Quantitative Pain Proxies

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Overview

This work collects published measurements of the quantitative values of five proxies that may relate the capacity to encode noxious stimuli to the capacity for the subjective experience of pain in five animal taxa, with the goal of inferring the likely variation in the capacity for pain that exists across taxa (without identifying that absolute magnitude, or providing a rank ordering of taxa, for such a capacity) based on comparing the range of variation that exists across taxa for each proxy. I have further separated proxies by modality of noxious stimulus where it made sense to do so (e.g. the maximum response of nociceptors to noxious heat, or the increase in stress hormones after application of a noxious chemical), in which case I calculated variation based on the stimulus modality with the widest range of variation for that proxy. These proxies are generally split into two groups, neurobiological indicators and physiological responses, and while the proxies in both groups showed a similar range of variation across taxa (between 1 and 1.5 orders of magnitude difference), I am generally more confident that the neurobiological indicators provide a reliable basis for comparing variation across taxa, at least in terms comparing their capacity to encode nociceptive information.

Based on the ranges of variation observed across taxa for the five proxies, it seems likely that there is at least an order of magnitude variation in their capacity for pain, but unlikely that there are more than two orders of magnitude variation. An argument could be put forward that differences should not be considered for each proxy in isolation but instead combined, perhaps in an additive or multiplicative manner (e.g. a weighted sum of proxy values). Regardless of whether such a combination would reflect a meaningful description of how the experience of pain occurs biologically, it should be noted that no taxa consistently had the highest, or lowest, values across all the proxies, and therefore any differences between taxa, and even their relative ordering, will mostly depend on the details of the combination method used, the choice of which would be relatively arbitrary.

One caveat of this work is that although most of the studies that values were sourced from studies performed in the context of studying nociception or pain (with the general exception of those measuring Substance P concentration), few were done in the context of making direct comparisons of capacities between species, particularly outside of single given taxonomic class, yet, this study compares taxa from 2 phyla and four classes. A study intended to make direct comparisons of one or more of these proxies between such a diverse range of taxa could probably use a design that would increase the comparability of values between species, although it is not clear if such a study would produce results with either a higher or lower range of variation between taxa.

Description of quantitative pain proxies

Nociceptive JNDs

The number of discrete intensities of noxious stimuli human subjects could discriminate during psychophysics experiments. Although this research is dated and has been criticised by contemporary pain scientists for not providing insight into the holistic experience of pain and suffering (discussed in [1], [2]), I believe it provides an objective reference for the capacity of the human nervous system to encode the intensity of a noxious stimulus. Measurements were highly repeatable within and between subjects [3], and the measurements of thermal stimuli appear to have reached the maximum limit of the ability of nociceptors to encode pain ([4]; incidentally, these measurements showed that this limit is reached by many women during labour [5]). Interestingly, nociception encodes relatively few intensity levels compared to other human senses: “there are approximately 570 barely distinguishable steps for vision in the range of intensity from complete darkness to the dazzle point and about 90 steps between the warmth threshold and the thermal pain threshold” [6] compared to the 21 distinguishable steps of noxious heat. Note that this method does not provide an insight into the capacity of humans to encode the intensity of their subjective experience of pain or suffering (indeed, this is one of the main criticisms of this work), as reports of nociceptive activity can vary widely from associated physiological responses and subjective reports of pain intensity [7]. As this technique relies on self-reports it has not been applied to any animal species, but we might assume if an animal is capable of nociception then it must be able to discriminate at least one intensity of noxious stimuli (i.e. discriminating between states of pain or no pain in a binary manner).

Neurobiological indicators

These proxies were selected as indicators of the capacity of an organism to encode the intensity of a noxious stimulus that could then result in the subjective perception of pain. I was surprised that the variation found for each proxy lay within a range of only 1.1 to 1.4 orders of magnitude.

Nociceptor response

The maximum (or saturation) firing rate that has been measured for nociceptors that are sensitive to a given modality of a noxious stimulus. A higher maximum firing rate would allow a nociceptor to encode a greater number of discrete stimulus intensity levels. Note that a higher firing rate does not necessarily imply that a wider range of absolute stimulus intensities are encoded, as the finer intensity steps could instead be encoded over the same (or a smaller) absolute intensity range. To illustrate, although fish have the highest nociceptor firing rate observed for vertebrates [8], both the intensity thresholds for activation (and the range of intensity encoded before saturation) are smaller than for other vertebrates, such that a fish’s nociceptors would likely be saturated at the same stimuli intensity at which, for example, the threshold is reached at which a chicken’s nociceptors begin firing [9].

Note that all species have some degree of nociceptor specialisation (which appears to generally be greater in vertebrates [where differentiation is also made between myelinated and unmyelinated nociceptors] than invertebrates [10], [11], and greater in mammals than other vertebrates [12]), where in addition to polymodal receptors that are sensitive to a variety of modalities of noxious stimuli (e.g. mechanical, thermal, and chemical) animals often also have some nociceptors which are sensitive to only a single modality of noxious stimuli. I have not directly considered nociceptor specialisation in this project and generally reported the higher firing rate where one was reported from both polymodal and specialised nociceptors for the same modality.

Nociceptor density

The density of cutaneous nociceptors fibres. For most vertebrates, nociceptors are free nerve fibres in the epidermis, besides for birds where nociceptors are situated in the dermis [13]. Nociceptor density varies across the human body [14], [15] and I have included values that appear to be representative of the median. Although anatomical variation does not appear to have been quantified for other species, I recall qualitative observations of varying densities reported in passing by studies on birds and fish. Interestingly, a clear relationship has not been found between nociceptor density and either the thermal pain threshold [14] or spatial discrimination ability [16] of different body parts in humans, but decreases in nerve fibre density are associated with changes in nociception (such as an increase in pain thresholds) that can occur with ageing [17] and neuropathies [18].

Substance P concentration

Substance P is a neuropeptide that modulates the nervous system's sensitivity to the intensity of noxious/aversive stimuli (although it is also involved in other physiological reflexes). Substance P (and its receptor NK1) are found in the dorsal horn of the spinal cord where the neuropeptide regulates the excitability of nociceptors, and also in the hypothalamus and amygdala, brain regions that integrate cues on physical pain from nociceptors as well as emotional pain from stress and anxiety [19]. Substance P mediates neurogenic inflammation and appears critical for the response to prolonged (rather than acute) physical (and emotional) pains in both nociceptors and the CNS. Studies that quantify Substance P concentrations in different brain regions were primarily conducted after the development of a radioimmunoassay in the mid-70s until the late 90s - recent work seems to have focused on using staining techniques to qualitatively map the distribution of Substance P in finer detail. As measurements indicate Substance P concentration (usually in ng/g,

measurements in pmols/g were converted to the former) in a brain region this is a size-independent value, although if two animals of different sizes have the same concentration, then the brain of larger will contain a greater quantity Substance P.

Physiological responses

These proxies were selected as indicators of how much an organism's physiology responds to a nociceptive event through the arousal of the autonomic nervous system. These responses could partly or entirely arise from nociceptive reflexes but are generally taken to indicate an experience of pain that goes beyond simple nociception. However, it is possible that an organism could suppress a physiological response to pain under some conditions, and physiological responses are also likely to habituate if an organism is repeatedly exposed to even intense noxious stimulus (demonstrated for the skin conduction in humans: [7]). Additionally, these physiological responses are not unique to the experience of pain but can result from other causes, both of a stressful and non-stressful nature. As the basal physiology varies widely between the taxa in this report, all responses are expressed as the relative changes that nociception causes from a control condition and, where possible, the physiological response is taken for a stimulus intensity that corresponds to the maximum nociceptor response.

Although the absolute magnitude of the physiological changes covered a similar range of variation as the neurobiological proxies (values for each proxy within a range of 1 to 1.5 orders of magnitude difference), I felt that the physiological responses seemed qualitatively less consistent. To elaborate, the direction of the change varied between taxa (e.g. noxious stimulation leads to heart rate arrest/reduction in fruit fly larvae but an increase the heart rate of all other species), for different noxious stimuli within the same taxa (e.g. the breathing rate of chicken increased response to feather plucking but stopped in response to noxious heat) and even for the same noxious stimuli applied to different individuals from the same species (in a minority of chickens feather plucking results in a heart rate decrease [20]). Further, in a few cases, the same noxious stimulus elicits no response from some species within a taxa whereas others respond (e.g. the respiration rate of several fish species increases in response to an acid application, but not for carp; see note ⁉️ for further information).

The finding that the physiological responses are generally lower for humans may reflect the fact that responses are measured in an experimental condition where participants are aware they will be exposed to painful stimuli and their physiological parameters are being measured. Conversely, for many animals, the handling stress associated with administering a noxious stimulus appears to be comparable to or even greater than that caused by the nociceptive event itself (see note ** for further information). I spent less time searching data on physiological responses than I did for the neurobiological indicators, and expect that additional measurements are available which could be used to fill in some missing values (however, it proved much harder to find values for cardiorespiratory responses in rodents than I expected).

Stress hormone

Corticosteroids are released in response to acute (including pain) and chronic stressors, and elevated levels of stress hormones after noxious stimulation are typically taken as an indicator that the subject experienced pain [21], [22]. The release of corticosteroids is typically delayed by some time (on the order of minutes) after a stressful event (adrenaline release happens first as part of the initial response) but may remain elevated above control levels for hours or even indefinitely. This delay can be problematic for measurement as stress hormones are usually sampled for blood or saliva at discrete time points (rather than continuously) and it is possible that the sampling interval misses the peak value of the hormone release, in which case the percentage change reported would be lower. I did not record the sampling time when collecting these measurements but recall that they varied from minutes to several hours after a noxious event.

Cardiorespiratory rates

A rapid increase in heart rate after a noxious stimulus is taken as an indicator of the initial flight-or-fight response to an experience of pain [21]–[23]. Respiration rate indicates a similar response and was primarily included as this is measured instead of heart rate in studies on fish pain.

Table of quantitative pain proxy values

Proxy	**Human**	**Rodent**	**Bird**	**Fish**	**Insect**	**Multiple between largest and smallest [Order of magnitude difference]**
Nociceptive JNDs	Mechanical (aching pain): >14 [24] Heat (pricking pain): **21** [3], [6] Cold: ? Chemical: ?	NT	NT	NT	NT	x21 (human vs. in principle minima) [1.3]

Maximum nociceptor response (spikes/s)	Mechanical[C]: 10 [25], [26] Heat[C]: 5 [25], [26] Cold[C]: 1 [26] Chemical: ?	Mechanical[C]: 8 (mouse [27]) Heat[C]: **2** (mouse [28]) Cold: ? Chemical: ?	Mechanical[A/C]: 15 (chicken [9]) ⁋ Heat[C]: 15 (chicken [9]) ⁋ Cold: ? Chemical: ?	Mechanical[A]: 55 (trout [8]) HeatI[A]: **50** (trout [8]) Cold: NS Chemical[A]: 40 (trout [8])	Mechanical[IV]: ? Heat[IV]: 15 (fruit fly - larvae [29], [30]) Cold[III]: ? Chemical[IV]: 10 (fruit fly - larvae [29]) Light[IV]: 10 (fruit fly - larvae [29])	x25 (heat: trout vs. mouse) [1.4]
Nociceptor density * (NF/mm or NF/mm^2)	5-20 NF/mm [16], [31]–[34] **100-200** NF/mm^2 [34], [35]	10 NF/mm (mouse [36], [37])	?	30-60 NF/mm^2 (zebrafish [38]) ## **<5-<20** NF/mm^2 (ray [39]) ##	6 NF/mm (fruit fly - larvae) ‡	>x12 (human### vs. ray###) [1.1]
Substance P concentration † (ng/g)	B: ? HL: 120-295 [40]–[42] h SC: 65 [40] h DH: **100** [43] h, f, #	B: 25 (rat [42]) # HL: 650-850 (rat [44]) f SC: 170 (rat [45]) f, # DH: 680-1440 (rat [44], [46], [47]) f, #	B: ? HL: 280 (pigeon [48]) f,# SC: ? DH: **1350** (pigeon [48]) f,#	B: 70 (trout [49]) f, #, †† HL: ? SC: ? DH: ?	B: 70 (blowfly - adult) ‡‡	x14 (DH: pigeon vs. human) [1.1]
Change in stress hormone (% from control)	Mechanical: **+30-40%** (cortisol [50]) § Heat: ? Cold: +25-35% (cortisol [51], [52]) s Chemical: ?	Mechanical: ? Heat: +500% (corticosterone, rat [53]) § Cold: ? Chemical: +600% (corticosterone, rat [54]) §	Mechanical: +125% (cortisol; chicken [55]) a Heat: +125% (corticosterone; pheasant [56]) a Cold: ? Chemical: ?	Mechanical: **+1200%** (cortisol; tilapia [57]) **, ⁋, a Heat: ? Cold: NS Chemical: +100% (cortisol; trout [58]) §	Mechanical: ? Heat: ? Cold: ? Chemical: +300-400% (AKH; cockroach, firebug - adults [59], [60]) §§ Light: ?	x34 (mechanical: trout vs. human###) [1.5]
Change in heart rate (% from control)	Mechanical: +10% [50], [61] § Heat: **+10%** [62] Cold: +10% [63] Chemical: ?	Mechanical: +10 (rat [64]) § Heat: ? Cold: ? Chemical: +25% (rat [54])	Mechanical: +20-30% (chicken [20], [65], [66]) **, a Heat: +20% (chicken [65]) **, § Cold: ? Chemical: ?	Respiration (opercular beat) rate appears more practical to measure than heart rate while fish are swimming.	Mechanical: -100% (fruit fly - larvae [67]) Heat: **-100%** (fruit fly - larvae [67]) Cold: -60% (fruit fly - larvae [68]) *** Chemical: ? Light: ?	x10 (heat: fruit fly larvae vs. human) [1]
Change in respiration rate (% from control)	Mechanical: +20% [69] § Heat: ? Cold: -5% [70] Chemical: ?	Mechanical: ? Heat: ? Cold: ? Chemical: ?	Mechanical: **+25%** (chicken [65]) § Heat: -100% (chicken [65]) § Cold: ? Chemical: ?	Mechanical: **+700%** (zebrafish [71]) **, a Heat: ? Cold: NS Chemical: +80% (trout, zebrafish [58], [72], [73]) §, ⁋	Respiration is not (usually ?) an active process that is distinct from the heart beat.	x35 (mechanical: zebrafish vs. human) [1.5]

A - Myelinated A-delta fibre.
C - Unmyelinated C fibre.
a - nociceptive event involved amputation of a body part or surgery. Chickens: Debeaking. Fish: Fin clipping.
f - Measured from frozen samples, Substance P concentration is likely to be reduced by 30% or more [74].
h - Substance P measurements on human brain regions were performed on tissues from elderly individuals, whereas measurements on the other species would have been performed on relatively young subjects. Aspects of human pain perception change with age [17], and the concentration of many neurotransmitters declines with age (although I have not found specific data on this for Substance P), so it is likely that the Substance P concentrations reported are somewhat lower than would be found in the brain tissue of young humans.
s - salivary cortisol measured instead of plasma/serum cortisol.
NT - Not testable for animals as this relies on a self-report, but a lower bound of 1 is assumed.
NS - Not sensitive.

* Nerve fibre density is taken as either a linear measurement (NF/mm) from sections through the epidermis or an area measurement (NF/mm^2) from whole mounts of the epidermis. The epidermal-dermal junction is folded rather than flat, and the former measurement takes this into account by measuring the total junction length, while the latter uses the skin surface area, which would be less than the total junction area. As such, despite their similar units, the two measurements cannot be directly compared without knowing the density of epidermal folds, which may vary between species. A multiplication factor of 20 has been proposed to convert NF/mm to NF/mm^2 for humans [31].
† CNS regions in which concentration and densities are measured: B - whole Brain. HL - Hypothalamus. SC - Spinal Column/cord. DH - Dorsal Horn of the spinal cord.
# Papers gave results in mol/g, which are converted to g/g based on a molar mass for Substance P of 1347 g/mol.
‡ Drosophila larvae have 8 nociceptors per hemisgment (5 cold-sensitive Type III and 3 polymodal Type IV; [75]) and have a width of 0.66 to 1 mm (depending on diet; [76]). Treating the larvae as having a circular cross-section with a radius of 0.4 mm gives a circumference of 2.5 mm, and the nociceptor density can be found as #nociceptor/(circumference/2).
§ Unclear if this value represents the maximal physiological response to this stressor.
⁋ Based on model fitting to the response curves of individual nociceptors
** Changes may be partially or entirely in response to non-nociceptive stressors (such as handling). Chicken: Both sham and real beak-trimming elicit an equal change in heart rate [66]. No change in heart rate in response to mechanical or thermal stressors are observed when chickens were placed under minimal anaesthesia (a technique used to separate nociceptive responses from handling stress in mammals), but it is not clear if this is an artefact of anaesthesia or because heart rate changes in awake animals are due to non-nociceptive stress [77]. Fish: Sham tail clipping of zebrafish elicits approximately 50% of the opercular beat rate increase observed for real tail clipping [71]. In tilapia, handling stress produces around 80% of the cortisol increase of tail clipping [57].
†† Some fish species contain a protein substitution that prevents reactivity to the antiserum used in the radioimmunoassay to quantify substance P concentration [49]. Studies using the radioimmunoassay on fish appear to have produced unreliable results [78], [79] and the technique has been applied less systematically to fish than to other vertebrate groups.
‡‡ Other tachykinins fulfil the role of substance P in many invertebrates [80]. This value was calculated from the callitachykinin-like immunoreactivity measured in blowfly brains (~45 fmol [81]) divided by the mass of a blowfly brain (volume of 0.8 mm^3 [82] multiplied by an assumed density of 1100 kg/m^3) and converted to concentration using the molar mass of Substance P (see note #).
Zebrafish [38]: This study provides the number of superficial axon bundles (3-6) per scale and scale area (0.75 mm^2; which I interpret as being the area covered by the epidermis, and is less than the total scale area) for adults and a single image of an axon bundle containing 7 axons, which was assumed to be the number contained within all bundles. However, axon bundle counting was done based on tubulin staining, which may stain axons that are not nociceptive. Ray [39]: This study counted all cutaneous nerve fibres not associated with lateral line mechanosensors, so these values would include any nociceptive free nerve fibres as well as other nerve fibres. Counts were converted to density assuming a skin area of 1.9 mm^2 was measured (counts were done on a section thickness of 0.19 mm from 100 mm^2 of excised tissue).
§§ Adipokinetic hormone (AKH) has been suggested to provide a general stress response for many insects that is analogous to glucocorticoid produced by the vertebrate HPA axis [83]–[86]. However, few studies have examined changes in AKH after nociceptive stimulus, and it is possible that other hormones serve this role in some insects. For example, diuretic hormone 44 (DH44) is structurally similar to vertebrate corticotropin-releasing hormone and appears to have a role in the stress response of Drosophila [87], but also does not appear to have been studied in relation to nociception. Note that the values reported are from experiments where venom was administered to insects in a similar manner to chemical nociception experiments performed on fish, however, it has not been established that these insects possess nociceptors to noxious chemicals.
⁋⁋ Species-dependent non-responsiveness has been observed. The respiration rate of carp did not change in response to acid application [72]. The whole body cortisol of zebrafish was measured at normal levels after tail-clipping [71], however, the cortisol was not measured until 90 minutes post-tail-clipping while the cortisol increase caused by handling returns to a normal level within 60 minutes [88], raising the possibility that a cortisol increase caused by tail-clipping was missed.
*** This study [68] chronically exposed larvae to a temperature that was cold enough (10 °C) to trigger cold-sensitive nociceptors, but it is likely that the observed change in heart rate was at least partly due to a change in metabolism reflecting cold stress rather than solely a nociceptive response.
Calculated from the centre of ranges.

Ideas for additional proxies

- Concentration of neurokinin 1 (receptor for Substance P) in same brain areas
- Concentration of endogenous opioids (β-endorphin, enkephalin, and dynorphin) and receptors (μ, δ, and κ) - which brain areas?
- Sensitivity to exogenous opioids (which?). Differences should be interpreted in the context of endogenous opioids/receptors and sensitivity to those (e.g. ectotherms tend to require higher analgesic doses than mammals). Reviewed in context of analgesics: Analgesia for non-mammalian vertebrates
- Activity of the sympathetic nervous system in response to stressors. For example: Sympathetic nervous system activity during skin cooling in humans: relationship to stimulus intensity and pain sensation
- EEG activity in response to stressors (often using minimal anaesthesia in animals), see: Pain perception at slaughter and Neurophysiological techniques to assess pain in animals
- Release of primary stress (flight-or-flight) hormones: Adrenalin/epinephrine in vertebrates and octopamine in invertebrates.
    - Also other hormones released by activity on the HPA axis, such as corticotropin-releasing hormone and adrenocorticotropic hormone.
- Other physiological indicators of pain (such as a change in blood pressure). Examples: Identifying and monitoring pain in farm animals: a review and Pain assessment in animal models: do we need further studies?
- Physiological responses to emotional stressors. For humans, see: Multivariate Brain Prediction of Heart Rate and Skin (particularly references at top of the second page) and Acute psychosocial stress: Does the emotional stress response correspond with physiological responses?

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