Animal (2019), **13:12**, pp 3000–3008 © The Animal Consortium 2019
doi:10.1017/S1751731119001885

Review: What innovations in pain measurement and control might be possible if we could quantify the neuroimmune synapse?

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(Received 18 May 2019; Accepted 8 July 2019; First published online 13 August 2019)

It has taken more than 40 years for the fields of immunology and neuroscience to capture the potential impact of the mechanistic understanding of how an active immune signalling brain might function. These developments have grown an appreciation for the immunocompetent cells of the central nervous system and their key role in the health and disease of the brain and spinal cord. Moreover, the understanding of the bidirectional communication between the brain and the peripheral immune system has evolved to capture an understanding of how mood can alter immune function and vice versa*. These concepts are rapidly evolving the field of psychiatry and medicine as a whole. However, the advances in human medicine have not been capitalised upon yet in animal husbandry practice. Of specific attention are the implications that these biological systems have for creating and maintaining heightened pain states. This review will outline the key concepts of brain–immune communication and the immediate opportunities targeting this biology can have for husbandry practices, with a specific focus on pain.*

Keywords: pain, glia, biophotonics, biomarker, animal welfare

Implications

The intimate relationship between neuronal processes and glial function is driving a neuroscience revolution. There is a growing appreciation for the active role glia and immune-like signalling within the central nervous system has in changing brain function and hence behaviour of the organism. This is of specific importance to the objective quantification and treatment of pain. Importantly, by targeting the neuroimmune synapse it may be possible to create disease-modifying pain treatments.

Introduction

How do we know we are sick and why is this concept important for quantifying behaviour?

Illness is actively avoided in human and animal population (Yirmiya *et al.*, 2000). All species implement a myriad of physiological and behavioural practices to diminish the likelihood of experiencing illness. In addition, multiple cellular and molecular capacities have evolved to protect and defend the host from pathogens (Yirmiya *et al.*, 2000). But if illness is encountered, how does the individual know it is sick? How does the organism know to adapt at a whole system behavioural level to the presence of a molecular toxin or cellular pathogen? This may seem like a simple question, yet applying the *Descartes* philosophy of the body as a machine, with each anatomical part able to work in isolation. However, this philosophical and experimental approach has not been successfully applied to solve this simple question. Nor does the *Descartes* approach provide the intellectual or mechanistic complexity required to explain such a multisystem-coordinated response.

The ability to quantify illness provides us with a key access point to the inner workings of biological systems. The body 'knows' when it is not in homeostasis and responds accordingly, but not always proportionally. The response to illness intentionally adapts multiple systemic systems and is not a random series of unlinked events. Therefore, being able to quantify this distributed response means a point measurement has the potential to give broad insights into the current physiology of multiple systems and would, therefore, be a critical diagnostic tool.

Recent advances in neuroscience and the convergence of scientific disciplines to create next-generation measurement and imaging technologies provide us with a unique opportunity to intervene and measure illness. This review will

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https://doi.org/10.1017/S1751731119001885 Published online by Cambridge University Press

introduce the principle concepts that underpin key cellular and molecular signals associated with the illness response, and the implications this has for future interventions and measurement approaches in areas of unmet need, such as pain. It will draw upon recent accumulated evidence from other reviews and primary research papers (Hutchinson *et al.*, 2007; Hutchinson *et al.*, 2011; Grace *et al.*, 2014; Nicotra *et al.*, 2014; Hutchinson, 2018a). As will be described here, harnessing the neuroimmune understanding of the interactions between, and within, complex physiological systems can literally allow the creation of windows into the body. Through these new insights, it is possible to measure and modulate disparate physiological systems. Moreover, it is also possible to form hypotheses that begin to explain biological coherence across multiple systems and point to mind–body convergence. These developments have significant implications for future animal husbandry practices. Evolution of the knowledge in this field will provide the tools that will allow the movement from empirical-based practice to precision and individualised interventions (Hutchinson *et al.*, 2018). In some cases, the quantification of these molecular events and the individualisation of husbandry practices may enable the reduction, and in some cases, the elimination of certain interventions.

The neuroimmune interface – the new building block of the central nervous system

In order to capture the full potential of recent developments in neuroscience, new foundational principles will now be introduced in order that the field of veterinary and animal sciences may gain insights into this rapidly developing field. This will allow appreciation of the cellular and molecular origins of the new era of mind–body convergence.

A fundamental dogma in neuroscience that the brain is immune privileged has been surpassed. It is now acknowledged that bidirectional signalling occurs between neuronal and immunocompetent cells (Buchanan *et al.*, 2010). Many immunocompetent cells are present in the central nervous system. These include glia (microglia, astrocytes and oligodendrocytes), endothelial cells, perivascular macrophages and infiltrating peripheral immune cells such as T cells and monocytes/macrophages (Eroglu and Barres, 2010; Grace *et al.*, 2011; Grace *et al.*, 2014). This neuronal and immune interfacing is not mere cellular cohabitation within anatomical compartments. Instead, immunocompetent cells engage intimately at the microscale and molecular level with neuronal processes to maintain brain homeostasis (Grace *et al.*, 2014). These cells form the structures that create and maintain the blood–brain barrier, neuronal myelination and encapsulate synapses (See the Primer of Psychoneuroimmunology Research (2016)).

The fundamental building blocks of the central nervous system should no longer be thought of as just the presynaptic and postsynaptic terminals forming the neuronal synapse. Instead, this basic building block in its simplest form is a tripartite relationship between an astrocyte and the neuronal processes (Grace *et al.*, 2014). In its more complex form, up to five cellular participants can contribute to this orchestrated relationship (Grace *et al.*, 2014). Importantly, at least one of these participants can be derived from the peripheral immune system. These cellular interactions allow for regular surveillance of brain disturbance, damage or infection, and can contribute to drug responses (Liu *et al.*, 2011; Northcutt *et al.*, 2015), stress and depression (Liu *et al.*, 2014) and exaggerated pain states (Hutchinson *et al.*, 2013). Given this profound new view of the brain, an intentional reframing of the brain's immune capacity will follow, including an introduction to the capabilities of these central-nervous-system-resident immunocompetent cells.

Immunocompetence of the brain – breaking the central immunoprivileged dogma

Until recently, glia were dismissed as to potentially contributing to the normal function of the brain and spinal cord (Allen and Barres, 2009). This conclusion was perpetuated owing to the principle dogma that the central nervous system was an immune-privileged organ. To be immune privileged is dictated by the exclusion of classical peripheral immune cells from crossing an anatomical barrier, in this case the blood–brain barrier, and hence preventing normal immune surveillance operations from occurring. However, it is now appreciated the brain and the spinal cord can host resident immunocompetent cells under states of both health and disease. Both glial cells and immune cells from the periphery are now appreciated to modulate neurotransmission and hence complex behavioural responses orchestrated by the central nervous system (Eroglu and Barres, 2010; Jacobsen *et al.*, 2014; Jacobsen *et al.*, 2016a).

The historical view of these cells being passive bystanders in the brain or mere components of the extracellular matrix must be surpassed (Allen and Barres, 2009). Instead, new terminology is needed to encompass the breadth of the molecular engagement, intimate spatial cellular connections, trophic and signalling relationships between the neuronal and immunocompetent cells. This can be best described as the neuroimmune interface (Grace *et al.*, 2014). The neuroimmune interface is not static. It is a dynamic multicellular functional unit which is replicated billions of times throughout the brain and spinal cord. It is comprised of neuronal processes (including both pre- and postsynaptic bodies), astrocytic projections and microglial surveillance (Allen and Barres, 2009). In some cases, chemotaxis facilitates selective migration of peripheral immune cells across the blood–brain barrier. This cellular movement also supports localised immunocompetent and neuronal cellular events

https://doi.org/10.1017/S1751731119001885 Published online by Cambridge University Press

that contribute to behavioural modulation. In sum, these responses protect the whole organism (Buchanan *et al.*, 2010). A description of some of the key cell types that create the neuroimmune interface follows provided with a specific focus on astrocytes and microglia, as the majority of research has focused on their role in brain health and disease.

Astrocytes – the stars of the central nervous system

Named after their star-shaped morphology, astrocytes are the most abundant cell type in the central nervous system (Allen and Barres, 2009). Unlike their neuronal cousins, astrocytes are not linear in their connectivity or signalling capacity. Their star-shaped morphology enables them to simultaneously provide structural support to neuronal systems and enable the formation of the blood–brain barrier. This is a dynamic process and as such astrocytes are critical to regulating cerebral blood flow. Other cellular projections of astrocytes can form the tripartite synapse structure. Via these projections and the molecular transporters which they express specifically within the synaptic cleft, astrocytes can contribute actively to synaptic transmission. Finally, astrocytes provide the neuronal energy supply through critical trophic support and are among the first responders to promote repair of neuronal systems. As has been briefly described here, astrocyte morphology and functions are highly polarised and heterogeneous (Watkins *et al.*, 2005). Changes in astrocyte function can rapidly impact multiple central nervous system outputs. As will be discussed, each of these features of astrocyte biology makes them exquisitely positioned to modify multiple complex cellular systems which can translate to alterations in behaviour (Jacobsen *et al.*, 2016a). If astrocyte phenotype can be harnessed through external interventions, a ready population of cellular mediators to change behaviour is available. In the animal husbandry context, modulation and augmentation of astrocyte function have the translational potential to modify all central nervous system outputs, including sympathetic and parasympathetic responses (Allen and Barres, 2009). Key developments in targeting selective astrocyte populations and discrete anatomical locations will allow the introduction of interventions that can move affective state in a positive direction and broadly control exaggerated pain states (Jacobsen *et al.*, 2016a).

Microglia – the macrophages of the brain (sort of)

Microglia are commonly thought of as the tissue-specific phagocytes of the central nervous system. They are highly reactive in their phenotype and display profound regional heterogeneity throughout the parenchyma, presumably to coordinate diverse responses to insult, or in dynamic response to the microenvironment of their local neuroimmune interface (Graeber and Streit, 2010). Under basal conditions, microglia are found in a surveillance state. Their cytoarchitecture enables them to continuously sample the extracellular space for perturbations in their microenvironment. They are spacially dynamic, with cellular processes reaching out into extracellular voids and making contact with adjacent cell membranes (Watkins and Maier, 2003).

When a signal or event is detected, microglia can rapidly transition to a state of reactive gliosis, resulting in changes in cell number, morphology, phenotype and motility (Hutchinson *et al.*, 2011). But microglia are not only a phagocytic cell. Microglia contribute extensively to the neurokine signalling environment, of the central nervous system (Dodds *et al.*, 2016), through the expression of membrane-bound and intracellular signalling proteins (e.g., mitogen-activated protein kinases), and the release of immunoregulatory products, such as cytokines and chemokines (Watkins *et al.*, 2007).

These sentinel and neurokine signalling roles of microglia make them perfectly positioned to be early reporters of change so that the whole of an organism's physiology can respond (Dodds *et al.*, 2016). Critically, these signalling events can occur during the asymptomatic phase of the pathology, meaning that if these signals can be externalised, it makes microglia and their signalling factors potential biomarkers of health and disease early enough to provide personalised interventions without a broader impact on the herd. However, each of these cellular processes can occur with significant speed and potency, making quantification of these events within specific neuroanatomical compartments challenging (Jacobsen *et al.*, 2016b). As such, we are presented with a similar case for astrocytes, with microglia providing an excellent target to change behaviour (Jalleh *et al.*, 2012; Jacobsen *et al.*, 2016a).

Neurokine signalling – a new signalling language of the central nervous system

Given the intimate relationship between neuronal and immunocompetent cells, the previous disconnect between the molecular signalling languages of the neuronal and immune systems has been overcome. It is now clear that classical neurotransmitters act at receptor and ion channel systems expressed by both neuronal and immune cells (Dodds *et al.*, 2016). Clearly, the functional consequences of these molecular mediators on each cell type are different. However, neurotransmitter release from a neuron can impact neuronal and immune cells alike when they express the given paired receptor (Dodds *et al.*, 2016). Likewise, factors considered immune in nature, such as cytokines and chemokines, can impact neuronal function through their receptors expressed by neuronal systems (Dodds *et al.*, 2016). Therefore, it is clear that naming molecules as neuro or immune has limited our imagination for the potential targets these molecules can have in the central nervous system. In reality, there are a range of factors which are derived from both immunocompetent and neuronal cells alike and can impact a disparate number of cellular targets often independent of their classical cellular assignment they have been given.

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However, a key distinction can, however, be drawn between the immune molecular mediators quantified in the periphery, which impact peripheral physiology, and those molecules that change brain function. Significantly, fewer (molar concentration) of the immune molecular mediators are required to change brain function and hence behaviour (Dodds *et al.*, 2016). Classically, a systemic immune response can result in log-fold increases in cytokines and chemokines which are required to coordinate a systemic immune response. In contrast, a doubling of already single-digit levels of immune mediator molecules is sufficient to trigger a behavioural change. An excellent example of the potency of the neurokine signalling is the impact that microglial-derived cytokine expression and release can have on pain processing, with below detection levels of Interluekin-1 beta (**IL-1β**) sufficient to elicit a painful response (Hutchinson *et al.*, 2007). Therefore, the immune signalling within the central nervous system has been termed neurokine, designating its uniqueness from that in the periphery.

How deep these differences may extend is still being determined. For example, the receptor co-localisation and ionic conditions that exist within the central nervous system are very different from the conditions that an immune receptor may operate under peripheral conditions (Hutchinson *et al.*, 2011). As such, co-localisation and tertiary peptide structures of the receptor systems may all be modified and contribute to the potency and efficacy differences.

Biological coherence through the neuroimmune interface

The unique spacial localisation of this constellation of immunocompetent cells within the critical anatomical structures of the central nervous system makes them pivotal to the health and disease of the brain and spinal cord (Hutchinson, 2018a). Complimenting this neuroscience revolution, and the movement away from viewing the brain as an immune-privileged organ, has been the acknowledgement of a key bidirectional communication and macro- and microanatomical colocation between the brain and the peripheral immune system (Grace *et al.*, 2014; Grace *et al.*, 2016). These discoveries have developed from an appreciation that how an organism behaves and interacts with its environment, can modify peripheral immune function (Prossin *et al.*, 2016b) and how the immune system functions can change the way the organism behaves and responds to environmental stimuli (Harrison *et al.*, 2009). In each of these cases, there is a key role for the neuroimmune interface in translating peripheral to central neuronal and immune stimuli. Importantly, this mind–body connectivity means that there is a distributed immune signal in the periphery which can be quantified to determine the status and functioning of the neuroimmune interface (Kwok *et al.*, 2012; Kwok *et al.*, 2013). This capacity to literally create a minimally invasive 'window into the body' provides unprecedented capacities to objectively diagnose and quantify brain and spinal cord states, including those relating to pain and affect (Hutchinson *et al.*, 2018).

So . . . how does an organism know that it is sick?

The apparently simple question of 'how does an organism know it is sick?' can now begin to be unravelled through an exploration of the underappreciated molecular signalling and structural morphology, that is, the micro- and nanoscale realms of the neuroimmune interface (Hutchinson, 2018a). It is the neuromodulatory capacity of these cells that allow the change of behaviour during times of illness (Miller and Raison, 2016). These behavioural adaptations have been linked to changes in cognitive function, mood, depression, anxiety, pain, addiction and reward (Thomas and Hutchinson, 2012; Liu *et al.*, 2014). Several key examples of this bidirectional communication will now be provided which have clear implications for animal husbandry practices and the management and welfare of animals.

The illness response – immune to brain communication

The illness response is a coordinated set of behavioural adaptations which develop during the course of an infection (Dantzer, 2001). During infection, the behaviour of an individual changes to express little motivation to achieve or perform normal daily functions, which include eating and drinking, socialising and they are often fatigued and have trouble maintaining normal sleep rhythms (Kelley *et al.*, 2003). In addition, other sensory disturbances are likely, including increased sensitivity to pain, trouble-performing cognitive tasks and the inability to experience pleasure (altered affective state) (Yirmiya *et al.*, 2000). This state has been replicated experimentally following both central and peripheral administration of bacterial endotoxin or recombinant proinflammatory cytokines, such as IL-1β. It is hypothesised that this innate central motivational state promotes recovery and is mediated by proinflammatory cytokines, such as IL-1β acting indirectly or directly in the brain to change the neuroimmune interface function (Kelley *et al.*, 2003; Dantzer and Kelley, 2007). Importantly, the response is heterogeneous across multiple brain and spinal cord centres, allowing for the presentation of each distinct behavioural outcome outlined above (Hutchinson *et al.*, 2007).

Critically, pharmacological blockade of these immune factors, or attenuation of the glial response, blocks the presentation of these adaptations. The similarities of these illness responses to chronic depressed mood and other psychiatric disturbances have now prompted some to hypothesise that the previously neuronal- and neurotransmitter-focused hypotheses for pathology presentation could actually have a neuroimmune interface origin (Dodds *et al.*, 2016). This suggests that rather than targeting neuronal processes to improve long-term mood, perhaps neuroimmune mechanisms should be considered (Hutchinson *et al.*, 2007).

https://doi.org/10.1017/S1751731119001885 Published online by Cambridge University Press

Moreover, when attempting to quantify these negative affective states, immune measures may prove a useful source of biomarkers (Buchanan *et al.*, 2010).

Brain to immune communication – behaviour can drive immune response

The first demonstration of brain control of immune function remains a key motivator for the growth of the entire neuroimmune interface field. Ader and Cohen (1975) employed Pavlovian conditioning in rats to elicit a conditioned immune response. Here, they paired a sweet-tasting solution with the systemic exposure to Cyclosporin A, a profound immune suppressor. Following repeated pairings and hence conditioning, a challenge of just the sweet-tasting solution was given. The immune response observed in these animals was as though they had just received the Cyclosporin A (Ader and Cohen, 1975). This discovery has subsequently been replicated in human populations (Kirchhof *et al.*, 2018). Therefore, it is anticipated it is conserved across multiple species.

It has taken some time for the scientific field to come to grips with the implications of this discovery. However, it is clear that the brain and therefore, the state of consciousness of the individual, can impact the peripheral immune system function. In the case of Ader and Cohen, the response was a conditioned immune response that took several days to build. Prossin *et al.* (2016a) have demonstrated that in humans the recall of an acutely distressing memory of the death of a loved one was sufficient to elicit a change in immune function. Critically, the change in immune function was proportional to the emotional memory-induced activation of brain function. Hence, connecting the physiological state and immune function, a biological coherence that bridges classically held discipline-based divides, is being observed (Hutchinson, 2018b).

In the case of immune–brain and brain–immune communication, the question should be asked as to where this cycle begins and ends. Clearly, if illness changes brain function to create depressed mood, then this has the ability to feed forward to enable greater peripheral immune modulation. It is also the case that if depressed mood creates a modified or dysregulated immune system that may be more susceptible to infection, which if illness occurs, may drive greater immune susceptibility. Therefore, when striving to maintain an illness-free husbandry environment, if infection control is the only intervention, a critical contributor has been overlooked. Could effective infection control be augmented if the status of the neuroimmune interface was known and modified to facilitate optimal peripheral immune surveillance, tissue repair and pathogen clearance? What else could be improved beyond infection control? What other unwanted outcomes does the neuroimmune interface contribute to that are critical to the animal husbandry field? We will now review the specific implications this approach has for acute and persistent pain in animals.

The pain problem – the pain opportunity

Pain in animals is an experience that we are unable to reliably diagnose or quantify (Grace *et al.*, 2010). Even when animals in pain are identified, verifying the success of interventional treatments is still ineffective (Williams and Page, 2014). These limitations arise from our inability to objectively measure pain (Nightingale, 2012). This is not a problem limited to animals. In human clinical populations, the individual can be questioned and spoken to, world class successful pain treatments are ranked at between 4 and 10 based on numbers of individuals needed to treat to achieve a 50% reduction in their pain score (Katz *et al.*, 2015).

In livestock production, acute pain is experienced due to management procedures, such as castration and tail docking, injuries from fighting or poor housing conditions, diseases such as mastitis or other infections and at birth (Williams and Page, 2014; Ison *et al.*, 2016). These acute injuries can transition into the persistence of pain, which has a profound impact on the wellbeing and resilience of the animal that cause increased costs and reduced productivity (Williams and Page, 2014; Ison *et al.*, 2016).

This transition to persistence of pain serves no benefit to the animal. Therefore, prevention, diagnosis and treatment of this persistence of pain are critical. For example, soon after birth, pain can interfere in mother–young bonding leading to malnutrition, infection or even death of the newborn (Mora-Medina *et al.*, 2016). Persistence of pain throughout life is a chronic stressor for the animal, leading to reduced food intake and hence lower daily average weight gain, producing less volume and lower quality product (Williams and Page, 2014; Ison *et al.*, 2016).

A growing consumer-driven pressure is also changing the markets available for sale of livestock products, which may limit the access of products to premium-priced markets serviced by welfare-sensitive companies. Therefore, there is a pressing need for tools that can objectively diagnose and measure pain in animals, with associated innovations in pain treatment options. Such innovations in pain measurement and treatment will directly benefit the afflicted animals and the industry as a whole by improving product quality.

An example of a potential trigger for the classical presentation of the persistence of pain in animals is amputation (Flor, 2002). While on the decline in livestock, surgical removal practices such as tail docking, castration and dehorning is still widespread. This practice itself causes pain, resulting from the resection of peripheral nerves and the possible formation of traumatic neuromas and causes significant ongoing sensitisation to mechanical stimuli (Castel *et al.*, 2014; Di Giminiani *et al.*, 2017). The parallel of these events in humans is considered to be significantly painful (Cravioto and Battista, 1981). This process causes adaptations in both peripheral and central sites (Flor, 2002) and is associated with the phenomenon of residual stump pain and phantom limb pain. Painful symptomatic neuromas following amputations are observed in up to a quarter of amputees (O'Reilly *et al.*, 2016).

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However, the solely neuronal view of persistence of pain has given way to an integrated neuroimmune hypothesis of exaggerated pain (Grace *et al.*, 2014). Glial cells, and peripheral immune cells circulating through the central nervous system, are now understood to be integral to creating and maintaining the neuroexcitatory states that underpin persistent pain (Nicotra *et al.*, 2012). The anatomical distribution of the disturbed neuroimmune interface contributes predictably to the behavioural consequence. That is, if the neuroimmune interface within the somatosensory system is perturbed then modified pain behaviours can be expected. Interestingly, the greater prevalence of exaggerated pain in females also appears to have its origins in this neuroimmune interface involvement, through oestrogenic priming of immune functions (Nicotra *et al.*, 2014). Hence, the persistent pain problem and the neuroimmune contributors are likely to be even more relevant in livestock owing to the predominance of female animals in many production settings such as the breeding herd.

Therefore, it is critical to understand this bidirectional communication between the peripheral immune, spinal immune and brain immune systems which maintain the signalling and neuronal reactivity that are sufficient to create heightened pain states in livestock. Moreover, while neuronal processes are critical for the conduction of heightened pain, it is hypothesised there is an anatomically distributed immune signal that triggers conduction of the exaggerated pain response.

The key role of the brain and spinal immune system in persistent pain

The neuronal mechanisms of persistent pain have been complemented in the last two decades by the heightened appreciation for the impact that central immunocompetent cells, glia, have in persistent pain. Until activated, glia are thought to have little-to-no role in the pain experience (Watkins *et al.*, 1997). However, both astrocytes and microglia in the spinal cord are activated (defined immunohistochemically by increased expression of reactivity markers) in response to inflammation and damage of peripheral tissues, peripheral nerves, spinal nerves and spinal cord (Watkins and Maier, 2003).

Enhanced pain, associated with every relevant chronic animal pain model examined to date, is blocked by disruption of glial activation and spinal cord proinflammatory cytokine actions (Watkins and Maier, 2003), thereby demonstrating the crucial role neuroinflammatory mechanisms play in multiple forms of persistent pain. Proinflammatory cytokines and other mediators like reactive oxygen and nitrogen species increase neuroexcitability in spinal nociceptive pathways by enhancing glutamate release, increasing AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor expression, phosphorylating NMDA (N-methyl-D-aspartate) receptor subunits and downregulating astrocyte glutamate transporters (Dodds *et al.*, 2016). Such mediators can also induce disinhibition of neuronal excitability by reducing the release of GABA (γ-aminobutyric acid) and glycine from interneurons and inhibitory descending projections, while glial-derived BDNF (brain-derived neurotrophic factor) downregulates the potassium-chloride cotransporter KCC2 (potassium-chloride transporter member 5) via TrkB (tropomyosin receptor kinase B) receptors, impairing $GABA_A$ channel hyperpolarisation (reviewed by Grace *et al.* (2014)). Thus, the immunocompetent cells of the central nervous system are perfectly situated to contribute substantially to the initiation and maintenance of multiple pain behaviour heightening mechanisms, but the anatomical location of these brain and spinal cord immune cells keep them hidden from peripheral sampling.

While healthy males and females do not have substantial differences in sensitivity to acute pain, females are significantly more susceptible to experience persistent pain than males (Nicotra *et al.*, 2012 and 2014). One of the reasons for this difference in the experience of persistent pain may be due to a difference in neuroimmune interface contributors (Nicotra *et al.*, 2012 and 2014) and in some cases possible differences in peripheral immune cellular mediators (reviewed by Dodds *et al.* (2016)). While the field is yet to reach consensus on the exact mediators and contributors to this component of female pain persistence, it is clear that neuroimmune interface contributors are an overlooked, yet profound participant in the effects, and raises the possible need for sex-specific pain treatments (Hutchinson *et al.*, 2011). An exploration of the neuroimmune changes and contribution to pain in livestock has not been performed, and therefore these innovations have yet to be capitalised upon in livestock husbandry practices.

The peripheral immune system – a novel hunting ground for biomarkers

Given the recognition that there is bidirectional communication between brain/spinal and peripheral immune cells, and that the reactivity status of peripheral immune responses is shared by their central nervous system counterparts, we have explored this hypothesis that peripheral immune cells which have the capacity to be represented at the neuroimmune interface mirror, what is occurring within spinal cord and brain sites. Hence, tapping into the biological coherence by sampling and assaying the peripheral blood is achievable. In recent breakthroughs in the persistent pain field, this concept has been confirmed in a series of preclinical and clinical proof-of-principle studies (Grace *et al.*, 2010 and 2011; Kwok *et al.*, 2012). It has established that brain and spinal neuroimmune interface pain mechanisms rely on a biological coherence supported by peripheral immune cells (Grace *et al.*, 2010 and 2011), and that the *ex vivo* activity of peripheral immune cells can stratify patients into chronic pain and healthy populations (Kwok *et al.*, 2012). A similar approach has been applied to other illness response and maladaptive illness responses like that occurring in depressed mood. Here, peripheral immune factors and responses have been quantified and linked to distinct disease states and may serve as objective diagnostic biomarkers in the future (see Liu *et al.* (2014) for review).

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Quantifying the illness response to guide practice

Two sets of tools are required to capitalise on this new discovery. Firstly, research grade measurement tools and analytical methods which are costly and time-consuming do have a place in this field (Jacobsen *et al.*, 2016a and 2016b). These technologies often have a heightened level of specificity and sensitivity and are, therefore, considered the gold standard for analysis. They are critical for benchmarking all other results. Moreover, these technologies can be used to establish best practice. However, these tools will not be able to inform day-to-day practice alone as they are often bulky and not physically fit for purpose owing to their size and complexity (Jacobsen *et al.*, 2016b; Hutchinson *et al.*, 2018). Instead rapid, rugged, cheap, reproducible and deployable technologies that measure the same or similar biological factors are needed. Moreover, these technologies need to deliver actionable information from analytical grade data.

In order to validate these putative peripheral biomarkers of biological coherence, a greater insight of the working of the neuroimmune interface is required. However, the current tools available to the neuroscience and immunology research fields are insufficient to explore the real-time function of these underappreciated cells of the neuroimmune interface (Jacobsen *et al.*, 2016b; Hutchinson, 2018a). As discussed, the signalling events that occur at the neuroimmune interface engage high potency large peptides, and short-lived reactive species which are not detectable with the quantitative or spacial resolutions needed to understand how this system functions. Therefore, a new generation of technologies is required (Jacobsen *et al.*, 2016b).

Such technological advances cannot be achieved by researchers acting in isolation (Hutchinson, 2018a; Hutchinson *et al.*, 2018). Instead, it is through transdisciplinary communities and through large-scale funding schemes like the Australian Government's Centres of Excellence programme that such challenges can be met. The Australian Research Council Centre of Excellence for Nanoscale BioPhotonics has a key mission to deliver new sensing and measurement tools to open new windows into the neuroimmune interface (Australian Research Council Centre of Excellence for Nanoscale BioPhotonics, 2019). Importantly, the field of biophotonics has the potential to deliver both gold standard research grade measurement technologies and rapid-cheap-deployable tools that enable decision-making in the field (Hutchinson, 2018a; Hutchinson *et al.*, 2018).

Why use light to examine the neuroimmune interface?

Multiple analytical approaches are available to researchers to obtain data across single molecule (nano), through secondary and tertiary biological structures (micro) to subcellular and cellular anatomy (macro). However, few of these have been translated to practical field uses that provide actionable information. Many times, it is the complexity and cost of the technology that limits their broader relevance. In contrast, light-based imaging and sensing tools have significant advantages in capturing the critically needed information in a non-destructively fashion from biological processes over the desired scales (Hutchinson, 2018a; Hutchinson *et al.*, 2018). Importantly, these light-based technologies allow the range of scales to be explored that need to be captured in order to understand the complexity and real-time functioning of the neuroimmune interface.

These multimodal imaging and sensing approaches are beginning to allow for complex biological events to be quantified (Jacobsen *et al.*, 2016b; Li *et al.*, 2018). It is clear that the cellular and molecular mechanisms that underpin biological coherence will require multiple systems to be working in concert. Quantifying each one of these processes in parallel is unlikely to provide the required contextual information to understand the whole system. Therefore, hypothesis-free, sample phenotyping or 'fingerprinting', approaches are key to unravelling the complex biology. A range of such methodologies exploit light-based measurements, such as Raman and tissue autofluorescence acquisition. In each case, the multidimensional data sets acquired using these approaches can provide complex information on hundreds and thousands of molecular features in parallel (Hutchinson, 2018a; Hutchinson *et al.*, 2018). However, alone these approaches may not yield sufficient specificity or sensitivity of actionable information.

Combining the aforementioned phenotyping technologies with specific measurement techniques is required. Historically, this has driven the creation of a great range of synthetic biology and chemically derived spectrally distinct fluorescent and luminescent probes that allow for parallel sensing and imaging (Jacobsen *et al.*, 2016b). However, the ability to separate these probes spectrally, that is, by their colour alone, is limited. As such, new tools that incorporate other forms of multidimensionality and specificity are required. An example of this is the use of the 4th dimension of time encoding, in addition to spectral information, which provides a future where tens of targets could be simultaneously measured with single excitation and detector pairs (Jacobsen *et al.*, 2016b). Here, 4D encoding utilises the 3D spatial information of where the molecule is in space, together with the precisely engineered timed release of photons from nanomaterials (Fan *et al.*, 2018). This 4D imaging approach allows for spectrally identical nanomaterials to be differentiated based on their individualised timed photoemission, providing unprecedented plexing of imaging information (Hutchinson, 2018a; Li *et al.*, 2018). It is through the use of a combination of these tools that measurement advances in the field can be made. But can scalable interventions also be sourced?

Targeting of the neuroimmune interface – an untapped field

The acknowledgement of the neuroimmune interface in a range of pathologies provides a range of new targets for

https://doi.org/10.1017/S1751731119001885 Published online by Cambridge University Press

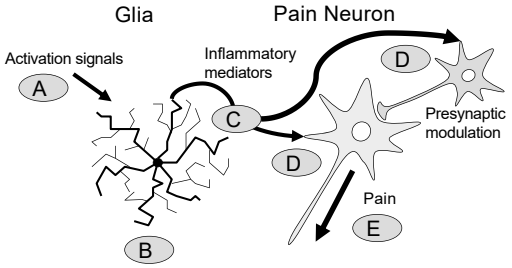

Figure 1 **Step A:** An activation or series of activation signals are required to activate glia often mediated via cell surface pattern recognition receptors that can be antagonised, such as innate immune receptors. These systems have the capacity to detect both endogenous and exogenous signals that allow cross-sensitisation and integration of complex physiological and environment stimuli into behavioural consequences. **Step B:** Glial activation and reactivity is an all-encompassing term used for the state in which glia release proinflammatory mediators and signalling primers. This state can be modulated or attenuated, thereby inhibiting various cellular events that block glial reactivity or its downstream consequences. An anti-inflammatory environment can also be produced, which increases the threshold that an activation signal has to overcome to activate the cells. **Step C:** Immune proinflammatory mediators, such as proinflammatory cytokines and chemokines, can be neutralised prior to reaching their intended receptor target (pre- and/or postsynaptic) using soluble receptors (which exist endogenously), neutralising antibodies, decreasing maturation of cytokines into their active form or increasing the rate of cytokine degradation. In some cases, generation of a second round of immune-derived innate immune pattern recognition signals can also be targeted. **Step D:** The action of many glial inflammatory mediators on neurons (pre- and/or postsynaptic) can also be antagonised at neuronal receptor sites. Importantly, it is also now acknowledged that under some conditions neuronal systems can bypass glial involvement by direct expression of innate immune pattern recognition systems and thereby become directly sensitised. **Step E:** Included here are the myriad of currently employed neuronal-targeted therapies that decrease the neuronal signalling of pain signals (pre- and/or postsynaptic). However, notice that if only Step E is targeted only a portion of the problem is addressed and the neuroimmune interface remains unbalanced. Unfortunately, the standard approaches to the management of human and animal acute and chronic pain continue to employ a Step E methodology likely contributing to the failure to adequately treat pain in humans and animals alike (Nightingale, 2012). Adapted from Hutchinson et al. (2007).

the pharmacological prevention and management of the condition, such as persistent pain. Moreover, these targets may reside within the systems that balance the biological coherence and as such may be highly tractable.

This process builds upon the hypothesised model of pharmacological interventions at the neuroimmune interface. Here the example for persistent pain is used, highlighting theoretical points (Figure 1: Steps A to E) where pharmacological targets can be directed to neuroimmune interface pain contributions.

Conclusions

It is clear that a greater understanding of the illness response and an ability to quantify it will have profound benefits across animal husbandry practices. The field exploring brain–immune communication is rapidly moving and will only make profound steps forward with new technologies and engagement across disciplines. Transdisciplinary collaborations that imagine, design, create and curate these technologies are needed. Therefore, a common language and alignment of expectations within the collaborations is needed. The convergence of human and animal biological sciences with common experimental and measurement technologies will enable a more rapid translation of clinical discoveries across both species, culminating in better health outcomes and productivity overall.

Acknowledgements

Hutchinson is the recipient of an ARC Future Fellowship (FT180100565). Australian Research Council Centre of Excellence for Nanoscale BioPhotonics (CE140100003).
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Declaration of interest

None.

Ethics statement

This work is a review and as such does not have direct ethics approval requirements.

Software and data repository resources

None.

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