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Regulation of sensorimotor gating via Disc1/Huntingtin-mediated Bdnf transport in the cortico-striatal circuit

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Abstract

Sensorimotor information processing underlies normal cognitive and behavioral traits and has classically been evaluated through prepulse inhibition (PPI) of a startle reflex. PPI is a behavioral dimension deregulated in several neurological and psychiatric disorders, yet the mechanisms underlying the cross-diagnostic nature of PPI deficits across these conditions remain to be understood. To identify circuitry mechanisms for PPI, we performed circuitry recording over the prefrontal cortex and striatum, two brain regions previously implicated in PPI, using wild-type (WT) mice compared to *Disc1*-locus-impairment (LI) mice, a model representing neuropsychiatric conditions. We demonstrated that the corticostriatal projection regulates neurophysiological responses during the PPI testing in WT, whereas these circuitry responses were disrupted in *Disc1*-LI mice. Because our biochemical analyses revealed attenuated brain-derived neurotrophic factor (Bdnf) transport along the corticostriatal circuit in *Disc1*-LI mice, we investigated the potential role of Bdnf in this circuitry for regulation of PPI. Virus-mediated delivery of Bdnf into the striatum rescued PPI deficits in *Disc1*-LI mice. Pharmacologically augmenting Bdnf transport by chronic lithium administration, partly via phosphorylation of Huntingtin (Htt) serine-421 and its integration into the motor machinery, restored striatal Bdnf levels and rescued PPI deficits in *Disc1*-LI mice. Furthermore, reducing the cortical Bdnf expression negated this rescuing effect of lithium, confirming the key role of Bdnf in lithium-mediated PPI rescuing. Collectively, the data suggest that striatal Bdnf supply, collaboratively regulated by Htt and Disc1 along the corticostriatal circuit, is involved in sensorimotor gating, highlighting the utility of dimensional approach in investigating pathophysiological mechanisms across neuropsychiatric disorders.

Keywords

Cortico-Striatal circuit; Sensorimotor gating; DISC1; Huntingtin; BDNF; Dimensional approach

Introduction

Historically, wide ranges of efforts have tried to address the fundamental biological question of how the brain drives behaviors under physiological vs. pathophysiological conditions. One popular approach is to focus on a specific neuropsychiatric disorder and analyze functional outcomes by perturbing a critical genetic factor or biological mediator for the disease. This approach is proven scientifically effective when the disease diagnosis is

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defined by biological and etiological evidence, *e.g.*, in the case of Huntington's disease (HD) caused by genetic alterations in *Huntingtin* (*HTT*) gene. However, in reality, the diagnostic criteria for most psychiatric disorders are formally defined to achieve clinical reliability while sacrificing etiological validity (1). As a result, biologically heterogeneous conditions are included in each diagnosis in this "categorical" approach. To overcome this limitation, recent discussion in the clinical nosology of brain disorders has brought a "dimensional" approach, in which the mechanism for a critical behavioral trait or dimension, independent of a diagnostic category, is addressed at multiple levels (*e.g.*, molecular, cellular and circuitry levels) (2). In this approach, behavioral dimensions that are directly translatable between humans and model animals, such as rodents, are particularly appreciated from both basic and clinical neuroscience viewpoints.

Sensorimotor gating is one of these representative dimensions; it has classically been measured using prepulse inhibition (PPI) of a startle response. During the test for PPI, neurophysiological responses obtained following presentation of a startle stimulus are compared to the responses obtained to the same startle stimulus when it is preceded by a lower amplitude prepulse stimulus (3). In healthy individuals, presentation of a prepulse stimulus suppresses both the normal neurophysiological and behavioral responses to the startle stimulus. Conversely, PPI is diminished in individuals with a range of neuropsychiatric disorders, including schizophrenia and HD (4, 5). Furthermore, PPI is conserved across multiple different species (6) and deficits in PPI are observed in a series of genetic- and pharmacological-based animal models that are developed to understand mechanisms for psychosis or mood dysregulation (7–9). Multiple circuits involving the forebrain are hypothesized to modulate the inhibitory functions of the prepulse (4). Preclinical studies have demonstrated that striatal lesions disrupt PPI (10). Other studies have suggested the involvement of the prefrontal cortex (PFC) in PPI regulation (11). Nevertheless, as far as we are aware, studies that directly address the role of corticostriatal circuitry for PPI regulation are unavailable. It remains elusive how PPI and sensorimotor gating are mechanistically explained as an integrative behavioral dimension at the circuitry, and down to the cellular and molecular levels. Given the PPI deficits reported across diverse brain dysfunctions, such as schizophrenia, psychotic disorders, mood disorder and neurodegenerative disorders (4), elucidating the mechanisms of PPI will have high clinical and social impacts.

Our ultimate goal is to comprehensively understand a key mechanism for PPI and sensorimotor gating at molecular, cellular, circuit and behavioral levels by taking a hypothesis-driven dimensional approach in an integrative manner. To address this long-term goal, the present study set out to test a key role of corticostriatal circuitry in the PPI regulation in mice. In the first half of the present study, we employed electrophysiology by using microwire arrays chronically implanted in this circuitry combined with optogenetics. The goal of the electrophysiological study was to provide a proof-of-concept that mice exhibiting PPI deficits have altered gating properties in the corticostriatal circuitry *in vivo*. We then obtained this proof at the functional level in mice deficient for DISC1, the molecule that participates in biological processes underlying a wide range of psychiatric conditions (12–14). Accordingly, we next addressed potential molecular and cellular mechanisms underlying PPI deficits in association with the corticostriatal circuitry. To explore the

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mechanisms for the PPI regulation mediated by DISC1, we turned our attention to the DISC1-HTT protein complex (15) for the following reasons: (i) *HTT* deficits lead to PPI deficits in HD patients and mouse models (5, 16, 17); (ii) Htt protein complex regulates corticostriatal transport of brain-derived neurotrophic factor (BDNF) (18); (iii) a reduction in BDNF causes PPI deficits (19); and (iv) the corticostriatal projection has a unique role for supplying BDNF to the striatum (20–22). Supported by the electrophysiological data for the validity of focusing on the corticostriatal projection in studying PPI regulation, we hypothesized that the DISC1-HTT complex regulates corticostriatal BDNF transport, thereby contributing to the corticostriatal circuitry mechanism for the sensorimotor gating responses. By using both molecular and pharmacological interventions, the current study tests the hypothesis to validate this molecular concept.

Methods

We addressed sensorimotor gating mechanisms at circuitry, cellular and molecular levels in *Disc1* locus-impairment (LI) mice, a rodent model representing pathophysiological aspects of neuropsychiatric disorders (23, 24), using the following three main approaches.

In vivo electrophysiology:

Microwire electrodes were implanted in male mice (3−4 months old) to record single unit activities and local field potentials (LFPs) from the prelimbic cortex (PrL) and dorsomedial striatum (DMS) during PPI testing, according to the procedure described (25).

Prepulse Inhibition (PPI):

Mice were tested in a startle chamber (SR-LAB, CA) either with or without neurophysiological recording microwires. The startle stimulus (120 dB) or the prepulse stimulus (74, 78, 82, 86 or 90 dB) followed by the startle stimulus were randomly given to each mouse to assess the degree of PPI, as described (26).

Adeno-associated virus (AAV):

AAV encoding Bdnf, mCherry or shRNAs to Bdnf, Akt1 or scramble control were stereotaxically infused bilaterally into DMS or PrL of mice (2−3 months old, male) and the mice were tested for PPI 3 to 4 weeks post-injection.

Additional details for these approaches as well as animal husbandry, regulatory guidelines, optogenetics, behavioral assays, *ex vivo* MRI, stereotaxic virus injection, histology, molecular and cell biology are described in Supplementary Information.

Statistical Analysis

Sample size for each animal experiment was predetermined to ensure adequate statistical power for drawing conclusion. Animal experiments were statistically analyzed either by Z-test, repeated measures two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests, Kruskal-Wallis test followed by Dunn’s multiple comparisons, or Student’s t-test, as specified in the figure legend. Refer to Supplementary Information for more details.

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Results

Critical roles for the PFC and DMS in sensorimotor gating: studies with wild-type mice

Previous studies reported that PPI was affected by manipulation of the PFC or the dorsomedial striatum (DMS) (10, 11). Thus, we hypothesized that the corticostriatal projection might regulate sensorimotor gating. To test this hypothesis, wild-type (WT) C57BL/6 mice were implanted with microwire arrays (25), allowing us to simultaneously record single unit activities as well as local field potentials (LFPs) from the PFC and DMS under awake, partially-restrained conditions (*i.e.*, in a plexi-glass tube) during PPI testing, in which either the startle stimulus (120 dB; Startle trial) or the prepulse stimulus followed by startle stimulus (PPI trial) were randomly given to each mouse (Figure 1a). The baseline firing patterns of a majority of single units shown in Figure 1a are representative of those in PFC and DMS, consistent with the firing properties (firing rates and amplitude) of cortical pyramidal neurons and striatal medium spiny neurons (MSNs), respectively (Figure S1). All implantation sites were verified histologically after completing the *in vivo* recording (Figure S2).

In the single unit activity recording, we observed that a significant proportion of neurons responded to the startle stimulus of the startle trials (32% of 102 PFC cells and 34% of 80 DMS cells) and a significant portion of neurons responded to the startle stimulus during the PPI trials (36% of 102 PFC cells and 33% of 80 DMS cells) (Figure 1b). While 26% of PFC and 25% of DMS cells responded to both trial types, 16% of PFC neurons and 17% of DMS neurons responded to the startle stimulus exclusively during one trial type (startle trial or PPI trial), but not the other. Thus, both PFC and DMS activities reflected a neurophysiological correlate of sensory gating processes taking place during PPI testing. We next quantified the effect of the gating stimulus (78 dB, low amplitude prepulse stimulus) on LFP responses to the startle stimulus. Specifically, we calculated the mean evoked potential for each trial type and normalized the mean amplitude for each frequency to the amplitude observed during the 1−5 second interval prior to delivering the startle pulse (Figure 1c,d). This frequency-wise analysis approach allowed us to quantify the impact of the prepulse stimulus on the oscillatory activity induced by the startle stimulus. Using this strategy, we found that the prepulse stimulus significantly reduced the induction of PFC beta oscillations (15−30 Hz) elicited by the initial startle stimulus (Figure 1e). Taken together with our single cell observations, these analyses provided evidence that both the cortex and striatum signal the sensorimotor gating response.

Critical role of the PFC→DMS projection in sensorimotor gating: comparative studies with wild-type and *Disc1* LI mice

Based on the results above, we next hypothesized that the corticostriatal circuitry directly regulates PPI and sensorimotor gating. To address this question, we compared WT mice with a genetically-engineered model that displays deficits in sensorimotor gating. In the present study, we chose a model with a disruption at the *Disc1* locus (*Disc1* LI model, −/−) (23, 24), and confirmed robust deficits in PPI in this model (Figure 2a).

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Next, we tested the functional role of the corticostriatal projection (or PFC→DMS projection) in PPI in *Disc1* LI mice compared with WT mice. To quantitatively address the function, we implanted them with microwire arrays and used the neurophysiological parameters of the cortical and striatal activities that were used in the analysis of WT mice (Figure 1). In response to the 120-dB cue in the startle alone trial, a similar percentage of PFC neurons were activated in *Disc1* LI vs. WT mice (33% of 108 PFC units in *Disc1* LI mice, Figure S3; 32% of 102 PFC units in WT mice, Figure 1b), implying that *Disc1* LI mice have similar levels of acoustic sensation compared to WT. When a low amplitude acoustic stimulus (78 dB) was provided during the PPI testing sequence, a similar percentage of PFC neurons were activated in both WT and *Disc1* LI mice (11/102 and 11/108 cells for WT and *Disc1* LI mice, respectively), whereas DMS activation following auditory stimulation was diminished in *Disc1* LI compared with WT mice (15/80 and 2/102 cells for WT and *Disc1* LI mice, respectively; Figure 2b), suggesting that *Disc1* LI mice have diminished striatal response of sensory gating compared to WT.

In neurophysiological measures, cellular response is the function of not only the proportion (number) of cells activated but also the intensity (degree) of response per cell. To further verify differences in neuronal sensory gating signals, we used a measurement based on receiver operator characteristic (ROC) analysis, which is widely used for classifying neurons' responses to stimuli (27). In contrast to the analysis applied to averages of populations of neurons (Figure 2b), ROC analysis provides an estimate of how the activity of a single neuron differs between two stimuli on a trial-by-trial basis (*i.e.*, the quality of signal detection; Figure 2c). Using this analysis, we found that *Disc1* LI mice showed diminished striatal signaling of sensory gating compared to WT littermates (Figure 2d). On the other hand, cortical sensory gating was not significantly different between the mutants and controls (Figure 2d). Taken together with our results obtained from population analysis data, the data demonstrate functional deficits in the corticostriatal circuit of *Disc1* LI mice, which may be causally related to the sensory gating deficits. We next quantified the effect of the gating stimulus (78 dB, low amplitude prepulse stimulus) on LFP responses to the startle stimulus in *Disc1* LI mice (the same way we did for WT mice in Figure 1c,d,e). In contrast to the gating effect seen on PFC beta oscillations in WT mice (Figure 1e), the LFP responses in *Disc1* LI showed no differences between the startle trial and the PPI trial (Figure 2e), *i.e.*, impaired gating.

To more directly test our hypothesis that *Disc1* LI mice may have deficits in the PFC→DMS corticostriatal projection, we probed the projection with concurrent optogenetic and *in vivo* recording approaches (Figure 2f). We injected adeno-associated virus (AAV) encoding CaMKII-ChR2 into the PFC of *Disc1* LI mice or WT littermates, and then stimulated the PFC with light to quantify neuronal activity at both the PFC and DMS (Figure S1). When light stimulation was delivered, a similar percentage of PFC neurons responded in both *Disc1* LI and WT mice (Figure 2g), demonstrating that there is no difference in the activation of the soma of PFC neurons between genotypes. On the other hand, striatal activation in response to cortical stimulation by light was significantly diminished in *Disc1* LI mice (Figure 2g). This shows that *Disc1* LI mice exhibit impaired function of the corticostriatal projection in response to direct stimulation. Taken together, the

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electrophysiology experiments suggest that the corticostriatal projection plays a role in sensorimotor gating and that *Disc1* LI mice have deficient corticostriatal gating.

Role for striatal Bdnf through the PFC→DMS projection in sensorimotor gating

To address the mechanisms underlying functional deficits in corticostriatal gating in *Disc1* LI mice, we set out to identify potential anatomical deficits in this model. Structural magnetic resonance imaging (sMRI), a technique that addresses gross anatomical changes in an unbiased manner, showed a significant volume reduction only in the striatum and cerebellum among multiple brain regions in *Disc1* LI mice compared with controls (Figure S4). In analogy to the modest but reproducible changes in brain anatomy found in patients with mental illnesses, the volume reduction in the striatum of *Disc1* LI mice is mild, but significantly different compared with littermate controls (Figure S4). The striatum does not express *Bdnf* transcripts and thus critically depends on a supply of Bdnf through projections from several brain regions (i.e., cortex, substantia nigra, amygdala and thalamus) with cortical neurons being the major source of BDNF for the striatum (20–22). Deficits in the supply of Bdnf to the striatum lead to a reduction in striatal volume (28). The selective reduction in striatal volume and functional deficits in corticostriatal circuitry therefore prompted us to test the amount of Bdnf in the striatum of *Disc1* LI mice. We measured Bdnf by enzyme-linked immunosorbent assay (ELISA) in 1.5-, 3-, and 6-month-old mice. While no significant differences in cortical Bdnf levels were observed between *Disc1* LI and WT at any age tested, the levels of striatal Bdnf were significantly lower in *Disc1* LI mice starting from 3 months of age (Figure 3a). Notably, the cortical and striatal *Bdnf* mRNA levels in *Disc1* LI were equivalent to those in WT (Figure 3b, Figure S5). Thus, the data suggest the possibility that the decreased striatal Bdnf may, at least in part, be due to deficient Bdnf transport from the cortex to the striatum in *Disc1* LI mice. To further examine this possibility, we analyzed Bdnf transport in primary cortical neurons from *Disc1* LI and WT mice. The velocity of anterograde and retrograde Bdnf transport was reduced in *Disc1* LI compared with WT and was rescued by transfection of full-length Disc1 (Figure 3c), consistent with previous findings that Disc1 plays a role in microtubule-dependent vesicle transport in axons (29, 30).

Our findings showing low striatal Bdnf levels and functional striatal deficits in *Disc1* LI mice suggest a mechanistic link between low Bdnf levels and deficient PPI. Several mouse models indirectly suggest the effect of BDNF on PPI. For example, heterozygous *Bdnf* mutant mice have attenuated PPI (19). Adult offspring of maternal immune activation mice show reduced BDNF-TrkB signaling and PPI deficits, which are rescued with a TrkB agonist (31). To address the extent to which the striatal Bdnf reduction underlies PPI deficits in *Disc1* LI mice, we supplemented Bdnf by bilateral stereotaxic injection of AAV-Bdnf to the striatum. Three to 4 weeks after the injection, expression of Bdnf, but not the control AAV, significantly rescued PPI to WT levels (Figure 3d). These data suggest that reduced Bdnf supply to the striatum, at least in part, underlies PPI deficits in *Disc1* LI mice.

Given reduced striatal Bdnf levels in *Disc1* LI mice (Figure 3a), we sought for possible consequences of such changes at the cellular level. Disruption of Bdnf signaling in striatum (*Dlx5/6-cTrkB*^{KO}) early in development (embryonic day 12.5) leads to a large

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loss (~50%) of MSNs at postnatal day 21, preferentially affecting dopamine receptor D2 (DRD2)-positive MSNs in mice (32). In contrast, mice lacking cortical Bdnf (*Emx-Bdnf*^{KO}), which results in postnatal Bdnf ablation in forebrain, show no significant loss of MSNs up to 4 months of age, while exhibiting reduced soma size and spine density (30−40%) of MSNs at 35d of age (33). Mild loss of striatal neurons was only observed beyond 1 year old in these mice (33). Therefore, we assessed the number, size and spine density of MSNs. We found no apparent loss of both DRD1^{+} and DRD2^{+} MSNs at 3, 7 and 12 months of age (Figure S6a), no significant change in spine density (Figure S6b), and a small but significant reduction in soma size in *Disc1* LI mice as compared to WT (Figure S6c), which may in part account for the reduced striatal volume in these mice (Figure S4). Together the data suggest that, unlike developmental ablation of striatal BDNF signaling (32), partial reduction (~25%) of postnatal striatal Bdnf observed in *Disc1* LI mice (Figure 3a) may not significantly alter cellular compositions and anatomical architecture of MSNs. Instead, the neurophysiological and behavioral deficits observed in *Disc1* LI mice are likely caused by attenuated Bdnf signaling at the functional level.

Functional interplay of Disc1 and Htt in Bdnf transport and sensorimotor gating

Given the role of Disc1 in facilitating BDNF transport (Figure 3c), we further addressed the mechanisms regulating this process. We recently reported that Disc1 physically interacts with Huntingtin (Htt) at the protein level (15). Htt is a multifunctional protein causally linked to HD, and plays a crucial role in BDNF transport (18). We therefore tested possible functional interplay of Htt and Disc1 in Bdnf transport using cortical neurons *in vitro*. In our previous study showing that the amino-terminal domain of Htt (aa.1−513) interacts with the amino acid stretch (aa.201−228) of Disc1, we developed a Disc1 construct that specifically lacks the Htt-binding domain (Disc1-△201−228) while preserving other functions of Disc1 (15). When introduced in cultured neurons prepared from *Disc1* LI mice, this Htt binding-defective mutant Disc1 facilitated Bdnf transport less efficiently than the full-length Disc1 (Figure S7): Note the significant difference in Bdnf transport speed achieved by the full-length Disc1 vs. the Disc1-△201−228, reflecting the contribution of Disc1−Htt association in Bdnf transport.

Phosphorylation of Htt serine (Ser)-421 by Akt1 is crucial for efficient Bdnf transport (34, 35). Akt1 is a component of multi-protein complex with Disc1 (36, 37), and Akt1-deficient mice are also reported to have PPI deficit (38, 39), suggesting a putative role of Akt1 in regulating Htt−Disc1 activity along the corticostriatal circuitry, hence regulation of PPI. This idea was directly tested in Akt1-knockdown in PFC, showing significantly reduced PPI in mice (Figure S8). Because Akt1 is effectively activated by lithium in neurons (39–41), we next used lithium as a tool to further study the mechanisms involving Akt1, Htt and Disc1 in the regulation of PPI. Chronic lithium treatment (100 mg/kg, i.p., daily, 14 days) normalized the PPI deficits in *Disc1* LI mice (Figure 4a), and knocking down cortical *Bdnf* expression abolished this rescuing effect (Figure 4b), suggesting that the PPI rescue by lithium was mediated by Bdnf supply from the cortex. This notion is supported by two lines of evidence: lithium treatment enhanced Bdnf transport in cortical neurons from *Disc1* LI mice *in vitro* (Figure 4c), and it increased striatal Bdnf levels in *Disc1* LI mice *in vivo* (Figure 4d). Collectively, the data suggest that the corticostriatal Bdnf transport machinery, sensitive

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to lithium-mediated regulation, at least in part underlies the PPI-associated corticostriatal circuitry function.

Disturbance of phospho-Htt at Ser-421: a mechanism for Bdnf transport deficits in *Disc1* LI mice

We further investigated a possible mechanism by which lithium augments Bdnf transport through Htt phosphorylation at Ser-421 in *Disc1* LI mice. We first confirmed that lithium (Li, 2 mM in the culture media, 16h) activated Akt1 and significantly upregulated the phosphorylation of Htt Ser-421 in primary neurons in culture (Figure S9). Importantly, we observed attenuated levels of Htt Ser-421 phosphorylation in the cortices of *Disc1* LI mice compared with WT (Figure 5a). Chronic lithium administration significantly upregulated levels of Htt Ser-421 phosphorylation in *Disc1* LI cortices (Figure 5b). These results suggest that reduced phosphorylation of Htt Ser-421 may account for Bdnf transport deficits in *Disc1* LI mice.

To address the mechanism more precisely, we next evaluated additional molecular components of the Bdnf transport machinery in *Disc1* LI cortices in the presence or absence of lithium. Co-immunoprecipitation assays confirmed that some components of the Bdnf transport machinery (*i.e.,* Kinesin heavy chain [Kif5], dynactin subunit $p150^{Glued}$) (18) are less tightly associated with Htt in *Disc1* LI brains compared with brains of normal controls (Figure 5c). Importantly, lithium treatment enhanced and normalized the interaction among these components (Figure 5c). Taken together, *Disc1* LI (or possibly depletion of key isoforms of the Disc1 protein) attenuates phosphorylation of Htt Ser-421 and impairs Htt integration into the motor machinery, which negatively impacts Bdnf transport. Disc1 likely mediates this integration mechanism, as Disc1 physically interacts with Htt (15), Kif5 (29), and $p150^{Glued}$ (42), all of which are components of the motor machinery responsible for Bdnf transport (18). This mechanism, as schematically illustrated in Figure 5d, is consistent with the finding that Htt Ser-421 is phosphorylated by Akt1 (34, 35), a molecular target of lithium (40, 41). In this model, both Disc1 and lithium can facilitate Htt Ser-421 phosphorylation either by increasing Akt1 accessibility to the motor complex or by upregulating Akt1 activity, which leads to the formation of a more stable motor complex responsible for Bdnf transport.

Note that the behavioral deficits of *Disc1* LI mice are not limited to the deficit in sensorimotor gating dimension. They are hypoactive in the open field and impaired in the rotarod test (Figure S10), similar to many HD mouse models (17). However, as far as we are aware, none of these behavioral deficits in other dimensions are likely to affect the PPI deficits. The present approach of focusing on one behavioral dimension with the *Disc1* LI model allowed us to specifically integrate the mechanisms underlying sensorimotor gating at molecular, cellular and circuitry levels, demonstrating the utility of dimensional approach in studying the pathophysiological mechanisms across neuropsychiatric disorders.

Discussion

Here we report a key mechanism underlying PPI and sensorimotor gating at the molecular, cellular, circuitry and behavioral levels in an integrated manner by using a hypothesis-driven

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dimensional approach. We provided evidence suggesting that the corticostriatal (*i.e.*, the PFC→DMS) projection plays a role in eliciting normal PPI at the circuitry level and that the Disc1-containing motor complex, including Htt and Bdnf, in the PFC→DMS projection accounts for a key mechanism at the molecular level. Amelioration of the PPI deficits in the *Disc1* LI model by lithium treatment is explained by augmentation of Bdnf transport via its pharmacological action on Akt1 activity and a specific phosphorylation of Htt at Ser-421. We propose this mechanism will provide a novel and deeper understanding of sensorimotor gating along with other regulatory mechanisms for PPI previously reported.

In addition to the contribution of this study to basic molecular and behavioral neuroscience, it may have clinical significance. Sensorimotor gating deficits are widely observed in neuropsychiatric conditions, including schizophrenia, bipolar disorder (in particular acutely manic stages) and post-traumatic stress disorder (43). Furthermore, these deficits are also observed in neurological conditions, such as HD (17). Sensory gating deficits likely underlie clinical problems in distractibility due to the impaired ability to screen out irrelevant cues, cognitive fragmentation, and disintegrated thought (44). The “dimensional” approach to address brain functions for a specific behavioral trait (*e.g.*, sensorimotor gating or PPI in the present study) may encompass a much more efficient and effective strategy for discovery of drug targets for translation. Thus, the National Institute of Mental Health states that such a dimensional approach, including the Research Domain Criteria (RDoC), may be a critical element in psychiatry in the overall scope of “Precision Medicine” (2).

Here we propose that Bdnf transport facilitated by Disc1 and Htt serves as a mechanism underlying sensorimotor gating. Because Disc1 and Htt are rather ubiquitously expressed in the brain, one may wonder why and how these proteins play a particularly important role in a specific behavior (*e.g.*, sensorimotor gating or PPI) via regulation of Bdnf transport in a specific neuronal projection. We attribute this specificity to the unique dependence of the striatum on the supply of Bdnf from the cortex. This uniqueness may emphasize the particular significance of this mechanism in the corticostriatal (PFC→DMS) projection and behavior. Based on the present data suggesting that corticostriatal Bdnf transport is necessary for the regulation of sensorimotor gating, several mechanisms for striatal Bdnf can be proposed. First, Bdnf may provide neurotrophic support for striatal neurons to maintain their functional maturity necessary for eliciting proper sensorimotor gating. Alternatively, Bdnf supply from the cortex may serve as a paracrine neuromodulatory factor that determines striatal neuronal firing rates, hence facilitating efficacy of downstream neural circuitries responsible for sensorimotor gating. The current study contributes to a dimensional understanding of PPI deficits observed in a range of psychiatric and neurological disorders beyond categorical boundaries in clinical diagnosis.

We used lithium as a pharmacological probe to address a key mechanism linking Bdnf transport and sensorimotor gating deficits in *Disc1* LI mice. Lithium is likely to have multiple molecular and cellular targets. For example, although the beneficial action of lithium on PPI deficits has been reported in more than one rodent models from multiple groups, the effects of lithium appear complex and may depend on the method of modeling: chronic lithium treatment prevented amphetamine-induced PPI disruption, but not ketamine-induced PPI disruption (45). Addressing the context-dependent efficacy of lithium on PPI

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will be a future research question. Likewise, although the efficacy of lithium in treating patients with bipolar disorder has been well known and was also reported for patients with HD as case reports (46, 47), its precise mechanisms regarding molecular targets and circuit specificity still remain elusive (48). Currently accepted putative targets of lithium include inositol monophosphatase and glycogen synthase kinase-3 (48), yet we have not integrated these known pathways in addressing the lithium-mediated PPI rescuing effect in this study. In addition, calcineurin, a phosphatase, is associated with schizophrenia (49, 50) and negatively regulates Htt (Ser-421) phosphorylation and Bdnf transport (51, 52); however, whether calcineurin plays a role in lithium-mediated PPI regulation remains to be studied. Although we acknowledge these as a potential limitation of the present study, further studies are warranted to address possible involvement of these known targets in lithium-mediated rescuing effects on BDNF transport and PPI, taking into account the cellular and circuit-wide functions of this compound. Of note, while some historical pharmacological evidence suggests a major contribution of the striatal indirect ($DRD2^+$) pathway in PPI regulation (53), involvement of both the direct ($DRD1^+$) and indirect pathways in PPI regulation is becoming evident (54, 55). Further studies will need to address the possible role of dopaminergic neurotransmission for DISC1-mediated PPI regulation. We believe that this will prove useful for future translational research, including clinical trials of lithium to improve cognitive deficits that are correlated with performance on PPI across multiple neuropsychiatric disorders. Given multiple molecules, circuits, and drugs that could variably regulate PPI, future research using a machine learning approach may help predict the impact of these variables on PPI and further decode the neural-behavioral coupling. Another important area of future investigation is the sex-dependent effect on BDNF transport machinery and associated behavioral/cognitive outcomes. Sex-dependent regulation of BDNF signaling and sex-associated vulnerability of corticolimbic circuitry have been suggested in several psychiatric conditions (56–58), and Disc1-mediated BDNF transport machinery described here may well be impacted by sex effects.

We have recently reported pathological interaction of Disc1 and Htt in mood-associated symptoms in HD (15): we showed a “gain-of-function” of mutant Htt proteins aberrantly sequestrated Disc1, resulting in altered enzymatic activity of phosphodiesterase 4 (Pde4) that is to be controlled by Disc1 under normal physiological conditions. In contrast, in the present study, we demonstrated that “loss-of-function” of Disc1/Htt interaction in the PFC→DMS projection leads to a distinct cellular deficit (*i.e.*, reduced Bdnf transport), thereby affecting a distinct behavioral dimension (*i.e.*, sensorimotor gating). This not only exemplifies multi-functional nature of both Disc1 and Htt, but also emphasizes a need to address each behavioral dimension based on specific molecular, cellular and circuit-wide mechanisms, demonstrating the validity of dimensional approach in investigating the mechanisms underlying neuropsychiatric disorders.

Sensorimotor gating or PPI may be regulated by circuitries other than the corticostriatal projection in which other molecular mediators possibly participate in additional contexts. Our hypothesis-driven approach does not exclude this possibility. For example, a recent study reported the involvement of the excitatory cortical neurons along the cortico-accumbal (PFC->NAc) circuitry in PPI regulation, but the study did not step in the investigation at the molecular levels (59). The PFC->DMS circuitry we report in the present study may

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cooperatively regulate PPI together with the PFC->NAc circuitry. Provided that BDNF is also transported along the PFC->NAc circuitry (20–22), Disc1-associated molecular mechanism may also play a role in this cascade. Elucidating all mechanisms for PPI is beyond the scope of the present study. Nevertheless, the central mechanism that we presented here at multiple levels in an integrated manner will provide novel insight into sensorimotor gating mechanisms in many brain disorders, such as schizophrenia and HD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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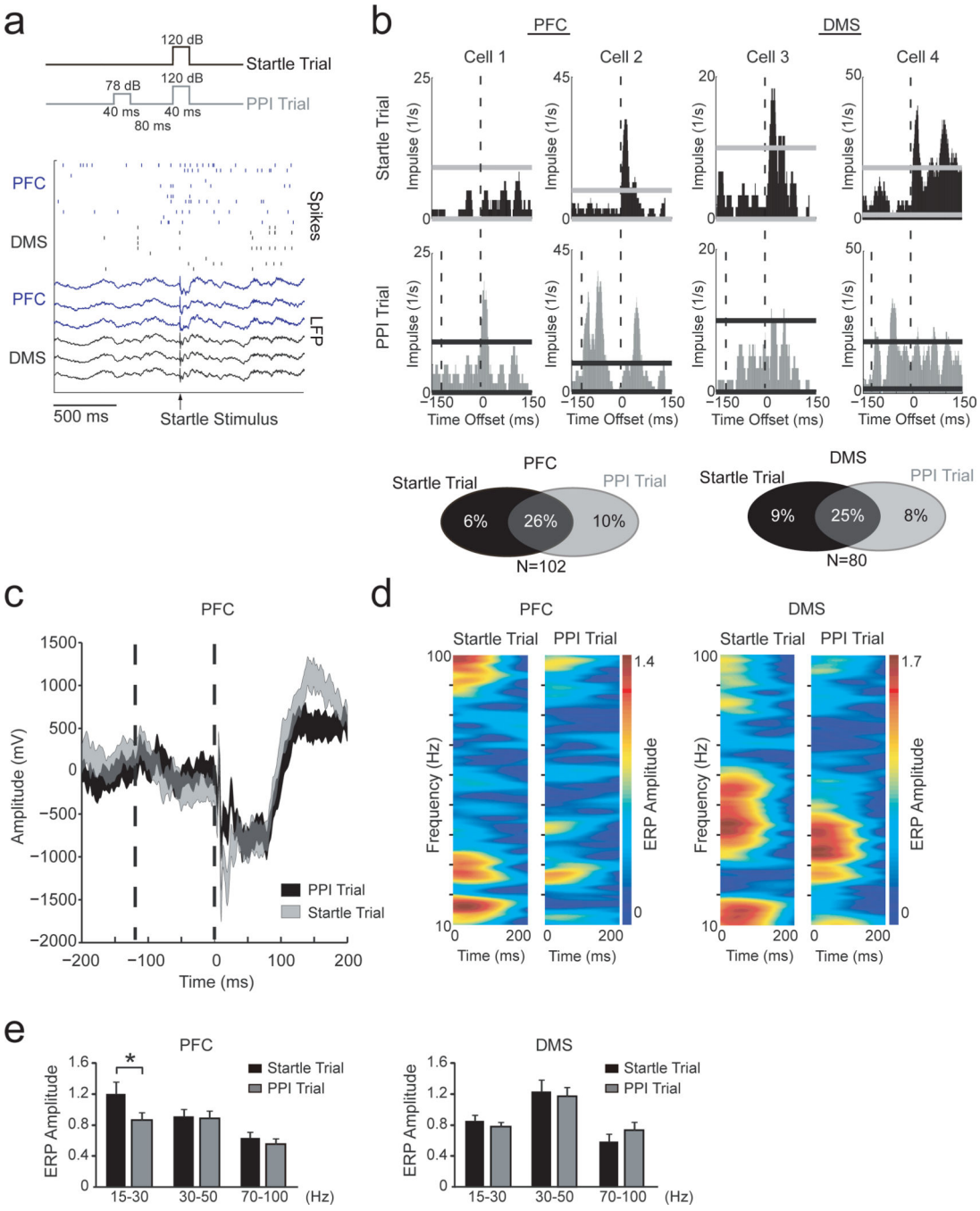

Figure 1. The prefrontal cortex and dorsomedial striatum in sensorimotor gating.

(**a**) Startle (120 dB) and pre-pulse (78 dB) stimuli used for testing PPI (top). Two-second trace of single neuron spikes and LFP (local field potential) activity recorded in a mouse during the PPI test (bottom). (**b**) Perievent time histograms (PETH) showing examples of the prefrontal cortex (PFC, left) and the dorsomedial striatum (DMS, right) unit responses during startle and PPI trials (n = 30 per trial, data are shown in 20 ms bins). Dashed lines correspond to the presentation of the 78 dB stimulus (−120 ms) and the 120 dB stimulus (0 ms). Horizontal bars represent the 95% confidence interval for mean firing rate during

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baseline. The percentage of neurons that responded to the startle stimulus during the startle and PPI trials are shown below. **(c)** PFC LFPs recorded during prepulse and startle trials. Zero millisecond (ms) corresponds to the time of presentation of the 120 dB pulse for both trial types. Data are shown as means ± SEM (n = 30 trials for each stimulus). **(d)** Amplitude-frequency components of the PFC (left) and DMS (right) LFP normalized to the mean LFP amplitudes observed during the −5 s to −1 s window prior to the presentation of the 120 dB pulse. **(e)** The prepulse significantly reduced the mean cortical (left), but not the DMS (right) beta response to the startle pulse (data were averaged within animals across 8−16 LFP channels per brain area). *P < 0.05 using mixed-model ANOVA followed by Bonferroni-corrected Wilcoxon signed-rank test.

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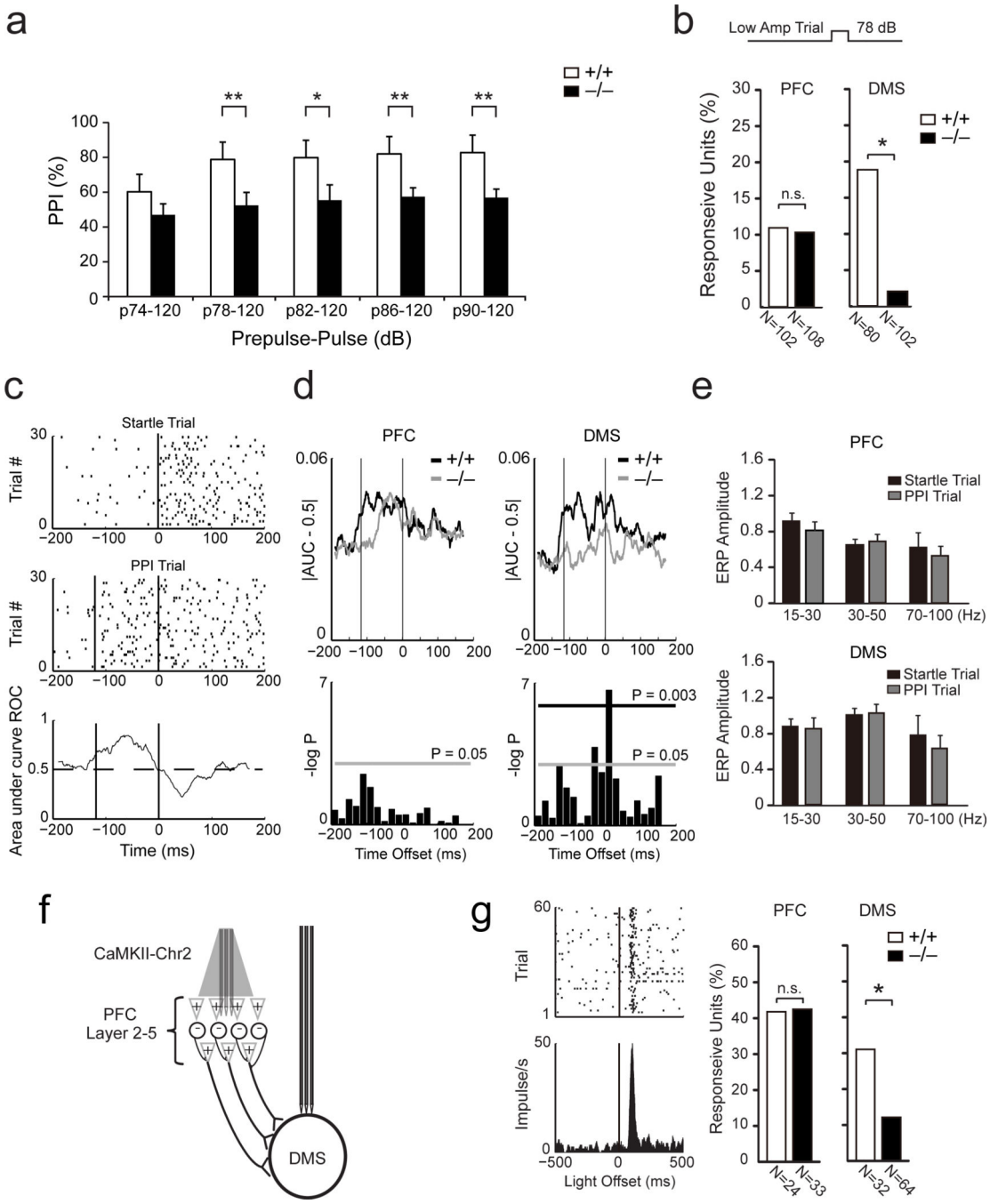

Figure 2. Sensorimotor gating in *Disc1* LI mice.

(a) *Disc1* LI (−/−) mice show reduced PPI. WT (+/+), $n = 7$; *Disc1* LI (−/−), $n = 8$. Data are shown as means ± SEM. *P < 0.05, **P < 0.01 (Student's t-test). **(b)** Startle and prepulse stimuli used for testing PPI (top). A similar portion of PFC neurons modulated their firing rates in response to the 78 dB low amplitude stimulus in *Disc1* LI mice and their WT littermates (P > 0.05 using Z-test). A significantly lower proportion of DMS neurons modulated their response to this stimulus in *Disc1* LI mice compared to their WT littermates (*P < 0.05 using Z-test). **(c)** Raster plot of DMS neuron response during startle

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and prepulse trials (top). Area under the ROC curve (AUC) demonstrating unit detection of gating across the stimulus interval (bottom). **(d)** Population AUC magnitude functions in WT and *Disc1* LI mice (top). Differences between genotypes were identified by comparing AUC functions averaged within 20-ms bins using a Wilcoxon rank-sum test (bottom). The gray line corresponds with P = 0.05. The black line corresponds with the significance threshold following Bonferroni correction for multiple comparisons (n = 102 PFC neurons and 80 DMS neurons in WT mice; n = 108 PFC neurons and 102 DMS neurons in *Disc1* LI mice). **(e)** The prepulse stimulus showed no effect on the mean PFC (top) or DMS (bottom) response to the startle stimulus across any frequency band examined (data was averaged within animal across 8−16 LFP channels per brain area, n = 10). **(f)** Schematic of concurrent optogenetic stimulation and neurophysiological recordings in *Disc1* LI and WT mice infected with AAV-CaMKII-Chr2 in PFC. **(g)** Sixty light pulses (10 ms pulse width) were delivered with a pseudorandomized inter-pulse-interval ranging between 8 s and 23 s. Left: Raster plot (top) and firing rate perievent time histogram (PETH) of a representative striatal neuron (bottom). Right: A similar proportion of PFC neurons modulated their firing rates in response to cortical stimulation (P > 0.05 using Z-test) in *Disc1* LI mice and WT littermates. A significantly lower proportion of DMS neurons modulated their firing rates in response to cortical stimulation (right; *P < 0.05 using Z-test) in *Disc1* LI mice compared to WT littermates.

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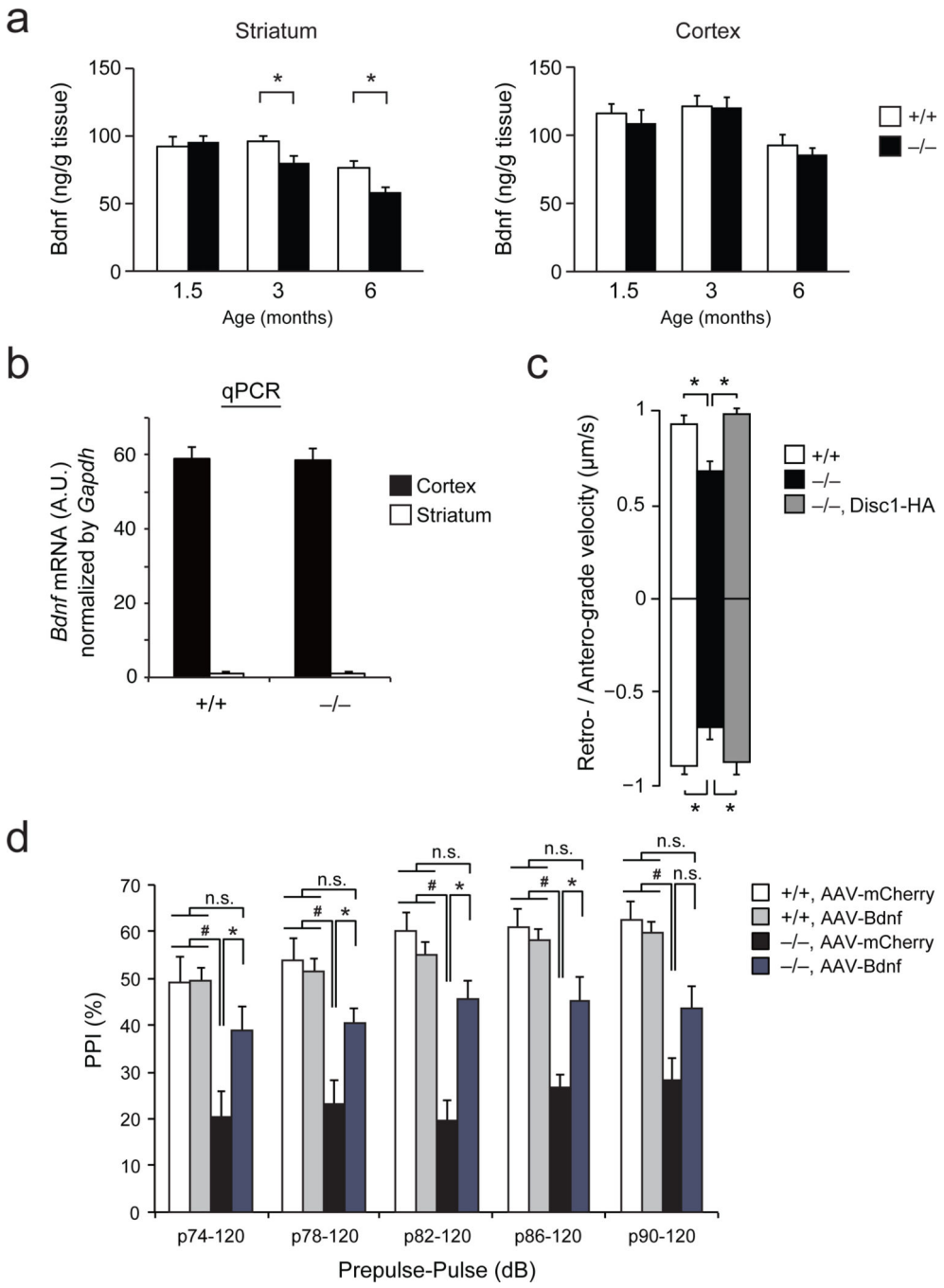

Figure 3. Deficits in corticostriatal Bdnf transport in *Disc1* LI mice.

(a) Age-dependent reduction in striatal, but not cortical, Bdnf in *Disc1* LI (−/−) mice as measured by ELISA. WT (+/+), $n = 9–10$; *Disc1* LI (−/−), $n = 9–10$. *P < 0.05 using Student's t-test. **(b)** No difference in *Bdnf* mRNA in the cortex and striatum between WT and *Disc1* LI mice at 3 months of age (WT striatum, 1.00 ± 0.05; *Disc1* LI striatum 0.97 ± 0.05; WT cortex, 59.15 ± 3.01; and *Disc1* LI cortex 58.72 ± 2.90). *Bdnf* mRNA in WT striatum was assigned as "1" to which the other results were normalized. WT, $n = 6$; *Disc1* LI, $n = 6$. **(c)** Impaired antero-/retro-grade transport velocity in *Disc1* LI primary cortical

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neurons was rescued by overexpression of full-length Disc1 (−/−, Disc1-HA). *P < 0.05 (Kruskal-Wallis test with Dunn's multiple comparisons). **(d)** *Disc1* LI mice injected with control AAV-mCherry showed significantly low PPI, as compared with WT mice injected with either AAV-mCherry or AAV-Bdnf (#P < 0.05). Injection of AAV-Bdnf significantly improved PPI in *Disc1* LI mice (*P < 0.05, as compared with control AAV-mCherry injection in *Disc1* LI; repeated measures two-way ANOVA followed by Bonferroni post-hoc tests). There were no statistically significant differences between "+/+, AAV-mCherry" and "−/−, AAV-Bdnf", nor between "+/+, AAV-Bdnf" and "−/−, AAV-Bdnf" (n.s.: not significant). WT+AAV-mCherry, $n = 10$; WT+AAV-Bdnf, $n = 10$; *Disc1* LI+AAV-mCherry, $n = 8$; *Disc1* LI+AAV-Bdnf, $n = 8$. Data are shown as means ± SEM.

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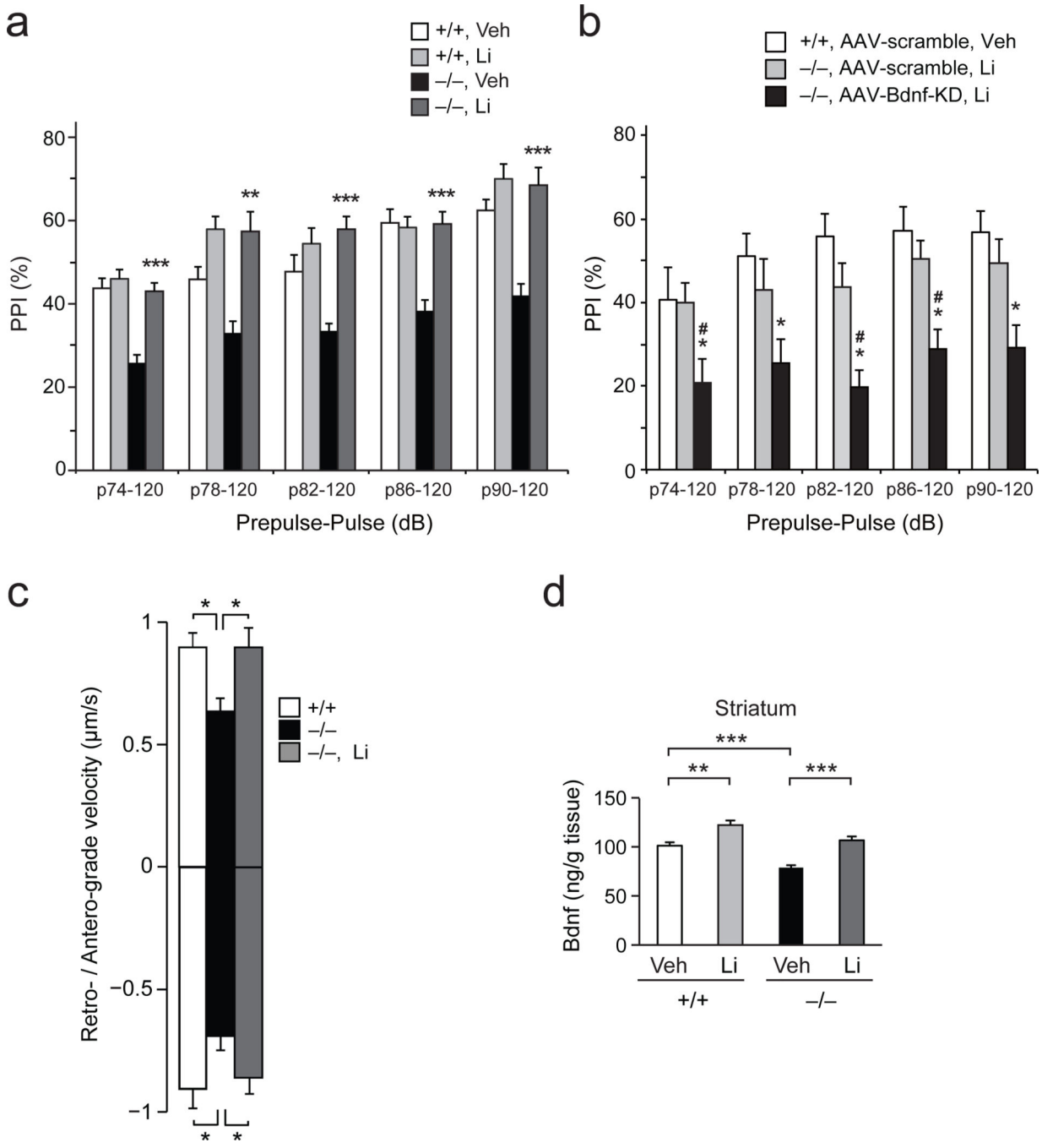

Figure 4. Lithium-mediated augmentation of Bdnf transport can rescue PPI deficits in *Disc1* LI mice.

(a) Lithium (Li, 100 mg/kg body weight, i.p., daily, 14 days) rescued the PPI deficits in *Disc1* LI (−/−) mice. $n = 8$ per cohort. Veh, vehicle. **P < 0.01, ***P < 0.001 as compared with *Disc1* LI cohort treated with Veh (repeated measures two-way ANOVA followed by Bonferroni post-hoc tests). **(b)** Li-mediated rescue of PPI was abolished by Bdnf knockdown. $n = 8$ per cohort. Veh, vehicle. *P < 0.05 as compared with WT (+/+) cohort injected with AAV-scramble and treated with Veh, #P < 0.05 as compared with

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Disc1 LI (−/−) cohort injected with AAV-scramble and treated with Li (repeated measures two-way ANOVA followed by Bonferroni post-hoc tests). **(c)** Li (2 mM in the culture media 30 min before imaging) rescued the antero-/retro-grade Bdnf transport speed in cultured primary cortical neurons prepared from *Disc1* LI (−/−) mice. *P < 0.05 (Kruskal-Wallis test with Dunn's multiple comparisons). **(d)** Li (100 mg/kg body weight, i.p., daily, 14 days) increased the levels of Bdnf in the striatum of *Disc1* LI (−/−) mice to levels equivalent to WT mice. $n = 8$ per cohort. The injections also increased Bdnf in WT, but the effects were more prominent in *Disc1* LI mice. **P < 0.01, ***P < 0.001 (Kruskal-Wallis test with Dunn's multiple comparisons).

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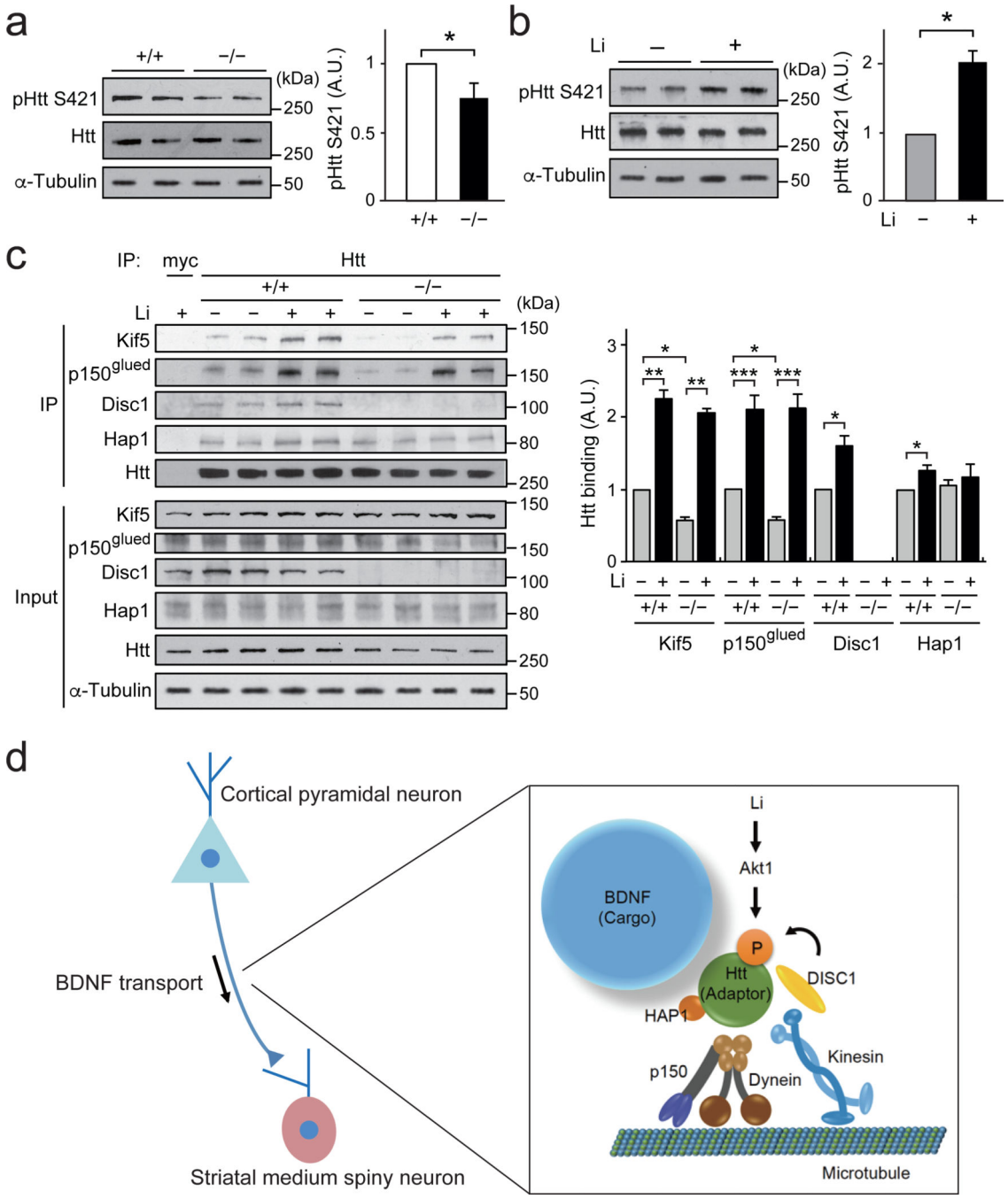

Figure 5. Lithium upregulates Htt Ser-421 phosphorylation and enhances assembly of the Bdnf transport machinery in *Disc1* LI mice.

(a) Levels of phospho-Htt Ser-421 normalized by total Htt levels in prefrontal cortex of *Disc1* LI (−/−) mice at 3 months of age, compared to WT (+/+) mice. *P < 0.05 (Student's t-test). **(b)** Levels of phospho-Htt Ser-421 normalized by total Htt levels in prefrontal cortex of *Disc1* LI (−/−) mice at 3 months of age after administration of Li (100 mg/kg body weight, i.p., daily, 14 days) (+) or vehicle (−). *P < 0.05 (Student's t-test). **(c)** Prefrontal cortical homogenates from WT (+/+) and *Disc1* LI (−/−) mice, chronically

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treated with Li (100 mg/kg body weight, i.p., daily, 14 days) (+) or with vehicle (−), were immunoprecipitated (IP) with anti-Htt antibody and analyzed by Western blots using the antibodies indicated. Equal amounts of protein extracts (100 μg/mouse) were used for each immunoprecipitation and lesser amounts (5−10%) of protein extracts were used as input (5 μg for Htt and α-Tubulin, 10 μg for the rest of the proteins) in order to allow quantitative evaluation of the signals. Graph: Relative binding capacity between Htt and each component of the Bdnf transport machinery. The ratio of the densitometry of a given protein band divided by the densitometry of Htt in IP blots for each condition was normalized to that for control condition (WT without Li). The experiments were done in triplicate. Data are shown as means ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001 (Student's t-test). **(d)** Schematic model for Htt-mediated BDNF transport facilitated by DISC1, Akt1 and Li. BDNF-containing cargo is linked to the motor machinery (kinesin and dynactin) via Htt adaptor protein and thereby transported along the cortico-striatal tract. DISC1 supports BDNF transport by facilitating the complex formation among Htt, cargo (BDNF) and motors, in part through augmentation of Ser-421 phosphorylation of Htt. Lithium (Li) could also enhance this complex formation via Ser-421 phosphorylation of Htt, possibly through upregulation of Akt1 activity or Akt1 recruitment.