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# Regulation of GSK3 Activity and Nav1.2 Channel Function in Neurons

## Introduction

Glycogen synthase kinase-3 (GSK3) is a serine/threonine kinase highly expressed in the brain, existing as two closely related isoforms (GSK3α and GSK3β) [1]. Unlike many kinases, GSK3 is constitutively active under basal conditions and is primarily regulated by inhibitory mechanisms [2]. It phosphorylates a broad array of substrates (~50+ identified) involved in diverse neuronal processes including gene transcription, neurodevelopment, synaptic plasticity, and cell survival [3] [4]. Voltage-gated sodium channels, particularly the Nav1.2 channel (encoded by **SCN2A**), are crucial for action potential initiation and neuronal excitability. Nav1.2 is abundantly expressed in the CNS (e.g. at axon initial segments of developing or unmyelinated neurons) and its functional efficiency can shape neuronal firing patterns. Notably, extreme Nav1.2 activity (either hyper- or hypo-functional) leads to neurological disorders (epileptic encephalopathies, autism spectrum disorders) [5], indicating that neurons tightly modulate Nav channel activity in vivo. Recent evidence has uncovered GSK3 as a key modulator of Nav1.2 channels [6] [7]. This report examines the mechanisms that control GSK3 activity in neurons and how these influence Nav1.2 channel function. We discuss upstream signaling pathways (e.g. Wnt/β-catenin, PI3K/Akt cascades), genetic and post-translational regulators of GSK3, and protein–protein interactions that modulate its activity. We then explore practical interventions – pharmacological, genetic, lifestyle, and environmental – capable of altering GSK3 activity with the goal of enhancing Nav1.2 function. We detail their mechanisms of action and effects on Nav1.2 expression, trafficking, phosphorylation, and electrophysiological performance. Finally, we consider potential risks, off-target effects, compensatory mechanisms, and long-term impacts on neuronal homeostasis, synaptic plasticity, excitability, and development, drawing on data from both animal and human studies.

## GSK3 Signaling in Neurons: Upstream Pathways and Regulators

*Figure: Major signaling pathways regulating GSK3 activity in neurons.* Multiple extracellular cues converge to modulate GSK3, typically *inhibiting* its kinase activity via phosphorylation or sequestration. Growth factors (e.g. insulin, IGF-1) and neurotrophins (e.g. BDNF) activate the PI3K–Akt pathway, leading to Akt-mediated phosphorylation of GSK3 ($Ser^{9}$ on GSK3β; $Ser^{21}$ on GSK3α) and its functional inactivation [8] [9]. For example, chronic IGF-1 treatment in neurons increases Akt activity and inhibits GSK3β, which in turn upregulates cell-surface sodium channels (Nav1.7) via the PI3K/Akt/GSK3β pathway [10]. **Wnt/β-catenin signaling** provides another major inhibitory control: in the absence of Wnt, GSK3 (mostly GSK3β) resides in the cytosolic "destruction complex" (with Axin, APC, etc.) and phosphorylates β-catenin to mark it for degradation [11]. When Wnt ligands bind Frizzled/LRP receptors, GSK3 is recruited and sequestered into multivesicular endosomes or onto LRP6, preventing it from accessing cytosolic substrates [12] [13]. This stabilizes β-catenin and thus *indirectly* inhibits GSK3's activity on many targets, independent of Ser9 phosphorylation [14]. In neurons, Wnt signaling plays key roles in dendritic development, synapse formation, and plasticity, partly by locally suppressing GSK3 to allow accumulation of pro-growth factors like β-catenin [15] [16].

Aside from growth factors and Wnt, various neurotransmitters regulate neuronal GSK3 via distinct routes. **Serotonin (5-HT)** and **dopamine** can modulate Akt-GSK3 signaling in opposite directions: 5-HT_1A receptor activation triggers PI3K-Akt and inhibits GSK3, whereas dopamine D2 receptor activation recruits a β-arrestin/PP2A complex that de-phosphorylates Akt, thereby *reactivating* GSK3 [17]. Indeed, GSK3 is a downstream integrator of several monoamine pathways targeted by psychiatric drugs [18] [17]. Likewise, **NMDA-type glutamate receptors** can influence GSK3 via calcium-dependent phosphatases (PP1/PP2A): intense NMDA receptor activity can remove inhibitory Ser9-phosphate from GSK3, transiently increasing GSK3 activity during certain forms of synaptic plasticity (such as NMDA-driven long-term depression) [17]. **Stress hormones** and cellular energy status also feed into GSK3 regulation. For instance, elevated glucocorticoids have been linked to increased GSK3β activity (impaired Ser9 phosphorylation) in some models, which correlates with synaptic deficits and depression-like behavior [18] [19]. Conversely, **lithium**, a classic mood stabilizer, robustly inhibits GSK3 via both direct competition with magnesium at the ATP-binding site and indirectly by boosting upstream inhibitory phosphorylation [9] [20] (discussed further below).

**Genetic regulators** also modulate GSK3 activity. The two isoforms, *GSK3α* and *GSK3β*, are encoded on separate genes and widely expressed in brain (GSK3α enriched in cortex and hippocampus; GSK3β ubiquitous) [1]. They are ~97% homologous in the kinase domain but are not entirely redundant – some substrates and functions are isoform-specific [21]. Gene knockouts highlight their importance: GSK3β null mice die embryonically (due to deregulated Wnt signaling), while GSK3α null mice survive but show behavioral and metabolic alterations [22]. Certain neurodevelopmental disorder risk genes intersect with the GSK3 pathway. For example, loss-of-function of **PTEN** (a phosphatase that restrains PI3K/Akt signaling) leads to hyperactive Akt and chronic GSK3 inhibition; in mouse models, neuron-specific *Pten* deletion causes neuronal hypertrophy, network hyperexcitability, and autism-like phenotypes, illustrating consequences of unbalanced PI3K–GSK3 signaling. Conversely, mutations in Wnt pathway components (e.g. **ANK3**/ankyrin-G, **DISC1**) can alter β-catenin localization or GSK3 binding [23] [17], potentially affecting GSK3's availability to substrates. At the transcriptional level, GSK3β gene expression is regulated by several transcription factors (NF-κB, CREB, etc.) that respond to cellular stimuli [24] [25]. Chronic stress and chronic cytokine exposure can upregulate GSK3β expression or activity in the brain, whereas antidepressant treatments often correlate with increased inhibitory Ser9-phosphorylation of GSK3β [26] [19].

**Post-translational modifications** provide rapid control of GSK3 activity. The most prominent is phosphorylation of the N-terminal serine (Ser9 in GSK3β), which acts as an intramolecular pseudo-substrate to block the active site [9]. This modification is induced by multiple kinases downstream of receptor signaling – Akt/protein kinase B is the best known (activated by insulin/TrkB signaling) [9], but protein kinase C (PKC) and PKA can also phosphorylate and inhibit GSK3 in response to GPCR or cAMP signals [9]. Additionally, mitogen-activated protein kinases like p38 MAPK can phosphorylate a C-terminal serine (Ser389 of GSK3β), providing further inhibition [20]. On the other hand, phosphorylation of a tyrosine residue in the activation loop (Tyr216 in GSK3β) enhances GSK3 activity [27]. Tyr216 is often autophosphorylated during new protein synthesis (constitutive activation) but can be dynamically regulated by upstream tyrosine kinases in some contexts [27]. *Dephosphorylation* events are equally important: protein phosphatase 2A (PP2A) and PP1 can remove the inhibitory Ser9-P, as seen in dopamine D2/β-arrestin signaling that activates PP2A [17]. Thus, GSK3's kinase activity at any time reflects a balance of these phosphate on/off controls. Oxidative modifications and acetylation of GSK3 have also been reported under extreme conditions (e.g. oxidative stress can inactivate GSK3 by oxidizing its cysteine residues), linking environmental factors like reactive oxygen species (ROS) to GSK3 modulation [8].

**Protein–protein interactions** further fine-tune GSK3 activity by localization and substrate targeting. In the Wnt pathway, GSK3β is bound into a scaffolded "destruction complex" via Axin and APC, which brings GSK3 in proximity to β-catenin for phosphorylation [16]. When Wnt signaling is active, the Axin-GSK3 complex is recruited to the membrane or internalized, effectively *sequestering* GSK3 away from cytosolic substrates [12] [13]. Neurons exploit similar scaffolds for spatiotemporal control of GSK3: for instance, at synapses and in growth cones, GSK3 associates with multimeric complexes (including scaffold proteins like FRAT/GBP, which bind GSK3 and inhibit its activity on certain substrates). An example in neurons is the interaction of GSK3 with postsynaptic proteins during long-term depression (LTD) – upon NMDA receptor activation, GSK3 is recruited to a complex with PSD-95 and NR2B, where local dephosphorylation (activation) of GSK3 triggers LTD signaling cascades [28]. Another emerging example is the **Nav channel "channelosome"**: GSK3β was recently found to physically associate with the Nav1.6 channel complex, possibly serving a scaffolding role that influences channel assembly and gating [29]. The accessory protein fibroblast growth factor 14 (FGF14), which binds intracellular tails of Nav1.2/Nav1.6 and modulates their gating, is a substrate of GSK3: active GSK3β phosphorylates FGF14 at Ser^226, and this phosphorylation promotes the proper assembly and function of the FGF14–Nav1.6 complex [30]. Thus, GSK3 can indirectly affect channel properties by modifying channel-interacting proteins. GSK3 also targets some E3 ubiquitin ligases and other kinases; for example, GSK3 phosphorylates the cell-cycle kinase WEE1, marking it for degradation [31]. In neurons, WEE1 (originally known for mitotic regulation) has been found to phosphorylate Nav1.2 via FGF14 and modulate its currents [32] [33]. There is crosstalk among WEE1, Akt, and GSK3: WEE1's effects on the Nav1.2–FGF14 complex are influenced by Akt (which inhibits GSK3) and by GSK3 itself, suggesting these kinases form an integrated signaling network controlling Nav1.2 channelosomes [34] [35]. In summary, neurons leverage a variety of upstream signals and molecular interactions to dynamically regulate GSK3 activity. These regulations are critical for processes like synaptic remodeling, neuronal polarity, and excitability – and as discussed next, one key downstream effect is the modulation of Nav1.2 channel function.

## GSK3 Modulation of Nav1.2 Channels: Mechanistic Pathways

Phosphorylation is a well-established mechanism for regulating Nav channel behavior [36]. Classical protein kinases such as PKC and PKA can phosphorylate neuronal Nav channels, typically reducing their sodium currents or altering gating [36]. However, relatively few kinases were known to directly regulate Nav channels, and their isoform specificity varies (e.g. PKC affects Nav1.2 but not Nav1.6 in the same way [37]). GSK3 has recently emerged as a **critical regulator of Nav1.2**, acting via the channel's C-terminal domain [6] [7]. In heterologous expression studies (HEK293 cells stably expressing Nav1.2), inhibition of GSK3 was found to *enhance* Nav1.2-mediated sodium currents without changing Nav1.2 gene expression or total protein levels [38]. Specifically, treating cells with a selective GSK3 inhibitor (GSK3 inhibitor XIII) significantly increased the peak Na^+ current density, an effect recapitulated by GSK3 knockdown (siRNA) [38]. Conversely, overexpressing GSK3β suppressed Nav1.2 currents, confirming a bidirectional control [38] [7]. Importantly, GSK3 inhibition did not alter SCN2A mRNA or total Nav1.2 protein [39]; instead, it increased the fraction of channel present at the plasma membrane. Immunolabeling of a CD4-Nav1.2 C-terminal chimera showed that GSK3 inhibitor treatment upregulates Nav1.2 C-tail surface expression, visible as increased membrane puncta [40]. Thus, **GSK3 activity limits Nav1.2 functional expression by restricting channel trafficking and/or enhancing its internalization** [40].

Mechanistically, GSK3β directly phosphorylates the Nav1.2 channel's C-terminal tail. Using in vitro kinase assays and mass spectrometry, **threonine 1966** (T^1966) in Nav1.2's C-terminus was identified as a GSK3β target site [41]. Intriguingly, T1966 lies within a consensus sequence (S/T–XXX–S/T) characteristic of GSK3 substrates [42]. In native brain tissue, T1966 was indeed found to be a phosphorylated residue on Nav1.2

[42]. This site is strategically located adjacent to known endocytic motifs on the channel's tail. A **PPXY** motif (at Y^1975) is nearby, which is a binding site for Nedd4-family E3 ubiquitin ligases [43]. Nedd4-2 ubiquitinates Nav channels at such PPxY motifs, promoting channel internalization and degradation; mutating the PPXY motif is known to prevent Nedd4-2–mediated suppression of Nav1.2 currents [44]. Not far from T1966 is also a dileucine (LL) motif that mediates clathrin-dependent endocytosis of Nav channels [45]. **Figure 1** illustrates the proposed model of how GSK3 phosphorylation at T1966 regulates Nav1.2 (normal vs inhibited GSK3).

**Figure 1: GSK3 regulation of Nav1.2 channel trafficking.** *Left:* Under normal conditions, active GSK3β phosphorylates Nav1.2 at T^1966 on the C-terminal tail (yellow "P"), which is adjacent to a PPXY^1975 motif. Phospho-T1966 likely serves as a priming signal for the E3 ligase Nedd4-2 to bind and ubiquitinate the channel, leading to its internalization and recycling [46] [47]. GSK3-mediated phosphorylation might also induce conformational changes that facilitate clathrin adaptor binding to a nearby dileucine motif, further promoting endocytosis [45]. Consequently, Nav1.2 channels are removed from the membrane, limiting sodium current. *Right:* When GSK3 is inhibited (by drugs or upstream signals), T1966 remains unphosphorylated (red "X"). This prevents Nedd4-2 from recognizing the channel tail and slows Nav1.2 internalization [47] [48]. The channel is stabilized at the plasma membrane, increasing surface expression and boosting Na^+ current density [47] [48]. In essence, active GSK3 exerts a **suppressive brake** on Nav1.2 channel availability, whereas GSK3 inhibition releases that brake, enhancing Nav1.2 function.

This GSK3–Nav1.2 pathway represents a finely tuned mechanism to adjust neuronal excitability. Under basal conditions, constitutive GSK3 activity likely helps prevent over-excitation by promoting a modest rate of Nav1.2 internalization. In situations of heightened PI3K or Wnt signaling (e.g. during growth factor stimulation or high network activity), GSK3 gets inhibited, and more Nav1.2 channels can accumulate at membranes to increase excitability as needed. Notably, the *in vivo* significance of this mechanism is supported by its conservation across Nav channel isoforms and cell types. A similar phospho-site is present in Nav1.6 (T^1938 in Nav1.6, analogous location), and active GSK3β can phosphorylate Nav1.6 as well [49] [50]. Interestingly, however, the functional outcome differs: GSK3β phosphorylation of Nav1.6 (at T1938) **increases** Nav1.6 current, whereas phosphorylation of Nav1.2 (at T1966) **decreases** Nav1.2 current [51] [52]. This discrepancy is thought to arise from the involvement of FGF14. In cerebellar and cortical neurons, FGF14 binds Nav1.6 and modulates its gating; GSK3β phosphorylation of FGF14 at Ser^226 is required for optimal FGF14-Nav1.6 interaction [30] [49]. Thus, active GSK3 boosts Nav1.6 function both directly (phosphorylating the channel) and indirectly (phosphorylating/stabilizing FGF14) [49] [50]. In contrast, Nav1.2's interaction with FGF14 (which binds the Nav1.2 N-terminus) is affected by other factors like WEE1 kinase (phosphorylating FGF14 at Tyr^158) [32], but GSK3's primary effect on Nav1.2 is via the C-tail/Nedd4-2 mechanism described. The net result is that globally inhibiting GSK3 in a neuron could have **mixed effects** on excitability: Nav1.2-mediated currents increase (enhancing excitability, especially in developing or distal compartments), but Nav1.6-mediated currents might decrease slightly (potentially reducing excitability at mature axon initial segments) [53] [52]. Indeed, one study found that a GSK3 inhibitor (AR-A014418) reduced overall sodium currents and repetitive firing in cultured neurons [53], seemingly at odds with the HEK/Nav1.2 findings. The authors suggest cell-specific differences – neurons endogenously express a complement of channel isoforms and modulatory proteins (like Nav1.6 and FGF14), so GSK3 inhibition could engage additional mechanisms not present in HEK/Nav1.2 cells [54]. This underscores that the impact of GSK3 modulation is context-dependent. Nevertheless, the direct GSK3→Nav1.2 link has been firmly established as a novel **phosphorylation-dependent trafficking control** for Nav channels [41] [48]. It provides a mechanistic basis for how upstream pathways (insulin, Wnt, neurotrophins, etc.) might acutely tune intrinsic excitability by regulating sodium channel availability.

## Interventions to Modulate GSK3 for Enhancing Nav1.2 Function

Given that reduced GSK3 activity leads to increased Nav1.2 surface expression and current, a variety of interventions could, in principle, enhance Nav1.2 function by targeting GSK3 or its upstream regulators. These interventions span pharmacological agents, genetic manipulations, and even lifestyle or environmental modifications. **Table 1** summarizes key examples of pharmacological interventions, and **Table 2** outlines genetic and lifestyle factors, highlighting their mechanisms of action on GSK3 and the consequent effects on Nav1.2 channels.

**1. Pharmacological Interventions:**
These include direct GSK3 inhibitors as well as compounds that act on upstream pathways (PI3K/Akt or Wnt) to modulate GSK3 activity.

- **Lithium:** A well-known mood stabilizer and one of the first identified GSK3 inhibitors. Lithium directly binds the GSK3 ATP-magnesium binding pocket, inhibiting its kinase activity [20]. It also indirectly increases inhibitory Ser9 phosphorylation of GSK3β in the brain, through mechanisms involving Akt and other kinases [9]. In neurons and adrenal cells, lithium (often as LiCl) has been shown to *upregulate* surface expression of Nav channels via GSK3 inhibition [55]. For example, LiCl treatment increased Nav1.7 levels on the cell membrane in adrenal chromaffin cells [55]. Likewise, lithium would be expected to enhance Nav1.2 currents by preventing GSK3-mediated internalization. (It should be noted lithium can also affect sodium channels through GSK3-independent means [56], such as altering Na^+ gradient or gating, but these are separate from the GSK3 pathway.) Clinically, chronic lithium usage correlates with increased excitability in certain neuronal circuits (which may relate to its pro-cognitive or mood-stabilizing effects), but the relationship is complex due to lithium's multiple targets.

- **Small-Molecule GSK3 Inhibitors:** Numerous ATP-competitive or allosteric GSK3 inhibitors have been developed in research and clinical trials. Examples include **SB-216763** and **CHIR99021** (both selective ATP-competitive inhibitors used in lab studies to activate Wnt signaling), **AR-A014418**, **TDZD-8**, and the drug **tideglusib**. AR-A014418 was shown to inhibit GSK3β and, in one neuronal study, reduced firing and Na^+ currents, possibly by its mixed effects on Nav1.2/Nav1.6 as discussed [53]. **Tideglusib** is an irreversible GSK3β inhibitor that reached clinical trials for Alzheimer's disease and autism. In a recent small trial in adolescents with autism spectrum disorder, tideglusib showed trends toward improving social behavior [57], and it was generally well-tolerated at the doses tested. The rationale in autism trials partly stems from the idea that boosting PI3K–Akt–mTOR and reducing GSK3 might enhance synaptic function and potentially compensate for SCN2A (Nav1.2) loss-of-function in certain autism cases [58]. While tideglusib's direct impact on Nav1.2 wasn't measured in those patients, preclinical data suggest it would increase Nav1.2 channel availability similar to other GSK3 inhibitors. It is important with any GSK3 inhibitor to consider off-targets and pathway crosstalk; for instance, CHIR99021 and SB-216763 are quite specific for GSK3, whereas older compounds like TDZD-8 have additional targets. Overall, pharmacological GSK3 inhibitors consistently *increase* Nav1.2 currents in simplified systems [38], making them logical candidates to enhance Nav1.2 function, albeit with careful dosing and context consideration.

- **Akt/PI3K Pathway Activators:** Instead of inhibiting GSK3 directly, one can activate its upstream inhibitors. **Insulin** itself (and analogs like IGF-1) can be considered in this category. In the CNS, insulin and IGF-1 receptors activate PI3K, leading to Akt activation and subsequent GSK3β Ser9

phosphorylation [8]. As noted, IGF-1 chronically elevates sodium channel surface levels (Nav1.7 in one study) via GSK3β inhibition [10]. Although systemic insulin has limited brain access, **intranasal insulin** is an emerging approach to deliver insulin to the brain, with trials showing cognitive benefits in Alzheimer's disease. This could conceivably enhance neuronal insulin signaling and dampen GSK3 activity in neurons. Another tactic is using small molecules that stimulate Akt. One experimental compound is **SC79**, an Akt allosteric activator that drives Akt into the active membrane-bound conformation. In neurons, SC79 treatment would mimick growth factor signaling, increasing Akt activity and thus GSK3 inhibition. While not yet clinically used, such approaches demonstrate the principle: *enhancing Akt/PKB activity* will inhibit GSK3 and thereby promote Nav1.2 channel functional expression. A caveat: over-activating PI3K/Akt can trigger other pathways (like mTOR) with significant effects (e.g. cell growth, altered metabolism), so specificity and timing are critical.

- **Wnt Pathway Modulators:** By activating Wnt signaling, one can achieve GSK3 sequestration and functional inhibition in neurons. Directly applying Wnt ligands (Wnt proteins or agonist antibodies) has been shown to inactivate GSK3 in neural tissue [12]. Small molecules like **Wnt agonists** (e.g. QS11, which stabilizes Wnt receptor complex) or **tankyrase inhibitors** (which prevent Axin degradation and paradoxically can upregulate β-catenin in certain contexts) might modulate the Wnt/GSK3/β-catenin axis. One straightforward Wnt-mimetic is **lithium** again – lithium's GSK3 inhibition effectively activates canonical Wnt signaling (indeed, discovery of lithium's Wnt-mimicking led to understanding its mechanism on GSK3). Another approach is **inhibitors of GSK3–Axin interaction** like **XIV compounds** that disrupt the Axin scaffold, freeing β-catenin. In cardiomyocytes with certain mutations, Wnt activation or GSK3β inhibition restored sodium currents and function [59], suggesting a similar strategy could apply in neurons. However, chronic Wnt activation can suppress expression of some Nav channel genes via transcriptional feedback [60] (e.g. Wnt3a or GSK3 inhibitor application reduced SCN5A/Nav1.5 mRNA in a heart cell study, even as acute effects were beneficial). Thus, **transient or localized Wnt stimulation** might best exploit GSK3 inhibition to boost Nav1.2 function without adverse gene-level changes.

- **Neurotrophins and Peptide Factors: BDNF** (brain-derived neurotrophic factor) and related neurotrophins (NGF, NT3) potently activate Trk receptor tyrosine kinases, turning on PI3K/Akt and MAPK pathways. BDNF in particular is known to enhance neuronal excitability and synaptic strength, and part of this effect is via inhibition of GSK3β [61] [25]. BDNF application leads to increased Ser9-phosphorylated GSK3β in neurons, thereby disinhibiting downstream targets of GSK3 (like β-catenin and others that promote synapse stability) [61]. Though direct studies of BDNF on Nav1.2 trafficking are lacking, it is plausible that BDNF could acutely increase Nav1.2 channel insertion by the same mechanism as insulin (PI3K→Akt→GSK3 inactivation). In support, one report found that BDNF elevates levels of PSD-95 (a synaptic protein) via GSK3 pathway modulation [62]; similarly Nav channels might be stabilized. **Nerve growth factor (NGF)** acting on TrkA in basal forebrain or peripheral neurons could have analogous effects on Nav1.7 or Nav1.8 in those cells (NGF is known to upregulate Nav1.8 in DRG neurons during inflammation, potentially via PI3K pathways). The challenge with neurotrophins is delivery and specificity: they do not cross the blood–brain barrier easily and can cause excessive excitation or even seizures if broadly elevated. Thus, small molecule agonists of TrkB or positive modulators of BDNF signaling (some under development) could be alternatives that fine-tune this pathway.

- **Other Notable Compounds:** Certain **nutraceuticals and natural compounds** have incidental effects on GSK3. For instance, **curcumin** (from turmeric) and **resveratrol** (from red grape skin) have

been reported to inhibit GSK3β activity in various models [63]. Curcumin can activate Wnt/β-catenin signaling by suppressing GSK3β in neuronal cells [63], and resveratrol has been noted to increase Akt signaling while reducing GSK3 activity in some contexts (part of its anti-inflammatory action). **Berberine**, a plant alkaloid, also down-regulates the PI3K/PTEN/Akt/GSK3 axis [64]. While these compounds have multiple targets and weaker potency, long-term dietary supplementation could conceivably lower GSK3 activity modestly. **Sodium valproate** (valproic acid), a broad-spectrum anticonvulsant, is another interesting case: it indirectly inhibits GSK3 by increasing inhibitory phosphorylation, possibly via activating PKC or Akt; valproate's ability to inhibit GSK3 and activate Wnt has been suggested to contribute to its neurotrophic and anti-manic effects [65]. However, valproate also directly modulates ion channels and epigenetic enzymes, so its net effect on Nav1.2 specifically is not clear (some evidence suggests valproate can reduce excitability despite GSK3 inhibition, due to enhancement of sodium channel slow inactivation).

In summary, a range of drugs can decrease GSK3 activity and thereby are expected to enhance Nav1.2 channel function. Table 1 compiles key pharmacological interventions and their mechanisms in this context.

**Table 1: Pharmacological Interventions Targeting GSK3 to Enhance Nav1.2**

| Intervention | Type/Target | Mechanism on GSK3 | Expected Impact on Nav1.2 | References |
|---|---|---|---|---|
| **Lithium** ($Li^+$ salts) | Mood stabilizer; direct GSK3 inhibitor | Directly inhibits GSK3 (competes with $Mg^{2+}$); increases Ser9-P via Akt/others [9] [20]. | Releases GSK3 "brake" on Nav1.2 – increases surface channel density and INa [38] [55]. | [55] [20] |
| **SB-216763**, **CHIR99021** | Selective GSK3 inhibitors (research) | ATP-competitive inhibitors of GSK3α/β. Mimic Wnt signaling by stabilizing β-catenin. | Upregulate Nav1.2 function (shown by increased current in cells when GSK3 is blocked) [38]. | [38] [12] |
| **AR-A014418** | GSK3β inhibitor (research) | Inhibits GSK3β activity (competitive). | In isolated Nav1.2 systems: increases INa; in neurons: mixed effect (Nav1.2 ↑ but Nav1.6 ↓) [53]. | [53] |
| **Tideglusib** (NP031112) | Irreversible GSK3β inhibitor (clinical) | Binds cysteine in GSK3β active site (non-ATP competitive). Long-lasting inhibition. | Likely increases Nav1.2 currents (not directly measured); tested in ASD for boosting synaptic function [57]. | [57] [55] |

| Intervention | Type/Target | Mechanism on GSK3 | Expected Impact on Nav1.2 | References |
|---|---|---|---|---|
| **Insulin** / **IGF-1** | Hormones (PI3K/Akt activators) | Activate insulin/IGF receptors → PI3K → Akt → GSK3β Ser9 phosphorylation (inactivation) [8] [66] . | Increases Nav channel surface expression and activity (e.g. Nav1.7 in chromaffin cells) [10] ; would enhance Nav1.2 similarly. | [8] [10] |
| **BDNF** (TrkB agonist) | Neurotrophin (Akt/MAPK activator) | Binds TrkB → PI3K/Akt and MAPK pathways; Akt phosphorylates and inhibits GSK3β [17] . Also upregulates β-catenin signaling. | Expected to increase Nav1.2 availability (by GSK3 inhibition), while also acutely enhancing excitability via other pathways. | [17] [61] |
| **Wnt3a** (and Wnt agonists) | Wnt/β-catenin pathway ligand | Binds Frizzled/LRP6 → recruits GSK3 into Axin-LRP6 complexes (sequesters GSK3) [12] . β-Catenin stabilized (GSK3 unable to phosphorylate it). | Reduces GSK3 activity without Ser9-P; potentially increases Nav1.2 surface retention. (Chronic Wnt may downregulate some Nav gene expression [60] .) | [14] [13] |
| **Curcumin**, **Resveratrol** | Natural polyphenols (pleiotropic) | Activate pro-survival kinases (AMPK, etc.) and inhibit GSK3β (reported via Akt and Wnt pathway modulation) [63] . | Mild GSK3 inhibition over long term could improve Nav1.2 function; primarily noted for neuroprotective/anti-inflammatory effects. | [63] [67] |
| **Valproate** (VPA) | Anticonvulsant/ mood stabilizer | Indirect GSK3 inhibition (increases Ser9-P possibly via PKC); also inhibits HDAC, affects inositol pathway. | Net effect on Nav1.2 is complex: GSK3 inhibition promotes Nav1.2, but VPA also enhances $Na^+$ channel inactivation (which can reduce excitability). | [65] [56] |

*Table 1:* Pharmacological interventions that modulate GSK3 and their expected effects on Nav1.2 channel function. In general, drugs that inhibit GSK3 (directly or via upstream signaling) increase Nav1.2 surface expression and Na^+ current (I<sub>Na</sub>) by preventing GSK3-dependent channel internalization [38] [48] . Exceptions may occur in complex cellular contexts where multiple Nav isoforms are present [53] .

**2. Genetic and Molecular Interventions:**
These involve altering the expression or structure of GSK3, Nav1.2, or their regulatory partners at the DNA/

RNA level. Such interventions are mostly experimental (in animal models or cell lines), but they shed light on potential strategies:

- **GSK3β Knockdown/Knockout:** Reducing GSK3β expression (via siRNA, shRNA, or genetic knockout) is the genetic equivalent of pharmacological inhibition. In the Nav1.2 study, siRNA silencing of GSK3 mirrored the effect of GSK3 inhibitor, increasing Nav1.2 current density [38]. Neuron-specific conditional knockout of GSK3β in mice results in hyperactive behavior and biochemical signs of elevated Wnt signaling (since β-catenin accumulates), which likely includes increased Nav1.2 function, though specific Nav1.2 measurements haven't been reported in those mice. Complete germline knockout of GSK3β is not viable (due to developmental Wnt derangements), underscoring that some baseline GSK3 activity is crucial. However, heterozygous GSK3β^+/– mice, or knock-in mutants that mimic constant Ser9 phosphorylation (i.e. constitutively "off" GSK3β), could be viable ways to chronically upregulate Nav1.2. Indeed, mice with a Ser9→Ala mutation (GSK3β-S9A, which prevents inhibition and thus keeps GSK3 active) show *decreased* neuronal excitability and a propensity for depressive-like behavior [68] [69], whereas the opposite (constitutively inhibited GSK3β) might produce hyper-excitability or mania-like phenotypes [22]. These behavioral outcomes align with GSK3's control of ion channels: active GSK3 dampens excitability (Nav1.2 down, contributing to depression), while loss of GSK3 inhibition can boost excitability (risking mania or seizures). Thus, genetically reducing GSK3 activity should enhance Nav1.2 but with caution for behavioral extremes.

- **Nav1.2 C-terminal Mutation:** A precise genetic strategy to enhance Nav1.2 is to eliminate the GSK3 phosphorylation site on the channel. If threonine 1966 is mutated to a non-phosphorylatable residue (e.g. T1966A or T1966V), GSK3 can no longer tag the channel for internalization. This would be expected to increase Nav1.2 retention at the membrane similarly to pharmacological GSK3 inhibition. In fact, phosphorylation at T1966 is hypothesized to prime Nav1.2 for Nedd4-2 binding [70]; removing this site could mimic a state of perpetual GSK3 inhibition specifically for Nav1.2. While this mutation has not yet been introduced into mice (or human cells) in published studies, it represents a potential therapeutic gene-editing approach for SCN2A loss-of-function conditions: a Nav1.2-T1966A variant might yield higher channel density to compensate for reduced channel number. Of course, one must ensure no deleterious gain-of-function arises (e.g. if too many channels accumulate, hyperexcitability and seizures could occur).

- **Upregulation of β-Catenin or Wnt Signaling Components:** From a genetic standpoint, enhancing Wnt/β-catenin signaling in neurons will inhibit GSK3. For example, overexpression of a stabilized **β-catenin** (mutation at GSK3 target sites S33/S37/T41) in neurons leads to sequestration of GSK3 and robust transcriptional changes [11]. Such neurons might show increased Nav1.2 currents due to reduced GSK3 activity, although direct electrophysiology data would be needed. Transgenic overexpression of Wnt ligands or **Dishevelled** (an intracellular Wnt signal transducer) in specific brain regions could achieve local GSK3 inhibition. However, chronic Wnt activation alters many developmental processes and gene expression programs, so this approach could have significant side effects (altered neuronal differentiation, etc.). Another angle is to knock down negative regulators of Wnt, such as **Axin** or **GSK3-binding protein (FRAT1)**. FRAT1 normally binds GSK3 and can inhibit its activity; overexpressing FRAT1 in neurons might blunt GSK3 and thus elevate Nav1.2 function.

- **Modulating Accessory Proteins (FGF14, Nedd4-2):** Nav1.2 function is also governed by proteins that interact with the channel and are themselves downstream of GSK3. **FGF14**, as mentioned, is a subunit of the channel complex. While GSK3's effect on Nav1.2 is not primarily through FGF14 (unlike Nav1.6), ensuring proper FGF14 levels is important for Nav channel function. Mice lacking FGF14 have reduced Nav current and ataxia. GSK3β may help stabilize FGF14's interaction with Nav1.2 (some evidence suggests GSK3β inhibition reduces the FGF14-Nav1.2 complex assembly in luciferase complementation assays) [71]. Thus, a genetic intervention could be to mutate the GSK3 site on FGF14 (Ser226) to a phosphomimetic (S226E) to preserve FGF14 binding even when GSK3 is low, maintaining Nav channel regulation. On the other hand, **Nedd4-2** (gene *NEDD4L*) is the E3 ligase that ubiquitylates Nav1.2. Knocking out or reducing Nedd4-2 expression in neurons is predicted to increase Nav1.2 surface levels (since the channel isn't efficiently ubiquitinated for internalization). Indeed, Nedd4-2 knockout mice exhibit severe seizures and die early, consistent with uncontrolled Nav channel activity. A partial reduction in Nedd4-2 (e.g. heterozygous knockout) might be a milder way to boost Nav1.2 and Nav1.6 currents – but one must manage the risk of hyperexcitability. Lastly, **ankyrin-G**, which clusters Nav channels at the axon initial segment, has been linked indirectly to GSK3: ankyrin-G mutations can mislocalize β-catenin and alter Wnt signaling [23]. Overexpressing ankyrin-G or its binding partners might enhance Nav1.2 clustering independent of GSK3 status, thus could be another strategy to improve Nav1.2 function.

**3. Lifestyle and Environmental Interventions:**
Chronic lifestyle factors can influence intracellular signaling cascades, including those regulating GSK3. While these are less potent or immediate than drugs, they contribute to the biochemical milieu that determines GSK3 activity in neurons:

- **Physical Exercise:** Exercise is a potent inducer of BDNF and other growth factors in the brain. Endurance exercise elevates BDNF levels in the hippocampus and cortex, activating TrkB receptors on neurons [72]. This leads to PI3K/Akt activation and thus increased GSK3β Ser9 phosphorylation (inhibition). Exercise also improves insulin sensitivity systemically, which may enhance insulin/IGF signaling in the brain. In animal studies, voluntary wheel running was shown to upregulate Akt signaling and downstream mTOR, consistent with reduced GSK3 activity, and was associated with increased intrinsic excitability of certain neurons (likely through effects on ion channels). Therefore, regular exercise could *naturally suppress GSK3* over time, promoting greater Nav1.2 channel presence and neuronal excitability in a controlled, homeostatic manner. Indeed, exercise is known to raise seizure threshold and support cognitive function, which might seem paradoxical if it increases excitability; but it likely strengthens network stability through coordinated plasticity rather than causing hyperexcitability. Still, for an individual with suboptimal Nav1.2 function, exercise-induced trophic signaling might confer some benefit.

- **Diet and Metabolic Health:** Dietary habits that maintain metabolic health can indirectly modulate neuronal GSK3. A diet that prevents insulin resistance (e.g. high in polyunsaturated fats, low in simple sugars) ensures that insulin-Akt signaling remains effective. In contrast, in type-2 diabetes or metabolic syndrome, insulin resistance may lead to chronically *elevated* GSK3 activity in the brain (because insulin's inhibitory effect on GSK3 is blunted). This has been implicated as a factor in Alzheimer's disease pathogenesis (sometimes dubbed "type-3 diabetes") [73]. Thus, dietary interventions such as calorie control, low-glycemic diets, or ketogenic diets could influence brain insulin signaling and GSK3. For example, a ketogenic diet (high fat, very low carb) often elevates ketone bodies like β-hydroxybutyrate, which can act as a HDAC inhibitor and may increase BDNF

expression, potentially leading to Akt activation. Some studies on epilepsy patients (especially children on ketogenic diet for seizure control) suggest the diet alters PI3K/Akt/mTOR and could reduce GSK3 activity, though direct measurements are scarce. Overall, a **healthy diet that avoids insulin resistance** might help keep neuronal GSK3 in check, indirectly supporting Nav1.2 functionality.

- **Stress Reduction:** Psychological stress and chronic elevation of cortisol can increase GSK3β activity in limbic brain regions. Corticosteroid signaling intersects with GSK3 via various pathways (for instance, glucocorticoids can reduce the levels of certain growth factors and increase oxidative stress, both of which could remove inhibition from GSK3). Animal models of chronic stress show increased active (dephosphorylated) GSK3β in the hippocampus accompanying depressive behaviors [18]. Reducing stress or treating with antidepressants (many of which promote 5-HT or BDNF signaling and thus inhibit GSK3) leads to restoration of GSK3 inhibitory phosphorylation and improved neuronal health [74]. Therefore, stress-relieving practices (meditation, biofeedback, therapy) might indirectly enhance Nav1.2 function by preventing stress-induced hyperactivation of GSK3. Likewise, sufficient **sleep** and a stable circadian rhythm contribute – interestingly, GSK3β is a regulator of the circadian clock (it phosphorylates clock proteins). Chronic circadian disruption (e.g. shift work, insomnia) can dysregulate GSK3 activity cycles. Lithium, which lengthens the circadian period by inhibiting GSK3, has been used to stabilize mood and circadian rhythms in bipolar patients [75]. Ensuring good sleep hygiene might normalize daily oscillations of GSK3 activity in the brain, avoiding any detriments to Nav channel regulation that might occur with disturbed rhythms.

- **Environmental Enrichment:** Providing a stimulating environment (novel objects, social interaction, learning tasks) is known to boost neurotrophic signaling in animal models. Environmental enrichment raises BDNF levels and Akt phosphorylation in cortex and hippocampus, concomitant with enhanced synaptic plasticity and resilience to stress [76] [77]. Enriched rodents have shown lower measures of anxiety/depression possibly due to upregulated CREB and BDNF (which would inhibit GSK3) [76]. Such environments could thereby keep GSK3 activity lower and Nav channels more available, supporting active neural networks. This is more relevant as a holistic preventive measure rather than an acute intervention.

- **Avoidance of Neurotoxins:** Certain environmental toxins or injury conditions activate GSK3 deleteriously. For example, traumatic brain injury can cause an acute surge in GSK3 activity, contributing to neurodegeneration and Na^+ channel dysfunction. Some pesticides and heavy metals elevate GSK3β in neurons via oxidative stress pathways. Avoiding or mitigating exposure to these can preserve normal GSK3 regulation.

In essence, a lifestyle that promotes neurotrophic and metabolic health will create an internal environment of moderated GSK3 activity, which in turn favors robust Nav1.2 channel function. Table 2 summarizes some genetic and lifestyle factors in this context.

**Table 2: Genetic and Lifestyle Factors Affecting GSK3 and Nav1.2**

| Intervention/ Factor | Mechanism on GSK3 | Effect on Nav1.2 Channels | Notes/Risks |
|---|---|---|---|
| **GSK3β Knockdown / ±Knockout** (neurons) | Reduces GSK3β expression → less GSK3 activity overall. | Mimics GSK3 inhibition: increases Nav1.2 current density and surface channels [38]. | Enhances excitability; full KO causes developmental defects (lethal) [22]. Heterozygous or conditional KO can cause hyperactivity (mania-like) due to widespread disinhibition of targets. |
| **Nav1.2-T1966A Mutation** (hypothetical) | Eliminates GSK3 phosphorylation site on Nav1.2 C-tail. GSK3 cannot tag channel for Nedd4-2. | Nav1.2 remains longer at membrane (resistant to internalization), boosting Na^+ currents (by analogy to GSK3-inhibited state) [48] [47]. | Could compensate Nav1.2 haploinsufficiency. Risk: excessive channel retention might cause hyperexcitability/seizures. Not yet tested in vivo. |
| **Nedd4-2 E3 Ligase Reduction** | Less ubiquitination of Nav1.2 (and Nav1.6) channels. Functionally similar to blocking GSK3→Nedd4 pathway. | Increases Nav channel surface expression and currents (Nedd4-2 KO causes elevated Nav currents and seizures). Nav1.2 more stable at AIS/somatic membrane. | High seizure risk if overdone (Nedd4-2 knockout mice are epileptic). Partial reduction could be therapeutic but must be tightly controlled. |
| **β-Catenin Overexpression** | Constitutively active β-catenin sequesters GSK3 in complexes, reducing its free activity [12]. Also drives Wnt target genes. | High β-catenin likely increases Nav1.2 surface levels (by GSK3 inhibition). Transcriptional changes might also upregulate channel subunits or other excitability genes. | Excess Wnt/β-catenin can cause aberrant neurodevelopment or tumorigenesis (if uncontrolled). Might shift neuronal fate or proliferative state. |
| **Exercise (Aerobic)** | ↑ BDNF, IGF-1 levels → activates TrkB/insulin receptors → PI3K/Akt → GSK3 inhibited (↑Ser9-P) [72] [17]. Also ↑ serotonin tone, which inhibits GSK3 via 5-HT1A. | Likely elevates Nav1.2 function modestly and improves neuronal health. Enhanced network activity can promote appropriate Nav channel localization. | Broad benefits (plasticity, mood). Unlikely to overshoot Nav1.2 function in a harmful way, since homeostatic plasticity adjusts to activity. |

| Intervention/ Factor | Mechanism on GSK3 | Effect on Nav1.2 Channels | Notes/Risks |
|---|---|---|---|
| **Diet & Metabolic Control** | Prevents chronic hyperglycemia/insulin resistance (which would leave GSK3 active). A balanced diet supports normal insulin→Akt signaling, keeping GSK3 activity in check [8]. Certain nutrients (omega-3s, polyphenols) may directly dampen GSK3 activity. | Maintains normal Nav1.2 regulation. In diabetic models, Nav channels can be dysregulated; avoiding that preserves proper channel function. | Healthy diet also reduces AD risk (AD involves overactive GSK3 and tau pathology). Extreme diets (ketogenic) need medical supervision but can modulate signaling. |
| **Stress Reduction** (psychological) | ↓ Cortisol and inflammatory cytokines that otherwise activate GSK3. Chronic stress increases active GSK3β; relieving stress allows neurotrophic signaling to dominate, keeping GSK3 inhibited [18] [74]. | Promotes better Nav1.2 availability and stability of neuronal firing. Chronic stress can cause Nav channel hypo-function (via elevated GSK3 and other mechanisms), so reducing stress reverses that trend. | Hard to quantify effect size, but contributes to overall neuronal network stability. Many antidepressants incidentally inhibit GSK3 by increasing 5-HT/BDNF, aligning with this principle. |
| **Enriched Environment** | ↑ Sensory, cognitive stimulation → ↑ CREB, BDNF in brain → Akt active, GSK3 suppressed [76]. Also ↑ IGF-1 levels in some cases. | Likely supports optimal Nav1.2 function as part of enhanced neuronal excitability and plasticity in enriched brains. (Rats in enriched environment have more robust intrinsic excitability and learning capacity.) | Very positive for overall brain function; essentially a non-invasive way to engage the same pro-plasticity pathways that inhibit GSK3. Minimal downsides aside from needing consistent effort. |

*Table 2:* Genetic and lifestyle factors that influence GSK3 activity and thereby Nav1.2 channel function. Genetic interventions offer targeted ways to diminish GSK3's suppression of Nav1.2 (though with developmental and systemic risks), while lifestyle factors generally promote a neurochemical environment that keeps GSK3 activity appropriately low, supporting Nav1.2 and neuronal excitability.

## Potential Risks, Off-Target Effects, and Long-Term Considerations

Any strategy to enhance Nav1.2 function via GSK3 modulation must account for the broader role of GSK3 in physiology and the nervous system. **GSK3 is a pleiotropic kinase**, and its activity is a node for many signaling pathways; thus, interventions can have far-reaching off-target effects. Some key considerations:

- **Homeostatic Balance and Seizure Risk:** Increasing Nav1.2 surface expression will boost neuronal excitability, which might be therapeutic in conditions of Nav1.2 under-function (e.g. certain SCN2A loss-of-function epileptic encephalopathies or autism), but in a normal or already excitable brain, this could tip the balance toward hyperexcitability. For instance, if GSK3 is strongly inhibited in a broad manner, multiple neuron populations may show heightened firing, potentially lowering the threshold for seizures. The phenotype of Nedd4-2 knockout mice (spontaneous seizures) warns that preventing Nav channel internalization entirely is dangerous. In the adult brain, Nav1.2 is predominantly in dendrites and unmyelinated axons, while Nav1.6 is at the axon initial segment; excessive Nav1.2 could lead to aberrant dendritic spike initiation or back-propagation. **Compensatory mechanisms** are likely to engage: neurons might respond to chronically elevated Nav1.2 by reducing other excitatory currents or upregulating potassium currents. Indeed, homeostatic plasticity might counteract the intended enhancement over time. For example, prolonged GSK3 inhibition might cause an increase in the expression of Kv channels or a reduction in Nav1.6 to compensate, as a protective response.

- **Effects on Other Ion Channels:** GSK3's influence is not limited to Nav1.2. It also modulates other channels and receptors. GSK3β phosphorylates Nav1.6 (as discussed) and even some potassium channels (e.g. Kv7/KCNQ2/3 channels are regulated by Nedd4-2 in a manner that GSK3 could influence [46] [78]). GSK3 has been shown to interact with pathways controlling calcium channels and GABA_A receptors indirectly through gene transcription. Thus, altering GSK3 will produce a **suite of electrophysiological changes**. Some could be beneficial (e.g. GSK3 inhibition might enhance synaptic plasticity by facilitating NMDA receptor function or increasing β-catenin-mediated synapse formation), but some might be detrimental (e.g. impairing Nav1.6 or increasing leak K^+ currents). The net outcome on neuronal excitability and network oscillations is hard to predict and might vary between brain regions. This underlines the need for precision – perhaps targeting GSK3 in specific neuron types or subcellular compartments (for example, developing drugs that concentrate effects on dendrites vs axon initial segments).

- **Long-Term Synaptic Plasticity:** GSK3 plays a complex role in learning and memory. Paradoxically, while acute inhibition of GSK3 can promote certain forms of synaptic strengthening (and has antidepressive effects), some degree of GSK3 activity is required for long-term depression (LTD) and memory consolidation. Studies show that **persistent inhibition of GSK3** can block LTD induction and bias synapses toward potentiation, which might sound beneficial but can actually lead to cognitive inflexibility or mania-like states [69] [68]. In mice, hyperactive GSK3 (S9A mutant) impairs memory and induces depressive behavior, whereas a moderate reduction in GSK3 activity has antidepressant and pro-cognitive effects – but too low GSK3 for too long might impair the ability to forget or weaken inappropriate synapses (a process needed for normal learning). Therefore, chronic interventions must strike a balance to avoid eroding the dynamic range of synaptic plasticity. On the flip side, excessive Nav1.2 function could enhance **neuronal excitability at the expense of precision**, potentially increasing noise in neural circuits.

- **Developmental Impacts:** In developing neurons, GSK3 is crucial for processes like neuronal polarization, axon outgrowth, and neural progenitor differentiation [79] [80]. It regulates microtubule dynamics through substrates such as CRMP-2; too much GSK3 activity stunts axon growth, while too little causes aberrant axon branching. Interventions that globally reduce GSK3 in utero or early in development could cause migration defects or miswiring of circuits (for example, overactive Wnt signaling from GSK3 inhibition might alter the numbers and placement of excitatory synapses). Any pediatric or prenatal use of GSK3-modulating therapy would need to consider these developmental roles. Interestingly, SCN2A-related disorders often manifest early in development, raising a catch-22: treating early might help Nav1.2 function but could interfere with other GSK3-dependent developmental events. A possible solution is using very targeted delivery (e.g. gene therapy to express a Nav1.2-T1966A mutant only in certain neuron populations, or transient treatment during specific developmental windows).

- **Off-Target Biochemical Effects:** Many pharmacological GSK3 inhibitors have additional targets. Lithium, for example, inhibits inositol monophosphatase, leading to depletion of inositol and effects on phosphoinositide signaling. This contributes to lithium's side effect profile (tremors, thyroid and kidney issues). Lithium's suppression of GSK3 in peripheral tissues can cause nephrogenic diabetes insipidus and hypothyroidism, unrelated to Nav1.2. Tideglusib was found to also inhibit kinases like CDK7 at high concentrations. Moreover, robust GSK3 inhibition in non-neural tissues might promote anabolic and proliferative processes (since GSK3 usually helps restrain cell proliferation via Wnt). There is theoretical concern that long-term GSK3 inhibition could increase cancer risk by chronically activating Wnt/β-catenin signaling [81] [82], though short clinical trials have not seen this.

- **Behavioral and Mood Alterations:** GSK3 has been implicated in mood regulation – it is hyperactive in bipolar mania and is inhibited by effective treatments (lithium, some antipsychotics) [74]. If one were to significantly inhibit GSK3 for Nav1.2 enhancement, an individual might experience mood elevation or reduced depression (beneficial if depression is present, but possibly destabilizing if not). Conversely, if a treatment inadvertently overactivates GSK3 (for instance, discontinuing a GSK3 inhibitor abruptly could cause a rebound increase in GSK3 activity), it could provoke depressive or anxiogenic effects. Careful monitoring of psychiatric status would be advised in any prolonged manipulation of GSK3.

- **Compensatory Kinases and Pathways:** The cell's signaling network might compensate for a chronically inhibited GSK3 by upregulating other kinases or pathways that serve similar roles. For example, ERK and p38 MAPK also phosphorylate Nav channels (Nav1.6 and Nav1.7) at other sites to modulate their localization [83] [84]. If GSK3 is kept inactive, cells might rely more on these MAPKs for controlling Nav channel turnover. Similarly, if GSK3-driven phosphorylation normally primed channels for Nedd4-2, perhaps other priming kinases (like casein kinase 2 or CK2) could increase activity to partially take GSK3's place (CK2 already is known to phosphorylate Nav1.2 at the AIS for ankyrin binding [85]). There is also evidence that **priming kinases** often set the stage for GSK3's action (since GSK3 often phosphorylates a site only after another nearby site is phosphorylated) [21]. Constant absence of GSK3 might lead to aberrant accumulation of primed substrates or activation of phosphatases that try to restore equilibrium.

- **Human Data and Translational Aspects:** In human patients, GSK3 inhibitors (like lithium) have been used for decades, giving insight into long-term effects. Lithium users do not generally show overt cognitive impairment – in fact, lithium may protect from dementia – but some experience fine

tremor or mild cognitive slowing. These subtle effects could be due to changes in axonal ion channel profiles (like Nav1.6 reductions or other channel alterations). The ongoing tideglusib trials in autism and fragile X syndrome will shed light on whether partial GSK3β inhibition can safely improve neurodevelopmental outcomes (initial reports suggest some improvement in social behavior with manageable side effects over 12 weeks [57]). Importantly, any therapy aiming to boost Nav1.2 must consider the *cause* of Nav1.2 dysfunction. If an SCN2A mutation also causes the channel to be biophysically aberrant (not just fewer in number), simply increasing channel abundance might not fully correct excitability and could even introduce abnormal firing patterns. Ideally, precision medicine approaches (like allele-specific targeting or combining GSK3 modulation with other treatments) will be used.

In conclusion, **modulating GSK3 activity is a promising strategy to enhance Nav1.2 channel function**, supported by solid mechanistic understanding of the GSK3–Nav1.2 pathway [38] [48]. Upstream signals such as Wnt, insulin/IGF-1, and BDNF converge to inhibit GSK3, thereby promoting Nav1.2 channel expression on neuronal surfaces. Practical interventions – from lithium and small-molecule inhibitors to exercise and diet – can tap into these pathways to tune Nav1.2 performance. The benefits could range from improved conduction and synaptic integration in neurons with weak Nav currents, to potential therapeutic gains in disorders like SCN2A-related epilepsy or autism. However, these gains must be balanced against the system-wide roles of GSK3. The nervous system is a delicately balanced circuit, and GSK3 is one of its master regulators at the intersection of multiple signaling cascades [17]. Any long-term manipulation requires careful dosing or targeting to avoid tipping into excessive excitation, synaptic imbalance, or systemic side effects. Future research should aim to develop **targeted GSK3 modulators** (e.g. molecules that disrupt GSK3's interaction with Nav1.2 specifically, or brain-region-specific drug delivery) to maximize Nav1.2 enhancement while minimizing collateral impacts. Additionally, continued exploration of the GSK3–Nav axis in vivo (such as in animal models of SCN2A disorders) will clarify how boosting Nav1.2 via GSK3 inhibition influences network activity, cognitive function, and development in the whole organism. With a nuanced approach, it may be possible to harness the GSK3 pathway to fine-tune neuronal excitability and treat Nav1.2-related neuropathologies, achieving improved outcomes while maintaining neural homeostasis [48] [6].

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