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The F-11 Sensory Neuron Model: A Scalable *In Vitro* Platform for Neuropathic Pain and Drug Screening

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HIGHLIGHTS

- The differentiated F-11 cell line is a scalable, mid-fidelity model positioned between simplistic lines and complex primary cultures
- A synergistic protocol using NGF and cAMP enhancers robustly generates a functional nociceptive neuron phenotype
- The model's validated nociceptive features offer a medium-throughput tool for early analgesic discovery

ABSTRACT

Neuropathic pain remains a major therapeutic challenge, largely due to the translational disconnect between preclinical animal models and clinical efficacy in humans. This review critically evaluates the differentiated F-11 cell line, a hybridoma of mouse neuroblastoma and rat embryonic dorsal root ganglion (DRG) neurons, as a scalable, reproducible, and physiologically relevant in vitro platform for neuropathic pain research and analgesic drug screening. A detailed analysis of differentiation strategies highlights the critical interplay of neurotrophic factors (notably NGF), intracellular signaling modulators (such as cAMP elevators), and extracellular matrix cues in driving neuronal maturation. Functional validation via calcium imaging and electrophysiology confirms capsaicin responsiveness and action potential generation, mirroring native nociceptors. Its compatibility with medium-to-high-throughput screening and mechanistic studies including investigation of silent nociceptor sensitization in chronic pain conditions along with emerging applications in neuropathy models, makes it a valuable tool for de-risking drug candidates before animal studies.

INTRODUCTION

Neuropathic pain constitutes a significant and escalating global health challenge, with a high prevalence arising from diverse etiologies, including diabetes, herpes zoster, nerve trauma, and chemotherapy (Leoni et al., 2025; Rosenberger et al., 2020). The clinical burden of this condition is profound; patients frequently experience refractory symptoms that severely diminish their quality of life. The underlying pathophysiology is exceptionally complex, involving a cascade of peripheral and central mechanisms, including peripheral sensitization, central disinhibition, glial cell activation, and ion channel modulation (Karcz et al., 2024). It is important to clarify that the term "pain" inherently denotes a subjective sensory and emotional experience processed within the central nervous system (Raja et al., 2020). In the context of the in vitro models discussed herein, the more precise terms “nociception” or “activation of nociceptive neurons” will be employed to refer specifically to the detection and transmission of noxious stimuli at the peripheral level (Salzer et al., 2019).

This pathophysiological complexity directly contributes to the severe limitations of existing pharmacotherapies. Conventional analgesics, such as NSAIDs and opioids, are largely ineffective against neuropathic pain and carry significant risk profiles. Meanwhile, first-line treatments including gabapentinoids and antidepressants provide only partial relief for a subset of patients, creating a critical bottleneck in effective clinical management (Cao et al., 2024). This therapeutic impasse is further compounded by a major translational gap in pain research. The development of novel analgesics remains heavily reliant on animal models; however, high costs, ethical constraints, and inherent species differences often lead promising drug candidates to fail to translate from preclinical efficacy to human clinical trials (Mouraux et al., 2021).

One emerging candidate platform is the differentiated F-11 cell line, a hybridoma of mouse neuroblastoma and rat embryonic dorsal root ganglion (DRG) neurons, which offers significant potential as a scalable and reproducible sensory neuron model (Haberberger et al., 2020).

This review provides a narrative synthesis of the current literature concerning differentiated F-11 cells. As this is not a systematic review, we acknowledge the potential for selection bias inherent in a narrative approach; however, efforts have been made to encompass both seminal and contemporary studies to provide a balanced assessment. This study assesses the current state of the field by comparing and contrasting published findings, with particular attention to methodological heterogeneity and its impact on reported neuronal phenotypes. This approach allows us to identify key knowledge gaps and evaluate whether claims regarding F-11 cells are substantiated by the available evidence. Specifically, this review aims to: (i) critically evaluate differentiation strategies for F-11 cells; (ii) provide a rigorous comparative analysis against the current ‘gold standard’, primary DRG neurons; and (iii) outline a clear roadmap for the integration of this platform into next-generation analgesic drug discovery pipelines.

Sensory neurons and primary dorsal root ganglion (DRG) as the gold standard

Primary sensory neurons, whose cell bodies reside in the Dorsal Root Ganglion (DRG), are the critical first responders of the peripheral nervous system, responsible for detecting and transmitting diverse stimuli from gentle touch to painful insults to the central nervous system (Jang & Garraway, 2024). Their functional specialization is reflected in a complex classification of subtypes, broadly categorized by cell diameter, molecular signature, and physiological role. Major populations include peptidergic nociceptors expressing the Nerve Growth Factor receptor TrkA and releasing neuropeptides such as CGRP; non-peptidergic nociceptors identified by IB4 binding; and various low-threshold mechanoreceptors

(Barabas et al., 2012; Lawson et al., 2019; Saeed & Ribeiro-da-Silva, 2012). This cellular heterogeneity underlies their ability to encode the multidimensional nature of sensory perception, particularly pain.

Because of their physiological relevance, primary DRG neurons isolated from animal models, especially rodents, are widely regarded as the gold standard in pain research (Chrysostomidou et al., 2021). These ex vivo cultures preserve the native expression of critical ion channels (e.g., Nav1.7, Nav1.8, TRPV1), receptors, and intracellular signaling pathways necessary for nociceptive transduction (Hutchings et al., 2019). However, despite their translational value, their use is constrained by limited tissue availability, donor variability, technically demanding culture protocols, low yield, and ethical concerns related to animal experimentation (Khosrowshahi et al., 2025).

Accordingly, the development of alternative in vitro models requires demonstration that key phenotypic and functional characteristics of primary DRG neurons are faithfully recapitulated (Chen et al., 2026). Validation typically involves assessing the expression of established neuronal and nociceptive markers, including TrkA (NGF responsiveness), MAP-2 (neuronal maturation and neurite development), CGRP and Substance P (peptidergic identity), Nav1.7/1.8 (pain-associated sodium channels), TRPV1 (capsaicin sensitivity), and IB4 binding (non-peptidergic phenotype) (Amaya-Rodriguez et al., 2024). The coordinated expression of these markers serves as a benchmark for evaluating the fidelity of emerging cell-based pain models.

In vitro cellular models

The F-11 cell line represents a pragmatic in vitro model that bridges the scalability of immortalized neuroblastoma lines with selected functional properties of dorsal root ganglion (DRG) neurons (Pastori et al., 2019). Derived from a hybridization of mouse neuroblastoma and embryonic rat DRG neurons, F-11 cells possess an inherent neuronal identity that distinguishes them from conventional cancer-derived lines. However, the acquisition of a mature sensory-like phenotype depends on appropriate differentiation protocols, which promote neurite outgrowth and the expression of functionally relevant ion channels and receptors (Bennison et al., 2020).

Following differentiation, F-11 cells provide a genetically stable and experimentally accessible platform exhibiting key nociceptive features, making them suitable for medium-throughput analgesic screening (Haberberger et al., 2020). Thus, they occupy an intermediate position between primary DRG cultures and immortalized cell lines, offering a practical balance between physiological relevance and experimental scalability (Orozco Morato et al., 2022).

F-11 cell differentiation strategies: A critical analysis of determinant factors

The F-11 cell line is a well-characterized hybrid derived from rat dorsal root ganglion (DRG) neurons and the mouse neuroblastoma cell line N18TG2 (Fan et al., 1992). This origin confers a dual phenotype: a proliferative, undifferentiated state suitable for routine expansion and a post-mitotic, neuron-like differentiated state marked by neurite outgrowth, electrophysiological excitability, and expression of sensory neuronal markers. Owing to this plasticity, F-11 cells are widely used as an in vitro model for neuropathic pain research, neuronal development studies, and neuropharmacological screening (Martínez et al., 2019).

Importantly, the physiological relevance of differentiated F-11 cells depends on the robustness and optimization of the differentiation protocol. Rather than being an intrinsic property, the mature sensory-like phenotype emerges only when key biological cues are appropriately replicated in vitro. These cues are

grounded in developmental neurobiology and can be grouped into three interconnected pillars (Blasa et al., 2021):

Growth factors

Neurotrophic factors such as Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), and Glial Cell Line-Derived Neurotrophic Factor (GDNF) function as extracellular ligands that bind to specific tyrosine kinase receptors (e.g., TrkA, TrkB) on the neuronal membrane (Erickson et al., 2001). Receptor activation triggers intracellular signaling cascades, including MAPK/ERK and PI3K/Akt pathways, ultimately modulating gene expression programs that promote neuronal survival, cell-cycle exit, neuritogenesis, and synaptic maturation (Artim et al., 2020).

Intracellular signaling modulators

The effectiveness of neurotrophic signaling is strongly influenced by intracellular second messengers, particularly cyclic AMP (cAMP) (Yan et al., 2016). Elevated cAMP levels activate Protein Kinase A (PKA), which phosphorylates transcription factors such as CREB. Activated CREB drives the transcription of genes encoding structural proteins, ion channels, and synaptic components required for neuronal maturation. In many neuronal precursor systems, sufficient cAMP signaling acts as a permissive trigger for differentiation.

Substrate and extracellular matrix cues

Neuronal differentiation is also shaped by the physical and biochemical properties of the culture substrate. Extracellular matrix proteins such as Laminin and Poly-D-Lysine enhance adhesion while simultaneously engaging integrins and other cell adhesion molecules (Morwood et al., 2023). These interactions initiate cytoskeletal remodeling, growth cone dynamics, and neuronal polarization processes essential for functional maturation (Dent et al., 2011).

Primary differentiation factors

Growth factors: NGF, GDNF, and BDNF

Nerve Growth Factor (NGF) is the central driver of most published F-11 differentiation protocols, and its absence generally results in minimal spontaneous neuronal maturation (Baldassarro et al., 2023). The primary variables influencing its efficacy are concentration and timing. Reported doses range widely (5–100 ng/mL), although concentrations between 10–50 ng/mL most consistently promote robust neurite outgrowth. Timing is equally important. Immediate NGF exposure at the time of plating when cells are still adapting and proliferating may be suboptimal. A more effective strategy allows 24 hours for cell adhesion in serum-containing medium before switching to a low-serum, NGF-supplemented differentiation medium (Gunning et al., 1981). This sequential approach prioritizes population stability before introducing the differentiation cue. Despite broad agreement on NGF's indispensability, direct comparative studies evaluating dosing regimens remain limited. It is unclear whether a single initial bolus is sufficient or whether NGF degradation necessitates replenishment. While many protocols rely on one-time supplementation, evidence from primary sensory neuron cultures suggests that sustained exposure enhances maturation (Katzenell et al., 2017). The absence of standardized dosing schedules likely contributes to inter-laboratory variability. Although NGF predominantly targets TrkA-expressing nociceptive neurons, F-11 cells may exhibit phenotypic heterogeneity. Other neurotrophic factors, such as Glial Cell Line-Derived Neurotrophic Factor (GDNF) signaling via Ret/GFRα receptors and Brain-Derived Neurotrophic Factor (BDNF) acting through TrkB are well-established modulators of distinct sensory neuron subpopulations (Zhang et al., 2014). However,

compared with NGF, systematic investigations of GDNF or BDNF in F-11 differentiation remain limited, representing a notable gap in protocol optimization and subtype-specific modeling.

Several studies have reported that brain-derived neurotrophic factor (BDNF) is capable of inducing differentiation in F-11 cells (Bathina & Das, 2015; Chen et al., 2013; Colucci-D'Amato et al., 2020); however, the resultant morphology is often distinct from that observed with nerve growth factor (NGF)-mediated differentiation (Camerino et al., 2016). This raises important questions regarding the specificity of neurotrophic factor signaling and the potential heterogeneity of cellular responses. It is plausible that a subpopulation of F-11 cells exhibits preferential responsiveness to glial cell line-derived neurotrophic factor (GDNF) or BDNF (Ferrini et al., 2021). Moreover, the potential synergistic effects of combinatorial neurotrophin treatment particularly the concomitant administration of NGF with other neurotrophic factors remain largely unexplored (Xiong et al., 2025). Accordingly, a systematic investigation comparing the morphological, electrophysiological, and transcriptomic profiles of F-11 cells differentiated under each condition, both alone and in combination, would be of considerable value in establishing the validity and optimizing the utility of this model system (Pastori et al., 2019).

Intracellular signaling enhancers

The observation that NGF alone may be insufficient to induce robust F-11 differentiation led to the incorporation of intracellular cAMP–elevating agents, most commonly forskolin and dibutyryl-cAMP (dbcAMP) (Sharma et al., 1990). Forskolin activates adenylate cyclase to increase endogenous cAMP production, whereas dbcAMP is a membrane-permeable, phosphodiesterase-resistant analog that directly elevates intracellular cAMP levels. By amplifying cAMP signaling, both agents potentiate the downstream transcriptional programs required for neuronal maturation (Wang et al., 2024). Experimental evidence consistently shows that combining NGF with forskolin or dbcAMP significantly increases the proportion of neurite-bearing cells, enhances neurite length and growth rate, and accelerates the functional maturation of voltage-gated sodium and potassium channels, resulting in more robust action potential firing (Richter-Landsberg & Jastorff, 1986). Despite their shared target pathway, important distinctions exist. Forskolin relies on cellular adenylate cyclase activity and may produce more transient cAMP elevation, whereas dbcAMP provides a more stable intracellular signal. Although dbcAMP is sometimes reported to yield stronger differentiation effects, it may also exhibit greater cytotoxicity at higher concentrations. Thus, the choice between these agents represents a balance between differentiation efficiency and cell viability. Collectively, the combination of NGF and a cAMP-elevating compound is now regarded as the operational standard for high-yield F-11 differentiation (Martínez et al., 2019). Mechanistically, this synergy is coherent: NGF supplies the trophic TrkA-mediated signal, while elevated cAMP functions as a permissive amplifier of the differentiation program (Yan et al., 2017).

Optimization parameters

The biochemical induction is only part of the equation. Physical and environmental parameters are equally critical for a successful outcome. Initial cell seeding density is a frequently underestimated yet critical variable. Plating cells too densely leads to overcrowding, intense competition for nutrients, and a buildup of metabolic waste, all of which stifle neurite outgrowth and can cause cells to cluster rather than differentiate as isolated, functional units. Conversely, seeding too sparsely deprives cells of vital autocrine and paracrine survival signals, resulting in poor viability and increased apoptosis (Kim et al., 2009).

Media composition, particularly serum concentration, is equally decisive. Standard growth media for F-11 cells (e.g., DMEM or Ham's F-12) are typically supplemented with 10–15% Fetal Bovine Serum

(FBS), a complex and undefined mixture of both growth promoters and inhibitors. Reducing serum concentration to 1–2% or employing serum-free conditions acts as a classic and potent trigger for differentiation across many neuronal cell lines. In F-11 cells, high serum sustains proliferation, while its withdrawal signals a decisive switch to a post-mitotic, differentiated state. Thus, combining serum reduction with specific differentiation agents such as NGF and forskolin creates a powerful "differentiation cocktail" that halts the cell cycle and actively drives the acquisition of a mature neuronal phenotype.

Culture substrate: laminin, poly-D-lysine, collagen

The choice of substrate is a decisive factor for neurite initiation and elongation. Poly-D-lysine (PDL) serves as a foundational, positively charged polymer that facilitates strong, non-specific electrostatic adhesion of cells to the culture surface (Stil et al., 2023). However, its role is largely mechanical, providing a sticky base with minimal inherent bioactive signaling. In contrast, laminin, a natural extracellular matrix (ECM) protein of the basal lamina, offers a far more dynamic interface. Cells adhere to laminin through specific integrin receptors, which in turn activate crucial pro-differentiation signaling pathways such as PI3K and FAK. For neurite outgrowth, laminin is unequivocally superior to PDL alone, particularly in promoting extensive, stable, and complex arbors (Perera et al., 2020). Consequently, the most effective and widely adopted strategy is sequential coating: a primary layer of PDL to establish strong adhesive anchorage, followed by a laminin overlay to present a bioactive surface. This combination leverages the mechanical reliability of PDL alongside the specific, growth-promoting signals of laminin. Other substrates, such as collagen, are used less frequently and are generally considered less effective than laminin for this application.

Impact of differentiation protocols on cell viability: The cytotoxicity tightrope

Perhaps the most critical consideration in optimizing an F-11 differentiation protocol is navigating the inherent tension between induction potency and cellular health (Martínez et al., 2019). The powerful differentiating agents required are, by their nature, potent signaling modulators that can place significant stress on the cells. This cytotoxicity is most evident with cAMP-elevating agents. While a concentration of 5–10 µM forskolin acts synergistically with other factors, exceeding 20–25 µM often becomes markedly cytotoxic, leading to cell rounding, detachment, and death over several days (Ho & Raw, 1992; Laurenza et al., 1989; Martínez et al., 2019). Similarly, high concentrations of dbcAMP (>1 mM) can disrupt osmotic balance and induce apoptosis (Honma et al., 1996).

The differentiation process itself is inherently taxing: cells must halt proliferation, undergo extensive cytoskeletal remodeling to extend neurites, and upregulate energy-intensive ion channels. Pushing them too aggressively can overwhelm their homeostatic capacity. This risk is compounded by serum reduction, which is essential for triggering differentiation, can deprive cells of survival signals and increase susceptibility to anoikis if they are not firmly anchored and supported by NGF (Gudbergsson et al., 2016). Thus, a successful protocol is not defined by the highest possible concentrations of inducers, but by the optimal window in which a maximal proportion of cells differentiates robustly while preserving long-term viability—a necessity for applications such as electrophysiology or chronic drug studies. Achieving this balance requires careful empirical titration. Accordingly, a key metric of success extends beyond neurite counts at 24–48 hours; it must include assessment of overall viability and morphological integrity after 5–7 days (Ramani et al., 2024).

Biomolecular and functional validation: A critical appraisal of F-11 cell differentiation as a model of DRG neuron phenotype

The utility of the F-11 cell line as a model system for pain research hinges upon its ability to function as a sensory neuron. While morphological and biomolecular characterization are essential for establishing cellular identity, the ultimate standard for validation lies in functional capacity. A rigorous validation of this model must, therefore, demonstrate that differentiated F-11 cells can transduce nociceptive signals, with biomolecular evidence serving to confirm the identity of the underlying machinery.

Morphological validation: The first line of evidence

The most immediate and visually compelling evidence of F-11 differentiation is a profound morphological shift. Undifferentiated cells exhibit a flat, fibroblast-like or polygonal appearance typical of proliferating cultures. Upon exposure to an effective differentiation cocktail—such as NGF and forskolin on Laminin—they undergo a dramatic transformation (Pastori et al., 2019). Cells cease division, retract lamellipodia, and adopt a phase-bright, rounded soma. Subsequently, they extend long, slender processes, definitively identifiable as neurites by positive immunostaining for neuronal markers such as β-III-tubulin and neurofilament (Morato et al., 2022). Differentiation can be further quantified by neurite length and branching complexity. Differentiated F-11 cells routinely extend processes exceeding 200–500 µm, with some reports of neurites over 1 mm—rivaling those of primary DRG neurons in culture (Martínez et al., 2019). While often bipolar, a significant proportion become multipolar, developing complex arbors. Metrics such as branch number and total outgrowth, quantifiable using software like ImageJ, provide additional measures of maturation (Pemberton et al., 2018). A critical perspective is nonetheless essential: dramatic morphological change, while necessary, is not sufficient to claim acquisition of a DRG-like identity. Many neuronal cell lines extend neurites upon differentiation. The key question is whether these F-11 neurites represent mere cytoskeletal rearrangement or are functionally and molecularly equipped as true dorsal root ganglion neurites (Shipley et al., 2016).

Functional validation: The proof of concept

The presence of specific molecules alone is not sufficient to establish functional relevance; rigorous functional assays therefore serve as the ultimate test of model validity. A primary line of evidence is the response to noxious stimuli. Differentiated cells consistently respond to capsaicin, a TRPV1 agonist, with a sharp increase in intracellular calcium, a response blocked by the antagonist capsazepine, mirroring native peptidergic nociceptors (Bertin et al., 2014). Responses to other algogens such as ATP (via P2X receptors) and acidic pH (via ASICs and TRPV1) have also been demonstrated, though they are typically less robust, reflecting the receptor expression profile of the line. Electrophysiological activity provides the most direct functional validation. Whole-cell patch-clamp recordings have shown that differentiated cells generate both tetrodotoxin-sensitive and tetrodotoxin-resistant action potentials, confirming functional integration of voltage-gated sodium and potassium channels essential for neuronal signaling (Pastori et al., 2019). Voltage-clamp experiments further detail fast-inactivating Na^+ currents, delayed-rectifier K^+ currents, and multiple Ca^{2+} current subtypes a biophysical profile consistent with immature or small-diameter DRG neurons. A critical validation of the model's utility in drug discovery is its predictable response to known analgesics. The local anesthetic lidocaine reliably inhibits evoked action potentials and voltage-gated sodium currents (Doan et al., 2014). Moreover, as a model for neuropathic pain mechanisms, these cells respond to gabapentin, which inhibits high-voltage-activated calcium currents and attenuates calcium

responses to various allergens mirroring its suspected mechanism of action in primary neurons (Sutton & Schuman, 2006).

Collectively, these functional data spanning from receptor activation to ion channel currents and modulation by clinically relevant drugs provide the definitive proof that differentiated F-11 cells are not merely a collection of neurons *in name*, but are indeed capable of the signal transduction that defines a functional nociceptor. This functional competency is the cornerstone of the model's utility in drug discovery.

Biomolecular validation: The core of cellular identity

Molecular characterization provides the most critical level of validation, offering mechanistic insight into the functional identity of differentiated F-11 cells (Zhu et al., 2023). A key biomarker is TrkA, the high-affinity NGF receptor and a hallmark of nociceptive DRG neurons (Fang et al., 2005). Differentiated F-11 cells consistently express TrkA, as detected by RT-qPCR, Western blot, and immunocytochemistry, with expression often increasing following NGF-based protocols and localizing to both the soma and neurites (Martínez et al., 2019). Expression levels are lower than in primary adult DRG neurons, as expected for a hybridoma line, and the population is skewed toward a TrkA-positive, NGF-responsive phenotype (Morato et al., 2022). Another well-established maturation marker is Microtubule-Associated Protein 2 (MAP-2), indicative of neuronal dendrites and cell bodies (Dehmelt & Halpain, 2005). Differentiated F-11 cells show strong MAP-2 upregulation, with expression intensity tightly correlating with morphological complexity, confirming that structural changes are underpinned by genuine neuronal scaffolding (Pastori et al., 2019). The most compelling biomolecular evidence, however, is the expression of functional sensory neuron-specific proteins. Differentiated F-11 cells express key pain-related ion channels, including the capsaicin receptor TRPV1 and the voltage-gated sodium channel isoforms Nav1.7 and Nav1.8 (Deng et al., 2023; Orozco Morato et al., 2022). They also express and release Calcitonin Gene-Related Peptide (CGRP), a neuropeptide quintessentially associated with neurogenic inflammation and pain transmission from DRG nociceptors (Haberberger et al., 2020).

Table 1. Comparison Between Differentiated F-11 Cells and Primary Rodent DRG Neurons

Characteristics	Differentiated F-11 Cells	Primary Rodent DRG Neurons	Conclusion on F-11 Validity
Morphology	Bipolar/Multipolar; long neurites (can be >500 µm) (Platika et al., 1985).	Bipolar/Pseudounipolar; variable neurite length (Lindsay, 1988).	Good resemblance in complexity, though primary neurons are pseudounipolar *in vivo*.
Proliferation	Post-mitotic after differentiation (Platika et al., 1985).	Constitutively post-mitotic (Scott et al., 1993).	Faithful mimicry after successful differentiation.
Key Marker: TrkA	Expressed, upregulated by NGF. Level is moderate (Francel et al., 1987).	Highly expressed in a large subset (~40-50%) (Averill et al., 1995).	Qualitatively similar, quantitatively lower. Represents a TrkA+ subpopulation.
Key Marker: MAP-2	Strongly expressed, correlates with morphology (Platika et al., 1985).	Strongly expressed in dendrites/soma (Averill et al., 1995).	Excellent match. Indicates true neuronal maturation.

Characteristics	Differentiated F-11 Cells	Primary Rodent DRG Neurons	Conclusion on F-11 Validity
Functional Channels: TRPV1	Expressed and functional (capsaicin response) (Caterina et al., 1997).	Expressed and functional in peptidergic nociceptors (Caterina et al., 1997).	Strong functional parallel. A key strength of the model.
Functional Channels: Nav	TTX-S and TTX-R currents documented (Francel et al., 1987).	Mix of TTX-S and TTX-R currents (Caterina et al., 1997).	Good functional match, supporting excitability.
Neurotransmitter: CGRP	Expressed and released upon stimulation (Francel et al., 1987).	Expressed and released from peptidergic nociceptors (Scott et al., 1993).	Strong similarity in neurochemical phenotype.
Electrophysiology	Can fire action potentials; exhibits key ionic currents (North et al., 2022).	Fire action potentials; rich repertoire of currents (Fang et al., 2005).	Good fundamental match, though primary neurons may have greater diversity.
Heterogeneity	Skewed toward a NGF-responsive, peptidergic phenotype (Binaschi et al., 2003).	Highly heterogeneous (peptidergic, non-peptidergic, proprioceptive) (Lawson, 2002).	Major limitation. F-11 is a simplified model of a DRG subpopulation.

Applications in drug discovery and toxicology

The differentiated F-11 cell line serves as a scalable, high-throughput-compatible platform for early-stage drug discovery and neurotoxicity assessment, effectively bridging the gap between recombinant systems and less tractable primary neuron cultures. Its utility spans large-scale compound library screening, typically using functional readouts such as inhibition of capsaicin-induced calcium influx to identify novel analgesics and high-content imaging for simultaneous quantification of neurite health and viability in models of chemotherapy-induced neuropathy. A paramount advantage is its exceptional suitability for mechanistic follow-up: unlike primary sensory neurons, F-11 cells are readily transfectable, enabling straightforward gene knockdown or overexpression to deconvolute a compound's mechanism of action and link specific molecular targets to functional phenotypes.

For instance, this platform could be employed to dissect the intracellular pathways that render 'silent' nociceptors responsive. By overexpressing or knocking down candidates like TRPV1, which is broadly distributed (~75%) in human DRG neurons and known to mediate capsaicin responses even in mechanically-insensitive afferents, researchers can directly test their necessity for the sensitization induced by inflammatory mediators such as prostaglandin E2 (Namer et al., 2019; Schmelz et al., 2000; Shiers et al., 2020).

Table 2. Comparative analysis of studies with therapeutic potential for neuropathic pain

Reference (Author, Year)	Main Application Potential	Key Features / Methodology	Limitations / Important Notes
(Cao et al., 2024)	In vitro model for diabetic neuropathy, molecular pathophysiology studies, and therapy validation (e.g., Ascl1 overexpression).	Differentiation into sensory neurons; replication of hyperglycemic conditions; analysis of pro-inflammatory cytokines and ion channels (TRPV1, TRPA1, Nav1.8); scalable for screening.	
(Morato et al., 2022)	Early-stage screening platform (HTS) for analgesic candidates, particularly ion channel and cannabinoid modulators.	Low-serum (LSM) differentiation increases expression of Nav, TRP, and cannabinoid receptors; suitable for screening thousands of compounds.	Target gene expression is lower compared to primary DRG neurons; more suitable as a pre-screening tool.
(Blasa et al., 2022)	Model for nerve regeneration studies, neuroprotective drug screening, and mechanisms of hyperexcitability in neuropathic pain.	Thermal stimulation (nanoparticle-based) induces long-term functional differentiation; neurite outgrowth; increased firing frequency and spontaneous activity.	
(Martínez et al., 2019)	Translational phenotypic model for HTS of new analgesic drugs.	Differentiated cells show increased TrkA, neurite outgrowth, and response to KCl (EC50 5 mM); successfully identified analgesic compounds from the Prestwick Chemical Library.	Bridges the gap between recombinant models and preclinical assays.
(Pastori et al., 2019)	Study of peripheral sensitization mechanisms, specifically the phenotypic switch of 'silent' C-mechanoinsensitive afferents, which become spontaneously active and mechanically sensitive in pathological states such as painful polyneuropathy (Kleggetveit et al., 2012; Schmidt et al., 1995; Schmidt et al., 2002; Serra et al., 2012).	Response to capsaicin, ATP, and acidic pH; can be differentiated to resemble mature sensory neurons with an algesic profile.	Lacks T-type calcium currents; significant transcriptional differences compared to primary DRG neurons; requires cross-validation.

Reference (Author, Year)	Main Application Potential	Key Features / Methodology	Limitations / Important Notes
(Prucha et al., 2018)	Study of therapeutic interventions (e.g., Low-Frequency Electromagnetic Fields, LF-EMF) on neuronal responses and pain signaling pathways.	Recapitulates spontaneous activity and calcium responses to bradykinin; used to investigate the role of voltage-gated calcium channels in hyperexcitability.	
(Martínez et al., 2021)	High-content microscopy-based drug screening platform for simultaneous measurement of dual phenotypes (neurite shortening and hyperexcitability).	Identified neuroprotective compounds (nitrendipine, felodipine); allows for simultaneous quantification.	Immortalized cells may not fully recapitulate the molecular and electrophysiological complexity of primary sensory neurons; needs further validation.
(Ambrosino et al., 2019)	Early-stage screening and mechanistic study platform for pain therapy, particularly targeting Kv7 channels.	Expresses all Kv7 subtypes; responds to bradykinin and capsaicin via TRPV1 activation; uses $[Ca^{2+}]$ i measurements to evaluate Kv7 modulators; highlights the role of Kv7.4.	

Relevance of the F-11 model to specific neuropathic pain mechanisms

Beyond its general application for analgesic screening, the differentiated F-11 cell line holds particular relevance for modeling disease-specific mechanisms underlying neuropathic pain. Neuropathic conditions such as diabetic neuropathy and chemotherapy-induced peripheral neuropathy (CIPN) are characterized by convergent molecular signatures, including pro-inflammatory cytokine signaling, ion-channel dysregulation, and neurotoxic stress, all of which can be experimentally interrogated in F-11 cells.

Emerging evidence from sensory-neuron–like in vitro systems indicates that exposure to pro-inflammatory cytokines such as TNF-α and IL-1β enhances nociceptor excitability through activation of intracellular signaling cascades that sensitize voltage-gated sodium channels and TRP receptors. Because differentiated F-11 cells express functional TRPV1, Nav1.7, and Nav1.8 channels and exhibit stimulus-evoked calcium influx and action-potential firing, they provide a tractable platform for mechanistic studies examining cytokine-induced peripheral sensitization, a hallmark of neuropathic pain.

This is particularly relevant for understanding the biology of 'silent' nociceptors, a subpopulation defined by their lack of response in naive states but whose sensitization is a hallmark of chronic pain (Middleton et al., 2021). Using F-11 cells, one could model the transition to hyperexcitability by exposing them to inflammatory mediators like prostaglandin E2 and then using transfection tools to probe the role of specific ion channels (e.g., Nav1.8, TRPV1) or neuropeptides (e.g., CGRP, whose gene CALCA shows strong overlap with P2X3 in human DRG) in this process (Middleton et al., 2021). This approach allows for the

deconvolution of mechanisms that are difficult to isolate in the heterogeneous environment of primary cultures or in vivo microneurography (Namer et al., 2019).

The model is also well suited for investigating metabolic and toxic neuropathies. Hyperglycemic stress central to diabetic neuropathy has been shown in neuronal cultures to induce oxidative stress, mitochondrial dysfunction, and altered ion-channel expression, particularly involving Nav and TRPV1 pathways. Differentiated F-11 cells, with stable electrophysiological properties and measurable neurite integrity, enable controlled simulation of these metabolic insults and quantitative assessment of downstream neuronal dysfunction.

Importantly, the utility of F-11 cells extends to chemotherapy-induced peripheral neuropathy, one of the most clinically prevalent forms of neuropathic pain. Neurotoxic agents such as paclitaxel and platinum-based compounds trigger axonal degeneration, calcium dysregulation, and inflammatory signaling in sensory neurons. High-content imaging of neurite morphology and viability in differentiated F-11 cultures provides a scalable approach for toxicity assessment directly linked to CIPN pathogenesis, thereby strengthening the translational relevance of this platform beyond generic cytotoxicity screening.

Although disease-specific studies using F-11 cells remain comparatively limited, the convergence between known neuropathic mechanisms and the validated molecular phenotype of differentiated F-11 neurons strongly supports their future application as a mechanistic neuropathic-pain model. Systematic integration of cytokine exposure paradigms, hyperglycemic culture conditions, and chemotherapeutic neurotoxicity assays represents a clear direction for advancing the model from general analgesic screening toward pathophysiology-driven neuropathic research.

Positioning F-11 cells in the modern drug discovery pipeline

In an era where human-induced pluripotent stem cell (iPSC)-derived neurons are increasingly accessible, "scalability" alone is insufficient to justify the use of any model (Dorota et al., 2025). The differentiated F-11 cell line must therefore be strategically positioned not as a competitor to human models, but as a high-value workhorse in the early, high-volume stages of the pipeline. Its primary utility lies in early-stage, medium-throughput screening. Following initial target identification and pure biochemical or recombinant assays (HTS), F-11 cells provide an essential first-tier cellular context (Morato et al., 2022). They serve as a cost-effective filter to triage "hits" by confirming activity in a neuronal environment before committing the significant time and expense required for complex models like iPSC-derived neurons or primary cultures. Furthermore, their ease of use makes them ideal for mechanistic lead optimization and preliminary safety pharmacology, enabling researchers to rapidly assess a compound's mode of action or potential neurotoxicity through high-content imaging, as highlighted in studies on chemotherapy-induced neuropathy (Martínez et al., 2021).

When compared directly to other prevalent models, the niche for F-11 cells becomes clear. Against standard neuroblastoma lines like SH-SY5Y or PC12, F-11 cells offer superior physiological relevance for pain research due to their DRG heritage, which grants them a native-like expression of key nociceptive ion channels (TRPV1, Nav1.8) and neuropeptides (CGRP) (Doran et al., 2015; Morato et al., 2022). Unlike these cancer-derived lines, differentiated F-11 cells more faithfully recapitulate the functional signature of a sensory neuron. While they cannot fully replicate the heterogeneity of primary cultures, F-11 cells are significantly more scalable, genetically consistent, and ethically straightforward, eliminating the issues of donor variability and continuous animal use that constrain primary cultures (Wieringa et al., 2012).

The most important distinction, however, is with human iPSC-derived sensory neurons. Human iPSC models are unparalleled for validating "human-relevance" and studying patient-specific genetics, but they

are costly, technically demanding, and require lengthy, complex differentiation protocols (Paik et al., 2020). The F-11 model, with its well-established protocols and rapid differentiation, occupies a critical and complementary position *upstream* of these sophisticated models. It is a robust, accessible, and reproducible platform for generating hypotheses, screening libraries, and refining leads. Promising candidates validated in F-11 cells can then be confidently advanced into the more physiologically complex and human-specific iPSC or primary culture systems for final preclinical validation, thereby de-risking the pipeline and ensuring that expensive late-stage models are used only for the most promising compounds (Ambrosino et al., 2019; Cao et al., 2024; Fan et al., 1992; Martínez et al., 2019; Prucha et al., 2018; Wieringa et al., 2012).

Limitations and physiological relevance

Despite its utility, the F-11 model possesses inherent limitations. Its hybridoma origin a fusion of mouse neuroblastoma and rat DRG neuron confers a residual proliferative and potentially immature genetic background that may not fully replicate the post-mitotic state of adult primary neurons. As a rodent-derived system, it also fails to capture species-specific nuances of human ion channel pharmacology and pain biology, a critical consideration for translational drug discovery. Perhaps the most significant constraint is its simplicity as a monoculture: it lacks dynamic interactions with glial and immune cells, now recognized as fundamental drivers of neuropathic pain pathogenesis. This absence of a complex microenvironment limits its utility for studying the paracrine signaling and neuro-immune crosstalk that define chronic pain states.

Future directions

Ongoing efforts aim to overcome these limitations through technological integration and increased model complexity. Guided by transcriptomic profiling to refine differentiation protocols, aiming to generate F-11 sub-lines that more faithfully recapitulate the molecular profile of human nociceptors, where the classical rodent distinction between peptidergic and non-peptidergic neurons is less segregated (Middleton et al., 2021). Furthermore, engineering reporter lines with fluorescent tags under pain-related promoters, such as TRPV1 or CGRP (CALCA), would enable real-time tracking of the 'silent' nociceptor phenotype and its sensitization. This would create a powerful high-content screening platform to identify compounds that can prevent or reverse the aberrant activity that correlates with pain reporting in patients, thereby directly linking in vitro mechanistic findings to clinical outcomes (Haroutounian et al., 2014; Namer et al., 2019).

The most ambitious direction involves integrating F-11 cells into microfluidic "organ-on-a-chip" devices to create a "DRG-on-a-chip." Such systems could model axonal transport, compartmentalize cell bodies from nerve terminals, and expose distinct cellular regions to different chemical environments—providing a more physiologically accurate platform for studying pain mechanisms and compound effects. Underpinning all these advances must be a commitment to rigorous, standardized validation to ensure the reliability and interpretability of data generated with each new iteration of the model.

CONCLUSIONS

The F-11 cell line, upon differentiation, emerges as a robust and pragmatic in vitro model that effectively bridges the gap between simplistic cancer lines and complex primary cultures. By recapitulating critical morphological, biomolecular, and functional hallmarks of sensory neurons—such as excitability, expression of key ion channels (TRPV1, Nav), and neuropeptide release—it provides a physiologically relevant yet scalable platform. While it does not fully capture the heterogeneity of native DRG or human-specific pharmacology, its utility for medium-throughput analgesic screening and mechanistic studies is significant. Future efforts to integrate F-11 cells into co-culture systems or microfluidic

organ-on-a-chip platforms will further enhance their physiological relevance, solidifying their role in the next generation of neuropathic pain research.

AUTHOR CONTRIBUTIONS

TAN: Conceptualization, and Writing manuscript. **RML**: Conceptualization, and Writing manuscript. **FB**: Investigation, Data curation, Formal analysis, and Writing – Review & Editing. **DWW**: Investigation, Data curation, Formal analysis, and Writing – Review & Editing. **FB**: Investigation, Data curation, Formal analysis, and Writing – Review & Editing.

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COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

Amaya-Rodriguez, C. A., Carvajal-Zamorano, K., Bustos, D., Alegría-Arcos, M., & Castillo, K. (2024). A journey from molecule to physiology and in silico tools for drug discovery targeting the transient receptor potential vanilloid type 1 (TRPV1) channel. *Frontiers in Pharmacology*, *14*, 1251061. https://doi.org/10.3389/fphar.2023.1251061

Ambrosino, P., Soldovieri, M. V., Di Zazzo, E., Paventi, G., Iannotti, F. A., Mosca, I., Miceli, F., Franco, C., Canzoniero, L. M. T., & Taglialatela, M. (2019). Activation of Kv7 Potassium Channels Inhibits Intracellular Ca2+ Increases Triggered By TRPV1-Mediated Pain-Inducing Stimuli in F11 Immortalized Sensory Neurons. *International Journal of Molecular Sciences*, *20*(18), 4322. https://doi.org/10.3390/ijms20184322

Artim, S. C., Kiyatkin, A., & Lemmon, M. A. (2020). Comparison of tyrosine kinase domain properties for the neurotrophin receptors TrkA and TrkB. *The Biochemical Journal*, *477*(20), 4053–4070. https://doi.org/10.1042/BCJ20200695

Averill, S., McMahon, S. B., Clary, D. O., Reichardt, L. F., & Priestley, J. V. (1995). Immunocytochemical localization of trkA receptors in chemically identified subgroups of adult rat sensory neurons. *The European Journal of Neuroscience*, *7*(7), 1484–1494. https://doi.org/10.1111/j.1460-9568.1995.tb01143.x

Baldassarro, V. A., Cescatti, M., Rocco, M. L., Aloe, L., Lorenzini, L., Giardino, L., & Calzà, L. (2023). Nerve growth factor promotes differentiation and protects the oligodendrocyte precursor cells from in vitro hypoxia/ischemia. *Frontiers in Neuroscience*, *17*, 1111170. https://doi.org/10.3389/fnins.2023.1111170

Barabas, M. E., Kossyreva, E. A., & Stucky, C. L. (2012). TRPA1 Is Functionally Expressed Primarily by IB4-Binding, Non-Peptidergic Mouse and Rat Sensory Neurons. *PLoS ONE*, *7*(10), e47988. https://doi.org/10.1371/journal.pone.0047988

Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Archives of Medical Science: AMS*, *11*(6), 1164–1178. https://doi.org/10.5114/aoms.2015.56342

Bennison, S. A., Blazejewski, S. M., Smith, T. H., & Toyo-Oka, K. (2020). Protein kinases: Master regulators of neuritogenesis and therapeutic targets for axon regeneration. *Cellular and Molecular Life Sciences: CMLS*, *77*, 1511–1530. https://doi.org/10.1007/s00018-019-03336-6

Bertin, S., Aoki-Nonaka, Y., de Jong, P. R., Nohara, L. L., Xu, H., Stanwood, S. R., Srikanth, S., Lee, J., To, K., Abramson, L., Yu, T., Han, T., Touma, R., Li, X., González-Navajas, J. M., Herdman, S., Corr, M., Fu, G., Dong, H., & Gwack, Y. (2014). The ion channel TRPV1 regulates the activation and proinflammatory properties of CD4+ T cells. *Nature Immunology*, *15*, 1055–1063. https://doi.org/10.1038/ni.3009

Binaschi, A., Bregola, G., & Simonato, M. (2003). On the role of somatostatin in seizure control: Clues from the hippocampus. *Reviews in the Neurosciences*, *14*(3), 285–301. https://doi.org/10.1515/revneuro.2003.14.3.285

Blasa, S., Borzenkov, M., Pastori, V., Doveri, L., Pallavicini, P., Chirico, G., Lecchi, M., & Collini, M. (2022). Prussian Blue Nanoparticle-Mediated Scalable Thermal Stimulation for In Vitro Neuronal Differentiation. *Nanomaterials*, *12*(13), 2304. https://doi.org/10.3390/nano12132304

Blasa, S., Pastori, V., D'Aloia, A., & Lecchi, M. M. (2021). Differentiated neuroblastoma F-11 cells as an alternative in-vitro model to dorsal root ganglion neurons. *Biomedical Science and Engineering*, *4*(s1), 153. https://www.boa.unimib.it/handle/10281/329249

Camerino, C., Conte, E., Cannone, M., Caloiero, R., Fonzino, A., & Tricarico, D. (2016). Nerve Growth Factor, Brain-Derived Neurotrophic Factor and Osteocalcin Gene Relationship in Energy Regulation, Bone Homeostasis and Reproductive Organs Analyzed by mRNA Quantitative Evaluation and Linear Correlation Analysis. *Frontiers in Physiology*, *7*, 456. https://doi.org/10.3389/fphys.2016.00456

Cao, B., Xu, Q., Shi, Y., Zhao, R., Li, H., Zheng, J., Liu, F., Wan, Y., & Wei, B. (2024). Pathology of pain and its implications for therapeutic interventions. *Signal Transduction and Targeted Therapy*, *9*, 155. https://doi.org/10.1038/s41392-024-01845-w

Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., & Julius, D. (1997). The capsaicin receptor: A heat-activated ion channel in the pain pathway. *Nature*, *389*, 816–824. https://doi.org/10.1038/39807

Chen, A., Xiong, L.-J., Tong, Y., & Mao, M. (2013). The neuroprotective roles of BDNF in hypoxic ischemic brain injury. *Biomedical Reports*, *1*(2), 167–176. https://doi.org/10.3892/br.2012.48

Chen, Y.-C., Bullock, B., Ogbeh, D. I., Ai, J., Okoh, J., Liu, J., Qiang, Y., & Hsia, S. V. (2026). In vitro modeling of human dorsal root ganglion neurons for GCaMP6-based calcium imaging of sensory responses to HSV-1 infection. *Journal of Virological Methods*, *339*, 115264. https://doi.org/10.1016/j.jviromet.2025.115264

Chrysostomidou, L., Cooper, A. H., & Weir, G. A. (2021). Cellular models of pain: New technologies and their potential to progress preclinical research. *Neurobiology of Pain*, *10*, 100063. https://doi.org/10.1016/j.ynpai.2021.100063

Colucci-D'Amato, L., Speranza, L., & Volpicelli, F. (2020). Neurotrophic Factor BDNF, Physiological Functions and Therapeutic Potential in Depression, Neurodegeneration and Brain Cancer. *International Journal of Molecular Sciences*, *21*(20), 7777. https://doi.org/10.3390/ijms21207777

Dehmelt, L., & Halpain, S. (2005). The MAP2/Tau family of microtubule-associated proteins. *Genome Biology*, *6*, 204. https://doi.org/10.1186/gb-2004-6-1-204

Deng, L., Dourado, M., Reese, R. M., Huang, K., Shields, S. D., Stark, K. L., Maksymetz, J., Lin, H., Kaminker, J. S., Jung, M., Foreman, O., Tao, J., Ngu, H., Joseph, V., Roose-Girma, M., Tam, L., Lardell, S., Orrhult, L. S., Karila, P., & Allard, J. (2023). Nav1.7 is essential for nociceptor action potentials in the mouse in a manner independent of endogenous opioids. *Neuron*, *111*(17), 2642-2659.e13. https://doi.org/10.1016/j.neuron.2023.05.024

Dent, E. W., Gupton, S. L., & Gertler, F. B. (2011). The growth cone cytoskeleton in axon outgrowth and guidance. *Cold Spring Harbor Perspectives in Biology*, *3*(3), a001800. https://doi.org/10.1101/cshperspect.a001800

Doan, T., Gay, C. L., Kennedy, H. P., Newman, J., & Lee, K. A. (2014). Nighttime breastfeeding behavior is associated with more nocturnal sleep among first-time mothers at one month postpartum. *Journal of Clinical Sleep Medicine*, *10*, 313–319. https://doi.org/10.5664/jcsm.3538

Doran, C., Chetrit, J., Holley, M. C., Grundy, D., & Nassar, M. A. (2015). Mouse DRG Cell Line with Properties of Nociceptors. *PLOS ONE*, *10*(6), e0128670. https://doi.org/10.1371/journal.pone.0128670

Dorota, A., Maryniak, N., Mariankowska, A., Milczarek, C., Dorota, M., Zywiec, W., Kozlowski, K., Koval, I., Czyzewski, B., & Czyzewska, J. (2025). Induced Pluripotent Stem Cells (iPSC) and Their Use in Disease Modeling. *Cureus*, *17*(10), e93999. https://doi.org/10.7759/cureus.93999

Erickson, J. T., Brosenitsch, T. A., & Katz, D. M. (2001). Brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor are required simultaneously for survival of dopaminergic primary sensory neurons in vivo. *The Journal of Neuroscience*, *21*(2), 581–589. https://doi.org/10.1523/JNEUROSCI.21-02-00581.2001

Fan, S. F., Shen, K. F., Scheideler, M. A., & Crain, S. M. (1992). F11 neuroblastoma x DRG neuron hybrid cells express inhibitory mu- and delta-opioid receptors which increase voltage-dependent K+ currents upon activation. *Brain Research*, *590*(1–2), 329–333. https://doi.org/10.1016/0006-8993(92)91116-v

Fang, X., McMullan, S., Lawson, S. N., & Djouhri, L. (2005). Electrophysiological differences between nociceptive and non-nociceptive dorsal root ganglion neurones in the rat in vivo. *The Journal of Physiology*, *565*(3), 927–943. https://doi.org/10.1113/jphysiol.2005.086199

Ferrini, F., Salio, C., Boggio, E. M., & Merighi, A. (2021). Interplay of BDNF and GDNF in the Mature Spinal Somatosensory System and Its Potential Therapeutic Relevance. *Current Neuropharmacology*, *19*(8), 1225–1245. https://doi.org/10.2174/1570159X18666201116143422

Francel, P. C., Harris, K., Smith, M., Fishman, M. C., Dawson, G., & Miller, R. J. (1987). Neurochemical Characteristics of a Novel Dorsal Root Ganglion X Neuroblastoma Hybrid Cell Line, F-11. *Journal of Neurochemistry*, *48*(5), 1624–1631. https://doi.org/10.1111/j.1471-4159.1987.tb05711.x

Gudbergsson, J. M., Johnsen, K. B., Skov, M. N., & Duroux, M. (2016). Systematic review of factors influencing extracellular vesicle yield from cell cultures. *Cytotechnology*, *68*, 579–592. https://doi.org/10.1007/s10616-015-9913-6

Gunning, P. W., Landreth, G. E., Layer, P., Ignatius, M., & Shooter, E. M. (1981). Nerve growth factor-induced differentiation of PC12 cells: Evaluation of changes in RNA and DNA metabolism. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *1*(4), 368–379. https://doi.org/10.1523/JNEUROSCI.01-04-00368.1981

Haberberger, R. V., Barry, C., & Matusica, D. (2020). Immortalized Dorsal Root Ganglion Neuron Cell Lines. *Frontiers in Cellular Neuroscience*, *14*, 184. https://doi.org/10.3389/fncel.2020.00184

Haroutounian, S., Nikolajsen, L., Bendtsen, T. F., Finnerup, N. B., Kristensen, A. D., Hasselstrøm, J. B., & Jensen, T. S. (2014). Primary afferent input critical for maintaining spontaneous pain in peripheral neuropathy. *Pain*, *155*(7), 1272–1279. https://doi.org/10.1016/j.pain.2014.03.022

Ho, P. L., & Raw, I. (1992). Cyclic AMP potentiates bFGF-induced neurite outgrowth in PC12 cells. *Journal of Cellular Physiology*, *150*(3), 647–656. https://doi.org/10.1002/jcp.1041500326

Honma, N., Uchida, A., Hirose, H., Srsen, V., Kishimoto, T., & Hisanaga, S. (1996). Two types of apoptotic cell death of rat central nervous system-derived neuroblastoma B50 and B104 cells: Apoptosis induced during proliferation and after differentiation. *Journal of Neurochemistry*, *67*(5), 1856–1865. https://doi.org/10.1046/j.1471-4159.1996.67051856.x

Hutchings, C. J., Colussi, P., & Clark, T. G. (2019). Ion channels as therapeutic antibody targets. *mAbs*, *11*(2), 265–296. https://doi.org/10.1080/19420862.2018.1548232

Jang, K., & Garraway, S. M. (2024). A review of dorsal root ganglia and primary sensory neuron plasticity mediating inflammatory and chronic neuropathic pain. *Neurobiology of Pain*, *15*, 100151. https://doi.org/10.1016/j.ynpai.2024.100151

Karcz, M., Abd-Elsayed, A., Chakravarthy, K., Aman, M. M., Strand, N., Malinowski, M. N., Latif, U., Dickerson, D., Suvar, T., Lubenow, T., Peskin, E., D'Souza, R., Cornidez, E., Dudas, A., Lam, C., Farrell Ii, M., Sim, G. Y., Sebai, M., Garcia, R., & Deer, T. (2024). Pathophysiology of Pain and Mechanisms of Neuromodulation: A Narrative Review (A Neuron Project). *Journal of Pain Research*, *17*, 3757–3790. https://doi.org/10.2147/JPR.S475351

Katzenell, S., Cabrera, J. R., North, B. J., & Leib, D. A. (2017). Isolation, Purification, and Culture of Primary Murine Sensory Neurons. *Methods in Molecular Biology*, *1656*, 229–251. https://doi.org/10.1007/978-1-4939-7237-1_15

Khosrowshahi, D., Lagae, L., & Bolander, J. (2025). Decoding Pain: Next-Generation In Vitro Systems for Mechanistic Insights and Drug Discovery. *The FASEB Journal*, *39*(16), e70914. https://doi.org/10.1096/fj.202501025RR

Kim, K., Dean, D., Mikos, A. G., & Fisher, J. P. (2009). Effect of initial cell seeding density on early osteogenic signal expression of rat bone marrow stromal cells cultured on cross-linked poly(propylene fumarate) disks. *Biomacromolecules*, *10*(7), 1810–1817. https://doi.org/10.1021/bm900240k

Kleggetveit, I. P., Namer, B., Schmidt, R., Helås, T., Rückel, M., Ørstavik, K., Schmelz, M., & Jørum, E. (2012). High spontaneous activity of C-nociceptors in painful polyneuropathy. *Pain*, *153*(10), 2040–2047. https://doi.org/10.1016/j.pain.2012.05.017

Laurenza, A., Sutkowski, E. M., & Seamon, K. B. (1989). Forskolin: A specific stimulator of adenylyl cyclase or a diterpene with multiple sites of action? *Trends in Pharmacological Sciences*, *10*(11), 442–447. https://doi.org/10.1016/s0165-6147(89)80008-2

Lawson, S. N. (2002). Phenotype and Function of Somatic Primary Afferent Nociceptive Neurones with C-, Aδ- or Aα/β-Fibres. *Experimental Physiology*, *87*(2), 239–244. https://doi.org/10.1113/eph8702350

Lawson, S. N., Fang, X., & Djouhri, L. (2019). Nociceptor subtypes and their incidence in rat lumbar dorsal root ganglia (DRGs): Focussing on C-polymodal nociceptors, Aβ-nociceptors, moderate pressure receptors and their receptive field depths. *Current Opinion in Physiology*, *11*, 125–146. https://doi.org/10.1016/j.cophys.2019.10.005

Leoni, M. L. G., Mercieri, M., Viswanath, O., Cascella, M., Rekatsina, M., Pasqualucci, A., Caruso, A., & Varrassi, G. (2025). Neuropathic Pain: A Comprehensive Bibliometric Analysis of Research Trends, Contributions, and Future Directions. *Current Pain and Headache Reports*, *29*, 73. https://doi.org/10.1007/s11916-025-01384-1

Lindsay, R. (1988). Nerve growth factors (NGF, BDNF) enhance axonal regeneration but are not required for survival of adult sensory neurons. *The Journal of Neuroscience*, *8*(7), 2394–2405. https://doi.org/10.1523/JNEUROSCI.08-07-02394.1988

Martínez, A. L., Brea, J., Barro, M., Monroy, X., Merlos, M., Burgueño, J., & Loza, M. I. (2021). Development of a novel in vitro assay to screen for neuroprotective drugs against iatrogenic neurite shortening. *PLOS ONE*, *16*(3), e0248139. https://doi.org/10.1371/journal.pone.0248139

Martínez, A. L., Brea, J., Monroy, X., Merlos, M., Burgueño, J., & Loza, M. I. (2019). A New Model of Sensorial Neuron-Like Cells for HTS of Novel Analgesics for Neuropathic Pain. *SLAS Discovery*, *24*(2), 158–168. https://doi.org/10.1177/2472555218810323

Middleton, S. J., Barry, A. M., Comini, M., Li, Y., Ray, P. R., Shiers, S., Themistocleous, A. C., Uhelski, M. L., Yang, X., Dougherty, P. M., Price, T. J., & Bennett, D. L. (2021). Studying human nociceptors: From fundamentals to clinic. *Brain: A Journal of Neurology*, *144*(5), 1312–1335. https://doi.org/10.1093/brain/awab048

Morato, E. O., Knight, B., & Nair, L. S. (2022). Transcriptional profiling of neuronal ion channels in dorsal root ganglion-derived immortal cell line (F-11) under different culture conditions. *In Vitro Models*, *1*, 385–395. https://doi.org/10.1007/s44164-022-00036-7

Morwood, A. J., El-Karim, I. A., Clarke, S. A., & Lundy, F. T. (2023). The Role of Extracellular Matrix (ECM) Adhesion Motifs in Functionalised Hydrogels. *Molecules*, *28*(12), 4616. https://doi.org/10.3390/molecules28124616

Mouraux, A., Bannister, K., Becker, S., Finn, D. P., Pickering, G., Pogatzki-Zahn, E., & Graven-Nielsen, T. (2021). Challenges and opportunities in translational pain research—An opinion paper of the working group on translational pain research of the European pain federation (EFIC). *European Journal of Pain*, *25*(4), 731–756. https://doi.org/10.1002/ejp.1730

Namer, B., Schmidt, D., Eberhardt, E., Maroni, M., Dorfmeister, E., Kleggetveit, I. P., Kaluza, L., Meents, J., Gerlach, A., Lin, Z., Winterpacht, A., Dragicevic, E., Kohl, Z., Schüttler, J., Kurth, I., Warncke, T., Jorum, E., Winner, B., & Lampert, A. (2019). Pain relief in a neuropathy patient by lacosamide: Proof of principle of clinical translation from patient-specific iPS cell-derived nociceptors. *EBioMedicine*, *39*, 401–408. https://doi.org/10.1016/j.ebiom.2018.11.042

North, R. Y., Odem, M. A., Li, Y., Tatsui, C. E., Cassidy, R. M., Dougherty, P. M., & Walters, E. T. (2022). Electrophysiological Alterations Driving Pain-Associated Spontaneous Activity in Human Sensory Neuron Somata Parallel Alterations Described in Spontaneously Active Rodent Nociceptors. *The Journal of Pain*, *23*(8), 1343–1357. https://doi.org/10.1016/j.jpain.2022.02.009

Orozco Morato, E., Knight, B., & Nair, L. S. (2022). Transcriptional profiling of neuronal ion channels in dorsal root ganglion–derived immortal cell line (F-11) under different culture conditions. *In Vitro Models*, *1*, 385–395. https://doi.org/10.1007/s44164-022-00036-7

Paik, D. T., Chandy, M., & Wu, J. C. (2020). Patient and Disease-Specific Induced Pluripotent Stem Cells for Discovery of Personalized Cardiovascular Drugs and Therapeutics. *Pharmacological Reviews*, *72*(1), 320–342. https://doi.org/10.1124/pr.116.013003

Pastori, V., D'Aloia, A., Blasa, S., & Lecchi, M. (2019). Serum-deprived differentiated neuroblastoma F-11 cells express functional dorsal root ganglion neuron properties. *PeerJ*, *7*, e7951. https://doi.org/10.7717/peerj.7951

Pemberton, K., Mersman, B., & Xu, F. (2018). Using ImageJ to assess neurite outgrowth in mammalian cell cultures: Research data quantification exercises in undergraduate neuroscience lab. *Journal of*

Undergraduate Neuroscience Education, *16*(2), A186–A194. https://pubmed.ncbi.nlm.nih.gov/30057501/

Perera, R. A. P. M., Tso, E., Tsang, O. T. Y., Tsang, D. N. C., Fung, K., Leung, Y. W. Y., Chin, A. W. H., Chu, D. K. W., Cheng, S. M. S., Poon, L. L. M., Chuang, V. W. M., & Peiris, M. (2020). SARS-CoV-2 virus culture and subgenomic RNA for respiratory specimens from patients with mild Coronavirus Disease. *Emerging Infectious Diseases*, *26*(11), 2701–2704. https://doi.org/10.3201/eid2611.203219

Platika, D., Boulos, M. H., Baizer, L., & Fishman, M. C. (1985). Neuronal traits of clonal cell lines derived by fusion of dorsal root ganglia neurons with neuroblastoma cells. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(10), 3499–3503. https://doi.org/10.1073/pnas.82.10.3499

Prucha, J., Krusek, J., Dittert, I., Sinica, V., Kadkova, A., & Vlachova, V. (2018). Acute exposure to high-induction electromagnetic field affects activity of model peripheral sensory neurons. *Journal of Cellular and Molecular Medicine*, *22*(2), 1355–1362. https://doi.org/10.1111/jcmm.13423

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., Keefe, F. J., Mogil, J. S., Ringkamp, M., Sluka, K. A., Song, X.-J., Stevens, B., Sullivan, M. D., Tutelman, P. R., Ushida, T., & Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: Concepts, challenges, and compromises. *Pain*, *161*(9), 1976–1982. https://doi.org/10.1097/j.pain.0000000000001939

Ramani, A., Pasquini, G., Gerkau, N. J., Jadhav, V., Vinchure, O. S., Altinisik, N., Windoffer, H., Muller, S., Rothenaigner, I., Lin, S., Mariappan, A., Rathinam, D., Mirsaidi, A., Goureau, O., Ricci-Vitiani, L., D'Alessandris, Q. G., Wollnik, B., Muotri, A., Freifeld, L., & Jurisch-Yaksi, N. (2024). Reliability of high-quantity human brain organoids for modeling microcephaly, glioma invasion and drug screening. *Nature Communications*, *15*, 10703. https://doi.org/10.1038/s41467-024-55226-6

Richter-Landsberg, C., & Jastorff, B. (1986). The role of cAMP in nerve growth factor-promoted neurite outgrowth in PC12 cells. *The Journal of Cell Biology*, *102*(3), 821–829. https://doi.org/10.1083/jcb.102.3.821

Rosenberger, D. C., Blechschmidt, V., Timmerman, H., Wolff, A., & Treede, R.-D. (2020). Challenges of neuropathic pain: Focus on diabetic neuropathy. *Journal of Neural Transmission*, *127*, 589–624. https://doi.org/10.1007/s00702-020-02145-7

Saeed, A. W., & Ribeiro-da-Silva, A. (2012). Non-peptidergic primary afferents are presynaptic to neurokinin-1 receptor immunoreactive lamina I projection neurons in rat spinal cord. *Molecular Pain*, *8*, 64. https://doi.org/10.1186/1744-8069-8-64

Salzer, I., Ray, S., Schicker, K., & Boehm, S. (2019). Nociceptor Signalling through ion Channel Regulation via GPCRs. *International Journal of Molecular Sciences*, *20*(10), 2488. https://doi.org/10.3390/ijms20102488

Schmelz, M., Schmid, R., Handwerker, H. O., & Torebjörk, H. E. (2000). Encoding of burning pain from capsaicin-treated human skin in two categories of unmyelinated nerve fibres. *Brain: A Journal of Neurology*, *123*(3), 560–571. https://doi.org/10.1093/brain/123.3.560

Schmidt, R., Schmelz, M., Forster, C., Ringkamp, M., Torebjörk, E., & Handwerker, H. (1995). Novel classes of responsive and unresponsive C nociceptors in human skin. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *15*(1), 333–341. https://doi.org/10.1523/JNEUROSCI.15-01-00333.1995

Schmidt, R., Schmelz, M., Weidner, C., Handwerker, H. O., & Torebjörk, H. E. (2002). Innervation territories of mechano-insensitive C nociceptors in human skin. *Journal of Neurophysiology*, *88*(4), 1859–1866. https://doi.org/10.1152/jn.2002.88.4.1859

Scott, C. W., Vulliet, P. R., & Caputo, C. B. (1993). Phosphorylation of tau by proline-directed protein kinase (p34cdc2/p58cyclin A) decreases tau-induced microtubule assembly and antibody SMI33 reactivity. *Brain Research*, *611*(2), 237–242. https://doi.org/10.1016/0006-8993(93)90508-k

Serra, J., Bostock, H., Solà, R., Aleu, J., García, E., Cokic, B., Navarro, X., & Quiles, C. (2012). Microneurographic identification of spontaneous activity in C-nociceptors in neuropathic pain states in humans and rats. *Pain*, *153*(1), 42–55. https://doi.org/10.1016/j.pain.2011.08.015

Sharma, S., Hansen, J. T., & Notter, M. F. (1990). Effects of NGF and dibutyryl cAMP on neuronal differentiation of embryonal carcinoma cells. *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience*, *8*(1), 33–45. https://doi.org/10.1016/0736-5748(90)90021-s

Shiers, S., Klein, R. M., & Price, T. J. (2020). Quantitative differences in neuronal subpopulations between mouse and human dorsal root ganglia demonstrated with RNAscope in situ hybridization. *Pain*, *161*(10), 2410–2424. https://doi.org/10.1097/j.pain.0000000000001973

Shipley, M. M., Mangold, C. A., & Szpara, M. L. (2016). Differentiation of the SH-SY5Y human neuroblastoma cell line. *Journal of Visualized Experiments*, *108*, e53193. https://doi.org/10.3791/53193

Stil, A., Liberelle, B., Bello, D. G., Lacomme, L., Arpin, L., Parent, P., Nanci, A., Dumont, É. C., Ould-Bachir, T., Vanni, M. P., De Crescenzo, G., & Bouchard, J.-F. (2023). A simple method for poly-D-lysine coating to enhance adhesion and maturation of primary cortical neuron cultures in vitro. *Frontiers in Cellular Neuroscience*, *17*, 1212097. https://doi.org/10.3389/fncel.2023.1212097

Sutton, M. A., & Schuman, E. M. (2006). Dendritic protein synthesis, synaptic plasticity, and memory. *Cell*, *127*(1), 49–58. https://doi.org/10.1016/j.cell.2006.09.014

Wang, G., Zhang, D., Qin, L., Liu, Q., Tang, W., Liu, M., Xu, F., Tang, F., Cheng, L., Mo, H., Yuan, X., Wang, Z., & Huang, B. (2024). Forskolin-driven conversion of human somatic cells into induced neurons through regulation of the cAMP-CREB1-JNK signaling. *Theranostics*, *14*(4), 1701–1719. https://doi.org/10.7150/thno.92700

Wieringa, P., Tonazzini, I., Micera, S., & Cecchini, M. (2012). Nanotopography induced contact guidance of the F11 cell line during neuronal differentiation: A neuronal model cell line for tissue scaffold development. *Nanotechnology*, *23*(27), 275102. https://doi.org/10.1088/0957-4484/23/27/275102

Xiong, F., Xiao, B.-L., Wang, Q., Liu, K., Wu, H.-W., Jing, C., Tong, K.-N., Zhang, Z.-T., & Guo, W. (2025). Nerve growth factor: What can surgeons and oncologists learn from a neurological and psychological biomarker? *Molecular Medicine*, *31*, 276. https://doi.org/10.1186/s10020-025-01333-z

Yan, K., Gao, L.-N., Cui, Y.-L., Zhang, Y., & Zhou, X. (2016). The cyclic AMP signaling pathway: Exploring targets for successful drug discovery (Review). *Molecular Medicine Reports*, *13*(5), 3715–3723. https://doi.org/10.3892/mmr.2016.5005

Yan, L., Wei, M., Gong, A. G., Song, P., Lou, J., Bi, C. W., Xu, S. L., Xiong, A., Dong, T. T., & Tsim, K. W. (2017). A Modified Chinese Herbal Decoction (Kai-Xin-San) Promotes NGF-Induced Neuronal Differentiation in PC12 Cells via Up-Regulating Trk A Signaling. *Frontiers in Cell and Developmental Biology*, *5*, 118. https://doi.org/10.3389/fcell.2017.00118

Zhang, H.-Y., Song, N., Jiang, H., Bi, M.-X., & Xie, J.-X. (2014). Brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor inhibit ferrous iron influx via divalent metal transporter 1 and iron regulatory protein 1 regulation in ventral mesencephalic neurons. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1843*(12), 2967–2975. https://doi.org/10.1016/j.bbamcr.2014.09.010

Zhu, H., Wang, G., Nguyen-Ngoc, K.-V., Kim, D., Miller, M., Goss, G., Kovsky, J., Harrington, A. R., Saunders, D. C., Hopkirk, A. L., Melton, R., Powers, A. C., Preissl, S., Spagnoli, F. M., Gaulton, K. J., & Sander, M. (2023). Understanding cell fate acquisition in stem-cell-derived pancreatic islets using single-cell multiome-inferred regulomes. *Developmental Cell*, *58*(9), 727-743.e11. https://doi.org/10.1016/j.devcel.2023.03.011