Rationalization for research on Shank genes affecting differential PNS synaptic transmission

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Summary

Differential synaptic transmission has been linked with phenotypic differences associated with autism spectrum disorder (ASD), especially sensory and motor abnormalities (Orefice et al., 2019; Schaffler et al., 2019; Vyas et al., 2021). Shank genes are multidomain scaffolding proteins expressed in both the central nervous system (CNS) and peripheral nervous system (PNS). Shanks are involved in the organizational efforts of the postsynaptic density concerning the development, function, and maturation of synapses (Jung and Park, 2022; Raab et al., 2010). The distinct role of Shank genes within the neural process of synaptic transmission is underresearched in the context of the PNS. While a specific candidate gene, Shank3, is linked with susceptibility to developing autism (Mashayekhi et al., 2021), the role of Shank genes in differential PNS synaptic transmission, and especially how Shank3 expressed in the PNS differentially contributes to ASD phenotypes caused by PNS synaptic dysfunction, is not well-understood. Comparison of synaptic electrical activity and observable behavioral, sensory, and motor differences between wild type and PNS- and CNS-specific Shank3 loss-of-function mouse models will allow for understanding of how disrupted Shank functioning contributes to sensory and motor deficits via mechanisms of dysfunctional peripheral synaptic transmission. New insights into how PNS disease mechanisms influence behavior, information processing, and quality of life for ASD patients is essential and can build upon prior research performed with Shank genes in the central nervous system.

Background

Phenotypic Characteristics of Autism Spectrum Disorder

The pathophysiological mechanisms characterizing autism spectrum disorder (ASD) are varied and remain elusive in many ways, inviting the need for continued research. Phenotypic differences characteristic of individuals with ASD include disruptions to social, behavioral, and sensory functioning, observable early in development and persistent into adolescence and adulthood. Hyperreactivity or hyporeactivity to sensory stimuli, defined by over-responsiveness or under-responsiveness to sensory input respectively, represent major symptoms of ASD (American Psychological Association, 2013). Phenotypic differences in motor movements characterizing ASD may be repetitive or stereotyped in that they are rhythmic, coordinated, suppressible, fixed, and not necessary for typical functioning (American Psychological Association, 2013; Ghanizadeh, 2010). It is these sensory and motor deficits that inspire further research and offer the initial empirical link between autism and the peripheral nervous system (PNS).
Further research of autism spectrum disorder specific to sensory and motor functioning and disrupted synaptic pathology is significant for understanding how autism differentially affects the brain during development and how disease mechanisms directly influence behavior, information processing, and quality of life into adulthood. While expansive research is needed to understand how the role of Shank genes in differential PNS synaptic transmission causes sensory and motor abnormalities, ASD is also characterized by social deficits, intellectual disability, and memory impairments, all influenced by disrupted information processing resulting from synaptic dysfunction (American Psychological Association, 2013; Jung and Park, 2022; Vyas et al., 2021). Social touching during development is correlated with secure attachment in relationships, cognitive development, language formation, and increased communicative ability in the form of nonverbal social rewards such as smiling, gesturing, and successful conversation (Cascio et al., 2019). Understanding disease mechanisms during development results in better understanding and treatment of disorders manifested in adulthood.

Peripheral Nervous System (PNS)

The peripheral nervous system consists of neural tissue not belonging to the brain or spinal cord, including nerves that project from muscles and sensory organs to the central nervous system (CNS) where that stimulus information is integrated, and nerves that project from the CNS to muscles to coordinate their contraction and movement (Striedter, 2016). Disruption to peripheral nervous system functioning is influenced by physiological and, as will be explored here, genetic factors. Genetic mutation of Shank genes is associated with autism spectrum disorder. Shank mutations have been shown to alter PNS mechanisms linked with sensory abnormalities such as tactile hypersensitivity (Orefice et al., 2019; Schaffler et al., 2019), as well as memory and motor impairments (Vyas et al., 2021). The influence of Shanks within the PNS may result in differential synaptic transmission that disrupts integration of sensory (especially tactile) information and motor outputs in ASD patients.

Shanks

Shank (multiple ankyrin repeat domain) family genes are multidomain scaffolding proteins known to be expressed within the CNS, largely in the hippocampus and sensory cortex. Shank function within the PNS is underresearched (Raab et al., 2010) though expression is evident in organs such as the kidneys, heart, liver, pancreas, testes, and spleen (Raab et al., 2010; Lim et al., 1999; Yao et al., 1999; Zitzer et al., 1999). Shank proteins largely reside in the postsynaptic density (PSD), a protein complex associated with the postsynaptic membranes of excitatory synapses that is important for neurotransmission, synaptic transmission, and synaptic plasticity (Kaizuka and Takumi, 2018). Shanks in the PSD contribute to the development, function, and maturation of synapses (Jung and Park, 2022; Raab et al., 2010). More specifically, Shanks have been observed to organize signal transduction within the postsynaptic density of excitatory glutamatergic synapses in the central nervous system (Boeckers et al., 1999; Kennedy, 2000; Vyas et al., 2021; Raab et al., 2010). Shanks in the CNS PSD especially impact the strength of synaptic transmission, thereby influencing the manner in which information is processed and encoded in the brain (Kennedy, 2000). Variability in information processing may contribute to the differences observed in ASD patients with disordered processing of sensory stimuli. It is therefore of interest to determine if this relationship between Shanks and strength of synaptic transmission exists outside of the CNS and within the PNS. A key member of this gene family, Shank3 (SH3 and multiple ankyrin repeat domain 3), is a leading candidate gene for autism spectrum disorder, as mutation of Shank3 is associated with increased susceptibility to developing autism and loss of Shank3 function has been shown to obstruct synaptic function and signaling through disruption of synaptic maturation (Mashayekhi et al., 2021; Jung and Park, 2022), as well as increase hypersensitivity to tactile stimuli when knocked-out of excitatory GABAergic synapses in the CNS (Chen et al., 2020).

Integrated Research Aim

Due to the presence of Shanks within the PNS (Raab et al., 2010), the characterization of Shank3 as a candidate gene for autism spectrum disorder (Jung and Park, 2022), and the ability of Shank3 to alter synaptic pathology (Vyas et al., 2021), the prospect of research on the role of Shank genes in differential PNS synaptic transmission associated with sensory and motor abnormalities characteristic of ASD pathophysiology is rational and important. While the relevance of Shanks is evident in prior literature, how Shanks influence synaptic transmission directly, specifically within the PNS, and how this causes observable sensory and motor phenotypic differences in ASD patients is lacking in current scientific understanding of ASD pathology.

Proposed Research

Rationale

In order to investigate the role of Shank genes in differential PNS synaptic transmission associated with sensory and motor abnormalities, it is necessary to determine how Shanks expressed in the PNS differentially contribute to ASD phenotypes caused by PNS synaptic dysfunction. Shank proteins and their influence on synaptic transmission is critically un-

derresearched in context of the peripheral nervous system. The potential for Shanks to differentially affect synaptic transmission in the PNS is key to understanding the strength of synaptic transmission and therefore how sensory information is processed and results in hyperreactivity to a tactile stimulus, hyporeactivity to a tactile stimulus, or abnormally-repetitive or stereotyped motor movements. I hypothesize that loss of Shank3 function will disrupt synaptic transmission in PNS neurons and result in ASD-related phenotypic differences, especially in sensory and motor functioning.

Experimental Approach

To address this shortcoming empirically, I propose a controlled manipulation of Shank3 in the peripheral nervous system of mouse models. While the scope of the proposed research entertains the PNS more broadly, it may be beneficial to focus on a specific region in the mouse models. Neuromuscular junctions offer a plethora of peripheral synapses (Raab et al., 2010) and relate directly to motor activity. A group of mice with a PNS-neuronal specific knockout (KO) of Shank3 can be compared against control models whereby Shank3 is not manipulated and no prior behavioral abnormalities characteristic of ASD are observed, as well as a CNS-specific knockout of Shank3 using exon deletion (Bey et al., 2018). Mice in the wildtype condition should not experience disrupted synaptic transmission. Comparison of synaptic transmission and behavioral outputs can be made between the PNS- and CNS-specific knockout groups, allowing for greater understanding of how Shanks differentially interact with PNS synaptic mechanisms. This will allow for direct testing of my proposed hypothesis whereby I predict that PNS-specific Shank3 knockout will result in disrupted synaptic transmission and ASD-associated behavioral differences.

To ensure successful knockout of Shank3 within PNS neurons in the experimental condition, immunohistochemical staining procedures can be utilized to verify the absence of Shank3 in the postsynaptic density. ProSAP2 (proline-rich synapse-associated protein 2) is associated with the family of Shank genes both in location within the postsynaptic density and in their function in regulation of the synaptic transmission of excitatory glutamatergic synapses (Kennedy, 2000; Vyas et al., 2021; Raab et al., 2010). ProSAP2 is a competent immunohistochemical antigen when produced against ProSAP2/Shank3 (Raab et al., 2010; Boeckers et al., 2002). The ProSAP2 antigen can be derived from polyclonal rabbit DNA and produced against the carboxyl-terminus, or tail-end of the amino acid chain, of ProSAP2/Shank3, as has been successfully demonstrated in prior research methodologies (Raab et al., 2010; Boeckers et al., 2002). Once successful knockout of Shank3 in the postsynaptic density of the PNS neurons has been verified, a measure of how loss of Shank3 impacts PNS synaptic transmission is necessary.

To analyze how loss of Shank3 function impacts synaptic transmission within peripheral neuromuscular synapses, a measure of synaptic electrical activity will be performed. The logic behind this potential methodology comes from how strength of synaptic transmission and Shank3 is measured in the CNS. Synaptic strength can be analyzed by determining the electrical activity in voltage across a synapse (Haas, 2015). Measuring synaptic strength between gap junctions (Haas, 2015) has been performed on Shanks in the CNS involved in the GABAergic synaptic and dendritic function. This methodology is therefore adaptable and useful for PNS-specific analyses. All statistical analyses will be performed and analyzed for significance using SPSS. A one-way between-subjects analysis of variance will indicate any statistically-significant differences between levels of synaptic electrical activity, and therefore any significant differences in the strength of synaptic transmission observed between groups.

The data I collect on differential synaptic transmission in the PNS-knockout models will be directly compared to the models' observable behavioral phenotypes. Analysis of ASD-associated phenotypic differences of behavior is informed by prior research (Jung and Park, 2022). While the behaviors I will examine will be ASD-associated and applicable to human research, they will also be specific to the mouse model. Measurement of observable motor abnormalities can be performed through scoring of mouse self-grooming, marble-burying, or digging behavior in a controlled environment in comparison with normal motor movement and activity in the Shank3 wild type mice, as well as the CNS-KO mice. These techniques allow me to observe any repetitive or stereotyped movements that are not necessary for typical functioning (American Psychological Association, 2013; Ghanizadeh, 2010). Measurement of sensory abnormalities, specifically hypersensitivity or hyposensitivity to tactile stimuli or tactile fixation, can be performed in an open-field test or with a light-dark anxiety measure. Relevant behavioral differences between mouse models can be analyzed using a three-chamber sociability test. Interest in determining abnormal social communication can be appeased using ultrasonic vocalization measurements. Disrupted social performance will highlight how ASD pathology contributes to deficits as the organism ages, impacting their quality of life and ability to have successful interactions with others. All statistical analyses will be performed and analyzed for significance using SPSS. A one-way between-subjects ANOVA will indicate any statistically-significant differences in motor outputs, reaction to sensory input, anxiety-related behaviors, or social communication observed between conditions.

Possible Outcomes and Interpretations

The potential of this study to illuminate how disrupted Shank functioning contributes to sensory and motor deficits is valuable for the expansion of research on autism spectrum disorder. Comparison of both PNS- and CNS-specific Shank3 knockout conditions will verify that any significant synaptic differences resulting in the observed ASD-related behavioral phenotypes can be attributed to Shank functioning in PNS or CNS mechanisms, respectively. This specificity will serve to support or challenge my proposed hypothesis, as well as justify the claim that extensive further research on Shanks in the peripheral nervous system is beneficial. Though the behaviors observed require coordination with the central nervous system as well, immunohistochemical staining and analysis of synaptic electrical activity layered with observable phenotypic differences will indicate a clear role of Shank3 in differential PNS synaptic transmission associated with sensory and motor abnormalities characteristic of ASD pathophysiology in the PNS-specific knockout condition. If PNS-specific Shank3 knockout reveals weaker synaptic transmission compared to the wild type and CNS-specific KO models and results in ASD-associated motor and sensory deficits, then I can conclude that Shanks in the PNS are important for successful synaptic transmission and that loss of Shank3 function contributes ASD-associated behavioral outcomes, as hypothesized. If PNS-specific Shank3 knockout reveals stronger synaptic transmission compared to the wild type and CNS-specific KO models, I can conclude that a different component of the PNS is more important for synaptic transmission, and that Shanks may potentially hinder synaptic transmission. If no difference is observed between conditions, I can conclude that Shanks do not play the hypothesized vital role in PNS synaptic transmission, and that another mechanistic component is contributing to the ASD-associated sensory and motor abnormalities.

In light of the anticipated and potential alternative results, the significance of, and justification for, expansive research on the role of Shank genes in differential PNS synaptic transmission is evident, and investigation of their contribution to ASD pathology is valuable.

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