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Interactive effect of maternal activation and DISC-1 knockout on pup preference of postpartum rats

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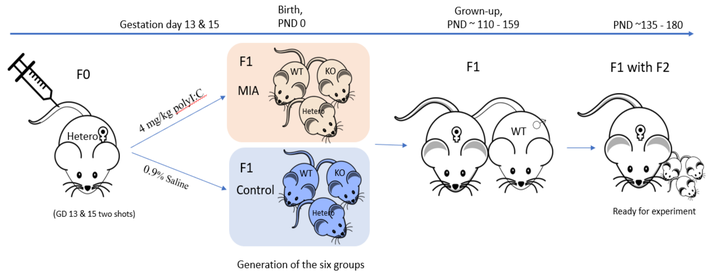
Introduction

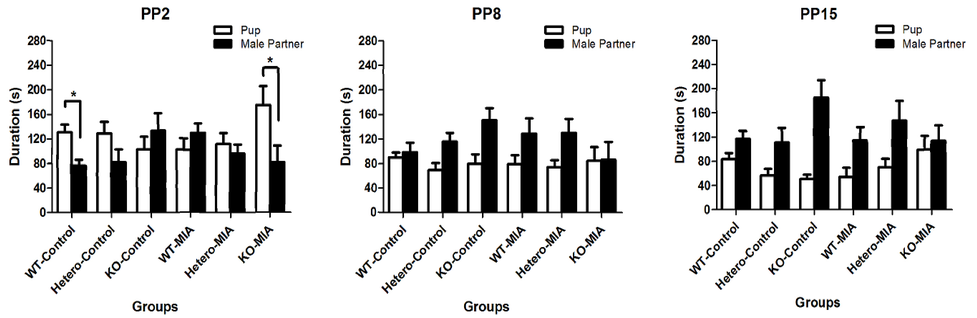
- Maternal immune activation (MIA) describes that infections during pregnancy activate immune elements that disrupt fetus normal brain behavior and results in an increased risk for psychiatric disorders like schizophrenia and autism spectrum disorder (Hsueh et al., 2018).
- In animal research, injection of polyinosinic:polycytidilic acid (PolyI:C) to mothers is commonly used to induce MIA, and consequently, induce the psychological deficits seen on schizophrenia (Patterson, 2009). MIA rats are shown to exhibit social behavior deficits, increased anxiety-like behaviors, and more fixed responses to objects than to strange mice (Hsueh et al., 2018;10). The abnormal social interactions might mimic the negative symptoms of schizophrenia and autism (Pletnikov et al., 2008).
- Disrupted-in-schizophrenia 1 (DISC1) gene DISC1 is involved in neurite outgrowth, neuronal migration, integration of newborn neurons, and cAMP signaling. It was originally identified in a unique Scottish pedigree, in which the gene is disrupted, resulting in the family having a spectrum of psychiatric disorders, including major depression, schizophrenia, and bipolar disease. (Blackwood *et al*, 2001; Jacobs *et al*, 1970; Muir *et al*, 2008; Thomson *et al*, 2013).
- Several DISC1 transgenic mouse lines have been established to assess the effect of the human DISC1 (hDISC1) truncation on behavior (Chubb *et al*, 2008). For example, frontal deficiency due to the truncated DISC1 resulted in deficient working memory (Li *et al*, 2007). Behavioral deficits such as prepulse inhibition (PPI) as a measure of sensorimotor gaiting, which is an endophenotype of schizophrenia, was also evident (Hikida *et al*, 2007; Niwa *et al*, 2010).
- Thus, rodents having DISC1 mutations are widely accepted disease models of schizophrenia and mood disorders [(Lipina and Roder, 2014; Randall *et al*, 2014) provide an overview].
- There is limited literature that considers the interaction between MIA and DISC1 risk factors of schizophrenia. The present study was designed to examine how MIA and DISC 1 knockout affect maternal behavior in rats as a way to asses social functioning deficit associated with MIA and DISC 1.
- It has been seen that transgenic mice with a DISC1 mutation showed social behavior deficits when born to mice exposed to polyI:C during pregnancy, demonstrating an interaction between genes and environment (Abazyan eta al., 2010; Patterson, 2011; Schwartzer et al., 2013)
- Rat maternal behavior serves as an excellent preclinical model for studying the interaction between MIA and DISC1 knockout because rats and humans share many aspects of mothering behaviors (e.g. intense maternal care is required to ensure survival of altricial infants)(Corter and Fleming, 2002; Numan et al., 2006).
- In order to evaluate maternal behavior, we used a pup preference test. This test examines the relative mother's motivation and affective response towards pups in comparison with the motivation to seek novelty, whether it is an object or another rat (Li et al., 2018). In this case, a male rat was used as competing stimulus for pups.

Methods

- The pup preference test was conducted in an open field arena, which was a 58.5 cm by 58.5cm square with Plexiglas walls of 45.0 cm high (CleverSys Inc., Reston, VA). At the beginning of the test, a male rat was confined in a cuboid restrainer (17.2 cm long × 9.0 cm wide) with an adjustable ceiling made of metal wire mesh.
- Two to four pups from the subject mother rat were restrained in a cylinder restrainer (diameter of 9.5 cm and a height of 9.7cm) with thin-metal-column fences (4 pups on postpartum day 2, PP2 and PP8, and 2 pups on PP15). Then, the subject dam was placed in the arena, and her activity was recorded by a monitoring system (Capture star, CleverSys Inc., Reston, VA). The behaviors were analyzed using Topscan Behavioral Analysis Software (CleverSys Inc., Reston, VA). These included the duration of time spent on exploring the male or the pups and frequency of visits to the male or pups.
- There were 6 experimental groups as shown in the image to the right.



Results



- On PP2, repeated measures ANOVA revealed that there was a three-way interaction among the interacted subjects (pups vs. male adults), the genotype (WT, HT or KO) and treatment (MIA, Control) [F (2, 50) = 4.696, p = 0.014]. The main effect of the three factors -- interacted subjects, genotype and treatment were not significant, nor were their two-way interactions (all p > .05).
- Further examination of the three-way interaction by the pairwise comparisons, however, revealed that the WT*Control rats spent more time with the pups compared to time spent with the male rat [F (2, 50) = 5.307, p = .025]. The KO*MIA rats spent more time with the pups compared to time spent with the male rat [F (2, 50) = 5.503, p = .023]. There was no significant difference in time spent with pup or male rat for the rest of the groups: WT*MIA [F (2, 50) = 0.491, p = .487], HT*Control [F (2, 50) = 1.634, p = .207], HT*MIA [F (2, 50) = 0.288, p = .594], and KO*Control [F (2, 50) = 0.880, p = .353]. There were no other significant main effects or interactions found on PP8 and PP15, thus, those statistics were not reported (p > .05).
- To examine genetic effect on pup preference for Control rats and for MIA rats, separate repeated measures ANOVAs were conducted. The interaction between genotype and interacted subjects was not significant in either case ([F(2,30) = 2.486, p = .100] for Control rats, and [F(2, 20) = 2.256, p = .131] for MIA rats).
- To examine environmental effect of MIA on pup preference for rats with the three different genotypes, separate repeated measures ANOVAs were conducted. The interaction between treatment and interacted subjects was significant for WT rats [F(1, 21) = 5.421, p = .030], but not for HT rats [F(1, 16) = 0.452, p = .511] nor for KO rats [F(1, 13) = 3.414, p = .088). Follow-up one-way repeated measures ANOVA revealed that the Control*WT rats interacted with pups (130.850 ± 12.797 s) longer than they interacted with the male rat (76.421 ± 9.506 s) [F(1, 16) = 7.848, p = .013]. Whereas, for the MIA*WT rats, there was no significant difference in the time spent with the pups (102.308 ± 18.442 s) or the male rat (103.190 ± 15.426 s) [F(1, 5) = 1.701, p = .249].

Discussion

- The main purpose of the study was to find a three-way interaction between genotype, treatment and interacted subjects. Interestingly, and not expected, the KO*MIA rats spent more time with the pups compared to time spent with the male rat.
- This unexpected results from this study are evidence that there is a complex spectrum of behavioral symptoms due to genetics, environment and their interactive effect (Schwartzer et al., 2013). For that reason, the interaction between genetics (DISC1) and environment (MIA) might be more complex than what is believed.
- It has been demonstrated that the timing of exposure to infection plays an important role.. The study by Abazyan et al. (2010) treated rats with poly I:C on gestation day 9, because it corresponds to the middle/end of the first trimester of human pregnancy. Another study by Ibi et al. (2010) injected with polyI:C for 5 days in a row (PP2-PP6). Since there is little information on the MIA effect on maternal behavior, the timing of exposure used in the present study (13 and 15) might have had an effect on the results.
- For the pup preference test used in this study, a male rat was used as the alternative to pups. This compares sex motivation vs. maternal behaviors. On the other hand, in a study by Hsueh et al. (2018), MIA offspring exhibited social deficits, including more anxiety-like behaviors, and more favorable responses to objects than to strange mice on the 3-CBT test. For future research, the use of an object could be used to answer a different question comparing social vs nonsocial behavior in a maternal behavior model.
- In addition, other measures/tests of maternal behavior could have yielded better/different results as well as a bigger sample size. This is just the beginning of a line of research that studies the interactive effects of genetics and environment in order to find more suitable treatments for psychiatric disorders that not only affect mother, but the way they raise their offspring.