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Effects of genetic and environmental risk for schizophrenia on hippocampal activity and psychosis-like behavior in mice

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Abstract

Schizophrenia is a serious mental illness most notably characterized by psychotic symptoms. In humans, psychotic disorders are associated with specific hippocampal pathology. However, animal model systems for psychosis often lack this pathology, and have been weak in providing a representation of psychosis. We utilized a double-risk model system combining genetic risk with environmental stress. We hypothesized these factors will induce hippocampal subfield pathology consistent with human findings, as well as behavioral phenotypes relevant to psychosis. To address this, we exposed wild-type and transgenic Disc1 dominant negative (Disc1-deficient) mice to maternal deprivation. In adulthood, hippocampal subfields were examined for signs of cellular and behavioral pathology associated with psychosis. Mice exposed to maternal deprivation showed a decrease in dentate gyrus activity, and an increase in CA3/CA1 activity. Furthermore, results demonstrated a differential behavioral effect between maternal deprivation and Disc1 deficiency, with maternal deprivation associated with a hyperactive phenotype and impaired prepulse inhibition, and Disc1 deficiency causing an impairment in fear conditioning. These results suggest distinct consequences of environmental and genetic risk factors contributing to psychosis, with maternal deprivation inducing a state more wholly consistent with schizophrenia psychosis. Further research is needed to determine if this pathology is causally related to a specific behavioral phenotype. The development of a strong inference animal model system for psychosis would satisfy a high medical need in schizophrenia research.

Keywords

Psychosis; Hippocampus; Maternal deprivation; Disc1

1. Introduction

Schizophrenia is the prototypical psychotic disorder whose pathophysiology has not yet been defined, but model systems for its dimensions have been proposed based on genetic, developmental, and pharmacological etiologies [1–3]. As these models include and as the dimensional syndromal emphasis of the Research Diagnostic Criteria (RDoC) has encouraged, these constructs hope to generate dimensional disease model systems associated with biological correlates, with psychosis being the critical disease dimension in

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schizophrenia [4–6]. Schizophrenia (SZ) develops in early adult years and symptoms continue throughout life [7]. Treatments are often effective but symptomatic and inevitably incomplete [8,9]. Thus, the medical need for a focused mechanistic therapy is high. To be successful with discovery for schizophrenia, dimensionally-related, regional, molecular and cellular targets are critical.

We have modeled human psychosis based on evidence that hippocampal subfield dysfunctions are critical elements of the temporo-prefrontal cortical network which supports psychotic manifestations [1,10,11], and hypothesize that hippocampal hyperactivity is an *in vivo* biomarker of psychosis [12–14]. We have examined the pathological elements of hippocampal subfields using human postmortem tissue, and identified, among other changes, reduced dentate gyrus GluN1, the obligate subunit of the NMDA receptor, and tissue markers of increased neuronal activity in CA3 and CA1 [15,16]. The model system hypothesizes that reduction in DG granular cell activity (based on low GluN1) will generate increased downstream hippocampal activity through metaplasticity processes at the insertions of the mossy fiber pathway in CA3, detectable in animals with synaptic reorganization, cFos immunohistochemistry, and mouse behaviors of psychosis [17]. Therefore we are hypothesizing a mechanistic disease model system for psychosis with DG hypofunction and downstream hippocampal hyperfunction, with psychosis generated by labile and increased positive feed-forward neuronal activity in CA3, which we postulate can contribute to hyper-associations, mistakes of memory and memories with delusional content, all psychotic phenomena.

To evaluate this hypothetical model system further, we questioned whether known genetic and environmental risks for SZ would similarly be associated with persistent hippocampal hyperactivity and with behaviors relevant to standard mouse models of psychosis. Would traditional animal models used to study SZ also show hippocampal hyper-activity? This led us to use a dominant-negative DISC1 (DN-DISC1) mouse model, as a genetic risk [18,19], and maternal deprivation, as an environmental risk [20,21] in male and female mice to see if both would show evidence of an association with hippocampal hyper-activity; and that their co-expression together would generate greater hippocampal pathology and animal behaviors of psychosis. We tested these postulates in wild-type mice and transgenic mice harboring a dominant negative Disc1 translocation with or without maternal separation.

In evaluating these outcomes and their interaction, we examined pyramidal cell activity within the hippocampus. This was done through analysis of cFos expression, separately analyzing the hippocampal subfields, as well as dorsal and ventral regions (corresponding to posterior and anterior hippocampus in humans, respectively [22]). Data were analyzed by genotype (wild-type and transgenic), by maternal deprivation (–/+ MD), and by the interaction of these factors. Because hippocampal hyperactivity, and associated psychosis, is a lifelong dimension of schizophrenia, we evaluated this measure in aged mice, long after the completion of any behavioral analysis. The purpose of this was to determine whether DISC1 deficiency and/or maternal deprivation induce long-lasting, persistent changes in the general basal levels of activity within the hippocampus.

We further evaluated behavioral outcomes, with emphasis on hippocampally-mediated and memory-related tasks (fear conditioning, Morris Water Maze) as well as tasks with some face validity for psychosis (locomotor activity, prepulse inhibition) [23], by genotype, maternal deprivation status, and their interaction.

Results suggest a general effect of maternal deprivation on hippocampal activity, with or without genetic manipulation. Moreover, we found different, non-overlapping effects of maternal deprivation and *Disc1* deficiency on behavior.

2. Methods and materials

2.1. Animals

We used transgenic mice carrying a dominant negative C'-truncated human *DISC1* gene, expressed under a prion protein promoter (TG-DN-PrP-*DISC1* (TG); see [24]). *DISC1* TG mice were provided by Dr. Akira Sawa at Johns Hopkins University. Mice heterozygous for this transgene were bred with wild-type (WT) mice to produce WT and TG (heterozygous) offspring. Mice were maintained on a C57BL/6J background, and following weaning, group housed up to four per cage with their same-sex littermates, on a 12 h:12 h light:dark cycle, with food and water available *ad libitum*. All behavioral experiments were performed during the light phase. For cFos analysis, $n = 10\text{--}12/\text{group}$. For behavioral experiments, $n = 18\text{--}29/\text{group}$. All studies were performed in accordance with protocols approved by the UT Southwestern Animal Resource Center and Institutional Animal Care and Use Committee.

2.2. Maternal deprivation

From P2 through P14, pups were removed from their home cage and placed into an empty cage in a separate room, on a heating pad (Conair, Stamford, CT), with the bedding maintained at approximately 30 °C. Pups were kept in this cage for one hour daily. Control mice were not separated from their home cages.

2.3. Behavioral experiments

Behavioral experiments were performed when the animals were 12–20 weeks old. Mice were exposed to a battery of tests assessing locomotor activity, then prepulse inhibition, then contextual and cued fear conditioning, or only a Morris Water Maze test. Mice exposed to multiple paradigms had each test separated by at least 48 h. Descriptions of behavioral paradigms are provided in the Supplementary Methods.

2.4. Immunohistochemistry

At least six months following behavioral testing, mice were habituated to a neutral environment, handling, and vehicle i.p. administration for four days. On the fifth day, mice were anesthetized with pentobarbital (40 mg/kg, i.p.), and perfused with 0.9% saline, followed by 4% paraformaldehyde. The brains were removed, stored in 4% paraformaldehyde at 4 °C for three days, passed through a sucrose gradient, and kept in 30% sucrose until slicing. Brains were sliced coronally at 50 μm , and sections were stored in 0.1 M PBS with 0.1% sodium azide. Every sixth free-floating section was stained for cFos. Staining procedure is presented in detail in the Supplementary Methods.

2.5. Statistics

Statistics were analyzed using GraphPad Prism software (GraphPad Software, La Jolla, CA) and SPSS (IBM, Armonk, NY). For all experiments, we first assessed whether there was a main effect of sex, using ANOVA. As we found no sex effect in any paradigm assessed, for all further analysis, the sexes were pooled, and ANOVAs were run with factors of genotype and maternal deprivation status, and when appropriate, a repeated measure of time, prepulse intensity, or hippocampal subfield. appropriate, Tukey's post-hoc comparisons were performed. Significance was set at $p < 0.05$.

3. Results

3.1. Hippocampal hyperactivity

To evaluate hyperactivity regionally in hippocampus by subfield in the mice, we stained for cFos protein using IHC and counted cFos-positive neurons to indicate cellular activity. We evaluated each risk separately (WT vs TG; with vs without maternal deprivation (+/-MD)) and their co-expression for changes in hippocampal neuronal activity. We aimed to determine the persistent effects of these risk factors hippocampal activity, so we assessed expression of cFos at least six months following the completion of any behavioral tests. Activity was assessed regionally within hippocampus, quantifying dorsal and ventral regions separately, given the distinct functions represented along the hippocampal long axis (homologous to posterior and anterior hippocampus in humans, respectively), as well as by subfield.

To determine how deprivation and genotype affects across the hippocampal subfields, we performed a three-way ANOVA with factors of genotype and deprivation, and the repeated measure of subfield. In both the dorsal and ventral hippocampus, we detected a significant main effect of deprivation (Dorsal: $F(1,36) = 26.625$, $p < 0.0001$; ventral: $F(1,37) = 28.182$, $p < 0.001$), and significant deprivation $\times$ subfield interactions (dorsal: $F(2,35) = 31.894$, $p < 0.001$; ventral: $F(2,36) = 65.69$, $p < 0.001$), suggesting maternal deprivation affects the excitatory tone similarly throughout the hippocampus, and the nature of this change differs based on the specific hippocampal subfield. Analysis revealed no effect due to Disc1 deficiency.

In order to more fully examine the nature of the effects of maternal deprivation on the baseline levels of hippocampal activity, exploratory two-way ANOVAs were performed for each subfield separately. In the dentrate gyrus, analysis indicated, due to main effects of maternal deprivation, that this environmental risk is associated with decreased activity in the dorsal DG ($F(1,36) = 21.09$, $P < 0.001$; Fig. 1A), and ventral DG ($F(1,37) = 18.204$, $p < 0.001$; Fig. 1B). We detected no effect of Disc1 genotype in DG cFos expression.

Following the hippocampal trisynaptic circuit, we next examined CA3. ANOVA demonstrated maternal deprivation caused an increase in the number of cFos expressing cells within both the dorsal CA3 ($F(1,36) = 25.055$, $p < 0.001$, Fig. 2A) and ventral CA3 ($F(1,37) = 36.36$, $p < 0.001$; Fig. 2B). Again, we detected no main effect or interaction involving genotype, suggesting maternal deprivation, but not Disc1 function, affects excitatory signaling within CA3.

In CA1, once again we detected a main effect of maternal deprivation in the dorsal division of this subfield ($F(1,41) = 34.49$, $p < 0.001$). Interestingly, we also found a significant genotype x deprivation effect ($F(1,41) = 5.736$, $p = 0.0213$), suggesting that lack of Disc1 function blunts the effect of maternal deprivation on CA1 activity. Indeed, post-hoc analysis revealed a significant decline in maternally deprived TG mice relative to similarly deprived WT mice, and no significant effect of maternal deprivation on TG mice within this subfield (Fig. 3A). Within the ventral CA1, though we did find a main effect of maternal deprivation ($F(1,37) = 26.813$, $p < 0.001$; Fig. 3B), there was no interaction with genotype, suggesting that Disc1 deficiency selectively diminishes the effects of maternal deprivation in the dorsal CA1.

3.2. Animal behaviors of psychosis

Here, we sought to determine whether the hippocampal hyper-activity changes associated with Disc1 deficiency and maternal deprivation can be associated with specific behavioral phenotypes often used to indicate psychosis in animal models.

3.2.1. Locomotor activity—Spontaneous locomotor activity was analyzed in five-minute bins over two hours (Fig. 4), using three-way ANOVA, with factors of genotype, deprivation status, and the repeated measure of time. Analysis revealed a main effect of maternal deprivation ($F(1,82) = 5.00$, $p = 0.028$), suggesting maternal deprivation alone is sufficient to induce spontaneous hyperactivity in adult mice.

3.2.2. Prepulse inhibition—Acoustic startle response (Supplementary Fig. 1) was analyzed using a two-way ANOVA and prepulse inhibition (PPI, Fig. 5), assessed at 72 dB, 74 dB, 78 dB, and 82 dB, analyzed with three-way ANOVA, using the repeated measure of prepulse intensity. We detected no main or interaction effect of genotype or deprivation on acoustic startle response.

Analysis of PPI revealed a significant prepulse x deprivation interaction ($F(3,81) = 3.244$, $p = 0.026$), suggesting maternal deprivation alters sensorimotor gating, depending on the relative salience of the prepulse. To determine the nature of this interaction, two-way ANOVAs were run at each prepulse intensity. We detected a main effect of deprivation at 72 dB (Fig. 5A), the lowest prepulse intensity ($F(1,87) = 7.168$, $p = 0.0089$). This effect disappears as the prepulse intensity increases.

3.2.3. Fear conditioning—Associative memory was evaluated by testing both contextual (Fig. 6A) and cued (Fig. 6B,C) fear conditioning. Two-way ANOVA demonstrated a main effect of genotype in contextual fear conditioning ($F(1,82) = 4.456$, $p = 0.038$), suggesting Disc1 deficiency decreases contextual fear recall. Moreover, we also detected a main effect of genotype in cued fear conditioning, both prior to the conditioned stimulus ($F(1,80) = 4.363$, $p = 0.04$) and during presentation of the CS ($F(1,80) = 5.116$, $p = 0.026$).

3.2.4. Morris water maze—Spatial learning and memory was assessed in WT and TG mice using the Morris Water Maze (Supplementary Fig. 2). Assessment of training, analyzing time to platform using three-way ANOVA revealed no main effect of genotype or maternal deprivation. During the probe trial, we saw no effect of genotype or maternal

deprivation, implying neither Disc1 deficiency nor maternal deprivation affects behavior in this paradigm.

In summary, the behavioral analysis shows a differential effect on behavior induced by genetic and environmental risk, with Disc1 deficiency impairing the fear response, while maternal deprivation induces spontaneous hyperactivity and an impairment in prepulse inhibition.

4. Discussion

These results demonstrate that two psychosis risk factors, Disc1 deficiency (genetic) and maternal deprivation (environmental), differentially affect behavior and psychosis-related biomarkers in mice. Interestingly, a known environmental risk factor, which seeks to model childhood neglect, induces a robust increase in basal hippocampal activity within CA3 and CA1, while also inducing a specific behavioral phenotype: spontaneous hyperactivity and impaired sensorimotor gating. Contrast this with the effects of a well-studied genetic risk factor, Disc1 deficiency, which attenuates the deprivation-induced hippocampal hyperactivity in dorsal CA1, lessens fear reactivity, and has no effect on the behaviors affected by maternal deprivation.

These results, when taken in the context of the construction of a valid animal model system of psychosis, provide some important considerations. First, our use of the genetic model (Disc1 translocation) fulfills a form of construct validity akin to what is referred to as “homological” or “strain” validity [25]. This is based on the similarity of the known genetic risk observed in humans and the specific genetic preparation used. On the other hand, our use of maternal deprivation satisfies a separate aspect of construct validity, pathogenic validity, which employs a preparation used to transform a healthy, unaffected organism into a vulnerable one. Because the specific causes of schizophrenia psychosis are not well understood, valid risk factors, such as these, provide a solid basis from which to verify a psychosis-like state. An important caveat to consider, though, is that maternal deprivation is not a specific environmental risk factor for psychosis; it also increases the risk of other mental illnesses, including bipolar disorder and major depression [26]. It is therefore striking that the results of this environmental challenge include brain pathology and a behavioral phenotype relevant to psychosis, though phenotypes relevant to these other disorders were not examined.

4.1. Hippocampal subfield activity

We attempted to verify this animal model system in two distinct ways, utilizing two facets of face validity. First, we aimed to demonstrate that Disc1 deficiency and/or maternal deprivation induce a state of biomarker validity, where pathological findings in humans with psychosis are represented in our preparation. To do this, we examined the resting state activity within the hippocampal subfields by examining cFos expression. It should be noted that analysis of cFos activity does not provide information on cellular excitability or synaptic function, but rather is taken here to represent a general marker of overall direct cellular activity in a particular region as a whole. Although cFos activity is taken to represent an acute measure of neuronal activity, we sought to assess activity in a basal state, when the

animal had not been treated with a drug or challenged with a task. Animals were habituated to handling and injection for several days prior to perfusion in order to further dampen the experimental effects of cFos activity. Studies on humans with schizophrenia have detected decreased DG excitability, as well as CA3 and CA1 resting-state hyperactivity [27–29]; the causes of this, however, remain unknown. Our results suggest that maternal deprivation alone is sufficient to induce this precise state throughout the hippocampus. Importantly, not only did *Disc1* deficiency not induce a similar state, but lack of *Disc1* function seemed to lessen the increased excitatory tone in the dorsal CA1. This would imply that maternal deprivation may induce a state with biomarker validity to schizophrenia, while genetic expression of a disease-related *Disc1* translocation does not. How, exactly, maternal deprivation causes these changes is still unknown.

In humans, a low cortisol response to stress is associated with increased hippocampal activity during picture encoding [30], and low levels of maternal care induce a lower cortisol response to stress [31]. Therefore, it is possible that maternal deprivation alters the stress response, setting a higher basal hippocampal activity. Alternatively, it has been demonstrated that in human schizophrenia post-mortem brains, there is a decrease in GluN1 within the DG, as well as increased excitatory synaptic markers in CA3 [16,28]. It is possible that this decreased activity within the DG, induced by maternal deprivation, induces metaplastic changes in downstream subfields, increasing their activity. Indeed, maternal deprivation has been shown to increase cell death within the dentate gyrus in rats [32] and decreases DG neurogenesis [33]. Moreover, it has been shown that prenatal methylazoxymethanol acetate (MAM) administration, another environmental model of schizophrenia, can induce hippocampal hyperactivity by decreasing inhibitory signaling within the region [34,35], suggesting multiple potential causes for overall hippocampal hyperactivity.

4.2. Animal behavioral phenotypes

The relationship between our animal model systems and psychosis-relevant behavioral deficits remains unclear. We therefore next sought to assess whether these factors can induce a behavioral phenotype consistent with psychosis; one that satisfies ethological validity, which aims to model symptoms similar to the human condition. We observed a range of phenotypes as a result of these manipulations. The psychosis risk factors employed in this study seem to have different, mostly non-overlapping effects on psychosis-like behaviors. Specifically, maternal deprivation alone increased spontaneous locomotor activity, and impaired sensorimotor gating. Meanwhile, *Disc1* deficiency had no effect on these measures. This is of particular interest, as of all the behavioral outcome we assessed, these two have the greatest face validity relative to psychosis. However, it must be acknowledged that psychosis is a very human condition, and the relevance of these specific phenotypes to psychosis remains controversial, and may in fact represent heightened anxiety as opposed to a psychotic state. However, as we found no difference in either thigmotaxis (data not shown) or startle response (Supplementary Fig. 1), which could indicate heightened anxiety, we believe these behavioral phenotypes are indeed related to psychosis. Heightened locomotor activity is thought to resemble psychotic agitation [36], and is associated with increased striatal dopamine, also found in schizophrenia [37]. Although in the context of schizophrenia, locomotor activity is often assessed after drug treatment, the goal of this

study was to examine the baseline state of the animal long after exposure to these risk factors, in the absence of exogenous agents. Much as we saw looking at resting state activity in the hippocampus, so too do we see a background effect in this behavioral measure. Prepulse inhibition deficits are also seen in humans with psychosis, and this phenotype is directly homologous between human and animal models [38,39]. Though any one of these phenotypes may be present in other conditions, the combination of phenotypes exhibited, within the specific experimental context employed, can give some validity as a psychosis model.

This is consistent with other studies demonstrating an effect of maternal separation on activity, both at baseline [40,41], as well as in response to cocaine challenge [42,43]. Maternal separation induced a deficit in PPI at the lowest prepulse intensity, which suggests impaired sensorimotor gating induced by a weak stimulus, which fades as the prepulse becomes more salient. Consistent with our results, it has been shown that maternal deprivation can reduce PPI in adult rats [44]; however, it is without effect in several mouse strains, including C57BL/6J, the background strain of our experimental line [45]. Moreover, other studies report that mice with *Disc1* deficiency show a deficit in this paradigm [46], and a single point mutation in *Disc1* is sufficient to reduce PPI [47], which is very much in contrast with our results. Thus, the variability inherent in this paradigm raises the possibility that this discrepancy may be due to a strain difference.

A lack of *Disc1* function seems to selectively affect fear conditioning. *Disc1* deficiency decreased both contextual and cued fear conditioning, while maternal deprivation had no effect on this behavior. It should be noted that we detected an impairment in cued fear conditioning both prior to, and during presentation of the CS. This suggests perhaps a generalized deficit in the behavioral expression of, or response to, fear, rather than strictly a deficit in learning and memory. It is important to interpret these results with caution, though; it has been demonstrated that deletion of *Disc1* reduced thermal pain sensitivity [48], perhaps introducing a confounding factor in the response to the foot shock. It should further be noted we saw no effect of either *Disc1* deficiency or maternal deprivation on spatial learning or memory, as assessed by the Morris Water Maze, so it remains a possibility that this phenotype is unrelated to the spatial/cognitive aspects of memory. However, if we assume that instead of a tangential behavioral abnormality, we are in fact observing a specific deficit in contextual and cued fear conditioning, this would not be wholly inconsistent with previous findings. On the other hand, working memory deficits have long been associated with schizophrenia [49], and mice with a similar *Disc1* truncation have been shown to display impairments in this function [50]. However, this form of memory, especially non-spatial working memory, seems to rely less on the hippocampus than the prefrontal cortex [51], and given our focus on the role of the hippocampus in the manifestation of psychosis, further analysis of working memory was beyond the scope of this study.

Our observed deficit in contextual fear conditioning is broadly consistent with previous findings indicating a loss of *Disc1* function impairs contextual fear conditioning [52], though that study found this effect only in male mice, while we found no significant sex effect. Moreover, trace fear conditioning and contextual fear conditioning have been

associated with increased dendritic spine density in CA1 [53,54]. Cultured hippocampal neurons from Disc1 mutant mice show decreased dendritic length, branching, and spine density [55], perhaps indicating an impairment in memory function in the mutant mice. Moreover, our analysis of cFos expression suggested a reduction in the responsiveness of dorsal CA1 to deprivation-induced hippocampal hyperactivity in TG mice, which could be due to changes in CA1 spine density, though these outcomes would need to be further studied for confirmation. It is well-established that contextual fear conditioning is hippocampally-mediated [56], and Disc1 is highly expressed in the hippocampus, both during development and adulthood [57]. Disc1 affects neurogenesis and neuronal migration [58]; that levels of neurogenesis seem to be positively associated with the degree of freezing in the contextual fear conditioning test [59,60] suggests a potential mechanism for the effects we observed. Moreover, it has been proposed that activity within the DG is reliant upon immature neurons [61], and therefore the reduced activity within this subfield we report may be due to a deficit in neurogenesis. Therefore it seems that the lack of Disc1 function may affect contextual fear conditioning through its impact on DG signaling, though further work is necessary to directly test this hypothesis.

Disc1 deficiency alone was sufficient to impair cued fear conditioning freezing. The cause of the effects of Disc1 deficiency on this behavior, which does not seem to require the hippocampus [56], may be due to alterations in axonal targeting and neuronal migration, which are known roles for Disc1 signaling [62,63]; suppression of Disc1 function can impair migration of neurons into CA1 [63], which is a key area of input from and output to the amygdala [64]. Thus it is possible that Disc1 deficiency alters the communication between the hippocampus and the amygdala, disrupting the expression of fear memory. Future studies are required to determine how disruption of Disc1 function affects neuronal development both within the hippocampus, as well as in afferent regions.

4.3. Conclusions

There is a body of literature examining gene x environment interactions in behaviors associated with schizophrenia. As there are a great number of genes which have been associated with schizophrenia and psychosis [65], the breadth of literature regarding gene x environment interactions is quite extensive. Overall, these interactions could provide new and emerging phenotypes, not observed when either genetic or environmental risk is present alone, a stronger phenotype observed with the combination of factors, or a protective phenotype, where one factor blunts the effect of the other [66]. Focusing on Disc1 dominant negative mutations, it has been shown that in combination with dietary lead, an assortment of behavioral phenotypes is revealed, including impaired PPI in female mice [67]. Disc1 deficient mice exposed to prenatal polyI:C displayed impaired social behaviors, decreased fear conditioning, increased anxiety, increased depressive-like behaviors, and increased MK-801-induced locomotor activity [68,69]; this latter behavior has further been shown to be blocked with antipsychotic administration [70]. Moreover, point mutations in the Disc1 gene alter the response to chronic social defeat in mice in a select group of behavioral tasks, including spontaneous locomotor activity and PPI [71]. However, we saw no such behavioral effect from the combination of these factors, though lack of Disc1 function did inhibit the increased dorsal CA1 activity induced by maternal deprivation. It is therefore likely that

these two factors, though both possessing some construct validity for psychosis, work via distinct mechanisms. Further research is warranted to delineate the specific pathological effects of these factors, to determine how they exert their specific effects.

In sum, the results demonstrate that maternal deprivation alters the resting state activity within the hippocampus, decreasing activity within the DG while increasing activity in the downstream subfields. These alterations are further associated with psychosis-relevant behavioral phenotypes, with the risk factors inducing quite different behavioral phenotypes. Most notably, maternal deprivation alone, which induces a state satisfying biomarker validity, also induces the behavioral phenotypes which most satisfy ethological validity. We can therefore conclude that the animal model system based on this construct gives us a relatively more faithful representation of psychosis in mice. Going forward, however, it is imperative that the relationship between network activity and behavioral effects be directly assessed, to determine what, if any, is the causal relationship between these effects in order to develop more precise medical diagnoses and intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bbr.2017.10.039>.

Fig. 1. cFos activity with the dentate gyrus. The number of cFos+ cells was counted in the dorsal (A) and ventral (B) DG. Indicated are relative numbers of cFos-positive nuclei (top), region analyzed (middle), and representative images (bottom). -/+MD represents without and with maternal deprivation, respectively. *, **, ***, **** represents $p < 0.05$, 0.01, 0.001, 0.0001, respectively. -MD: WT $n = 9$; TG $n = 11$. +MD: WT $n = 12$; TG $n = 12$.

Fig. 2. cFos activity with CA3. The number of cFos+ cells was counted in the dorsal (A) and ventral (B) CA3. Indicated are relative numbers of cFos-positive nuclei (top), region analyzed (middle), and representative images (bottom). -/+MD represents without and with maternal deprivation, respectively. *, **, ***, **** represents $p < 0.05$, 0.01, 0.001, 0.0001, respectively. -MD: WT $n = 10$; TG $n = 11$. +MD: WT $n = 12$; TG $n = 12$.

Fig. 3. cFos activity with CA1. The number of cFos+ cells was counted in the dorsal (A) and ventral (B) CA1. Indicated are relative numbers of cFos-positive nuclei (top), region analyzed (middle), and representative images (bottom). -/+MD represents without and with maternal deprivation, respectively. *, **, ***, **** represents $p < 0.05$, 0.01, 0.001, 0.0001, respectively. -MD: WT $n = 10$; TG $n = 11$. +MD: WT $n = 12$; TG $n = 12$.

Fig. 4. Locomotor activity. Spontaneous locomotor activity was measured over two hours. -/+MD represents without and with maternal deprivation, respectively. * represents a main effect of deprivation at $p < 0.05$. -MD: WT $n = 20$; TG $n = 23$. +MD: WT $n = 29$; TG $n = 18$.

Fig. 5. Prepulse inhibition. Prepulse inhibition was assessed at prepulse intensities of 72 dB (A), 74 dB (B), 78 dB (C), and 82 dB (D). -/+MD represents without and with maternal deprivation, respectively. * represents $p < 0.05$, Fisher's LSD post-hoc test. -MD: WT $n = 20$; TG $n = 23$. +MD: WT $n = 29$; TG $n = 18$.

Fig. 6. Fear Conditioning. Contextual (A) and cued (B,C) fear conditioning was assessed. A) Contextual fear conditioning. B) Pre-CS freezing in a cued fear conditioning paradigm. C) CS freezing. -/+MD represents without and with maternal deprivation, respectively. * represents a main effect of genotype at $p < 0.05$., -MD: WT $n = 20$; TG $n = 23$. +MD: WT $n = 29$; TG $n = 18$.