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Emerging neurotechnology for antinoceptive mechanisms and therapeutics discovery

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ABSTRACT

The tolerance, abuse, and potential exacerbation associated with classical chronic pain medications such as opioids creates a need for alternative therapeutics. Phenotypic screening provides a complementary approach to traditional target-based drug discovery. Profiling cellular phenotypes enables quantification of physiologically relevant traits central to a disease pathology without prior identification of a specific drug target. For complex disorders such as chronic pain, which likely involves many molecular targets, this approach may identify novel treatments. Sensory neurons, termed nociceptors, are derived from dorsal root ganglia (DRG) and can undergo changes in membrane excitability during chronic pain. In this review, we describe phenotypic screening paradigms that make use of nociceptor electrophysiology. The purpose of this paper is to review the bioelectrical behavior of DRG neurons, signaling complexity in sensory neurons, various sensory neuron models, assays for bioelectrical behavior, and emerging efforts to leverage microfabrication and microfluidics for assay development. We discuss limitations and advantages of these various approaches and offer perspectives on opportunities for future development.

1. Introduction

Chronic pain is the most common form of long-term disability in the world, affecting 100 million people in the United States alone (Larner, 2014). The situation becomes dire for aged populations, with more than 60% of elderly housed in U.S. care facilities suffering from chronic pain (Miu and Chan, 2014). Pharmacological treatment options for chronic pain are limited and problematic. The most prevalent approach involves prescription opioids, which are associated with a high incidence of adverse effects including long-term tolerance and abuse (Ballantyne and LaForge, 2007; Chang et al., 2015). As a result, novel therapeutics are urgently needed that offer improved efficacy and reduced side-effects.

Contemporary drug discovery strategies rely on the identification of molecular targets (i.e., druggable targets) associated with a particular pathway and subsequently probe the role of a given gene product in disease progression. Unfortunately, the yield for this type of target-directed non-opioid analgesic drug discovery has been low. Subsequent to the identification of a relevant bioactive compound, it is common to use of animal models (Munro et al., 2017) for further compound screening. As an alternative or supplemental strategy, there is increasing recognition that phenotypic screens may serve as means for discovering drugs which ameliorate chronic pain (Pruss, 2010; Zhang et al., 2014). Rather than focusing on the activity of a molecular target, modern phenotypic screens capture complex cellular level behaviors or observable traits that are physiologically relevant and central to a pathology without relying on the identification of a specific drug target or a corresponding hypothesis concerning its pathological role. Phenotypic screens are not a panacea for drug discovery. However, this approach can generate entirely novel treatments for disorders which have either complex or unknown mechanisms (Moffat et al., 2017).

To serve as a basis of a phenotypic screen, the selection of cell or tissue type should be based on pathological relevance. In addition, the assay or screening method must be capable of quantifying signals associated with pathological outcomes or cellular behavior. Sensory neurons derived from dorsal root ganglia (DRG) undergo changes in membrane excitability during chronic pain conditions and are integral to the development of chronic pain (Waxman et al., 1999; Zhang et al., 1999). Therefore, they are an excellent candidate cell type for a phenotypic screen to identify new pain therapeutics. Furthermore, it has

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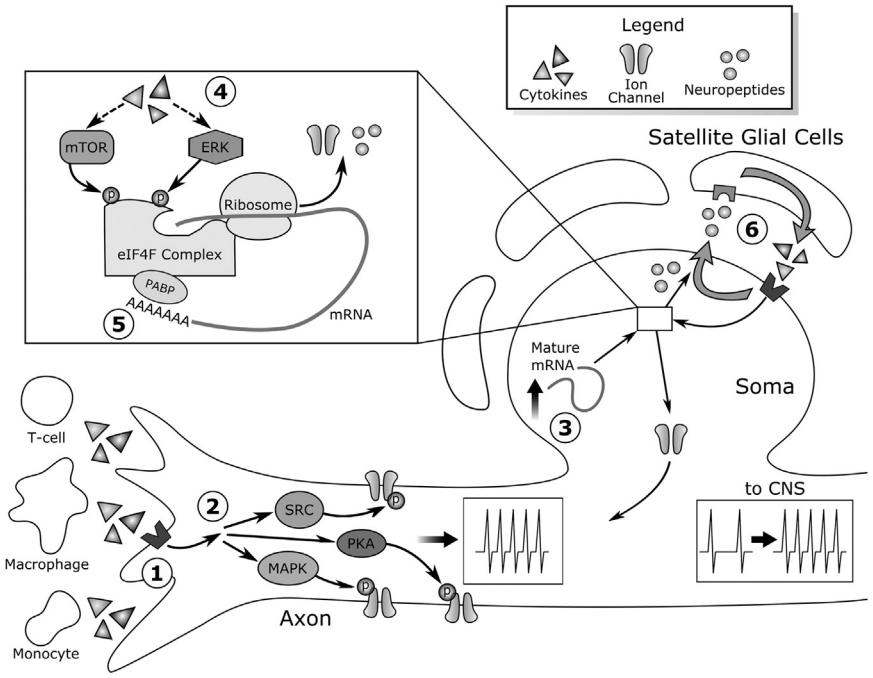

Fig. 1. Schematic illustrating the signaling complexity associated with establishing and maintaining chronic neuropathic pain. Numbered actions are expounded in Section 2.2. Inset summarizes the cytokine-mediated translational regulation exerted at the 5′ and 3′ UTR end of mature mRNA. The outcome of cytokine-mediated eIF4F cap complex formation and polyadenylation is increased translation efficiency.

been suggested that therapeutic strategies which target peripheral DRG neurons rather than tissues in the central nervous system may be "better and safer" (Berta et al., 2017) as peripheral input is ultimately necessary for the maintenance of neuropathic pain states (Haroutounian et al., 2014) and such strategies may not modulate reward circuitry in the brain. Here, we review the current state-of-the-art in the design and development of in vitro sensory neuron-based phenotypic assays for chronic peripheral neuronal hyperexcitability. Emergent technologies are described that are enabling for more advanced, higher throughput, and ultimately more physiologically relevant, phenotypic screening paradigms.

2. Discussion

2.1. Bioelectrical behavior of DRG neurons

DRG are clusters of sensory neurons, each projecting a single bifurcated axon toward both the dorsal spinal cord as well as peripheral targets (skin, muscle, viscera) (Fig. 1). In the periphery, axon terminals detect both noxious and innocuous thermal, chemical, and mechanical stimuli, and transduce these signals in the form of all-or-nothing action potentials toward the spinal cord (Wall and Devor, 1981). The type and threshold of stimulus necessary to initiate afferent signals, as well as the speed and fidelity with which these signals are processed, depends on the type of sensory neuron. Small-diameter neurons with unmyelinated axons have relatively high stimulus thresholds and are called nociceptors (or C-fibers). Small to medium-diameter neurons with thinly myelinated axons, lower thresholds and higher conduction velocity are also referred to as nociceptors, but specifically as Aδ-fibers. The largest-diameter sensory neurons with relatively thick myelin are low threshold and called proprioceptors since they are largely responsible for afferent feedback from muscles for position, movement, and reflex (Basbaum and Woolf, 1999). Under normal conditions, nociceptive DRG neurons exhibit relatively low levels of spontaneous spike activity (Weng et al., 2012). However, inflammation due to disease or injury may lead to increased intrinsic activity and increased sensitivity to external stimuli (Davidson et al., 2014; Djouhri, 2006; White et al., 2005). This sensitization of peripheral nociceptors significantly contributes to the manifestation of chronic pain (Krames, 2015).

2.2. Signaling complexity in sensory neurons

Whether in vitro or in vivo, sensory neuron excitability is ultimately mediated by ion channel expression and function. These include multiple classes of sodium channels, voltage-gated and two-pore potassium channels, hyperpolarization-activated cyclic nucleotide-gated channels, voltage-gated calcium channels, ligand-mediated cation channels, and gap junctions (Benarroch, 2015b; Kim et al., 2016; Ratté and Prescott, 2016; Waxman and Zamponi, 2014). All of these independently serve as potential pharmacological targets for pain (Moldovan et al., 2013). However, the means by which ion channels may be regulated, either directly or indirectly, also involves a multitude of molecular signaling pathways (Price et al., 2016) as well as multiple cell types (Costa and Neto, 2015). Fig. 1 illustrates only some of the pathways by which ion channel expression and function are known to be regulated in an inflammatory or neuropathic pain state. These include (1) direct activation of ligand-gated ion channels by inflammatory cytokines, neurotrophins, ATP, etc., which leads to increased depolarization and activates kinase pathways. (2) Direct phosphorylation of voltage-gated sodium channel (VGSC) or transient receptor potential (TRP) ion channels by cytokine-activated kinases, including p38 MAPK (Hudmon et al., 2008), PKC (Vellani et al., 2001), cAMP-dependent PKA (Bhave et al., 2002; Fitzgerald et al., 1999), and c-Src kinases (Jin et al., 2004). (3) Kinase-activated transcription regulation via repression/activation of transcription mediators such as members of the cAMP response element binding protein (CREB) family (Cheng and Penninger, 2002) as well as activity-dependent epigenetic regulation of transcription via increased/decreased DNA methylation and histone acetylation (Benarroch, 2015a). (4) Cytokine-mediated activation of axonal and/or somal pre-translational pathways, including mTOR (Kang et al., 2009; Melemedjian et al., 2010a; Moy et al., 2017; Obara et al., 2012; Obara and Hunt, 2014) and phosphorylation of the eIF4F complex (Barragán-Iglesias et al., 2018; Khoutorsky and Price, 2017), resulting in increased cap-dependent ion channel protein synthesis. (5) Translational repression/activation via CPEB-mediated increased/decreased polyadenylation (Richter, 2007). (6) Maintenance of the inflammatory state via increased neuronal secretion of neuropeptides, which can create an inflammatory positive-feedback loop via intraganglionic paracrine signaling between DRG soma and adjacent satellite glial cells (Huang et al., 2013; Takeda et al., 2009).

Due to this complexity, the challenge for therapeutic interventions for chronic pain is to effectively reverse a rise in excitability when there are so many distinct ion channels or molecular targets that may be sufficient to trigger underlying pathology - a situation (Ratté and Prescott, 2016) have referred to as “degeneracy”. The molecular target complexity for chronic pain suggests that assays wherein measured outcomes are more integrative and based on cellular behavior represented by phenotypic assays may have an emerging role for therapeutic discovery.

Importantly, cytokine-induced changes in sensory neuron excitability and protein synthesis appear to be conserved in vitro. For example, cultured adult DRG and trigeminal ganglion neurons treated with IL-6 exhibit dose-dependent increases in the phosphorylation of extracellular signal-regulated kinase (ERK), an upstream signaling protein involved in nascent protein synthesis via Mnk1 and eukaryotic initiation factor activation (Melemedjian et al., 2010b; Tillu et al., 2012; Yan et al., 2012). Moreover, combinations of IL-6 and NGF have been shown to rapidly increase global nascent protein synthesis in vitro (Melemedjian et al., 2010b; Moy et al., 2017), and our group has recently demonstrated that adult mouse DRG neurons achieve and maintain a sensitized phenotype following IL-6 incubation when cultured on MEAs (Black et al., 2018).

2.3. Sensory neuron models

To date, the gold standard tissue model for both in vitro and in vivo pathological pain studies has been rodents. While studies with rodent sources of primary sensory neurons have greatly informed the understanding of molecular mechanisms underlying chronic pain, there are electrophysiological differences between species (Davidson et al., 2014) which may limit the interpretation of phenotypic screening results. For example, there are differences in the pharmacological sensitivity of GABA channels between human and rodent species in DRG neurons (Sheahan et al., 2018; Valeyev et al., 1996). In addition, human and rodent DRG exhibit differential expression levels of various voltage-gated sodium channels, which also exhibit different activation/inactivation kinetics (Chang et al., 2018; Dib-Hajj et al., 1999). However, even with access to primary human specimens through organizations such as the National Disease Research Interchange, the prospect of operating high-content, high-throughput screening with donated human tissue would be very challenging given the low anticipated cell yield (Valtcheva et al., 2016). Alternative sources of human tissue include human embryonic stem cells (hESCs) (Boisvert et al., 2015) and human induced pluripotent stem cells (hiPSCs) (Wainger et al., 2014) that can be guided to differentiate into sensory-like neurons. These methods potentially enable virtually unlimited sources of non-tumor-derived human tissue, several of which are now commercially available. However, a recent large-scale study of common genetic effects in a neuronal cell type differentiated from hiPSC-derived sensory neurons was carried out by (Schwartzentruber et al., 2018). This study concluded that hiPSC-derived sensory neurons are transcriptionally distinct from human DRG, implying that current in vitro differentiation protocols may not be functionally or transcriptionally mature. With that in mind, we will discuss various phenotypic and functional assays which have utilized these tissue models. Our hope is that future protocols for hiPSC-derived sensory neuron differentiation and culture may yield physiologically relevant pathological pain models, and that methodologies developed using primary tissues and immortalized cell lines may be successfully extended to incorporate these tissues.

2.4. Phenotypic assays based on sensory neurons

2.4.1. Calcium and voltage imaging

Imaging intracellular ion concentrations enables either direct or indirect measures of cellular excitability from dense populations with high temporal and single cell resolution (Hempel et al., 2017). By far, the most widely used method of optically interrogating single cell or network activity is calcium imaging. The dye chemistries are well established, imaging is relatively straightforward, and calcium is broadly appreciated as integral to cellular signaling and subsequent excitability. Imaging based on either calcium-sensitive fluorescent dyes or genetically-encoded calcium indicators (GECIs) function on the basis of calcium chelation via direct ion or calmodulin protein binding (Grienberger and Konnerth, 2012). Direct or indirect calcium chelation initiates the associated fluorophore’s conformational state change to either enhance the quantum yield of the fluorophore (increase/decrease brightness) or shifts its emission spectrum for quantitative ratiometric measurements. In the context of sensory neuron excitability, calcium imaging using commercial dyes has been shown compatible with primary rodent DRG (Von Banchet et al., 2005), hiPSC sensory neurons (Wainger et al., 2014), immortalized cell lines expressing relevant ion channels (De La Torre-Martínez et al., 2017), and even primary human DRG in vitro (Valtcheva et al., 2016) to study phases of Wallerian degeneration subsequent to injury (Vargas et al., 2015), anti-hyperalgesic drug mechanisms (Sutton et al., 2002), and functional expression of pain-relevant ion channels (Fouillet et al., 2017), among other endpoints. Recently, demonstrations of increased imaging throughput have been carried to characterize cold- and heat-sensing ion channel (TRPM8 and TRPV1) responsiveness using immortalized cell lines in a 96-well format (De La Torre-Martínez et al., 2017). Additionally, (Fouillet et al., 2017) have developed a high-throughput method based on calcium dynamics to optically interrogate Nav1.7 channel function using primary rat DRG neurons.

However, calcium imaging has limited applications due to some inherent disadvantages. (1) Calcium activity is an indirect measure of neural activity due to calcium’s slow kinetics relative to cell membrane potential, and correlating fluorescent signals to action potential in vivo or vitro patterns remains a significant challenge. (2) Since calcium is an important secondary messenger which mediates numerous intracellular processes, including maintenance of transmembrane potential, persistent synthetic calcium chelation may alter cell signaling and viability (Smith et al., 2018). Additionally, studies of Cre-based transgenic mouse models expressing GCaMP6 report aberrant cortical network activity tantamount to seizure (Steinmetz et al., 2017) which may be due to Cre toxicity or genetic background. (3) Long-term optical monitoring of cellular excitability at visible/near-infrared wavelengths may lead to phototoxicity (Denk et al., 1994) or stimulation of photobiological processes.

Dyes associated with other intracellular ion concentrations (e.g., sodium and potassium) are also available, as are indicators of transmembrane potential changes (i.e., voltage indicator dyes). However, current sodium and potassium indicators suffer from lack of binding specificity as compared to calcium indicators (Meuwis et al., 1995; Minta and Tsien, 1989). Voltage imaging also faces inherent challenges associated with the short duration of action potentials, requiring high frame rate acquisitions from expensive detectors and leading to relatively low fluorescence photon yields (i.e., low signal-to-noise ratios) (Kulkarni and Miller, 2017). Still, moderate throughput voltage imaging experiments have been carried out in HEK-293 cells expressing human Nav1.7 alpha subunits in 96 well fluorescent imaging plate reader (FLIPR) formats using voltage dyes to screen for VGSC antagonists (Zhao et al., 2016). However, FLIPR blue dye assays do not enable sodium response times on the order of single action potentials (1–10 ms), instead offering changes in fluorescence on the order of tens of seconds. Surrogate measures of specific sodium channels have been proposed to overcome these challenges, such as thallium flux-based functional assays for the sodium channel Nav1.7 (Du et al., 2015). While the fluorescence yield and dynamics of these surrogate measures are more amenable to high-throughput screening, they are still an indirect measure of membrane excitability.

More recently, label-free methods of optically detecting single action potentials based on minute changes cell membrane deformations

(electromotility) via interferometry (Batabyal et al., 2017) or changes in near-membrane protein methyl resonance peaks via vibrational spectroscopic imaging have also been reported (Lee et al., 2017). These methods offer the advantages of reduced phototoxicity, no risk of photobleaching, and high temporal resolution as compared to calcium imaging. While these outcomes are highly promising, especially for in vitro applications, these methods are still in their infancy and the cellular mechanisms that influence these measurements need to be further investigated. For example, in the case of vibrational spectroscopic imaging, the contributions of molecular reorientation and relative protein densities during electromotility are unknown. These methods also potentially suffer from similar inherent noise and acquisition rate challenges as label-based voltage imaging.

2.4.2. Patch-clamp electrophysiology

In terms of high-content measurements of cellular excitability and interrogation of individual membrane channel properties, manual patch-clamp (MPC) electrophysiology remains the gold standard. However, MPC is highly limited in regards to assay development due to its relative invasiveness, low-throughput, and technical difficulty. To overcome the challenges of throughput and technicality, automated patch clamp (APC) technologies have been developed. For an excellent review on 1st and 2nd generation automated patch clamp technologies for pain-related ion channel research, see (Bell and Dallas, 2017). Briefly, the most widely adopted and successful APC format involves the application of cell suspensions to planar recording chips, where a combination of gravity and negative pressure draw cells into contact with micropores (or apertures) centered on recording sites embedded within the planar chip. Once in contact, negative pressure is maintained until the system reports a threshold impedance necessary for potential 'clamp'. To date, these formats have largely been used by pharmaceutical companies to screen for human ether-a-gogo-related gene (hERG) induced cardiac ion channel blockade (Möller and Witchel, 2011). hERG channel (Kv11.1) blockade can lead to cardiac arrhythmia, and hERG channel block has become a frequent cause for drug discovery program delays or withdrawals from clinical trial (Danker and Möller, 2014). In the same way, APC is now being used to screen for antagonists of Nav1.7, an ion channel highly implicated in nociception and chronic pain development. One such technology (SynchroPatch 768PE) successfully screened 10,000 Nav1.7 reference compounds against heterologous cell lines expressing human Nav1.7 variants with a throughput of approximately 6000 data points per day with a Z' factor of 0.72 (Li et al., 2017); highlighting the potential of APC formats for high-throughput screening targeting pain-relevant ion channels, if not nociceptor excitability.

2.4.3. Substrate-integrated microelectrode arrays

Similar to patch-clamp electrophysiology, substrate-integrated microelectrode arrays (MEAs) may be used to resolve and record individual action potentials, or spikes (Gross et al., 1985; Pancrazio et al., 1999). However, these extracellular action potentials are recorded non-invasively, enabling long-term and/or repeated monitoring of large cell populations. For excellent reviews of fundamental principles and applications of extracellular microelectrode arrays, see (Heinricher, 2004) and (Spira and Hai, 2013). Briefly, MEAs function on the principles of volume conductor theory, where regions of cell membrane serve as a current sink, or sources, both embedded within a conductive medium. As an action potential propagates, so does the region of membrane depolarization, with sink becoming source (and vice versa). Relative to stationary recording and reference electrodes, this is manifest as time-dependent potential increases/decreases, giving rise to characteristic extracellular waveforms with temporal resolution approaching or equal to patch-clamp recordings (1–10 ms). By this means, MEAs enable high-content quantitative measures of cellular excitability, such as spike rate, amplitude, bursting rates, and neuron firing synchronization (Gross et al., 1995; Selinger et al., 2004). The quality of these recordings, in terms of signal-to-noise ratio, is largely dependent on the impedance of the recording electrodes and the quality of the cell coupling. A large number of substrate materials have been successfully used to fabricate MEAs and facilitate cell adhesion, including silicon dioxide (Mastrototaro et al., 1992), silicon nitride (Connolly et al., 1990), SU-8 (K. Zhang et al., 2010; Y. Zhang et al., 2010), polystyrene (Hammack et al., 2018), parylene (Charkhkar et al., 2016, 2012; Omaki et al., 2017), liquid crystal elastomer (Rihani et al., 2018), and others. Reduction in electrode impedance is dependent on several factors, but recent advances in electrodeposition, metallic 3D printing, and electrospinning have enabled nanofabrication of electrode sites; potentially enhancing long-term cell-to-electrode coupling and effectively reducing electrode impedance, resulting in increased signal-to-noise ratios (Heim et al., 2012; Jao et al., 2015; Krishnamoorthy and Zoski, 2005; Santoro et al., 2014; Weidlich et al., 2017).

A large body of previous work has demonstrated that primary neurons, particularly those that are embryonically-derived, can be reliably cultured on MEAs, and are a valuable tool for neurotoxicology (Johnstone et al., 2010) and neuropharmacology (Accardi et al., 2016; Kulagina et al., 2004; Novellino et al., 2011; Parenti et al., 2013). Commercially available systems from Axion Biosystems, ALA Scientific, and Multichannel Systems utilize multi-well MEA plates for moderate throughput, high content screening based on extracellular action potentials (Fig. 2). These systems have been used to assess the neural effects of putative toxicants (Valdivia et al., 2014), illicit drugs (Hondebrink et al., 2016), marine neurotoxins (Nicolas et al., 2014), insecticides (Dingemans et al., 2016), as well as patient-specific genetic predispositions for epilepsy and related disorders (Tidball and Parent, 2015; Vessoni et al., 2016).

Extension of single- and multi-well MEA technology to explorations of pain-related mechanisms and therapeutics has begun. (Pearce et al., 2005) demonstrated an integrated MEA-microfluidic system for creating a precise 'temperature clamp' on mouse DRG neurons seeded on an MEA and examined cold sensitivity. (Yang et al., 2016) recapitulated functional effects of a sodium channel mutation associated with inherited erythromelaglia using adult rat DRG neurons on multi-well MEA plates. Cells expressing the gain-of-function mutation showed an increased level of spontaneous firing in response to temperature elevations, consistent with the warmth-induced manifestation of pain. However, the fraction of microelectrode sites showing any discernible spike activity for the cultured wild-type DRG, even at elevated temperatures, was less than 2%, a level far too sparse to support high-throughput screening. By relying on embryonically-derived tissue, Newberry et al. (2016) demonstrated cultured DRG neurons on multi-well MEAs where activity could be detected on 53% of the microelectrode sites, an observation we have largely confirmed in our laboratory. Furthermore, spontaneous spike activity could be modulated by exposure to capsaicin, a ligand for the transient receptor potential cation channel subfamily V1 (TRPV1), bradykinin, and inhibitors of voltage gated sodium and calcium channels (Newberry et al., 2016). While embryonic tissue is amenable to in vitro culture due to increased viability (Eide and McMurray, 2005), there are significant disadvantages due to differences in gene expression and related function as compared to later developmental stages. These include changes linked to the voltage-gated Na channel Nav 1.8/1.9 (Benn et al., 2001), mRNA associated with axonal transport, vesicle trafficking, and axonal protein synthesis (Gumy et al., 2011). Additionally, dissociated embryonic and neonatal DRG cultures show little or no apparent NGF-induced sensitization to capsaicin (Zhu and Oxford, 2011), unlike adult DRG in vivo and in vitro. This raises the possible concern that embryonic DRG neurons are insensitive to cytokine mediated sensitization, which would be in stark contrast to the outcomes of studies using adult DRG neurons for patch-clamp (Von Banchet et al., 2005) and MEAs (Moy et al., 2017). With optimization of adult DRG culture conditions, it may be possible to greatly enhance the yield of active microelectrodes by a factor of 10–20 to enable cell-based assay use. Recent work in our lab

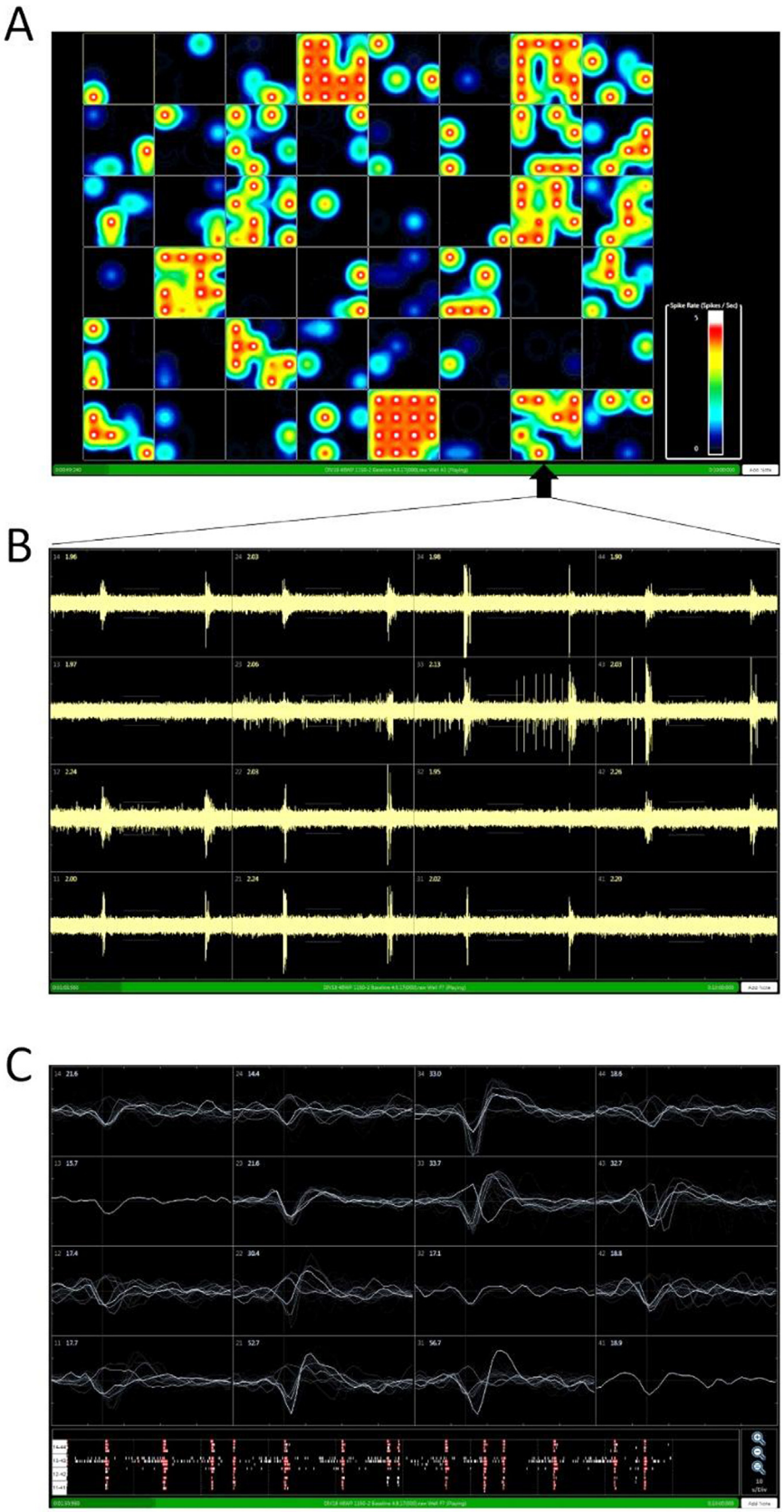

Fig. 2. Screen images from the Axion Biosystems AxiS data acquisition system depicting extracellular neural activity from a 48-well microelectrode array plate cultured with primary mouse cortical neurons 19 days in vitro. A) Representative heat map showing detected spikes across the multi-well plate. The arrow identifies well F7. B) Real time recording from 16 microelectrode contacts within well F7. C) Event detection within well F7 illustrating the features of the detected spikes.

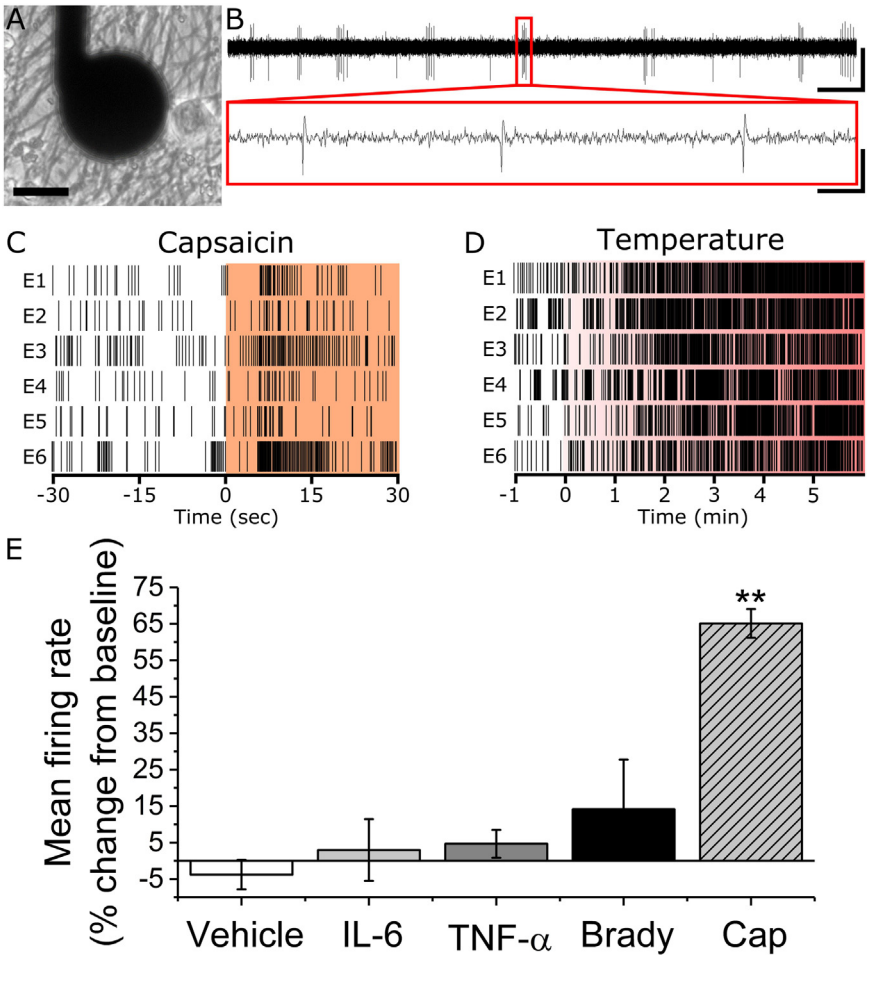

Fig. 3. Extracellular MEA-based recordings from hiPSC sensory neurons. (A) Phase contrast image of dense cellular and axonal network on MEA substrate after 48 days in vitro. Scale bar represents 20 μm. (B) Filtered continuous recording (12.5 kHz sampling rate) from a representative electrode. Vertical and horizontal scale bars represent 30 μV and 500 ms, respectively. Red inset illustrates resolution of single action potential waveforms. Vertical and horizontal scale bars represent 30 μV and 3 ms, respectively. (C) and (D) Raster plots of hiPSC sensory neuron activity in the absence and presence of 100 nM capsaicin and temperature increase (from 37 to 42 °C). (E) hiPSC sensory neuron firing rates were insensitive to human recombinant IL-6, TNF-α, and bradykinin (Brady), but were significantly increased by addition of 100 nM capsaicin.

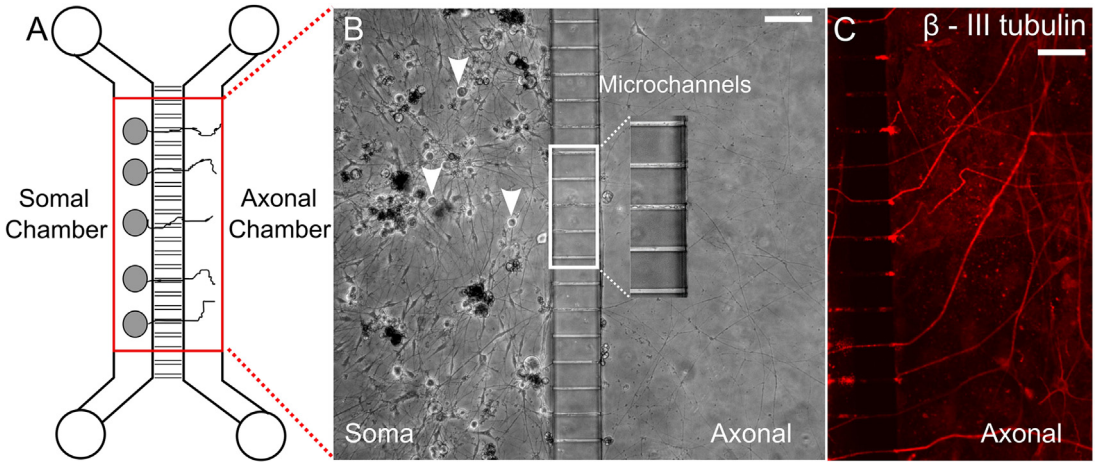

Fig. 4. (A) Schematic of PDMS-based compartmentalized culture device. (B) Phase image of DRG sensory neuron culture (day 4) extending processes from the soma into the axonal compartment. White arrows indicate examples of cell soma. (C) β-III tubulin staining of axonal compartment highlights axon density by day 8. Scale bar represents 100 μm.

indicates that higher yield of spontaneous activity may be associated with maintaining a relatively high density of non-neuronal cell types (satellite glia, Schwann cells, and fibroblasts).

Importantly, the multiwell MEA platform does not inherently exclude the use of primary human DRG or hiPSC sensory neuron models. Proof-of-concept extracellular recordings from custom MEA devices using human DRG have been demonstrated (Enright et al., 2016), as has the use of hiPSC derived sensory neurons (Odawara et al., 2017; Wainger et al., 2014), suggesting that MEAs allow maximal physiological relevance in a moderate throughput assay platform. Our group has used commercially available hiPSC derived sensory neuron progenitors (Axol Bioscience) to monitor spontaneous and evoked activity in a multiwell MEA format. While we observed spontaneous baseline activity with excellent signal-to-noise ratios as well as electrically, chemically, and thermally evoked activity, we did not observe any culture sensitivity to human-recombinant inflammatory cytokines IL-6 or TNF-

α (Fig. 3). This suggests that either culture conditions or differentiation protocols need to be further optimized to lead to cytokine-sensitive hiPSC sensory models and/or genetically modified models for studies of chronic pain mechanisms and pharmacology.

2.4.4. Compartmentalized cultures and higher-order co-cultures

As previously discussed, each DRG sensory neuron extends a single bifurcated axon toward both the dorsal spinal cord and the periphery. In the periphery, nerve terminals form intimate contact with different tissues and have differing electrophysiological and pharmacological profiles than the soma. Perhaps most importantly, it is the peripheral nerve terminals - not the cell soma - that will be directly and immediately exposed to the previously discussed 'inflammatory soup' following tissue injury. It is this exposure that initiates the inflammatory cascade which can lead to chronic pain conditions. Therefore, there is considerable interest in monitoring excitability changes and pharmacological profiles in physically and/or fluidically isolated soma and axons, as shown in Fig. 4. Various methods have been described to physically compartmentalize sensory neuron soma and axons, including PDMS microfluidic channels (Dworak and Wheeler, 2009; Park et al., 2006), Campenot chambers (Campenot, 1977; Pazyra-Murphy and Segal, 2008), and porous filters (Unsain et al., 2014). To date, a majority of studies using sensory neurons in compartmentalized cultures have focused on monitoring rates and extent of neurite outgrowth and myelination (Gornstein and Schwartz, 2017; Ravula et al., 2007; Wheeler et al., 2014; Yang et al., 2012), de/regeneration following axotomy (Kilinc et al., 2011; Unsain et al., 2014), and anterograde/retrograde transport or signaling (Cohen et al., 2011; Gluska et al., 2015; Y. Zhang et al., 2010; K. Zhang et al., 2010; Zweifel et al., 2005), among others (Gumy et al., 2011).

The use of compartmentalized cultures has been extended to studies of sensory neuron excitability in only a few cases, wherein somatic or axonal activity were monitored independently via calcium imaging (Jonas et al., 2015) and/or MPC electrophysiology (Tsantoulas et al., 2013). However, standard or commercial microfluidic devices are not designed to accommodate MPC electrodes. Moreover, MPC on single axons remains a high technical barrier. The integration of single or multi-well MEAs with PDMS microchannel devices may prove more amenable to simultaneous stimulation and recording applications. While PDMS-based microfluidics have been used previously with MEAs for drug (Enright et al., 2016) or temperature delivery (Pearce et al., 2005), to date, no study has demonstrated compartmentalized cultures on MEAs.

In addition to isolating neuronal soma from axons, compartmentalized culture platforms may also be used to isolate multiple pathophysiologically relevant cell types. For example, previous studies have incorporated DRG neurons and keratinocytes (Griscom et al., 2005; Kumamoto et al., 2014; Roggenkamp et al., 2012) or osteoblasts (Neto et al., 2014; Silva et al., 2017) in separate compartments of PDMS microsystems or Campenot chambers to mimic skin or bone innervation and to study interactions between neurons and the cell types native to the innervated tissue. Additionally, sensory communication between DRG neurons and dorsal horn neurons has been monitored via calcium imaging in an open well format (Ohshiro et al., 2007), but not in a compartmentalized culture to date.

While compartmentalized cultures may be advantageous for separating cell types and components, careful consideration needs to be given to the microchannel design. It has been noted that standard microchannel device designs create high-shear fluid flows during seeding or medium exchange. Since relatively low fluid flow rates have been observed to drive the directionality of neurite outgrowth (Gu et al., 2014), there is concern that similar designs will suffer from microchannel crossing failure due to directionality or 'bundling' (van de Wijdeven et al., 2018). Additionally, both microchannel width and length need to be optimized to accommodate axonal outgrowth rates (Tsantoulas et al., 2013), mitigate the non-neuronal cell migration (Park et al., 2006), and control passive diffusion of growth factors or other pharmacological treatments. Some of these potential issues have driven the design of novel 'open-well seeding' platforms for generating multi-nodal compartmentalized cultures and/or maintaining custody of cells and biochemical agents (Park et al., 2009; van de Wijdeven et al., 2018) without generating high-shear stresses which may limit application or potentially modulate function.

3. Perspective

Modern cell-based pharmacological screening strategies monitor changes in cellular phenotype as an integrative measure of a drug's activity. Ideally, the phenotypic endpoint is highly relevant to the indication of interest and provides some insight into the underlying mechanistic pathways. However, the successful identification of a 'hit' ideally would not require full mechanistic understanding of the drug-tissue interaction. An important primary phenotype of neural cells is excitability. The first-order neuronal cell type involved in the transduction and transmission of peripherally-mediated pain are sensory neurons of the DRG. Therefore, future cell-based phenotypic screening paradigms targeting the identification of peripheral analgesics should enable direct measurement of DRG sensory neuron excitability. Strategies should be developed to maximize content (cellular as well as temporal resolution and intercellular communication), throughput, and ease-of-use while minimizing invasiveness. Furthermore, the mechanistic complexity of chronic pain demands a cellular model capable of recapitulating responsiveness and/or sensitization due to compounds known to be involved in human chronic pain conditions. This has driven the ongoing development of hiPSC sensory neuron models, which provide the opportunity for maximal human relevancy while also being scalable for high-throughput platforms. However, to date, no published study has demonstrated functional sensitivity of hiPSC sensory neurons to inflammatory cytokines such as IL-6, IL-1β, TNFα, or NGF. Therefore, more work needs to be done related to expression of sensitization pathways in hiPSC-based models as well as standardization of differentiation protocols (Schwartzentruber et al., 2018). Along these lines, it may be worthwhile to investigate phenotypic contributions of either primary or hiPSC-derived non-neuronal cell types (satellite glia, Schwann cells) in heterogeneous culture models, as it has been demonstrated in other hiPSC neuron models that the presence of support cells (glia, oligodendrocytes) facilitates synaptogenesis, phenotypically relevant mRNA expression, and functional maturation (Ishii et al., 2017; Kayama et al., 2018; Odawara et al., 2014; Schutte et al., 2018; Tang et al., 2013). In the case of chronic pain development, satellite glia and Schwann cells have been shown to facilitate sensitization and intercellular signaling via gap junctions and transmitter release (e.g., ATP) (Campana, 2007; De Logu et al., 2017; Hartlehnert et al., 2017; Huang et al., 2013; Kodama et al., 2017; Poplawski et al., 2018; Takeda et al., 2009). Therefore, developing combinatorial imaging-electrophysiological approaches may add highly relevant content without diminishing density or throughput. This may be achievable using field effect transistor-based MEAs, which have been fabricated from silicon (Fromherz et al., 1991), graphene (Blaschke et al., 2016), and organic materials (Benfenati et al., 2013) among others. In the case of graphene and organics, this offers transparent substrates and electrodes in combination with high electrode density. Furthermore, the feasibility of both high-density MEAs (Musick et al., 2009) and functional imaging (Pasca et al., 2015) has already been demonstrated using 3D culture platforms. While it is the authors' opinion that not all pharmacological screening outcomes demand the reported advantages of 3D culture, it seems to be the natural convergence for advances in both functional recording technology and tissue engineering.

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Authorship statement

BJB and JJP organized and interpreted the core ideas and contributed the main text of the manuscript. TJP and GD contributed concepts of high content screening through electrophysiological endpoints. ZTC, TJP, and GD contributed concepts on electrophysiology measures for study of molecular mechanisms underlying pain. BJB, RA, and SP collected and analyzed data and created the manuscript figures.

Conflict of interest statement

None.

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