Research article

FAK family kinases in brain health and disease

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Abstract

Brain disorders are now identified as one of the largest and costliest health risks throughout human life. While most brain disorders are not life threatening *per se*, their chronic and incurable nature renders the overall burden from these disorders much greater than would be suggested by mortality figures alone. Several neurodevelopmental conditions, including autism and dyslexia, are being diagnosed at increasing rates throughout the last few decades. Adolescence is now well recognized as a vulnerable brain developmental phase, in which mental disorders such as schizophrenia, depression, and bipolar disorder first appear. Additionally, the constant increase in life expectancy has led to a significant rise in the risk of several neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD). A primary research goal of neuroscience is to decipher the molecular mechanisms that play direct roles in the pathophysiology of brain disorders, including those of the young and old alike. Research into these mechanisms will have the most significant impact on brain diseases and mental health. The focal adhesion kinase (FAK) and its homologous FAK-related proline-rich tyrosine kinase 2 (Pyk2) define a distinct family of non-receptor tyrosine kinases that are predominantly expressed in the developing as well as in the adult brain. Despite their high similarity, they are believed to fulfill distinct roles within the brain, which are partially determined by their different expression patterns, localization, and interacting proteins. Here, we provide a comprehensive and up-to-date overview of all known neuronal interactors and signaling pathways in which Pyk2 and FAK are involved. Using bioinformatics analysis and statistical tools, we validate, for the first time, the long-term hypothesis by which FAK is involved in axonal guidance and neurodevelopmental signaling, while Pyk2 has a more prominent role in functions of the adult brain, such as memory and learning. We also characterize two new and previously unidentified roles of Pyk2 in neuropathic pain signaling and neuroinflammation. Correlation of the most significant pathways for each kinase with human brain disorders revealed the involvement of Pyk2 in neurodegenerative diseases such as PD, AD, Huntington's disease (HD), and schizophrenia, while FAK was found to be mostly related to neurodevelopmental disorders in which axonal guidance plays a major role, and to a lesser extent to amyotrophic lateral sclerosis (ALS), schizophrenia, mood disorders, and AD. The involvement of FAK in these non-developmental pathways may suggest its possible role in compensating for Pyk2 in specific processes and/or brain disorders. Understanding the molecular mechanisms underlying regulation of FAK family proteins in brain and behavior may lead to novel therapeutic approaches for preventing or treating the underlying causes of neurodevelopmental abnormalities, psychiatric disorders, and neurodegenerative diseases.

Keywords

Brain disorder; Signaling pathway; Tyrosine kinase; Pyk2; FAK; Neurodevelopmental; Neuropsychiatric; Neurodegenerative

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1. Introduction

Our brain is prone to multiple distinct disorders that may emerge at every stage of life. Neurodevelopmental disorders, which result from impairments in the growth and development of the brain or central nervous system (CNS), such as autism, dyslexia, and fragile X, first emerge in early childhood [1]. Psychiatric diseases such as depression and schizophrenia are commonly diagnosed in teenagers or during early adulthood, albeit their origins may lay much earlier in life [2]. Moreover, as we age, we become increasingly susceptible to neurodegenerative disorders, which are caused by the progressive death of neurons in different regions of the nervous system, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) [2]. Brain disorders, including neurodevelopmental, psychiatric, and neurodegenerative diseases, are among the most severe health problems which our society faces, causing immense human suffering and imposing an enormous economic burden [3]. Much of brain disorders are chronic and incurable, and their disabling effects may last years or even decades. Thus, the overall disease burden from these disorders is much greater than would be proposed by mortality figures alone.

According to the US National Institute of Mental Health, one in every five American adults suffers from a mental disorder in any given year, with depression among the leading causes of disability worldwide (https://www.nimh.nih.gov/index.shtml). The underlying causes of most brain disorders remain poorly understood, and existing drug or behavioral therapies are at best only partially effective.

Therefore, a deeper understanding of the molecular and cellular basis of these disorders may allow the design of new behavioral and pharmacological therapies that will produce better outcomes. Despite the rapid progress in the past two decades in the development of non-invasive technologies to study human brain structure and function, significant limitations in our ability to investigate details of the physiology and molecular biology of the human brain still exist. The absence of appropriate animal models and the lack of knowledge of the biological mechanisms that cause these disorders is a fundamental obstacle to the development of new treatments.

In this review, we summarize the current knowledge regarding the molecular mechanisms of brain and behavior that are mediated by the focal adhesion kinase family of non-receptor tyrosine kinases. Several recent and previous publications may suggest a pivotal role for these kinases in regulating both the normal developing brain and contributing to brain disorders [4].

2. The focal adhesion kinase family: it takes two to tango

Neurons in the human brain use action potentials and neurotransmitters to communicate with each other and to transfer information. Apart from their immediate effects, which account for the fast functioning of the nervous system, these action potentials and neurotransmitters exert actions whose consequences can last from fractions of seconds to days. One major mechanism by which the long-lasting effects of action potentials and neurotransmitters are brought about is protein phosphorylation, which regulates ion channel properties, enzymatic activities, cytoskeletal organization, and gene expression [5]. Protein tyrosine phosphorylation was initially identified as a major step in the action of growth factors and oncogenes, but it is now recognized as a critical post-translational modification for regulation of mature cell functions [6]. The adult brain exhibits high levels of tyrosine kinase activity, and the neuronal synapses are particularly enriched in both tyrosine kinases and tyrosine-phosphorylated proteins. The process of tyrosine phosphorylation is involved in regulation of synaptic activity such as depolarization, long-term potentiation (LTP), long-term depression (LTD), and ischemia [7]. Tyrosine kinase- and tyrosine phosphatase-mediated changes in phosphorylation of proteins that reside in the neuronal synapse mediate both short-term and long-lasting changes in synaptic and neuronal functions [8, 9]. Among the proteins that are phosphorylated on tyrosine residues in response to action potentials and neurotransmitters, are focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2) [9].

FAK and its homologous FAK-related proline-rich tyrosine kinase 2 define a distinct family of non-receptor tyrosine kinases that exhibit approximately 48% amino acid sequence identity, common phosphorylation sites, and a similar domain structure, that includes an N-terminal four-point-one, ezrin, radixin, moesin (FERM) domain, a kinase domain, three proline-rich regions (PRRs), and a C-terminal focal adhesion targeting (FAT) domain. Following integrin or growth factor stimulation, Pyk2 and FAK are auto-phosphorylated on a tyrosine residue (Y402 or Y397, respectively) which provides a critical binding site for Src. Following its binding, Src phosphorylates additional tyrosines on Pyk2 or FAK, which are essential for full activation of the kinases and for their binding to downstream signaling proteins [10] (Fig. 1). Although FAK is expressed in most cells, Pyk2 exhibits a more restricted expression pattern with strongest expression in the CNS and hematopoietic cells [11].

While FAK is associated with signaling pathways that are activated by integrins and growth factor receptors, Pyk2 is activated by a variety of other extracellular cues and receptors, including G-protein coupled receptors, inflammatory cytokine receptors, increase in extracellular or intracellular Ca^{+2} concentrations, as well as integrins in certain cell types [11, 12]. The different expression patterns, localization, and non-kinase domain-dependent binding activities of Pyk2 and FAK may limit their functional redundancy and propose distinct roles within cells.

3. One brain, two kinases

FAK and Pyk2 are predominantly expressed in neurons of rat forebrain regions, including cerebral cortex, hippocampus, and striatum. FAK expression is highest in embryonic brain and decreases during postnatal development. Pyk2 is barely detectable in the prenatal stage, and its levels dramatically increase during the postnatal period, suggesting it might have a specific role in the adult brain. FAK localizes to point contacts at the edge of the growth cone in developing cultured neurons, while Pyk2 is mainly found in the cell body as well as in the dendritic tree of mature neurons. The different developmental patterns and cellular distributions suggest that FAK may be necessary for axon guidance during brain development, while Pyk2 may be involved in regulating synaptic plasticity in the adult brain [9, 13, 14].

Pyk2 is highly expressed in the CNS, where it is activated in response to increase in intracellular and extracellular calcium levels, neuronal membrane depolarization, hyperosmolarity, and activation of protein kinase C (PKC) [9, 11]. Among all tyrosine kinases, Pyk2 is unique in its sensitivity to increases in intracellular Ca^{2+}. It was previously demonstrated that calmodulin-bound calcium (Ca^{2+}/CaM) binds the FERM domain of Pyk2 to induce dimerization and consequent autophosphorylation and activation of the kinase [15]. While the molecular basis of how Pyk2 translates changes in cytoplasmic calcium levels into biological responses remains to be established, it was speculated that binding of Ca^{2+}/CaM to Pyk2 may stabilize the weak FERM:FERM interaction for productive autophosphorylation [16].

Experiments using rat hippocampus slices showed that Pyk2 is involved in the induction of LTP, a central process for learning and memory. Pyk2, but not FAK, is recruited to *N*-methyl-D-aspartate receptor (NMDAR) by the post-synaptic density 95 protein (PSD-95), where it regulates the receptor through binding and activation of Src and Fyn kinases [17, 18]. Activated Src and Fyn phosphorylate the NMDAR subunit GluN2B on tyrosine 1472, leading to exocytosis of GluN1-GluN2B receptors to synaptic membranes as well as to calcium influx through NMDAR, which further activates Pyk2 [17]. A recent publication by Giralt *et al.* [19], using Pyk2 knockout mice confirmed that Pyk2 is essential for LTP and consequent hippocampal-dependent spatial memory acquisition, as well as for the modulation of dendritic spine density and organization of post-synaptic regions.

In contrast, another group has shown that Pyk2 is activated by NMDAR during LTD of cultured neurons and suppresses, through its kinase domain and Src binding site, NMDAR-induced extracellular signal-regulated kinase (ERK) phosphorylation and activation. Moreover, the authors suggested that alteration of ERK signaling

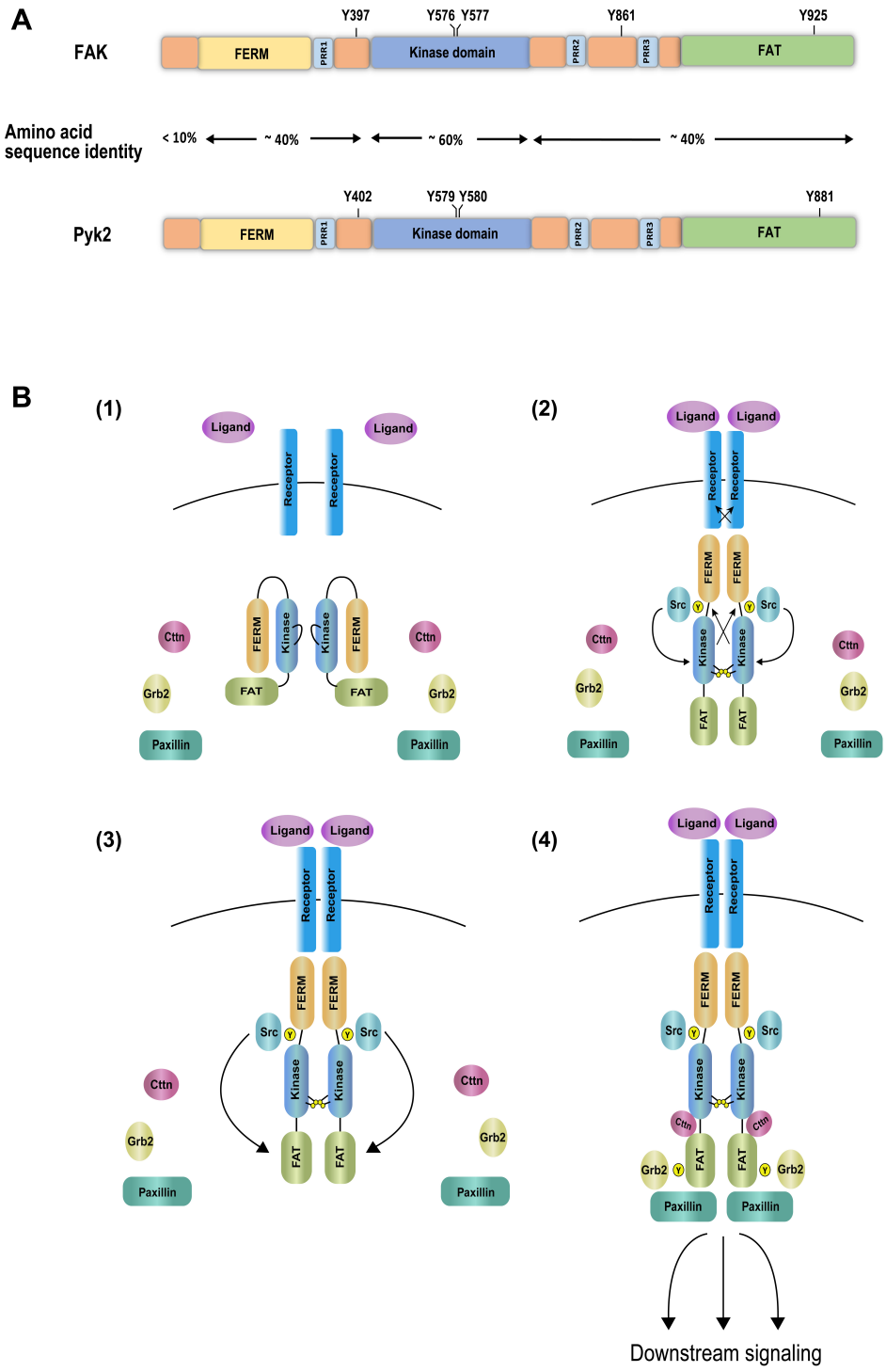

Fig. 1. Structural organization and activation mechanisms of FAK family proteins. (A) Domain structure of FAK family proteins. FAK and Pyk2 share a similar domain arrangement with 60% amino acid sequence identity within the central kinase domain, and 40% amino acid sequence identity within other protein regions, which contain three conserved proline-rich regions (PRRs), a FERM domain, and a FAT domain. In addition, the two kinases share identical positions of three tyrosine phosphorylation sites: auto-phosphorylation and Src binding site (Y397 in FAK and Y402 in Pyk2), kinase activation loop phosphorylation sites (Y576-577 in FAK and Y579-580 in Pyk2), and Grb2 binding site (Y925 in FAK and Y881 in Pyk2). (B) Activation mechanism of FAK family proteins. (1) In their inactive state, FAK/Pyk2 adopt a folded conformation. (2) Following ligand stimulation (i.e. growth factor or extracellular matrix protein), FAK/Pyk2 are unfolded and homodimerize via their FERM domains. Following dimerization and auto-phosphorylation, Src is recruited and phosphorylates the kinase activation loop in FAK/Pyk2, leading to complete activation of the kinases. (3) Src then phosphorylates additional tyrosine residues outside of the kinase domain of FAK/Pyk2. (4) Src-mediated phosphorylation and activation of FAK/Pyk2 leads to binding of downstream signaling proteins such as cortactin (Cttn), Grb2, and paxillin, which bind in the PRRs, FAT domain tyrosine, or FAT domain, respectively, and mediate downstream signaling.

dynamics could affect the activation of LTP versus LTD pathways, with Pyk2 playing a role in determining this balance [20].

The implications of Pyk2 in hippocampus-dependent cognitive functions to disease pathology was recently demonstrated in Huntington's disease (HD), an inherited neurodegenerative disorder which results from the expansion of a tri-nucleotide CAG repeat within the huntingtin (*HTT*) gene [21]. In their recent paper, Giralt *et al.* [19], showed that Pyk2 levels are decreased in post-mortem hippocampus of human HD patients as well as in the hippocampus of R6/1 mice, an HD mouse model transgenic for the first exon of the human *HTT* gene with amplified CAG repeats [22]. Their data suggest that deficits in Pyk2 levels contribute to the hippocampus-associated cognitive deficits, dendritic spine loss, and PSD-95 alterations in R6/1 HD mice. Increasing expression levels of Pyk2 in the hippocampi of these mice restored their synaptic and cognitive abilities, however it was not sufficient to restore synaptic plasticity under the specific experimental conditions that were used in this study [19]. Based on these data, Giralt *et al.* [19], suggested that Pyk2 expression levels might be a limiting factor for the function of excitatory synapses in the hippocampus and raised the question of possible implications of decreased Pyk2 protein levels in other pathological conditions, in which NMDAR dysfunction is thought to be directly or indirectly involved.

This hypothesis has been recently validated in a subsequent publication from the same group, which used genetically-depleted as well as overexpression mouse models to elucidate the role and downstream signaling events of Pyk2 in AD. Although it has long been assumed that a deletion of Pyk2 should improve the prognosis or the symptoms of AD, no change in synaptic functions or in hippocampus-dependent learning and memory was observed in an AD mouse model in which Pyk2 was genetically depleted. Nevertheless, overexpression of Pyk2 in the hippocampus of AD mice significantly improved their memory and learning ability due to increase in Pyk2 activation levels and a consequent decrease in Src cleavage which results in the formation of a neurotoxic form of Src kinase [23].

Targeted deletion of the mouse *Fak* gene in the developing dorsal forebrain, resulted in local disruption of the cortical basement membrane, suggesting that FAK plays a key signaling role in cortical basement membrane assembly and remodeling [24]. FAK is abundantly expressed in the nervous system and is particularly enriched in the cortex and hippocampus, brain regions that are pivotal for learning and memory [9, 13, 14]. A central role for FAK in regulation of the neuronal growth cone has been suggested, but conflicting data exist regarding its specific role in neuritic outgrowth and axonal guidance during brain development. Along these lines, cell-specific deletion of FAK increases the number of axonal terminals and synapses formed by neurons *in vivo* and induces a larger number of axonal branches in cultured neurons, indicating FAK as a negative regulator of axon branching and synapse formation [25]. In contrast, other studies suggest that FAK promotes neuritic extensions, possibly through inhibition of ATP-gated P2X7 receptor [26], and that netrin recruits and activates FAK, which mediates netrin downstream signaling and consequent positive effect on axonal guidance and outgrowth [27]. To explain this controversy, it was suggested that FAK integrates various axonal guidance cues and, depending on the sum of all specific inputs, activates different corresponding signaling pathways [28].

The role of FAK in the regulation of synaptic plasticity and consequent cognitive behavior has never been examined in a genetic knockout model, but a role in these processes *in vivo* was demonstrated by using 1,2,4,5-benzenetetraamine tetrahydrochloride (Y15), a specific FAK inhibitor that targets the autophosphorylation site of the protein. Using this inhibitor, the authors demonstrated that FAK is involved in regulation of hippocampal neuritic outgrowth, synaptic function, and consequently hippocampus-mediated spatial learning and memory *in vivo* [29].

Although the role of FAK in AD has yet to be determined, evidence suggesting its involvement in the disease does exist. For example, it has been previously demonstrated that amyloid β oligomer (Aβo) treatment of primary human and rat cortical cultures leads to the association of Fyn with FAK and consequent increase in FAK tyrosine phosphorylation and activation [30]. Activation of FAK was also observed in the olfactory bulb of AD patients, where dysfunction is considered as an early event in disease prognosis [31]. These data suggest that FAK may have a compensatory role for Pyk2 in AD, and that the lack of synaptic and behavioral phenotype in AD mice in which Pyk2 was genetically deleted [23] may be explained, at least in part, by a compensatory effect of FAK [32].

4. FAK family kinases and the neuronal cytoskeleton

Neuronal dendrites are responsible for receiving, processing, and integrating all the information that the neuron receives in the form of internal and external cues to generate pertinent responses. The branching pattern and the extent of dendrite branching are directly associated with the number and distribution of inputs that the neuron receives and processes [33]. Hence, dendritic branching, synapse formation and stabilization play critical roles in the structural and functional plasticity of the brain. In the mature nervous system, the cytoskeleton within dendritic branches consists of a packed network of microtubules, which provide structural stability, anchors organelles and serves as a highway for the transport of cargoes which include building materials for dendrites and organelles [34]. Dendritic spines are enriched in filamentous (F) actin, which provides shape to the spine, organizes the postsynaptic signaling machinery, regulates changes in spine structure, and maintains spine stability [35–39]. Although the structure of dendritic spines and branches is stable for months or years, the individual actin and microtubule filaments that make up these structures turn over in minutes to hours in both the developing as well as in the mature nervous systems. Structural stability and functional integrity are achieved by proper regulation of the molecules that control dendritic cytoskeletal signaling and dynamics. Dendritic spines contain signaling proteins that promote the formation and dissociation of actin filaments and microtubule networks and stabilize these cytoskeletal structures. In addition to shaping the dendritic spine cytoskeleton, these proteins ensure that only a fraction of the cytoskeleton undergoes remodeling at any given time, and that existing actin filaments and microtubule networks can both maintain dendritic structure and serve as scaffolds for their own replenishment [40].

Alterations in dendrite morphology or defects in neuronal development, including changes in dendritic branching patterns, fragmentation of dendrites, retraction or loss of dendrite branching, as well as changes in spine morphology and number, contribute to several neurological and neurodevelopmental disorders such as autism spectrum disorders, AD, schizophrenia, Down syndrome, fragile X syndrome, Rett syndrome, anxiety, and depression [41]. Various

studies report that many neuropsychiatric disorders are characterized by dendritic and synaptic pathology, including abnormal spine density and morphology, synapse loss, and aberrant synaptic signaling and plasticity [6]. The altered synaptic connectivity resulting from dendrite arbor and spine destabilization is thought to contribute to the impaired perception, cognition, memory, mood, and decision-making that characterize these pathological conditions [42]. The identification of pathways that mediate stabilization of dendritic arbors and spines in normal brain and their mis-regulation in brain disorders raise their profiles as targets for therapeutic intervention [40].

Both Pyk2 and FAK are well known as regulators of cytoskeletal signaling, however very little information exists to date regarding their role in dendritic branching and spine stability. Epistasis experiments in cultured neurons suggest that Pyk2 inhibits, while FAK promotes, dendritic branching during adhesion-dependent, protocadherin-mediated self-avoidance of sister dendrites [43]. In another study, FAK was shown to act downstream of Ephrin B receptors in hippocampal neurons to control the stability of mature dendritic spines, by activating the RhoA-ROCK-LIMK1 pathway, that promotes cofilin phosphorylation and its consequent inhibition [44].

Co-localization of Pyk2 with PSD-95 positive puncta within dendritic spines has been recently shown by immunofluorescence and electron microscopy [19]. This study demonstrates a reciprocal interaction between Pyk2 and PSD-95, each enhancing the function of the other, thereby regulating NMDAR function and the organization of the post-synaptic density within hippocampal neurons. Accordingly, the number of PSD-95 positive puncta and the density of dendritic spines were significantly decreased in the hippocampi of mice lacking Pyk2. While spine head size was not changed, the spine neck length was decreased. This phenotype is reminiscent of the one observed in the HD mouse model, which was used in the same paper [19]. Overall, these data support a role of Pyk2 in the regulation of post-synaptic density organization and dendritic spine morphology, which may explain the cognitive phenotype of Pyk2 knockout and HD transgenic mice.

5. FAK family, neuronal signaling pathways, and brain disorders: a bioinformatics landscape

To investigate the role of Pyk2 and FAK in neuronal signaling pathways from a bioinformatics perspective, we retrieved Pyk2- and FAK-associated protein relationships using Ingenuity Pathway Analysis (IPA; QIAGEN Bioinformatics). A list of Pyk2- and FAK-interacting proteins was retrieved based on experimental validation and direct relationship comprising protein-protein interactions and upstream/downstream phosphorylation from IPA. Additionally, a set of protein-protein interactions and kinase substrates of Pyk2, which was recently published [45], was added to the combined list of Pyk2-interacting proteins (Fig. 2 and Supplementary Table 1).

Analysis of the network connectivity map of Pyk2 and FAK with their respective neuronal signaling proteins revealed several directly interacting proteins that are shared by both Pyk2 and FAK, such as Src family kinases (SRC, FYN, YES, and LYN), EGFR and ERBB2, integrin β3 (ITGB3), regulatory subunits of phosphoinositide-3 kinase (PI3K) (PIK3R1 and PIK3R3), several adaptors and scaffolding proteins such as GRB2, SHC1, NCK1, NCK2, paxillin (PXN), Crk family proteins (CRK and CRKL), and BCAR1, the GTPase activating proteins RASA1 and GIT1, the tyrosine phosphatase SHP2 (PTPN11), the dual specificity and lipid phosphatase PTEN, the serine/threonine kinase GSK3B, and the molecular chaperone HSP90AA1.

Among the proteins that uniquely and physically interact with Pyk2, we found glutamate metabotropic receptors 1 and 5 (GRM1, GRM5), glutamate ionotropic receptors 2A and 2B (GRIN2A and GRIN2B), fibroblast growth factors 2 and 3 (FGFR2, FGFR3), and integrin subunit β2, which is most abundant in hematopoietic cells (ITGB2). Other unique proteins that interact directly with Pyk2 include the post-synaptic density scaffolding proteins HOMER2 and HOMER3, the phospholipase Cγ2 (PLCγ2), the regulatory subunit 2 of PI3K (PIK3R2), JAK1 and JAK3 which are non-receptor tyrosine kinases involved in cytokine receptor-mediated signal transduction, dynamin 1 (DNM1) which is a GTPase involved in clathrin-mediated endocytosis and vesicular transport processes, and WAS/WASL-interacting protein family member 1 (WIPF1). For FAK, we found uniquely interacting receptors and isoforms, such as the insulin-like growth factor receptor 1 (IGF1R), RET receptor, integrin subunits β1 (ITGB1) and α4 (ITGA4), the ephrin receptors EPHA2 and EPHB2, the netrin 1 receptor DCC, and other directly interacting proteins such as the phospholipase Cγ1 (PLCγ1), the protein phosphatase 1 catalytic subunits α and β (PPP1CA and PPP1CB), MAPK8 and MAPK8 interacting protein 3 (MAPK8IP3), JAK2 and STAT1, which are involved in cytokine signaling, RHOU and the Rac1 guanine nucleotide exchange factor 7 (ARHGEF7). The unique interactors of each kinase may be responsible for regulation of specific neuronal signaling pathways by Pyk2 or FAK, while the common interactors may be related to compensatory activities of the two kinases. Overall, these data suggest that Pyk2 and FAK have some unique and other overlapping activities in the brain.

Next, we performed canonical pathway enrichment using IPA for each Pyk2 and FAK and manually extracted neuronal signaling pathways. To explore which neuronal pathways are the most significant for Pyk2 or for FAK, we performed comparative core analysis in which we analyzed the difference in the significance of the enriched neuronal pathways for each (Fig. 3 and Supplementary Table 2). Our analysis revealed that for Pyk2, the top significant pathways were glutamate receptor signaling ($\Delta \log (p\text{-value}) = 3.16$) (Fig. 4), neuropathic pain signaling in dorsal horn neurons ($\Delta \log (p\text{-value}) = 2$) (Fig. 5), and synaptic long-term potentiation ($\Delta \log (p\text{-value}) = 1.62$) (Fig. 6). For FAK, the most significant pathways were axonal guidance signaling ($\Delta \log (p\text{-value}) = -17$) (Fig. 7A and Fig. 7B), Reelin signaling in neurons ($\Delta \log (p\text{-value}) = -10$) (Fig. 8), and semaphorin signaling in neurons ($\Delta \log (p\text{-value}) = -4.63$) (Fig. 9).

To connect the Pyk2- and FAK-associated neuronal signaling pathways to brain disorders, we performed extensive literature-mining on the significant canonical pathways associated with each kinase (Table 1). For Pyk2, most of the disorders associated with the top three significant canonical pathways relate to neurodegenerative diseases such as PD, HD, AD, and schizophrenia, all of which are associated with LTP [50]. Additionally, we identified a novel role for Pyk2 in neuropathic pain, a type of pain that arises from damage to the nervous system, which may result from surgery, diabetic neuropathy, amputation, viral infection, neuronal trauma, and nerve compression. Inflammation or disrupted neuronal activities induce several signaling pathways in post-synaptic dorsal horn neurons, which mediate induction and maintenance of neuropathic pain by both transcriptional and post-translational mechanisms. The most common expression of neuropathic pain is mechanical allodynia,

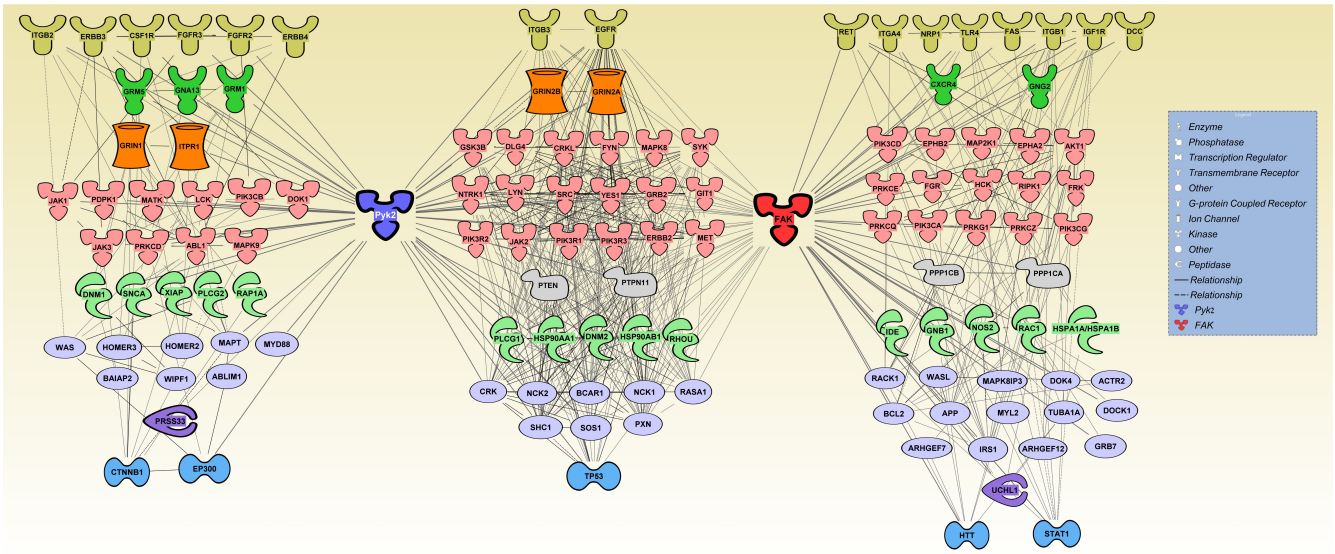

Fig. 2. Network maps of Pyk2, FAK, and their respective neuronal interacting proteins. A list of neuronal proteins that interact with Pyk2 (left), FAK (right) or both was extracted from the top corresponding neuronal canonical pathways that were obtained from IPA. The connection of Pyk2 or FAK to each protein was manually validated by using Google Scholar and PubMed search (see Supplementary Table 1). Solid lines represent direct relationship between two proteins, whereas dashed lines represent indirect relationship.

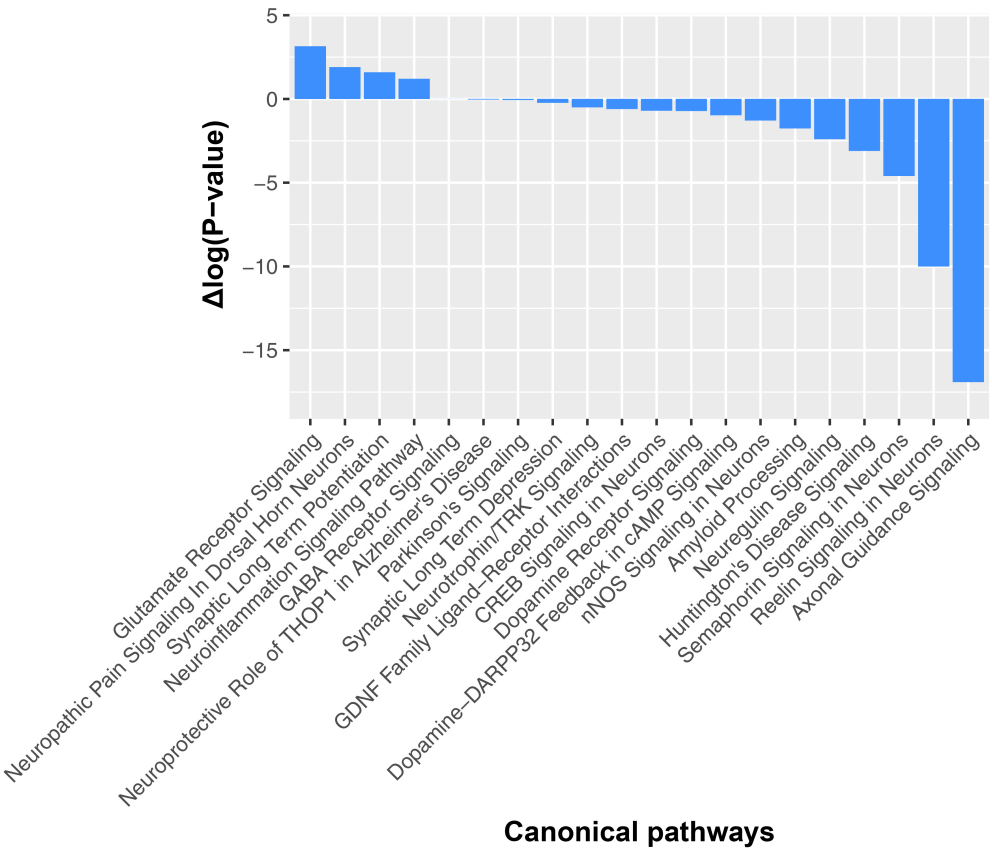

Fig. 3. A comparative core analysis of top canonical pathways for Pyk2 and FAK. Experimentally validated phosphorylation and protein-protein interaction data were obtained from IPA and data previously published [45], from which neuronal canonical pathway enrichments for Pyk2 and FAK associated proteins was performed. The bar plot represents the difference in significance of the enriched pathways based on Pyk2 versus FAK relationships. Significance was examined using the Fisher's exact test.

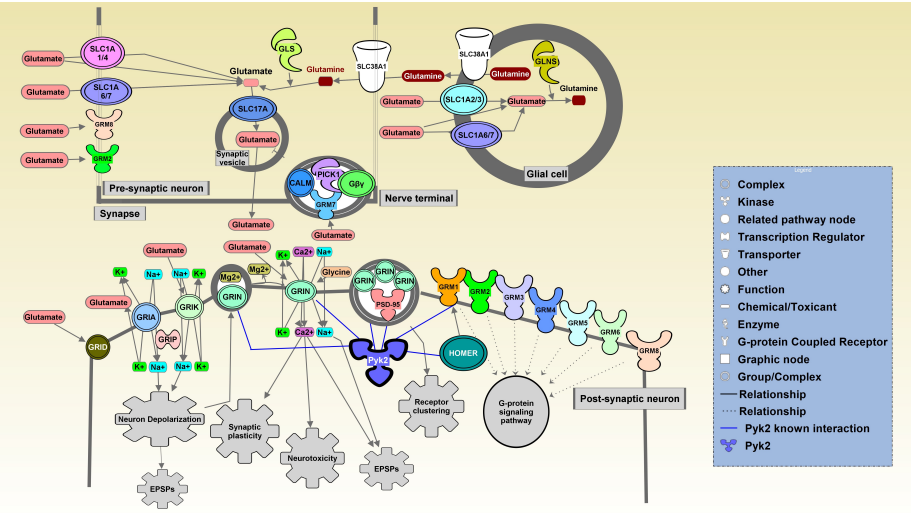

Fig. 4. Glutamate receptor signaling. Glutamate is a non-essential amino acid and a predominant excitatory neurotransmitter in the brain. In the nervous system, glutamate plays a crucial role in learning and memory of an organism. It also has a critical role in LTP and synaptic plasticity of neurons. Glutamate receptors are classified into two types: metabotropic receptors (GRM) and ionotropic receptors (GRIN). Metabotropic receptors are involved in the metabolic formation of secondary messengers whereas ionotropic receptors are ligand-gated ion channels. Ionotropic receptors are further classified into NMDA and AMPA receptors. The receptors are activated by glutamate, which results in the Na^+ ion-mediated depolarization in the neuron and development of excitatory post-synaptic potential (EPSP). Shown is a representative image from IPA depicting the pathway of glutamate receptor signaling with known Pyk2 interactions. Blue lines indicate the connection between Pyk2 and its interactors in the glutamate pathway. Other relevant interactions for this pathway, which do not connect with Pyk2 directly, are shown in gray. The connections of Pyk2 to each of the proteins were manually validated by using Google Scholar and PubMed search.

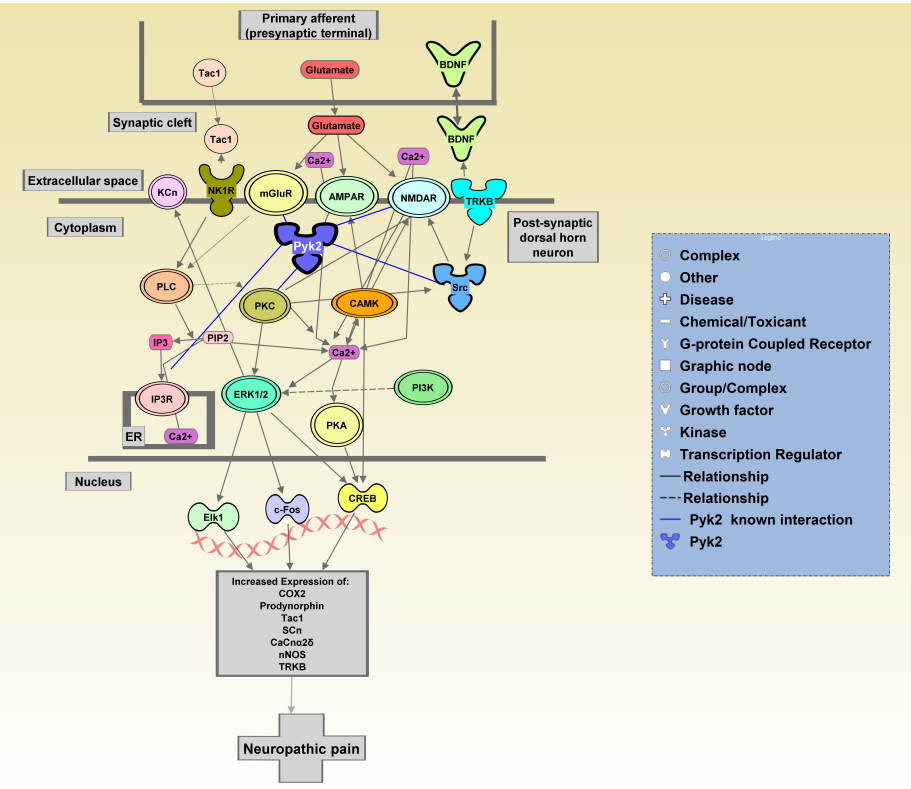

Fig. 5. Neuropathic pain signaling in dorsal horn neurons. Neuropathic pain refers to the pain that originates from pathology of the nervous system. These pathologies may result from surgery, diabetic neuropathy, amputation, viral infection, nerve trauma and nerve compression. The most common symptom of neuropathic pain is mechanical allodynia, which is a painful sensation caused by innocuous stimuli such as light touch [46]. The pathway depicts the signaling cascade of neuropathic pain and the possible involvement of Pyk2. Blue lines indicate the known interactions between Pyk2 and other proteins involved in the pathway. Other interactions that do not involve direct interaction with Pyk2 are depicted in gray. The connections of Pyk2 to each of the proteins were manually validated by using Google Scholar and PubMed search.

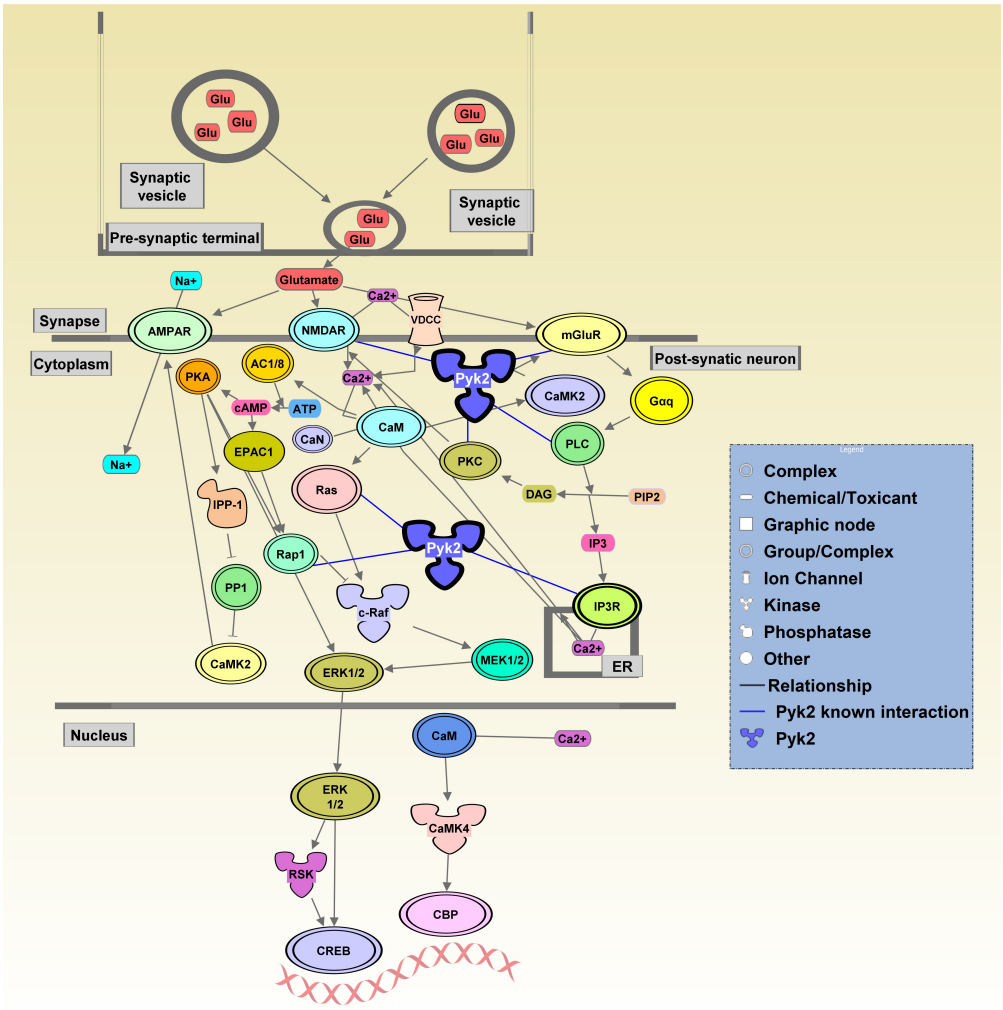

Fig. 6. Synaptic long-term potentiation (LTP). LTP is an increase in the strength of synapse between two neurons followed by high stimulation. It plays an important role in learning and memory and in synaptic plasticity. In the hippocampus, LTP induction requires the activation of post-synaptic NMDA receptors (GRIN). Ca^{+2} influx through NMDA receptors results in the activation of ERK, cAMP signal transduction pathways and calcium/calmodulin-dependent protein kinase II (CamKII). Activation of these pathways induces a rapid increase in the number of AMPA receptors at the synapse. In addition to the ionotropic receptors, the metabotropic glutamate receptors (GRM) also play a role in LTP. These receptors activate, via G-protein coupled receptors (GPCRs), the phospholipase C (PLC)/protein kinase C (PKC) pathway, which triggers the NMDA receptor and thus increase Ca^{+2} influx [47–49]. The pathway highlights the important components of long-term potentiation and the involvement of Pyk2 in the pathway. Shown is a representative image of the pathway depicting direct interactions between Pyk2 and other proteins involved in this pathway (blue lines). Other interactions that do not involve direct interaction with Pyk2 are depicted in gray. The connections of Pyk2 to each of the proteins were manually validated by using Google Scholar and PubMed search.

which results in painful sensations caused by innocuous stimuli such as light touch [46].

Importantly, another signaling pathway that appeared as significant for Pyk2 over FAK was neuroinflammation (Δ log (*p*-value) = 1.2) (Fig. 3). Neuroinflammation is an inflammatory process of the nervous tissue that may result from a variety of cues such as infection, traumatic brain injury, toxic metabolites, and autoimmunity. Microglia are innate immune cells within the CNS, that are initially activated in response to these cues, followed by recruitment of peripheral immune cells that penetrate the brain through the compromised blood-brain-barrier (BBB) [73]. As Pyk2 is highly expressed in hematopoietic cells including microglia, which are immune cells originating from the myeloid lineage, it is not surprising that this kinase is involved in inflammatory processes including that of the brain and spinal cord. Neuroinflammation may be relevant to several disorders such as multiple sclerosis, AD, HD, PD, as well as to autism [54] (Table 1).

For FAK, most of the disorders associated with the top three significant canonical pathways relate to neurodevelopmental disorders such as neuronal development, corpus callosum dysgenesis, cystic fibrosis, and Joubert syndrome and related disorders (JSRD), in which axonal guidance plays a major role. These data strongly support the suggested role of FAK in neuronal development. Furthermore, we found a connection between reelin and semaphorin signaling pathways to ALS, schizophrenia, mood disorders, and AD. Interestingly, among the less significantly different pathways for FAK, we found HD-related signaling (log (*p*-value) = −3.1), and amyloid processing (log (*p*-value) = −1.769), which is directly related to AD [64] (Fig. 3 and Table 1). The involvement of FAK in these pathways may suggest a possible role in compensation for Pyk2, which could

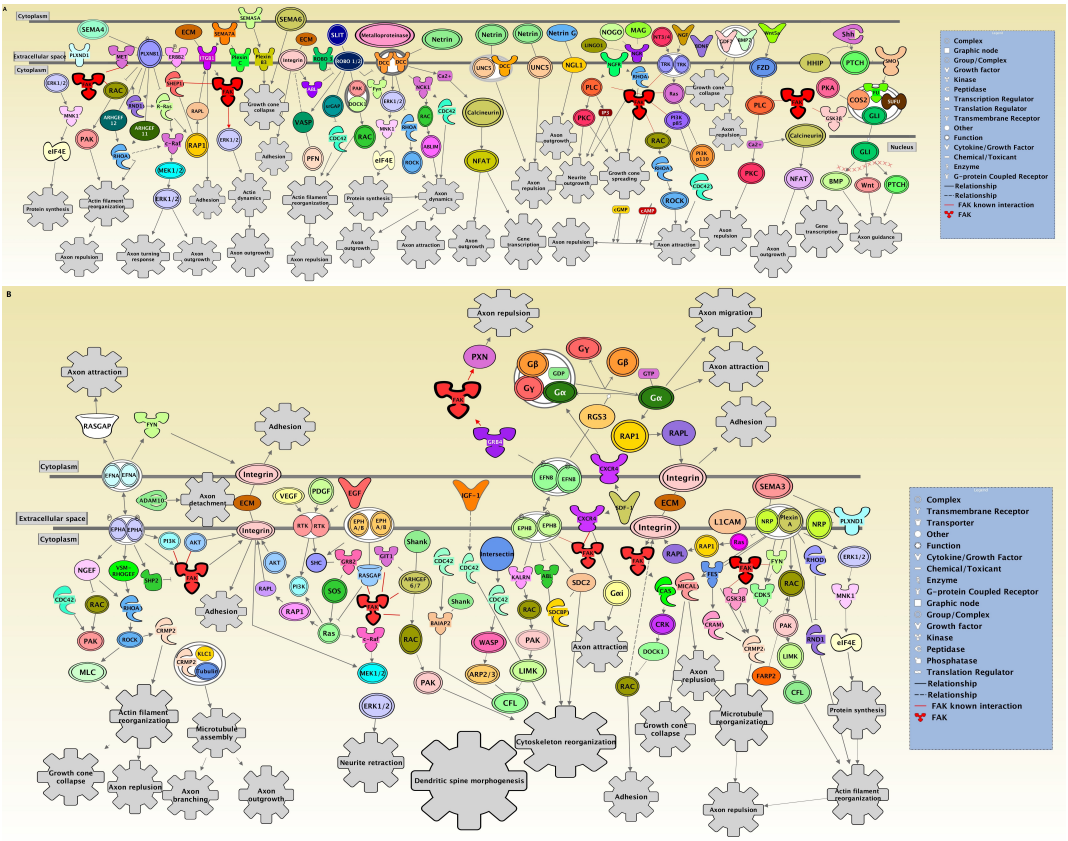

Fig. 7. (A, B), Axonal guidance signaling. Axonal guidance, also known as axon pathfinding, is a process by which growing nerve fibers find their targets in the developing brain. The axonal growth cone, located at the axon leading edge, contains receptors that sense attractive and repulsive guidance cues, which help navigate the axon to its destination. These attractive and repulsive guidance cues are guided through four major families of guidance molecules and receptors including: 1) Netrins, DCC, and UNC-5 receptors, 2) Slits and Robo receptors, 3) Semaphorins, plexin, and neurophilin receptors, and 4) Ephrins and ephrin receptors. Shown is the IPA canonical pathway of axonal guidance with known protein-FAK interactions. Red lines indicate the connection between FAK and other proteins involved in the pathway. Other relevant protein interactions for this pathway, which do not involve FAK, are shown in gray. The connections of FAK to each of the proteins were manually validated by using Google Scholar and PubMed search.

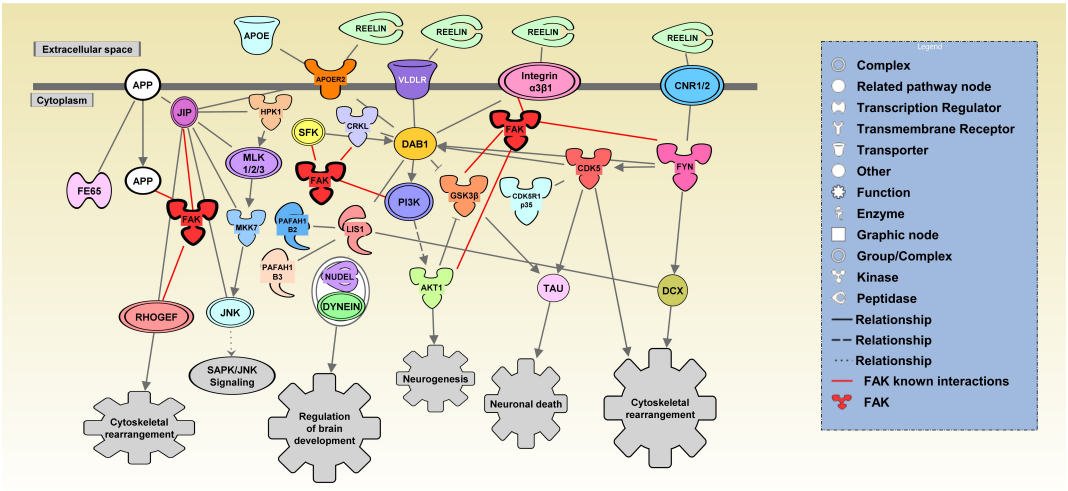

Fig. 8. Reelin signaling in neurons. Reelin is a large extracellular glycoprotein that plays a crucial role in regulating migration of neurons and proper positioning of the cortical layers in the developing brain. In the adult brain, it assists in the maintenance of synapses and helps in the stimulation of dendrites and dendritic spines. Shown is a representative IPA canonical pathway of Reelin signaling in neurons. Red lines indicate the connection between FAK and other proteins involved in the pathway. Other relevant protein interactions for this pathway not involving FAK, are shown in gray. The connections of FAK to each of the proteins were manually validated by using Google Scholar and PubMed search.

Table 1. Top neuronal canonical pathways of Pyk2 and FAK and their related brain disorders.

Canonical pathway	Associated disorders	References
Glutamate receptor signaling	Parkinson's disease Huntington's disease Alzheimer disease	[51]
Neuropathic pain signaling in dorsal horn neurons	Mechanical allodynia	[52]
Synaptic long-term potentiation	Parkinson's disease Alzheimer's disease Huntington's disease Schizophrenia	[53]
Neuroinflammation signaling pathway	Multiple sclerosis Alzheimer's disease Huntington disease Parkinson's disease Autism	[54]
GABA receptor signaling	Autism Alzheimer's disease Parkinson's disease Hyperactivity disorder	[55, 56]
Neuroprotective role of THOP1 in Alzheimer's disease	Alzheimer's disease	[57]
Parkinson's signaling	Parkinson's disease	[58]
Dopamine receptor signaling	Parkinson's disease Huntington's disease	[59]
Synaptic long-term depression	Parkinson's disease Alzheimer's disease Huntington's disease	[60]
Dopamine-DARPP32 feedback in cAMP signaling	Schizophrenia Obsessive-compulsive disorder	[61]
NOS signaling in neurons	Alzheimer's disease Huntington's disease	[62]
CREB signaling in neurons	Rett syndrome Cognitive impairments	[63]
Amyloid processing	Alzheimer's Disease	[64]
Neurotrophin/TRK signaling	Alzheimer's disease Huntington's disease	[65]
GDNF family ligand-receptor interactions	Epilepsy Alzheimer's disease Parkinson's disease	[66, 67]
Huntington's disease signaling	Huntington's disease	[19]
Neuregulin signaling	Alzheimer's disease Myelination	[68]
Semaphorin signaling in neurons	Amyotrophic lateral sclerosis Alzheimer's disease	[69]
Reelin signaling in neurons	Alzheimer's disease Schizophrenia Mood disorders	[70, 71]
Axonal guidance signaling	Neuronal Development Corpus callosum dysgenesis Cystic fibrosis Joubert syndrome and related disorders (JSRD)	[72]

While FAK is associated with signaling pathways that are activated by integrins and growth factor receptors, Pyk2 is activated by a variety of other extracellular cues and receptors, including G-protein coupled receptors, inflammatory cytokine receptors, increase in extracellular or intracellular Ca^{+2} concentrations, as well as integrins in certain cell types [11, 12]. The different expression patterns, localization, and non-kinase domain-dependent binding activities of Pyk2 and FAK may limit their functional redundancy and propose distinct roles within cells.

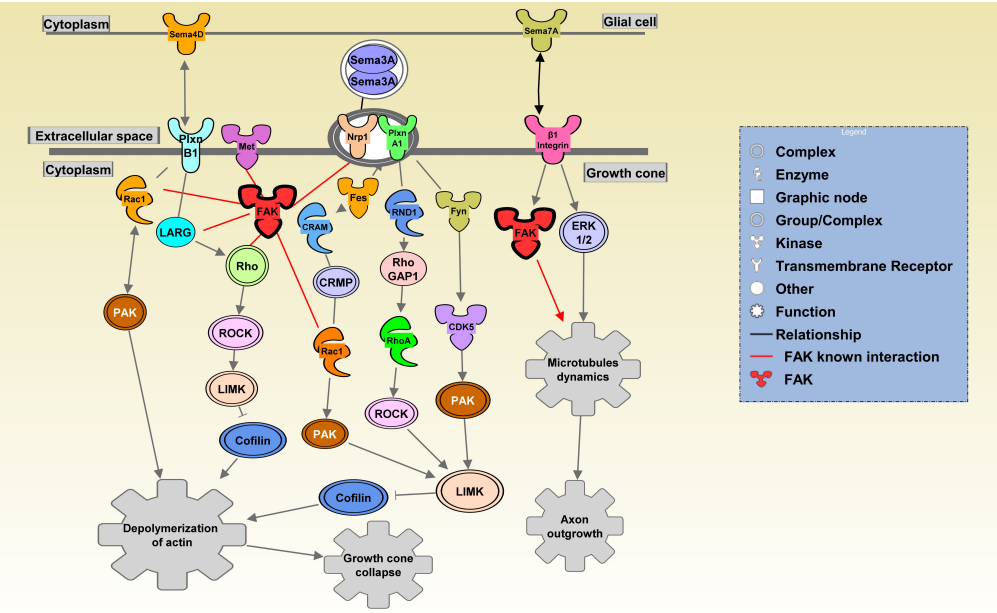

Fig. 9. Semaphorin signaling in neurons. Semaphorins are a large family of membrane-associated proteins that play a crucial role in the regulation of diverse developmental processes. Semaphorins are known for their ability to provide attractive or repulsive cues for migrating cells and growing neurites, i.e. dendrites and axons. One of the important downstream outputs of semaphorin signaling is actin depolymerization, which is enhanced by cytoskeletal proteins such as cofilin and PAK. Shown is a representative IPA canonical pathway of semaphorin signaling in neurons. Red lines indicate the connection between FAK and other proteins involved in the pathway. Other relevant protein interactions for this pathway not involving FAK are shown in gray. The connections of FAK to each of the proteins were manually validated by using Google Scholar and PubMed search.

explain the relatively mild phenotypes that were observed for Pyk2 in HD and AD mouse models in two recent publications [19, 23].

6. Conclusions and future perspectives

Brain disorders are a major health concern at all stages of human life. From neurodevelopmental disorders at early age, to neuropsychiatric disorders among adolescents and adults, and to dementia among the elderly, brain disorders affect up to 20% of the population. Because existing pharmacological and behavioral treatments are at best only partially effective, a transformational research approach that will provide fundamental insights into the molecular basis of these diseases and ultimately into the development of new therapies, is of urgent need.

Pyk2 and FAK are highly expressed in both the developing and adult brain, but not much is known about their specific roles in brain and behavior as well as their contribution to brain disorders. Using literature mining and bioinformatics, we have summarized all existing knowledge regarding the roles of FAK family in the brain, and correlated their known neurological signaling pathways and specific brain disorders. Despite several recent publications that used genetic mouse models to explore the role of FAK family members in brain and behavior, several gaps in our knowledge and understanding still exist. For example, Pyk2 regulates dendritic spine density and shape in hippocampal neurons, however the molecular mechanism by which it does so, remains undefined. Furthermore, the role of FAK in regulating hippocampal synaptic plasticity and consequent memory and learning was demonstrated by using a small molecule kinase inhibitor, however the effect of a complete deletion of the gene in a specific region of the brain, was not explored to date.

Our literature search and bioinformatic analysis suggests distinct roles for Pyk2 and FAK in the brain, which may partake in different developmental stages. However, the high similarity between the two proteins, together with their partially overlapping patterns of expression and activity within the brain, and the low severity of the Pyk2 knockout brain phenotype, suggest some degree of compensation between the two kinases. Research into the molecular mechanisms which are regulated by each of the two kinases, and into their unique and overlapping functions in brain and behavior, would be an attractive subject for future investigation. Such research may lead to new therapeutic strategies for preventing or treating the underlying causes of neurodevelopmental defects, neuropsychiatric disorders, and neurodegenerative diseases.

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Conflict of Interest

The authors declare that they have no conflict of interests.

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