their behavior in the presence of threats, including threatening conspecifics. Large male lions, dominant alpha baboons, jack kangaroos are at near-constant risk of violence from others of their kind, not as acts of predation but as acts of social stress. They too have cause to hide any weakness, though as with mice, that could be modulated through a stress-induced response rather than a consciously deliberated calculation that it is time to look tough. Male mice may perceive other male mice as threatening, and show the same evidence of stress-induced analgesia when exposed to unfamiliar male mice as they do around male humans (Langford et al., 2011).

If virtually all species in the close confines of a laboratory setting would innately see humans as threatening potential predators, then all animals are “prey species” and all should be unreadable in their pain. Change the word

"prey" to "quarry" and it is obvious that where humans are concerned, most species have reason to see us as dangerous. Blue whales, the largest animal ever to live, have been the victims of human predation and humans are the main killers of most large carnivores and herbivores on land and sea. Apex predators will hide—not just their pain, but their entire bodies—when humans come on the scene. Even just playback recordings of humans speaking will create what Suraci calls "a landscape of fear" (Suraci et al., 2019). In a captive or laboratory situation, where prey, predators or meso-predators cannot get their distance from us, we should expect them to freeze in place, hide if they can, tense up or counter-attack if restrained for an exam.

A laudable response to the concern that prey animals hide their pain are the efforts to better standardize quantifiable, reproducible pain measures, and those efforts can certainly be extended to all laboratory species. Currently, standardized tables of "pain faces" or grimace scores are gaining traction, in mice, rats, rabbits, horses, sheep, pigs (McLennan et al., 2019). Validated grimace scales are available for mesopredator cats and ferrets, and for preverbal human infants. Their value is both to allow quick valid real-time pain diagnosis for individuals' clinical care as well as for readouts in pain biology experiments. Grimace scales can also be part of assessing the severity of standardized experimental models with the various species (Jirkof et al., 2020; Keubler et al., 2019). Interestingly, though this could be a simple project for a student, and though guides to dog facial expressions have been available for decades, there are no validated published dog grimace scales or pain faces in the literature (Darwin, 1896; Reid, Nolan & Scott, 2018). Does this reflect a belief that dog pain is in fact much easier to score and not in need of such a scale?

Almost no one writing that prey species hide their pain names the non-prey animals who are more easily diagnosed. I assume most would say, "Dogs," rather than a more general claim that lions, wolves, crocodiles or other predators in pain are easy to read. My experience as a veterinary clinician is to agree, or at least to say, "Some dogs, some of the time, in some circumstances." Allweller attributes this to dogs' predatory nature (Allweller, 2019). As a veterinary clinician, I have found notable individual differences within species as much as between species, and veterinary practitioners and scientists reveal similar concern about how easily dog pain is diagnosed (Conzemius et al., 1997; Weber, Morton & Keates, 2012; Reid, Nolan & Scott, 2018). I have worked with timid laboratory dogs and scared shelter dogs; both tense as the human hand approaches and make a reliable examination difficult. To complicate things yet further, even very friendly human-oriented dogs will act differently with a person present or absent, showing, and perhaps feeling, differences in painfulness

when there is a nurturing human present to interact with (Hardie, Hansen & Carroll, 1997; Hansen, 2003).

Though not extensively studied, it seems clear that some animals—prey, predator, and mesopredator—will sometimes change their pain-related and other behaviors in the presence of humans in ways that make pain less amenable to diagnosis. This may be especially problematic in the animal laboratory, where a person who may not be a trusted caregiver conducts a seconds-long visual exam of an animal in a cage, possibly with a hands-on exam of an animal who can only be handled and palpated against his or her will.

4 Dogs: The Animals Who Only Sometimes Hide Their Pain?

It is worth looking more closely at pain diagnosis in dogs and if it is true that they are more easily diagnosed, to examine why that is so, and why they too are sometimes as challenging as any other species. I propose that laboratory workers can learn from canine pain diagnosis to improve pain recognition in their research animals.

Dogs are predators and occasionally prey but there are other features of their evolutionary biology and their place in human domains that may make for more important differences from mice, sheep, rabbits, and even cats. Dogs have a long history of co-evolution with people in which both species have developed abilities to communicate with each other (Grimm, 2014). In addition to human familiarity with dogs as a species, in the Western world, many dogs and humans share a household, nurturing a mutual individual familiarity. How much an individual dog will fear and avoid people, or trust and bond with them depends on that dog's history and what she or he has learned about individual people, and about people as a species.

See how these three features—domesticity, extensive contact with their guardians, and learned trust of humans—shape what a companion animal veterinarian can expect, for example, when an aging dog develops painful arthritis. The dog's guardian spends many hours with the animal and can report on episodes of vocalizing, abnormal walking or eating, reluctance to run, slower movements up the stairs or onto the bed, changed behaviors in urinating and defecating. The vet can then complement these reports with a hands-on examination of the cooperative animal, with the vet and the guardian then working together over several days to evaluate the response to analgesics. I consider this multimodal approach the gold standard of animal pain diagnosis, but it requires the time and effort of teaching dogs that they can trust people, the time

and effort to observe multiple behaviors over hours or days, and the dog's willingness to allow a physical examination at the hands of a stranger. Note that this scenario describes a multimodal diagnostic approach as outlined above that combines historical evidence with "real time" evaluations:

- Decreases in normal behaviors, such as reluctance to run or hop onto the bed
- Increases in emergent pain behaviors such as limping and vocalizing
- Palpation of the suspected painful joints
- Dynamic evaluation of the dog walking and of the clinician's movement of the animal's limbs
- Evaluation of the above in response to analgesic treatments

5 It's Not Them; It's Our Relationship with Them

Pain biologists usually need simple, quantifiable data points of painfulness in their study animals. For much of their work, if they acclimate their animals to the laboratory and the procedures, they collect behavioral and other data on pain and pain medicines without the complex information the clinician and dog guardian are working with. They are looking to *measure* pain, to extrapolate from their animal models to humans, whereas the clinician is looking to *diagnose* her veterinary patient.

Laboratory animal veterinarians and researchers likewise seek simple quick pain assessment tools, though their goal is animal welfare rather than pain data. They want to come in the morning after an animal surgery and evaluate the need for analgesia or examine an animal on a longer-term project and decide on euthanasia of animals who have reached study endpoints. At a time when pain biologists are looking to go outside of the rodent lab to learn what they can in the complexity of companion animal veterinary patient diagnosis, laboratory animal veterinary clinicians continue to seek standardized uncomplicated assays from the pain biologist's toolkit (Klinck et al., 2017; Mogil, 2019; Turner, Pang & Lofgren, 2019). Where this fails, it may have less to do with species and their place in a food chain and more to do with the human-animal relationships in the laboratory.

Dogs and humans have co-evolved a disposition to focus on each other and form relationships, but learning fine-tunes that relationship. Many dogs never learn to trust people while many other animals do and develop strong relationships with their humans even in the laboratory; both outcomes affect the success of clinical pain diagnosis (Davis & Balfour, 1992; Russow, 2002; Driel &

Talling, 2005). One factor for quality animal welfare assessments may be how tame or well-acclimated to human presence the individual animal is. Surely, some species are more inherently tamable than others. Normally, a minimally acclimated rat will resist any restraint, so holding a tense or struggling rat to palpate for pain becomes a useless diagnostic. It distresses the animal without informing pain management decisions. However, in my experience, a rat well-accustomed to handling will sit cradled in the veterinarian's arm eating treats while the veterinarian palpates for evident sore spots. A simple test for pain in caged monkeys is to distribute raisins on the top bars of the cage to see if they feel good enough to stretch or climb up to retrieve them. Some monkeys will not do this with humans, at least, unfamiliar veterinarians, present. An unacclimated dog or cat will cower in a corner when humans are present, whether or not he is in pain, and if the cat has a box, he will hide, not just his signs of pain, but any sign there is a cat in the cage at all. It is a testable hypothesis using any of these scenarios as models, to move beyond anecdote and quantify acclimated versus non-acclimated animals' pain signs, and to score ability of known versus unfamiliar human observers to diagnose the animals' pain.

Species membership is immutable; a rabbit will never be a predator. But fear of humans and other perceived predators can diminish if the humans put in the effort (Evans et al., 2019). Animals' flight distance is not an essential fixed number for a species, but can change with acclimation and training (Stankowich, 2008). This happens in free-ranging situations, where predators and prey will both learn to approach humans for handouts, even if they will not tolerate being touched or held. And it certainly happens in a confined environment, but it requires time and effort. Pain biologists standardly acclimate animals to the facility for several days prior to any pain measurements and may acclimate them to a specific testing apparatus for half an hour or more prior to the test (Mogil, Wilson & Wan, 2001). This is common practice but I have found no literature testing the hypothesis that the degree of taming or acclimation to human handling affects how easily pain can be assessed in any species. Indirectly supporting the need for such information, Chesler et al. found that the investigator's identity was as important as a mouse's genes in predicting individual mice's pain behaviors, just as Sorge later found the difference in mouse pain data in the hands of male and female scientists (Chesler, Wilson & Wan, 2002; Sorge et al., 2014). A next step for these studies could be somehow quantifying the amount of acclimation an animal receives or some scale of the animal's approachability or tameness and correlating those indices with pain behaviors.

Standard mouse husbandry includes moving mice to a clean and unfamiliar cage every week or so. The first step is to chase the mouse to grab the tail

by fingers or forceps and then lift the animal by the tail into the fresh cage. Welfare scientists are reporting that switching to a gentler method (using a tube, or an open hand to scoop up the mouse) may reduce anxiety and stress and improve behavioral testing data (Chesler et al., 2002; Gouveia & Hurst, 2017; Clarkson et al., 2018; Mertens et al., 2019). Shifting the human-mouse relationship by changing this one simple aspect of standard mouse care could potentially affect how readily mouse pain can be evaluated. This too is a testable hypothesis: are mice who are handled more gently for routine husbandry more approachable, and can familiar or unfamiliar humans evaluate their signs of pain more reliably than they can mice who are chased and grabbed by the tail?

Scientists frequently fail to publish animal welfare-relevant information such as animal husbandry, pain management or environmental enrichment as part of their Materials and Methods descriptions in their work (Wurzel, 2007; Carbone & Austin, 2016; Sert et al., 2020). Steps they take to acclimate their animals to human handling and shift from prey-predator toward a neutral or even nurturing relationship could similarly be relevant when publishing data, improving animal welfare, improving science, and improving data-sharing and reproducibility along the way.

6 Implications for Laboratory Animal Workers and Ethics Committees

How we treat animals reflects what we think we know about them, including what we infer about their pain and suffering (Carbone, 2004). What should ethics oversight committees do if mice, rabbits, sheep and other “prey species” truly hide pain from human observers?

My sense at this point is that for most species—predator, mesopredator, and non-predator—human presence can influence how easily pain is detected and diagnosed, and that this likely differs depending on how acclimated the animals are generally to human presence, as well as to individual, familiar people. Equally important, clinical care of dogs is a paradigmatic illustration that pain diagnosis is a multimodal time-consuming task, and of course can vary with the type and anatomic location of the pain. How should these facts shape ethics committee decisions about painful procedures in the animal laboratory?

Consider the current worst-case, but hardly rare, scenario: a committee approves animal surgeries with follow-up evaluations of minimally-acclimated animals performed by scientists or veterinary staff unfamiliar with and unfamiliar to the individual animal, with pain assessed at but a few time points.

Rather than applying the principle that surgeries that are painful to humans should be considered *a priori* painful to nonhumans, the committee and veterinarians hope to do better, by evaluating the animals in real time and treating pain as needed. Thus in this case, the animals' access to analgesic medicines depends entirely on this quick interaction between strangers, with or without standardized quantified criteria. When the person gets close enough for a close visual and then physical examination, s/he likely can affect the animal's behavior and signs of pain. Poor pain diagnosis may combine with a stressful examination to actually decrease animal welfare. Ethics committees can demand better for the animals, incidentally improving the quality of the data they yield as well.

Options to improve pain management in protocols could include:

- Substituting mice with dogs or monkeys if their pain is truly easier to diagnose and manage
- Allowing protocols with “real time” pain monitoring if investigators develop multimodal assessments that factor in acclimation of the animals to the humans scoring pain
- Relying more on published severity assessments for the particular model, with the assumption that most animals in the protocol under review will experience similar severity of pain
- Require detailed multimodal assessments of pilot animals, ideally robust enough to be publishable as a severity assessment of the model

If mice hide their pain in a way that dogs or monkeys do not, should the committee urge researchers to study animals they can better evaluate and manage? Should the committee require all scientists to use the analgesiometric tools that pain biologists use, skirting the animals' fear response as much as possible through video monitoring of grimaces, activity levels or other data that do not require a hands-on or in-person evaluation? Should the committee instead decide that mouse pain is undiagnosable, and proceed with the belief that every experiment is more painful than it appears, requiring aggressive treatment with analgesics on the few painful projects it is willing to approve? Certainly, they should not allow the worst-case approach in which pain management is based on casual unstructured evaluations done by people the animals might be afraid of, lulling themselves into believing that if animals in pain, the occasional observation would detect that.

Or should an ethics committee look to the clinician's approach to individual patient pain management and require that standard of care for all laboratory animals on invasive or painful studies? Perhaps the reason we have no published validated pain grimace scales for dogs and nonhuman primates is that in the laboratory, people often do invest the time into individual animals

on invasive experiments and thus rely on a more extensive multimodal pain assessment, more similar to the clinician's approach to patient care than to the pain biologist's quick efficient single-assay scoresheet. No one knows just how many mice and rats are used in research laboratories but they are legion, and an individual approach to laboratory rodents as individual patients would be enormously time-consuming (Carbone, 2004; Goodman, Chandna & Roe, 2015).

Formal severity assessments offer a third option between the intensive pain diagnosis of the clinician and the focal pain measurements of the pain biologist (UK Home Office, 2014; Zintzsch et al., 2017; Keubler et al., 2019). A rigorous severity assessment of an experimental model includes the efforts of trained animal welfare scientists using a combination of physical, physiological and behavioral measures of animal welfare studying a few dozen animals. The severity profile and evidence-based refinements and analgesic regimens can then be applied to future cohorts of animals undergoing that model. Severity assessments with refinement protocols must be rigorous, and sufficiently powered to justify peer-reviewed publication. Scientists may then operate in accord with the protocols and may achieve better pain management (or avoid high-severity experiments) than pilot work or doing the experiment with *ad hoc* monitoring by the scientist and her veterinarian.

If animal ethics committees accept that individual pain assessments in the laboratory are rarely reliable, regardless of species, some recommendations are:

- As a first step, ethics committees must maintain skepticism about scientists' and veterinarians' ability to claim that an experiment is of low welfare severity or that animals are faring well and getting analgesics appropriate to their needs.
- The protocol should be based on published severity assessments that include evaluation of analgesics and refinements that can decrease severity and animal suffering. When a published severity assessment is not available for a particular model, adequately powered pilot studies can be of use, but they will require frequent evaluations of multiple pre-identified parameters.
- Animal behavioral management must be part of every protocol. Extensive training and acclimating all animals on all studies may not be feasible. Routine husbandry and handling should be based on best practices and current science, such as avoiding chasing and tail-restraint for mouse cage cleaning. More invasive protocols that require frequent close evaluation of animal health and welfare should include more extensive training and acclimation, to "cooperate with research procedures" and to submit comfortably to examination (Institute for Laboratory Animal Research, 2011).

- The protocol must include criteria and schedules for identifying animals who do not fare well on the pre-established pain management regimen, removing or treating animals who meet criteria. Establishing these intervention criteria requires consideration of observer effects on animal pain behavior.
- Publications should include animal welfare-relevant information in their Methods, including information on animal training and acclimation and other aspects of human-animal interaction.

7 Conclusion

Animals of most species find humans threatening. Even species such as dogs and cats, not usually labeled “prey species,” will alter their behavior in the presence of people. Intermittent examinations at the hands of unfamiliar, frightening people are thus both stressful to the animal and of limited value for managing animal welfare. Scientists, veterinarians and ethics committees will likely serve animals better if they rely less on individual evaluation of animals as an experiment progresses and more on establishing evidence-based model-specific pain management regimens derived from high quality severity assessments. Fostering a human-animal bond may improve pain management and should be a high priority for animals on high-severity experiments.

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