

## RESEARCH ARTICLE

# Chronic *Toxoplasma gondii* in Nurr1-Null Heterozygous Mice Exacerbates Elevated Open Field Activity

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## Abstract

Latent infection with *Toxoplasma gondii* is common in humans (approximately 30% of the global population) and is a significant risk factor for schizophrenia. Since prevalence of *T. gondii* infection is far greater than prevalence of schizophrenia (0.5-1%), genetic risk factors are likely also necessary to contribute to schizophrenia. To test this concept in an animal model, Nurr1-null heterozygous (+/-) mice and wild-type (+/+) mice were evaluate using an emergence test, activity in an open field and with a novel object, response to bobcat urine and prepulse inhibition of the acoustic startle response (PPI) prior to and 6 weeks after infection with *T. gondii*. In the emergence test, *T. gondii* infection significantly decreased the amount of time spent in the cylinder. *Toxoplasma gondii* infection significantly elevated open field activity in both +/+ and +/- mice but this increase was significantly exacerbated in +/- mice. *T. gondii* infection reduced PPI in male +/- mice but this was not statistically significant. Aversion to bobcat urine was abolished by *T. gondii* infection in +/+ mice. In female +/- mice, aversion to bobcat urine remained after *T. gondii* infection while the male +/- mice showed no aversion to bobcat urine. Antibody titers of infected mice were a critical variable associated with changes in open field activity, such that an inverted U shaped relationship existed between antibody titers and the percent change in open field activity with a significant increase in activity at low and medium antibody titers but no effect at high antibody titers. These data demonstrate that the Nurr1 +/- genotype predisposes mice to *T. gondii*-induced alterations in behaviors that involve dopamine neurotransmission and are associated with symptoms of schizophrenia. We propose that these alterations in murine behavior were due to further exacerbation of the altered dopamine neurotransmission in Nurr1 +/- mice.

## OPEN ACCESS

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## Introduction

Schizophrenia is a neuropsychiatric illness that is diagnosed based on positive symptoms such as delusions, paranoia and hallucination and negative symptoms such as blunted emotions. Recent gene wide association studies have identified gene variants that have only a small association with increased risk of schizophrenia [1]. Copy number variations in some genes are associated with a greater risk for schizophrenia; however, these are very rare [2]. A number of environmental and developmental stressors, such as being raised in an urban area, juvenile cannabis use, obstetric complications, maternal infection during pregnancy and infection with *Toxoplasma gondii* have all been shown to increase the risk of developing schizophrenia [3,4]. One prevailing hypothesis is that genetic susceptibility and environmental stressors interact to potentiate the risk of schizophrenia.

*Toxoplasma gondii* is an obligate intracellular protozoan parasite that undergoes sexual reproduction in a cat host, where it is shed as oocysts into the environment through feces. *Toxoplasma gondii* also infects most warm-blooded vertebrates, reproducing asexually during an acute phase that triggers an immune response and the production of antibodies to the parasite [5]. Following the initial immune response, the parasite enters a chronic phase whereby it forms quiescent tissue cysts with bradyzoite stages, primarily in brain and muscle tissues. Globally, the rate of human infection has been estimated at 30%, with rates as high as 80% in some countries [5]. While these infections have been considered benign, evidence is accumulating that suggests that *T. gondii* infection alters human behavior and, in some cases, contributes to or exacerbates mental illness [6–8]. Interestingly, slower reaction time, an increase in traffic accidents and associations with specific personality traits were reported in individuals with antibody titers to *T. gondii* [8]. High antibody titers to this parasite also increase the incidence of schizophrenia (average odds ratio of ~2.6), increase severity of schizophrenia symptoms [4,9–13] and exacerbate gray matter reductions in schizophrenia patients [14]. Additionally, children born to a mother with high antibody titers have a similar elevated risk for developing schizophrenia [4,15–17]. This relatively high risk factor, combined with the high prevalence of this parasite suggests that it could be a substantial contributor to the number of schizophrenia cases [18,19].

One proposed mechanism by which *T. gondii* affects behavior is through elevating dopamine neurotransmission. An initial study found elevated tissue dopamine levels (14%) in whole brain of mice after *T. gondii* infection [20]. The strongest support for this dopamine hypothesis was provided by the discovery that *T. gondii* expresses two tyrosine hydroxylase enzymes that can synthesize DOPA *in vitro* using enzymatic assays and ultimately dopamine *in vivo* based on immunohistochemical labeling of dopamine [21,22]. In addition open field activity, a behavior closely linked with dopamine neurotransmission, is commonly found to be elevated after infection with *T. gondii* [23–26]. This hypothesized mechanism is particularly relevant to schizophrenia as elevated subcortical dopamine neurotransmission has been implicated as an important factor in the positive symptoms of schizophrenia and antipsychotics work by blocking the dopamine D2 receptor [27–29].

Although infection with *T. gondii* does not cause schizophrenia itself, as evidenced by much greater rates of *T. gondii* infection (~30%) compared to schizophrenia (~1%), *T. gondii* infection could serve as a perturbation that combines with a genetic predisposition to lead to schizophrenia. Nurr1 (NR4A2) is an orphan nuclear receptor that is essential for the development and continued survival of mesencephalic dopamine neurons [30–33]. This receptor is implicated as a potential contributor to the development of schizophrenia as rare mutations in Nurr1 have been reported in schizophrenia patients [34,35] and were associated with attention deficits in schizophrenia patients [36]. Although the homozygous deletion of Nurr1 is lethal at

birth, Nurr1-null heterozygous (+/-) mice survive normally. The heterozygous genotype, however, causes alterations in mesoaccumbens and mesocortical dopamine levels and elevated open field activity [37]. Because of these changes, the Nurr1 +/- mice have been investigated as a model for schizophrenia-related behaviors [37–40]. Furthermore, these mice are sensitive to the developmental stressor of post-weaning isolation, an experimental treatment used as a model for early life stressors that contribute to the risk of schizophrenia. Post-weaning isolation of +/- mice specifically disrupted sensorimotor gating as measured by prepulse inhibition of the acoustic startle response (PPI), a parameter that is also disrupted in patients with schizophrenia and correlates with positive symptoms [37–47]. Additionally, these mice had elevated amphetamine-stimulated dopamine release in the shell of the nucleus accumbens, a parameter that is also similar to what has been found in patients with schizophrenia [48]. Therefore, these mice represent a condition of susceptibility to alterations in a behavior disrupted in schizophrenia that requires an environmental insult.

Based on the observations that the Nurr1 +/- genotype alters dopamine neurotransmission and open field activity and infection with *T. gondii* is proposed to alter dopamine neurotransmission, the goals of the current study were to determine how *T. gondii* infection interacts with the +/- genotype to affect dopamine related behaviors. Our results demonstrate a particular susceptibility of +/- mice to the behavioral effects of *T. gondii* infection, specifically elevated open field activity and a trend toward disrupted sensorimotor gating. Additionally, we demonstrate an inverted U relationship between antibody titers and the effect on open field activity.

## Materials and Methods

### Ethics Statement

All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and study protocols were approved by the Institutional Animal Care and Use Committee at Mississippi State University. All animals used in this project were housed in the AAALAC accredited facilities of the College of Veterinary Medicine, Mississippi State University. The individual room temperatures were maintained between 18–22°C with food and water *ad libitum*. Care of the mice was overseen by a laboratory animal veterinarian.

### Animals

Animals were housed at Mississippi State University in an AAALAC accredited facility on 12 hour light/dark cycle with food and water available *ad libitum*. Nurr1 +/- and wild-type (+/+) mice used were obtained from a colony currently bred at Mississippi State University and originally produced in the laboratory of Dr. Vera Nikodem at the National Institute for Diabetes and Digestive and Kidney Diseases [30]. Mice were genotyped as previously described to distinguish +/- and +/+ mice [30]. The Nurr1 +/- mice were bred into a C57BL6 background. At 19–21 days of age, mice were weaned and housed in groups of 3–5/cage. All mice were housed in cages with steel grid lids and all cages were located in the same room.

### Behavioral tests

The following behavior tests were performed on 120-day old Nurr1 +/- and +/+ mice prior to and six-weeks after infection on separate days with each test period 3–4 days apart: (1) PPI; (2) Emergence, open field and novel object measured sequentially in 20 min intervals; (3) open field and bobcat urine response measured sequentially in 20 min intervals. The PPI, emergence, open field, and novel object and bobcat urine tests were all performed between 0800

and 1200 on different days with each test 3â 4 days apart. Body weights were recorded following the PPI test. After initial behavior testing, mice were injected subcutaneously with 200  $\mu$ l Hank's Buffered Salt Solution (HBSS) containing 1000 tachyzoites (*T. gondii* ME49) or HBSS without tachyzoites. *Toxoplasma gondii* ME49 is a type II strain and was chosen for these studies because type II strains are most commonly reported in human *T. gondii* infections in the United States. Six weeks post-injection, behavior testing was repeated. After the completion of testing, the mice were euthanized by CO<sub>2</sub> asphyxiation. Trunk blood samples were collected from sacrificed mice into Vacutainer tubes with heparin. Blood samples were centrifuged at 2000 x g for 5 min and plasma collected for serological testing. *Toxoplasma gondii* antibody titers were determined using an indirect immunofluorescent antibody test (IFAT) with *T. gondii* (RH) antigen, murine plasma diluted 2-fold from 1:25, and a fluorescein-isothiocyanate-labeled secondary antibody. Titers were calculated as the reciprocal dilution producing the last visible fluorescence on the IFAT. A cut-off titer of 50 was used to determine seroconversion to *T. gondii*.

Prepulse inhibition of the acoustic startle response was preformed as previously described [38]. Mice were placed in a startle chamber on a transducer, calibrated to 1 Newton to measure the startle response (Kinder Scientific, Poway CA). Each procedure consisted of a 5 min acclimation period followed by 65 stimulation trials presented in a pseudorandom order using a 70 dB background white noise. The first five and the last five stimulations were the 120 dB startle. The other 56 trials were presented in pseudo-random order and consisted of 10 trials of a 120 dB startle, and 10 trials each of a 72 dB prepulse, 74 dB prepulse and 78 dB prepulse (2 dB, 4 dB and 8 dB above background, respectively) 100 ms prior to the 120 dB startle, as well as 4 trials each of a 72 dB, 74 dB, and 78 dB prepulse alone and 4 trials of no stimulation. An average of 15 seconds separated each trial (between 13â 17 seconds). The startle response was calculated as the average startle magnitude from the 10 120 dB trials presented in random order. PPI was calculated as the percent change between the average startle response alone and the average startle response after the prepulse. Acclimation was measured as the percent change between the first five startle tests and the last five startle tests. Following the PPI test, the weight of each mouse was recorded.

The emergence test, open field and novel object tests were performed similar to those described by [49]. This test was performed 3â 4 days after PPI. Three separate open field chambers 25 cm x 25 cm with a gray floor and clear plexiglass walls and each equipped with a video camera attached to a computer were used to record animal behaviors. Each experimental mouse was first tested in the emergence test. The mouse was placed inside a stainless steel cylinder (10 cm deep and 6.5 cm in diameter) and video recorded for 20 min. A trained observer blind to genotype and treatment watched the video and scored the following behaviors: the latency to exit the cylinder, the number of exits from the cylinder to the open field, and time spent in the cylinder during each entry. An exit or entrance was defined as placement of all four paws into the open field or into the cylinder, respectively. A maximum of 300s was used for the latency to exit. Additionally, the total time spend inside the cylinder was calculated.

At the end of the emergence test, the mouse was placed in the center of the next open field chamber and recorded for 20 min. Distance traveled was measured using LimeLight, a video tracking software that tracks the movement of the center of the mouse. For analysis, the open field arena was divided into 16 squares (4X4 arrangement) and the time spent within the inner 4 squares (time in center) was measured. Following the open field test, the mouse was moved to the third open field chamber with a small plastic toy secured to the center of the open field. Distance traveled and time in the center of the open field was determined. Between each subject, the chambers were thoroughly cleaned and wiped down with 70% ethanol.

The bobcat urine test was conducted 3â 4 days following the emergence/open field/novel object test. Using two of the open field chambers described above, each mouse was first placed

in the center of the first open field chamber and recorded for 20 min. The mouse was then moved to the next open field chamber that contained a plastic receptacle secured to the floor of the chamber in each of the 4 corners with one that contained 50  $\mu$ l of bobcat urine. Diagonal to the bobcat urine was 50  $\mu$ l of rabbit urine and water was placed in the two receptacles in the remaining corners. Each mouse was recorded for 20 min. Following each test, the chambers were cleaned as described above. The location of the bobcat urine was changed for every test to avoid any possible bias within the open field chamber. Time spent in each of the 4 quadrants was measured and a percent calculated for the preceding open field test and the test containing bobcat urine. Comparisons were made between the percent time spent in the bobcat urine quadrant and the corresponding quadrant in the preceding open field test. This comparison accounts for any bias in the time spend among quadrants.

## Data Analysis

Data from behavioral tests prior to infection were analyzed using parametric analysis of variance (ANOVA) across genotype and sex with Fisher's PLSD post hoc comparisons where appropriate. Post treatment data from emergence test, open field, novel object, and body weight were analyzed using ANOVA across treatment, genotype and sex. Fisher's PLSD post hoc comparisons were used where appropriate. For the PPI data, the effects of treatment, genotype, sex and prepulse intensity were analyzed using ANOVA with repeated measures design. For bobcat urine avoidance, a paired t-test was used to compare, for each experimental group, the percent time spent in the bobcat urine quadrant and the corresponding quadrant in the preceding open field test. For comparison of effects of antibody titers and change in open field activity an ANOVA was calculated across the control, low, medium and high antibody categories followed by Fisher's PLSD post hoc comparisons. All statistical analysis was performed using StatView Software. Results were considered statistically significance at  $p < 0.05$ .

## Results

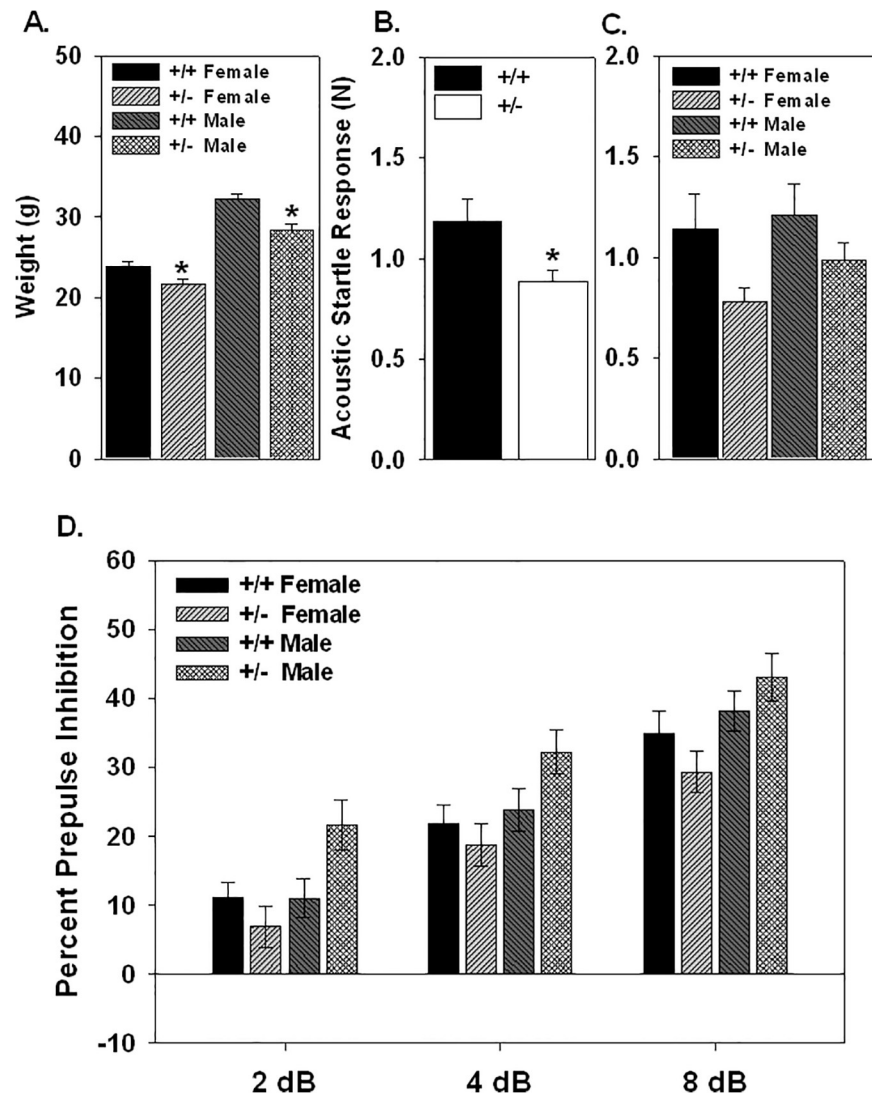
### Reduced Body Weight Due to the Nurr1 +/- Genotype

A number of behavioral studies have been carried out on Nurr1 null mice [38, 40, 50, 51]. Here we found that the +/- genotype significantly reduced body weight ( $F_{1,85} = 9.89$ ,  $p = 0.0023$ ). Female and male +/- mice had a lower body weight than their +/+ littermates based on the pre-test weight at 120d of age with an 8.7% and 12.1% lower body weight, respectively (Fig 1A). This difference appears to be due to a reduction in weight gain with aging as weight gain was also reduced in the +/- control mice weighed 6 weeks later (see below).

### Prepulse inhibition in the Nurr1 +/- Mice

In the pretest, PPI and acoustic startle response data was analyzed across genotype, sex and prepulse intensity using a repeated measures ANOVA. A significant genotype effect was observed in the acoustic startle response where the +/- genotype resulted in a reduction in magnitude of the startle response ( $F_{1,89} = 5.33$ ,  $p = 0.023$ ; Fig 1B.). No significant interaction was found between genotype and sex (Fig 1C.). No difference in PPI was observed due to the +/- genotype in either males or females. There was an overall trend toward a sex effect ( $F_{1,77} = 3.51$ ,  $p = 0.065$ ) much of which was due to differences between male and female +/- mice (Fig 1D.). The results from the male mice are very similar to what was previously reported [38]. Although Vuillermot et al. reported significant reduction in PPI in +/- mice, testing was done during the dark phase of the light cycle as compared to the light phase done here [40]. No difference in habituation to the startle response based on genotype was observed (data not





**Fig 1. Body weight, acoustic startle response and prepulse inhibition of the acoustic startle response was measured in 120 day old female and male Nurr1 +/+ and +/- mice.** Body weight (A.) was significantly reduced due to the +/- genotype and sex with significant reductions in both the female +/- ( $p = 0.037$ ) and male +/- mice ( $p = 0.0002$ ) as compared to +/+ mice. There was a significant reduction in acoustic startle response due to the +/- genotype (B.) that was not affected by sex (C.). No significant differences in percent prepulse inhibition of the acoustic startle response was found due to the +/- genotype or sex. Body weight and acoustic startle: \*genotype difference  $p \leq 0.05$  based on ANOVA with Fischer's post hoc comparison.  $n = 19$  female +/+; 21 female +/-; 29 male +/+; 18 male +/-.

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shown). Additionally, there were no differences in PPI, acoustic startle response or habituation in the pretest results between control mice later injected with a vehicle and treatment mice later injected with *T. gondii* (data not shown).

### Emergence Test, Open Field Activity and Novel Object in Nurr1 +/- Mice

In the emergence test, no significant differences in latency to exit the cylinder or time in the cylinder were found due to the +/- genotype (Table 1). The most obvious difference in the emergence test was that the +/- mice entered and exited the cylinder more frequently than

Table 1. Emergence test latency, time in cylinder and total exits.

	Latency (s)	Time in Cylinder (s)	Exits
<b>Pretest</b>			
♀♀ Female +/+ (n = 18)	40.40 $\hat{A}\pm$ 12.76	445.32 $\hat{A}\pm$ 104.93	13.63 $\hat{A}\pm$ 2.18
♀♀ Female +/- (n = 19)	49.20 $\hat{A}\pm$ 17.55	372.12 $\hat{A}\pm$ 62.38	18.78 $\hat{A}\pm$ 1.68
♂♂ Male +/+ (n = 29)	75.44 $\hat{A}\pm$ 16.89	448.32 $\hat{A}\pm$ 71.10	11.76 $\hat{A}\pm$ 1.29
♂♂ Male +/- (n = 18)	57.97 $\hat{A}\pm$ 14.79	299.54 $\hat{A}\pm$ 40.68	18.00 $\hat{A}\pm$ 1.44
♀♀ +/+	61.55 $\hat{A}\pm$ 11.56	447.13 $\hat{A}\pm$ 59.06	12.50 $\hat{A}\pm$ 8.01
♀♀ +/-	53.46 $\hat{A}\pm$ 11.40	336.81 $\hat{A}\pm$ 37.63	18.39 $\hat{A}\pm$ 1.09*
<b>Control</b>			
♀♀ Female +/+ (n = 9)	16.13 $\hat{A}\pm$ 4.23	226.98 $\hat{A}\pm$ 59.78	17.78 $\hat{A}\pm$ 1.15
♀♀ Female +/- (n = 7)	13.19 $\hat{A}\pm$ 3.16	437.14 $\hat{A}\pm$ 137.08	19.25 $\hat{A}\pm$ 3.39
♂♂ Male +/+ (n = 17)	10.11 $\hat{A}\pm$ 2.24	342.68 $\hat{A}\pm$ 57.95	15.59 $\hat{A}\pm$ 1.47
♂♂ Male +/- (n = 10)	6.76 $\hat{A}\pm$ 2.03	223.81 $\hat{A}\pm$ 53.19	19.60 $\hat{A}\pm$ 3.08
♀♀ +/+	12.20 $\hat{A}\pm$ 2.10	282.89 $\hat{A}\pm$ 39.52	16.21 $\hat{A}\pm$ 0.96
♀♀ +/-	9.62 $\hat{A}\pm$ 1.91	316.83 $\hat{A}\pm$ 60.96	21.57 $\hat{A}\pm$ 2.27*
♀♀ All Control	11.14 $\hat{A}\pm$ 1.46	297.15 $\hat{A}\pm$ 34.07	18.46 $\hat{A}\pm$ 1.15
<b>T. gondii infection</b>			
♀♀ Female +/+ (n = 5)	8.50 $\hat{A}\pm$ 2.86	129.74 $\hat{A}\pm$ 43.33	13.60 $\hat{A}\pm$ 3.61
♀♀ Female +/- (n = 10)	15.85 $\hat{A}\pm$ 5.64	203.31 $\hat{A}\pm$ 42.36	21.10 $\hat{A}\pm$ 3.10
♂♂ Male +/+ (n = 9)	14.89 $\hat{A}\pm$ 3.66	133.68 $\hat{A}\pm$ 40.49	13.56 $\hat{A}\pm$ 1.62
♂♂ Male +/- (n = 8)	10.95 $\hat{A}\pm$ 2.48	137.07 $\hat{A}\pm$ 28.69	19.25 $\hat{A}\pm$ 4.32
♀♀ +/+	12.60 $\hat{A}\pm$ 2.63	132.27 $\hat{A}\pm$ 29.24	13.86 $\hat{A}\pm$ 1.67
♀♀ +/-	13.68 $\hat{A}\pm$ 2.29	173.87 $\hat{A}\pm$ 27.24	20.28 $\hat{A}\pm$ 2.51*
♀♀ All T. gondii infection	13.21 $\hat{A}\pm$ 2.15	155.67 $\hat{A}\pm$ 19.99 <sup>#</sup>	17.47 $\hat{A}\pm$ 1.67

\*Genotype effect

# T. gondii infection effect after ANOVA and post-hoc comparison. p&lt;0.05.

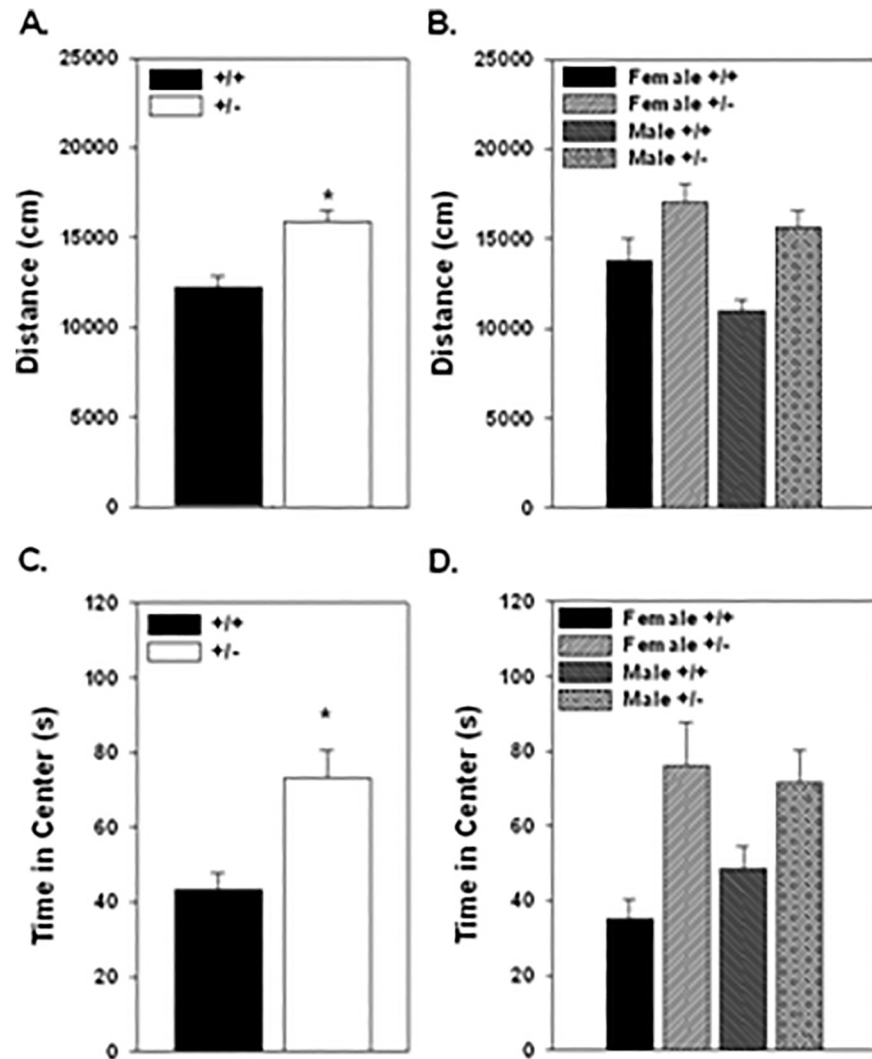
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the +/+ mice as shown by a greater number of exits ( $F_{1,81} = 9.335$ ,  $p = 0.003$ ). This effect was similar across male and female +/- mice.

Parameters associated with open field activity analyzed included distance traveled and time in the center of the chamber. As previously reported, the +/- mice were significantly more active in the open field than the +/+ mice ( $F_{1,80} = 16.76$ ,  $p = 0.0001$ ; Fig 2A.). This was similar for both the male and female mice. Additionally, the +/- mice spent more time in the center of the open field chamber than the +/+ mice with similar effects in both the males and females ( $F_{1,80} = 15.17$ ,  $p = 0.0002$ ; Fig 2B.). No differences in the interaction with the novel object, as measured by time in the center of the chamber, were found due to the genotype (data not shown).

### Bobcat Urine Avoidance in the Nurr1 +/- Mice

In the bobcat urine avoidance, time spent in each of the 4 quadrants of the open field was measured with bobcat and rabbit urine present and in the preceding open field test. The percent of time spent in the bobcat urine quadrant was compared to the percent of time spent in corresponding quadrant from the preceding open field test. There was a significant aversion to bobcat urine in the female mice (+/+,  $p = 0.036$ ; +/-,  $p = 0.037$ ; Fig 3A.). No significant effect of bobcat urine was found in the male mice. All pretest measures were also compared based on either later receiving the vehicle injection or T. gondii infection. No differences in any parameters were found in the groups prior to treatment (data not shown).



**Fig 2. Behavior in an open field was tested in male and female Nurr1 +/+ and +/- mice.** Total distance traveled (A., B.) and time in the center (C., D.) are shown based on genotype (A., C.) and genotype and sex (B., D.). The +/- mice were significantly more active than the +/+ mice as indicated by distance traveled, however, there was no significant interaction between genotype and sex (B.). Additionally, the +/- mice spent significantly more time in the center of the chamber than the +/+ mice (C.), which also showed no interaction between genotype and sex (D.). \*significant difference based on ANOVA with Fischer's post hoc comparison. n = 18 female +/+; 19 female +/-; 29 male +/+; 18 male +/-.

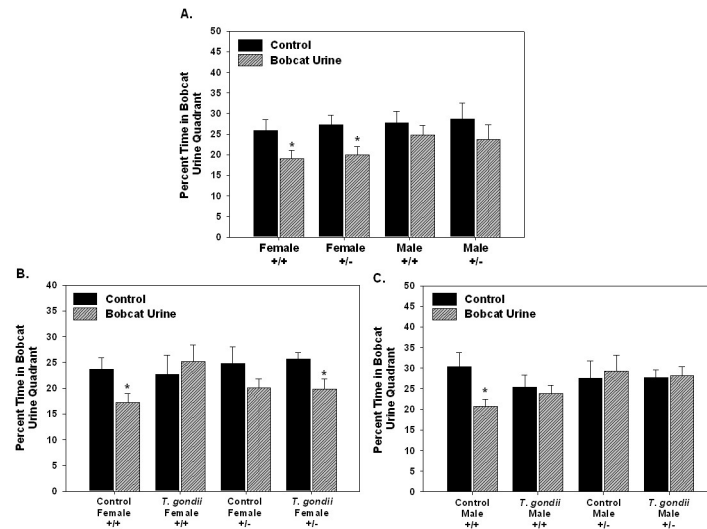
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### Seroconversion to *T. gondii* antigen

A total of 86 mice were used to begin these experiments with 18 female +/+ mice, 21 female +/- mice, 29 male +/+ mice and 18 male +/- mice. Of those mice, data files were lost due to equipment malfunction in 2 emergence tests, 2 bobcat urine avoidance and 1 open field test. For *T. gondii* injection, 8 female +/+ mice, 13 female +/- mice, 12 male +/+ mice and 8 male +/- mice were injected. Three female +/+ mice, 3 female +/- mice and 3 male +/+ mice were seronegative for *T. gondii* and were excluded from the behavioral analysis. Additionally, 1 control female +/+ mouse died before the second round of behavioral tests.

Infection of mice was confirmed by seroconversion to *T. gondii* antigen. There was a wide range of antibody titers measured, with a titer of 50 as the lowest for inclusion as antibody





**Fig 3. To demonstrate aversion to bobcat urine, percent time spent in the quadrant with bobcat urine (Bobcat Urine) was compared to the percent time spent in the same quadrant from the preceding open field test (Control) for each treatment group.** In the first test (A.), female mice (both +/+ and +/-) showed significant aversion to bobcat urine as they spent significantly less time in the bobcat urine quadrant than in the corresponding quadrant from the open field test. No significant aversion was found using these parameters for the male mice. In the second test, after either a vehicle injection (Control) or *T. gondii* injection, the control female +/+ mice showed a significant aversion to bobcat urine that was abolished by injection with *T. gondii* (B). In contrast the control female +/- mice showed a trend toward aversion to bobcat urine but significant aversion to bobcat urine after *T. gondii* injection (B.). The control male +/+ mice showed significant aversion to bobcat urine that was also abolished by *T. gondii* injection (C.). In contrast, the male +/- mice showed no aversion to bobcat urine either in the control condition or after *T. gondii* injection. Comparisons based on paired t-test with  $p < 0.05$ . Figure A:  $n = 18$  female +/+; 21 female +/-; 29 male +/+; 18 male +/- . Figure B:  $n = 10$  female +/+ controls; 6 female +/- controls; 5 female +/+ *T. gondii*; 10 female +/- *T. gondii*. Figure C: 17 male +/+ control; 10 male +/- controls; 10 male +/+ *T. gondii*; 8 male +/- *T. gondii*.

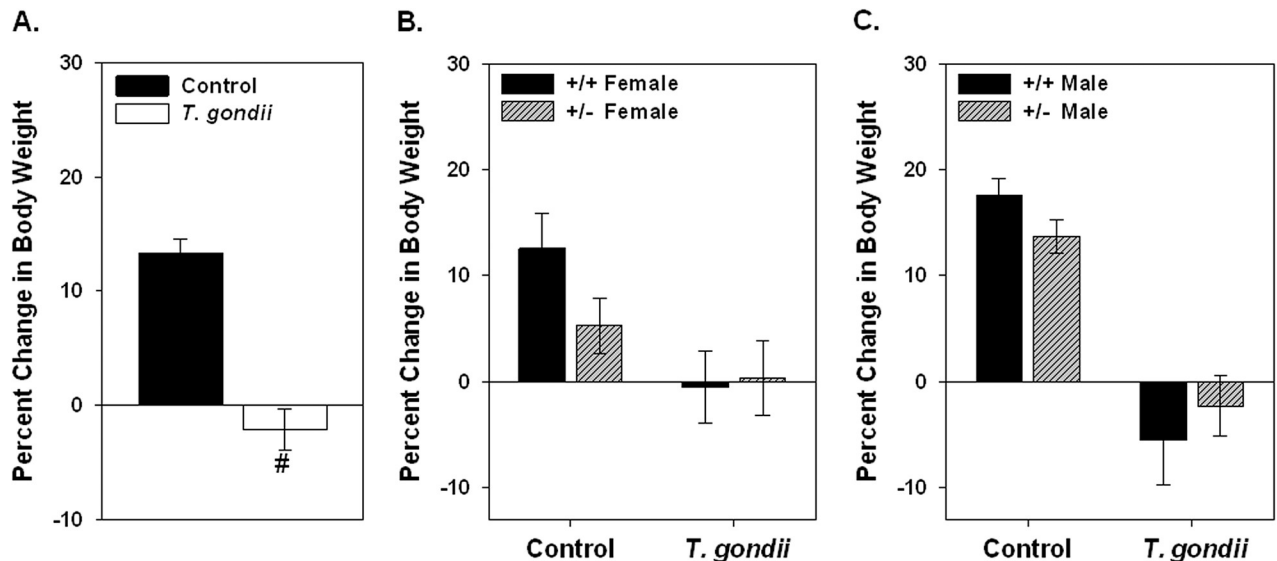
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positive, and up to 6400 measured in some mice. Geometric mean titers and range for seropositive mice based on sex and genotype are shown in Table 2. Nine mice injected with *T. gondii* had antibody titers below 50. These mice were excluded from the behavioral analysis. The +/- mice had an overall lower average antibody titer than the +/+ mice, but this was not statistically significant. Mice injected with *T. gondii* that seroconverted showed a significant reduction in weight gain (Fig 4A.). No significant interactions between *T. gondii* infection, genotype or sex on weight gain were found. Overall, there was a reduction in weight gain in female mice and net weight loss in the male mice (Fig 4B and 4C.). The female +/+ control mice gained  $12.59 \pm 3.30\%$  of their original body weight with the female +/- control mice gaining  $5.26 \pm 2.58\%$  of their body weight. In contrast, the +/+ female *T. gondii* infected mice averaged a reduction in the percent change in body weight with a  $-0.51 \pm 3.39\%$  reduction in body weight. The +/- *T. gondii* injected female mice also had a lower percent change in body weight ( $0.32 \pm 3.50\%$ ). The *T. gondii* infected +/+ and +/- male mice had a  $-5.5 \pm 4.15\%$  and  $-2.30 \pm$

**Table 2. Antibody titers to *T. gondii*.**

Sex-Genotype	Geometric mean titer	Range in titers for seropositive mice
Female +/+	919	50–3200
Female +/-	696.4	50–3200
Male +/+	1371.6	500–6400
Male +/-	1131.4	100–6400

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**Fig 4.** Effect of *T. gondii* infection on body weight is shown in female and male Nurr1 +/+ and +/- mice as percent change in body weight from weight prior to infection to body weight 6 weeks after infection or vehicle injection (Control). Infection with *T. gondii* significantly reduced weight gain (A.). There was no significant interaction in the change in body weight due to either the genotype or sex, however, the +/- mice gained less weight over this time (B., C.) and the infected male mice, on average, lost weight (C.) while the infected female mice showed no change in body weight (B.). #significant difference due to treatment based on ANOVA with Fischer's post hoc comparison. n = 10 female +/+ controls; 9 female +/- controls; 5 female +/+ *T. gondii*; 10 female +/- *T. gondii*; 18 male +/+ control; 11 male +/- controls; 9 male +/+ *T. gondii*; 8 male +/- *T. gondii*.

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$\pm 2.83\%$  change in body weight as compared to  $17.56 \pm 1.57\%$  and  $13.68 \pm 1.57\%$  increase in body weight in male +/+ and +/- control mice, respectively.

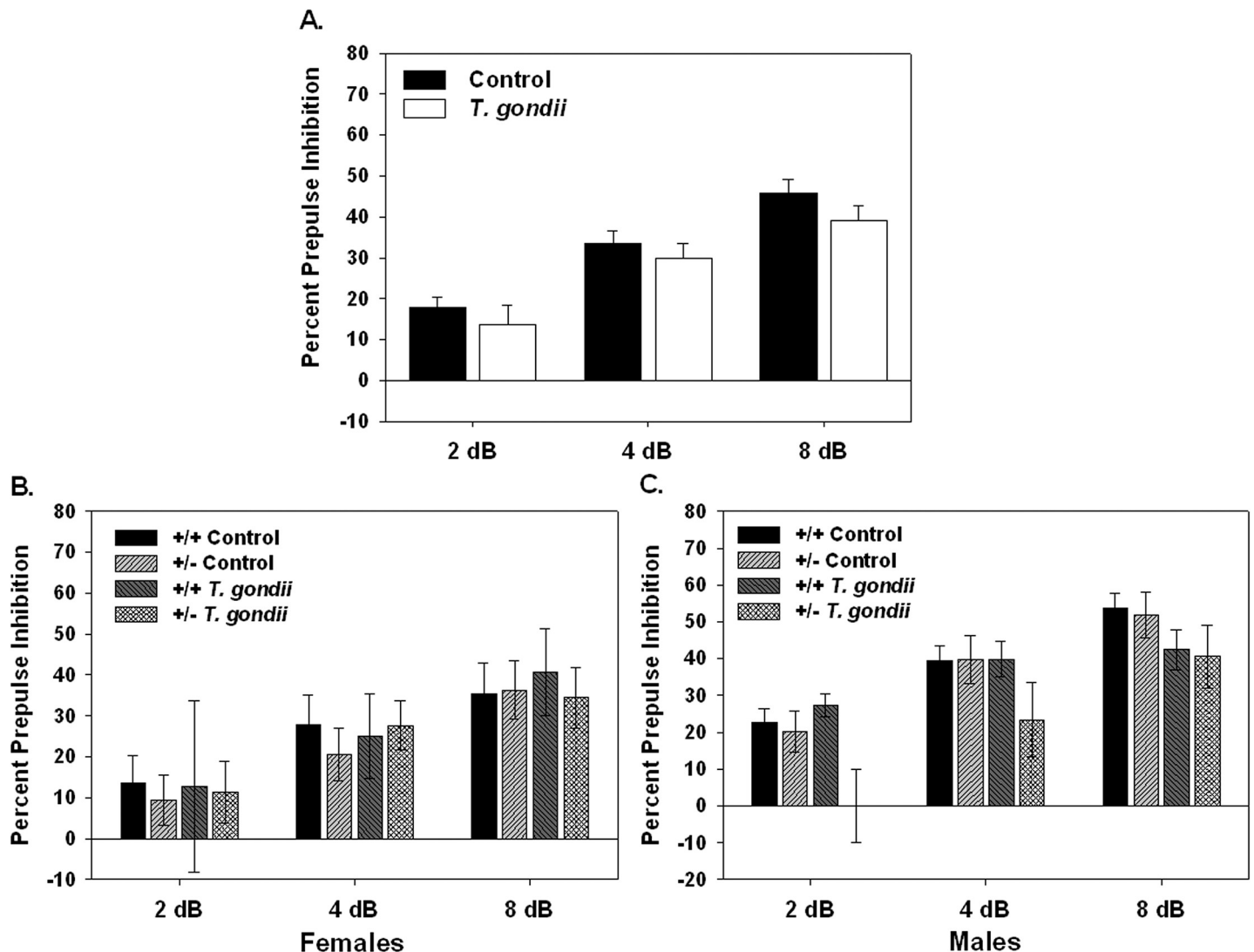
### Effect of *T. gondii* infection on behavior â PPI, emergence test, open field and novel object

Infection with *T. gondii* had no effect on acoustic startle response or habituation of startle (data not shown). Additionally, no effect on PPI was found in the female mice after infection among any groups. In the male mice, *T. gondii* infection resulted in a decrease in PPI in the +/- mice as compared to both *T. gondii* infected +/+ mice and control +/- mice, particularly at the 2 dB prepulse intensity (Fig 5), however, this did not reach statistical significance.

No difference in latency to exit was found among groups in the emergence test performed six weeks after injection with *T. gondii* or vehicle (Table 1), however, latency to exit was significantly reduced in the post-injection test compared to the initial test. Infection with *T. gondii* did not affect latency to exit or total exits. *Toxoplasma gondii* infection significantly reduced the time spent in the cylinder ( $F_{1,72} = 9.18$ ,  $p = 0.034$ ) but had no effect on the latency to exit or number of exits.

Infection with *T. gondii* significantly elevated the distance traveled in the open field ( $F_{1,65} = 6.56$ ,  $p = 0.0031$ ; Fig 6A.) and had a significantly greater effect on the +/- mice in elevating activity than on the +/+ mice ( $F_{1,65} = 4.573$ ,  $p = 0.036$ ; Fig 6B.). No effect of sex was found as female and male +/+ and +/- mice showed similar elevation of open field distance (Fig 6C and 6D.). Similarly, the time in the center was significantly elevated by infection with *T. gondii* ( $F_{1,66} = 8.407$ ,  $p = 0.0056$ ; Fig 7A.). No significant interactions were found with genotype or sex (Fig 7B, 7C and 7D.).

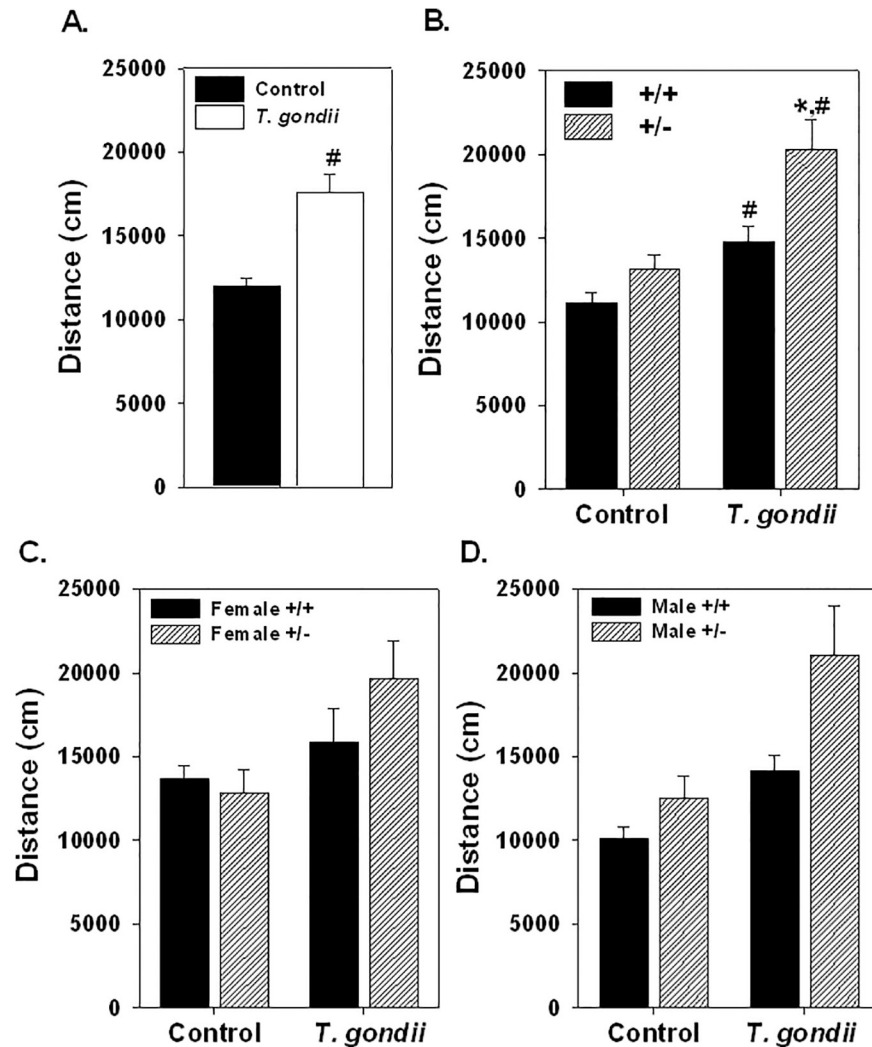
In order to understand the variables associated with changes in the behavior of mice infected with *T. gondii*, the relationship between reciprocal antibody titers with changes in



**Fig 5. Percent prepulse inhibition in Nurr1 +/+ and +/- mice after vehicle injection (Control) or *T. gondii* infection (A).** Additionally, percent prepulse inhibition is shown in female (B.) and male (C.) +/+ and +/- mice that received a vehicle injection (Control) or infection with *T. gondii*. *Toxoplasma gondii* infection had no significant effect on prepulse inhibition. There was a trend towards reduced percent prepulse inhibition in male +/- mice after chronic infection with *T. gondii* (C.). n = 10 female +/+ controls; 9 female +/- controls; 5 female +/+ *T. gondii*; 10 female +/- *T. gondii*; 17 male +/+ control; 12 male +/- controls; 9 male +/+ *T. gondii*; 7 male +/- *T. gondii*.

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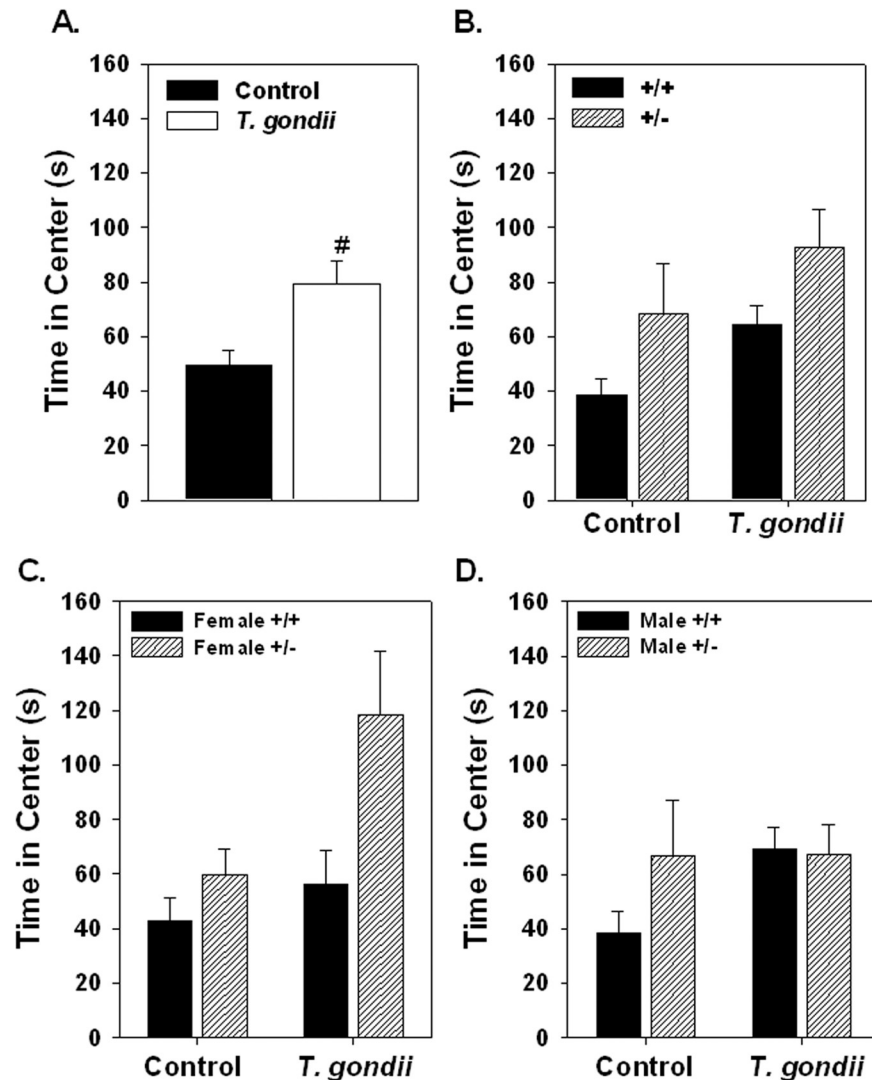
body weight and open field activity were determined. Based on our measurements, antibody titers were categorized into low (50–400), medium (800–1600) and high (≥3200) levels and compared to percent change in body weight and percent change in distance traveled in the open field tests (Fig 8). All mice (male and female, +/+ and +/-) were combined for this analysis. Data from the mice injected with *T. gondii* that did not seroconvert (reciprocal antibody titers less than 50 and labeled “Zero”) were included in the graph but not in the statistical analysis. There was a significant effect of antibody levels on change in body weight ( $F_{3,70} = 19.842$ ,  $p < 0.0001$ ) where body weight was reduced in all seropositive groups compared to control mice (Fig 8A.). A similar change in body weight was observed in mice injected with *T. gondii* that were seronegative (“Zero” group) and control mice. When the percent change in distance traveled in the open field between the



**Fig 6. Open field activity is shown in female and male Nurr1 +/+ and +/- mice after *T. gondii* infection or vehicle injection (Control).** Infection with *T. gondii* significantly elevated open field activity with all groups (sex and genotype) combined (A.). There was a significant interaction between infection with *T. gondii* and the Nurr1 +/- genotype on open field activity such that the +/- mice were significantly more active than infected +/+ mice and uninfected +/- control mice (B.). Both female and male +/- mice infected with *T. gondii* were more active in the open field than the control +/- mice (C., D.). Much of the increase in activity in the infected +/+ mice was the result of increased activity in the male mice. Significant genotype (\*) and treatment (#) effects based on ANOVA with Fischer's post hoc comparisons. n = 10 female +/+ controls; 8 female +/- controls; 5 female +/+ *T. gondii*; 10 female +/- *T. gondii*; 17 male +/+ control; 10 male +/- controls; 9 male +/+ *T. gondii*; 8 male +/- *T. gondii*.

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pretest and the post-test was graphed based on antibody levels, an inverted U shaped distribution was found. Statistical analysis revealed a significant effect of antibody titer level and change in open field activity ( $F_{3,70} = 5.175$ ,  $p = 0.0027$ ). Low and medium antibody level groups had a significant increase in activity, however, activity of mice in the high reciprocal antibody level ( $\approx 3200$ ) group was not different from control and appeared only slightly elevated (Fig 8B.). Mice that were injected with *T. gondii*, but were seronegative (Zero) showed a similar change in activity to the control mice.

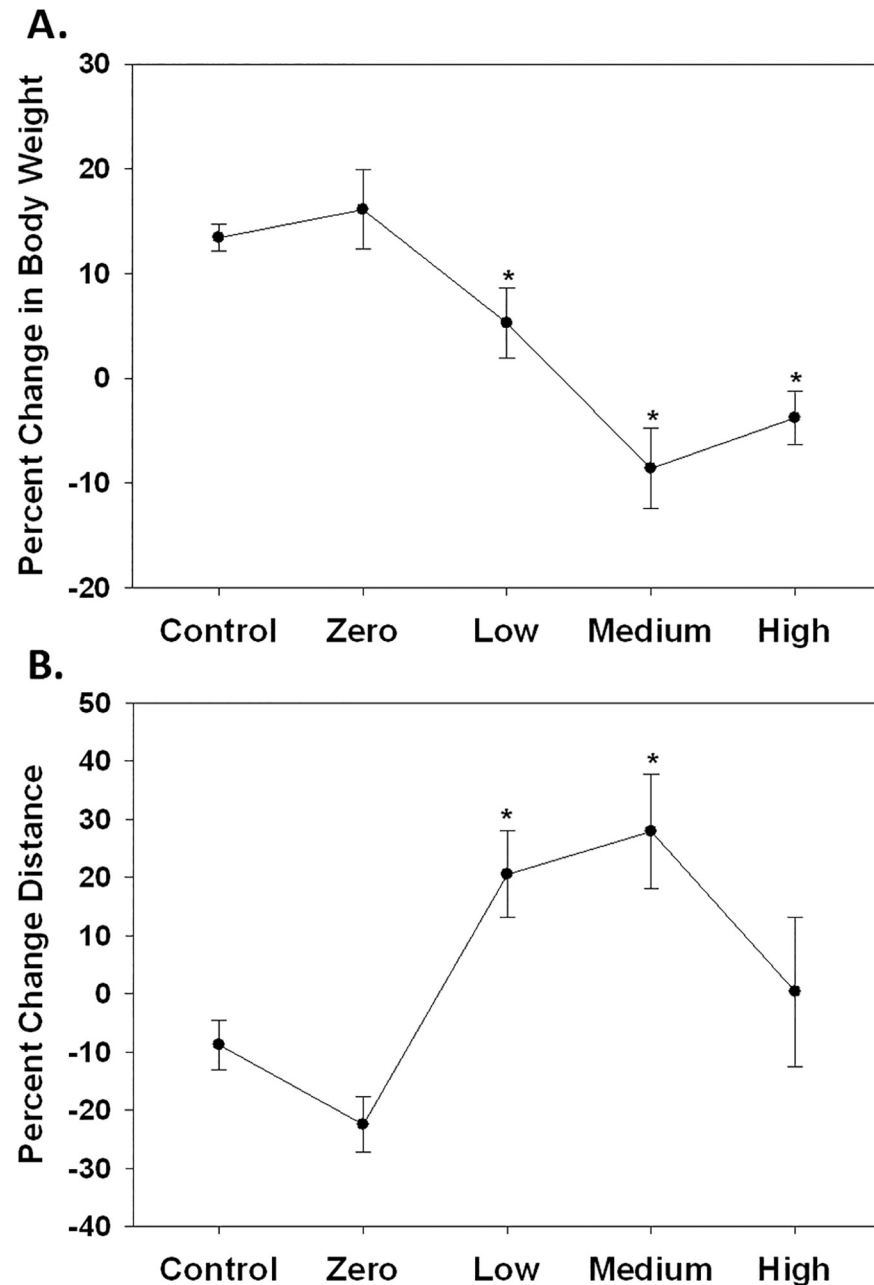


**Fig 7. Time spent in the center of the open field is shown in female and male Nurr1 +/+ and +/- mice after *T. gondii* infection or vehicle injection (Control).** Infection with *T. gondii* significantly elevated the time in the center of the open field with all groups (sex and genotype) combined (A.). Although, no significant interaction between *T. gondii* infection and genotype was found related to time in the center, much of the increase in time in center was the result of an increase time spent in the center for the +/+ mice. Significant treatment (#) effect based on ANOVA with Fischer's post hoc comparisons. n = 10 female +/+ controls; 8 female +/- controls; 5 female +/+ *T. gondii*; 10 female +/- *T. gondii*; 17 male +/+ control; 10 male +/- controls; 9 male +/+ *T. gondii*; 8 male +/- *T. gondii*.

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### ***T. gondii* infection, Nurr1 +/- genotype and Bobcat Urine Avoidance**

In the bobcat urine avoidance test, there was a significant aversion to bobcat urine in both the male and female +/+ control mice, that was abolished in the *T. gondii* injected mice (Fig 3B and 3C.). In contrast, there was a trend toward aversion to bobcat urine in the control female +/- mice which became a significant aversion to bobcat urine in the *T. gondii* injected female +/- mice. The male +/- mice showed no aversion to bobcat urine as behavior was similar in control mice and *T. gondii* injected mice.



**Fig 8. Percent change in body weight and change in distance traveled in the open field are shown in relation to the level of *T. gondii* antibodies.** For this analysis, male and female Nurr1  $+/+$  and  $+/-$  mice are combined. Control refers to all mice injected with vehicle. Zero refers to mice injected with *T. gondii* but with no detectable antibody titers to *T. gondii*. The Low group consists of mice with antibody titers between 50 and 400. The Medium group consists of mice with antibody titers between 800 and 1600. The High group consists of the mice with antibody titers  $>3200$ . For body weight, there were significant reductions in body weight at all three antibody levels, Low, Medium and High (A.). For change in distance traveled in the open field, there was an inverted U-relationship consisting of a significant increase in distance traveled for mice in the Low and Medium antibody levels, but no significant effect on distance in mice at High antibody levels (B.). Significant antibody (\*) effect based on ANOVA with Fischer's post hoc comparisons.  $n = 47$  Control; 4 Zero; 11 Low; 8 Medium; 11 High.

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## Summary of Interactions between *T. gondii* Infection and the Nurr1 +/- Genotype

Collectively these behavioral data were consistent with those previously reported in the Nurr1 +/- mice with elevated exploratory behavior demonstrated by the greater exits during the emergence test and greater distance traveled in the open field. Additionally, the +/- mice showed reduced anxiety related behaviors consisting of decreased time spent in the cylinder in the emergence test and increased time spent in the center of the open field. *Toxoplasma gondii* infection produced effects in +/+ mice that mimicked some of the effects due to the +/- genotype such as a reduction in age related weight gain, elevated open field activity, and increased time spent in the center of the open field. Similarly, *T. gondii* infection alone had no effect on PPI just as the +/- genotype alone does not alter PPI. However, the combination of the +/- genotype and *T. gondii* infection had a cumulative effect, specifically seen as a greater effect on elevating open field activity. Additionally, preliminary evidence suggests that *T. gondii* infection and the +/- genotype may disrupt PPI in male +/- mice.

## Discussion

Understanding the genetic mutations that predispose individuals to schizophrenia and how these genetic components interact with environmental factors to lead to schizophrenia are important in order to provide interventions that could prevent the development of schizophrenia. Because infection with *T. gondii* is an important risk factor for developing schizophrenia, here we investigated how this infection may interact with the heterozygous deletion of the Nurr1 gene, which is necessary for dopamine neuron development, important for dopamine neurotransmission and also implicated to have a role in the development of schizophrenia [30–35].

The current investigation includes additional data on how the Nurr1 +/- genotype alters behavior and the interaction with a chronic, adult-acquired infection with *T. gondii*. A consistent behavioral consequence of the +/- genotype is an increase in seeking behavior as demonstrated by elevated activity in an open field. Previous studies have reported similar elevated open field activity in +/- male and female mice [39,40]. This effect is greatest with a novel open field or with a mild-stressor such as a vehicle injection [39,40,50]. Our data illustrates that repeated open field tests can eliminate the difference between +/- and +/+ mice as seen by the lack of difference in distance traveled between the vehicle injected +/- and +/+ mice. Also of note is that the +/- mice showed decreased anxiety related behavior based on a significant increase in the time in the center of the open field. These data from the open field and emergence test demonstrate that the Nurr1 +/- genotype has effects that are mostly independent of sex. Here we also found no significant effect on PPI between male +/+ and +/- or female +/+ and +/- mice, however, there was a trend towards differences in PPI between male and female +/- mice.

Attenuated Nurr1 expression, as occurs in the Nurr1 +/- mice, has been proposed as a rodent model for behaviors related to symptoms of schizophrenia based on elevated open field activity in these mice [38–40]. Open field activity is a behavior that is associated with dopamine neurotransmission because amphetamine and other dopamine stimulating drugs increase open field activity while dopamine D2 receptor antagonists have a strong effect on decreasing open field activity [50,52–54]. Additionally, transgenic mice with altered dopamine neurotransmission genes that increase dopamine neurotransmission, such as in the dopamine transporter or vesicular monoamine transporter 2 knockout/knockdown mice, show a profound elevation in open field activity [55–58]. The effects of the +/- genotype on open field activity are most likely due to dopamine neurotransmission as the need for Nurr1 in the development and continued survival of mesencephalic dopamine neurons has been demonstrated by gene deletion studies [30,32,33,59]. Additionally, the Nurr1 +/- genotype results in reduced

expression of several dopamine neurotransmission genes and altered dopamine neurotransmission, which includes reduced tissue dopamine levels in the nucleus accumbens and elevated basal dopamine levels in the shell of the nucleus accumbens [48,50,60].

The current data revealed that *T. gondii* infection significantly elevated open field activity in both +/+ and +/- mice. This effect was exacerbated in the +/- mice. Time spent in the center of the open field was also significantly increased by *T. gondii* infection, but did not differ across genotypes. An increase in open field activity has been previously reported in adult mice chronically infected with *T. gondii* [23,24,26,61,62]. Other studies, however, have found either no effect on activity or even a decrease in activity after chronic *T. gondii* infection in mice [63–65]. Xiao et al. reported sex-specific effects on open field activity, with an increase in female mice and a decrease in male mice chronically infected with *T. gondii* [26]. Some of these differences may be due to differences in testing parameters. Specifically Skalova et al., who found a decrease in activity in infected female mice, used a dark arena with a red light, which reduces the anxiety related response to a bright open field [64]. Differences in infection routes and protozoal stages (either peroral introduction of cysts or parenteral injection of tachyzoites) and the strain of mice used could also contribute to these discrepancies. In general, data from the current study found that infection with *T. gondii* elevated open field activity and reduced anxiety related behaviors, as evidenced by distance traveled in open field test and time in the center of the open field, respectively. These data indicate that the +/- mice are especially susceptible to the effects of a chronic, adult acquired infection with *T. gondii*, specifically illustrated by effects on elevated open field activity and reduced anxiety related behaviors.

Avoidance of bobcat urine that was abolished by *T. gondii* infection was only found in the +/+ mice. Vyas et al. found that avoidance is dose dependent, such that an optimal amount of bobcat urine is necessary to demonstrate aversion and attraction, but it appeared the +/+ female mice were the most sensitive to this effect [66]. Interestingly, the female +/- mice showed significant bobcat urine avoidance after *T. gondii* infection while the male +/- showed no aversion to bobcat urine. These data show differences in susceptibility to aversion to bobcat urine, reversal of aversion with *T. gondii*, and effects on open field activity across the groups tested. This suggests that separate mechanisms may exist that underlie these behaviors. Additionally, these data show that genetic predisposition may influence the extent of altered behavior due to *T. gondii* infection in animals.

PPI is the decrease in startle response when the startle stimulus is preceded by a low intensity stimulus, which is a commonly used measurement of sensorimotor gating. Deficits in sensorimotor gating are consistently observed in schizophrenia patients and these deficits correlate with positive schizophrenia symptoms [41,42,44–47]. PPI is regulated by dopamine neurotransmission as systemic amphetamine and infusion of dopamine in the nucleus accumbens disrupts PPI and disrupted PPI can be normalized by dopamine receptor antagonists. Since PPI can be measured in similar ways between rodents and humans, PPI has become a commonly used model for investigating parameters associated with schizophrenia symptoms [67–70]. To date, no studies have demonstrated deficits in sensorimotor gating after infection with *T. gondii*. We corroborate these findings as we found no effect on PPI in the +/+ mice, however, when *T. gondii* was combined with +/- genotype in male mice, we found a trend toward disruption of PPI. The male +/- mice were also the group with the highest open field activity after *T. gondii* infection. This reduction in PPI, although not significant, was similar in magnitude to the significant reduction in PPI found in male +/- mice when isolated at weaning although the post-weaning isolation study had a larger sample size than the current one [38]. Elevated dopamine turnover and elevated amphetamine stimulated dopamine release was also found in isolated +/- mice, as compared to group raised +/- mice and isolate +/+ mice [48]. That isolated +/- mice were observed to have altered dopamine neurotransmission concurrent

with disrupted PPI suggests that *T. gondii* is also enhancing dopamine neurotransmission in the +/- mice, causing altered PPI and elevated open field activity.

Since elevated open field activity is linked to elevated dopamine neurotransmission and *T. gondii* has been shown to express tyrosine hydroxylase enzymes leading to *in vivo* synthesis of dopamine, an attractive hypothesis is that *T. gondii* cysts in the brain directly elevate dopamine levels to alter these dopamine related behaviors [21,22]. This hypothesis is intriguing since abnormal dopamine neurotransmission has been implicated in schizophrenia and infection with *T. gondii* increases the risk for developing schizophrenia [28,71–76]. Unfortunately, no studies to date have measured markers of dopamine neurotransmission, dopamine electrophysiology or determined if the dopamine produced by cysts is sufficient to impact overall extracellular or stimulated dopamine levels.

The problem with the dopamine hypothesis of chronic *T. gondii* infection for elevating the risk of schizophrenia is that alterations in dopamine neurotransmission in schizophrenia are considerably more complicated than simply an increase in tissue and/or extracellular dopamine levels [73]. Alterations in dopamine neurotransmission found in schizophrenia suggest that there is hypofunction in mesocortical dopamine neurotransmission, but elevated subcortical dopamine neurotransmission. Clinical PET studies reported a significantly greater dopamine response to amphetamine in the striatum, but a decrease in markers of dopamine innervations in the prefrontal cortex in patients with schizophrenia [77]. Presynaptic effects in dopamine neurons in schizophrenia include elevated dopamine synthesis and dopamine release in the striatum [78]. Furthermore, the super-sensitivity to dopamine, as demonstrated by the increased response to dopamine stimulating drugs such as amphetamine in patients with schizophrenia, is also associated with a dramatic increase in the high affinity state of the dopamine D2 receptors with only modest effects on overall dopamine receptor expression [29]. Currently, it is unclear how *T. gondii* affects the various parameters of dopamine neurotransmission across these different dopaminergic pathways.

Neuroinflammation, including infiltration of macrophages, activation of astrocytes and cuffing around vessels, is a consistent feature reported in the brains of rodents infected with *T. gondii* [23,63,79,80]. Also associated with these pathological processes are the release of inflammatory cytokines and the enhancement of the kynurenine pathway. Within astrocytes, tryptophan is degraded via this metabolic pathway to kynurenine which is further degraded to the neuroactive metabolite kynurenic acid [81]. Kynurenic acid is an endogenous antagonist to the  $\alpha 7$  nicotinic acid receptor and the N-methyl-D-aspartate receptor [82–85]. Systemic kynurenine, which results in conversion to kynurenic acid in the brain, increases amphetamine stimulated dopamine release in the striatum, increases dopamine neuron activity, and disrupts PPI in rats [86–88]. Furthermore, ventral tegmental dopamine neurons are activated (i.e. increase firing rate and percent burst firing) by systemic kynurenine [89]. Infection with *T. gondii* has been shown to stimulate the kynurenine pathway and elevate kynurenic acid levels in the brains of mice [90,91]. Collectively, these observations provide a reasonable mechanistic hypothesis that infection with *T. gondii* alters dopamine related behavior via elevating kynurenic acid synthesis. Of note are the observations that kynurenic acid and kynurenine levels are elevated in the brains and cerebral spinal fluid of schizophrenia patients [92–97]. Additionally, anti-inflammatory drugs, such as cyclooxygenase inhibitors, have been shown to have some efficacy at treating schizophrenia symptoms [98,99]. Based on these data, inflammation and enhancement of kynurenic acid associated with *T. gondii* infection could be an important factor in mediating the changes in behavior found in rodent models and in contributing to an increase in the risk of schizophrenia.

A likely mechanism by which inflammation (i.e. cytokines, kynurenic acid etc.) could alter dopamine neurotransmission is by altering the function of dopamine neuron afferents. The

regulation and control of mesoaccumbens dopamine neuron electrophysiology by afferents is well categorized. These areas include excitatory innervations from the pedunculopontine tegmentum that stimulate burst firing of these dopamine neurons and inhibitory innervations from the ventral pallidum neurons that regulate the number of spontaneously active dopamine neurons [98,99]. The ventral subiculum and prefrontal cortex can regulate the nucleus accumbens which has inhibitory input to the ventral pallidum [74]. Additionally, pyramidal neurons in the prefrontal cortex innervate the ventral tegmental dopamine neurons in such a way as to provide excitatory (glutamate) innervation to GABAergic interneurons that then synapse on dopamine neurons that project to the nucleus accumbens but direct excitatory innervation to dopamine neurons that have reciprocal innervation back to the prefrontal cortex [100]. Therefore, a condition that inhibits neuron output from the prefrontal cortex would produce elevated subcortical dopamine neurotransmission and attenuated cortical dopamine neurotransmission, reproducing the condition of subcortical hyperdopaminergic neurotransmission and cortical hypodopaminergic neurotransmission described in schizophrenia. Similarly, impairment in ventral pallidum neurons innervating mesoaccumbens dopamine neurons could increase the number of spontaneously active dopamine neurons, resulting in elevated dopamine neurotransmission and an increase in the number of dopamine neurons that can be induced into burst firing, which occurs in response to behaviorally relevant stimuli. Both of these conditions would result in elevated open field activity and possibly disrupted PPI.

Another possible mechanism by which inflammation could impact dopamine neurotransmission is a direct effect on dopamine neuron function. The predominant effect of cytokines on dopamine neurotransmission, however, is a reduction in dopamine neurotransmission and depression-like behaviors [101]. Since the behaviors measured in the current investigation occurred after chronic *T. gondii* infection, reduced dopamine neurotransmission early during the infection could set up a delayed condition of elevated dopamine neurotransmission via dopamine receptor super-sensitivity, for example [102].

A growing number of animal studies are demonstrating how a genetic predisposition can potentiate the effects of an environmental stressor [101,103–105]. Previously we found that post-weaning isolation, which is used to model the effect of early life stressors that increase schizophrenia risk, interacts with the Nurr1 +/- genotype. Here we find that another risk factor for schizophrenia, *T. gondii* infection, is also potentiated in Nurr1 +/- mice. The elevated susceptibility of the Nurr1 +/- mice to the altered behaviors resulting from *T. gondii* likely results from either, 1) dysregulation of the dopamine system and/or 2) increased propensity for inflammation due to a reduced or attenuated induction of Nurr1 levels. Nurr1 expression in dopamine neurons appears to be regulated by dopamine neuron activity and, as a transcription factor, Nurr1 has been found to regulate expression of various dopamine neurotransmission genes including dopamine transporter, vesicular monoamine transporter 2, GTP cyclohydrolase and tyrosine hydroxylase [60,106–108]. Collectively, these data, combined with the effects in the +/- mice, suggest that Nurr1 serves as an important regulatory step between dopamine neuron activity and subsequent expression and function of dopamine neurotransmission proteins. If *T. gondii* infection alters dopamine neuron activity, the regulatory response to this change would be different in the +/- mice. Post-weaning isolation alters the response in +/- mice resulting in an elevated amphetamine-stimulated dopamine release [48].

*Toxoplasma gondii* infection could exert effects on dopamine neurotransmission directly by the regulation of Nurr1 expression in neurons. A recent finding by Xiao et al. reported that acute infection with *T. gondii* resulted in an increase in expression of MiR-132, a cyclic AMP-responsive element binding regulated microRNA [109]. MiR-132 was also recently found to negatively regulate Nurr1 expression and inhibit the differentiation of embryonic stem cells into dopamine neurons [110]. If *T. gondii* increases MiR-132 in dopamine neurons resulting

in repression of Nurr1 expression, then the +/- mice would be more vulnerable to attenuated Nurr1 expression, which would likely have a greater effect on dopamine neuron gene expression and dopamine neurotransmission. Since Nurr1 is also expressed in neurons in other brain regions, the effect of the +/- genotype combined with a possible direct effect of *T. gondii* on Nurr1 expression via MiR-132 could result in abnormal function of other brain regions, such as the ventral subiculum or prefrontal cortex.

Nurr1 expression and function is also regulated by and has a role in the downstream effects of inflammation. Specifically, Nurr1 expression was shown to be anti-inflammatory as reduced levels of Nurr1 in astrocytes enhance inflammation and neuronal degeneration resulting from lipopolysaccharide treatment [111]. This could potentially cause increased inflammatory responses in the Nurr1 +/- mice and make them more vulnerable to the effects of inflammation on dopamine neurotransmission. Collectively, these data provide several potential mechanisms through which chronic *T. gondii* infection can interact with the Nurr1 +/- genotype to precipitate susceptibility for disrupted dopamine related behaviors.

Another important finding in the current data is that a differential effect on open field activity was present with an inverted U relationship between activity and the level of humoral immune response based on antibody titers to *T. gondii*. This could be of considerable relevance to other studies on the behavioral effects of *T. gondii*. Previous studies using rodents did not report *T. gondii* antibody titers, only seropositivity to *T. gondii*. The current study found reduced weight gain after *T. gondii* infection which is similar to what was previously reported [63,65,112]. It is unclear the relationship between effects on weight and activity as a reduction in weight could lead to greater activity or greater activity could produce weight loss. Although we found a relationship between weight and open field activity, there was not a significant correlation between change in weight and change in open field activity. Additionally, a change in body weight at all antibody titers was observed, whereas high antibody titers were not associated with an increase in activity.

These data suggest that the level of active (or humoral) immune response and antibodies produced to *T. gondii* could be an important indicator of the effects on behavior. This is of particular relevance as the increased risk of schizophrenia or severity of schizophrenia symptoms are related to levels of antibodies to *T. gondii*. Specifically, Brown et al. found an association between high IgG antibody titers (endpoint dilutions  $\geq 1:128$ ) in the mother and increased risk of schizophrenia spectrum disorders (odds ratio of 2.6 when adjusted for maternal age) in their offspring [16]. Blomström et al., investigating risk of schizophrenia associated with IgG antibody levels in mothers to *T. gondii* in relation to the general population, found that antibody levels greater than the 75<sup>th</sup> percentile was associated with significant increased risk of schizophrenia based on an odds ratio of 2.1 [15]. These authors also found an antibody-associated effect such that increased risk was related to antibody titers, with an odds ratio of 1.6 when antibody titers were between the 75<sup>th</sup> and 90<sup>th</sup> percentile and an odds ratio of 3.2 when antibody titers were greater than the 90<sup>th</sup> percentile. Hinze-Selch found significantly higher *T. gondii* antibodies in patients with schizophrenia, although no differences in frequency of patients with schizophrenia and controls [113]. These levels of antibodies in human patients are likely equivalent to the low and medium levels in the murine groups from the current study. Although most previous studies tested for the presence of detectable antibodies to determine infection, none, to our knowledge, have compared behavioral effects and antibody titers, as has been done in human studies investigating risk of mental illness. Some of the differences in the effect of *T. gondii* infection on behavior, particularly open field activity, could be due to differences in the immune response [26,64,65]. If mice or rats had very high levels of antibodies, this could diminish the effects of *T. gondii* infection on behavior. Further investigation of antibody titers and behavior could provide additional insight into how *T. gondii* infection



alters behaviors in rodents. In future studies, it will be interesting and prudent to investigate how additional parameters, such as other markers for immune activation including cytokine levels, could add to the predictive value of *T. gondii*-induced alterations on behavior. The use of both  $+/+$  and  $+/-$  mice should enhance this modeling as we get a graded response with infection depending on genotype. Finally, direct effects of *T. gondii* antibodies remain a possibility as autoantibodies to several proteins in the brain have been described that result in changes in behavior and can lead to psychotic symptoms [114].

## Conclusions

These data demonstrate that reduced Nurr1 function, as found in the Nurr1  $+/-$  genotype, makes mice more susceptible to *T. gondii* induced alterations in the dopamine related behaviors of open field activity. Additionally, the levels of immune response to *T. gondii* infection, particularly humoral response as measured by antibody titers to this protozoan, is not a linear relationship, but an inverted U relationship. This model reproduces the genetic-environmental hypothesis of schizophrenia where a specific gene mutation or environmental stressors alone are not sufficient to cause disease, however, the combination of the two are capable of causing the disease. Understanding the mechanism(s) through which chronic *T. gondii* infection alters behaviors in rodents and interacts with the Nurr1  $+/-$  genotype could provide insight into how it can modify behaviors in humans and interact with other predisposing gene mutations to contribute to mental illnesses. Results from this study generate novel hypotheses and raise additional questions regarding the role of the immune system as related to *T. gondii* infection in the development of schizophrenia.

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## Author Contributions

Conceived and designed the experiments: JBE ASV DSL. Performed the experiments: JBE ASV SXG EK. Analyzed the data: JBE ASV SXG HMS ADC. Contributed reagents/materials/analysis tools: DSL. Wrote the paper: JBE ASV.

## References

1. Need AC, McEvoy JP, Gennarelli M, Heinzen EL, Ge D, Maia JM, et al. Exome sequencing followed by large-scale genotyping suggests a limited role for moderately rare risk factors of strong effect in schizophrenia. *Am J Hum Genet.* 2012; 91: 303–312. <https://doi.org/10.1016/j.ajhg.2012.06.018> PMID: 22863191
2. Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, et al. Common variants conferring risk of schizophrenia. *Nature.* 2009; 460: 744–747. <https://doi.org/10.1038/nature08186> PMID: 19571808
3. Brown AS (2011) The environment and susceptibility to schizophrenia. *Prog Neurobiol* 93: 23–58. <https://doi.org/10.1016/j.pneurobio.2010.09.003> PMID: 20955757
4. Torrey EF, Bartko JJ, Yolken RH. Toxoplasma gondii and other risk factors for schizophrenia: an update. *Schizophr Bull.* 2012; 38: 642–647. <https://doi.org/10.1093/schbul/sbs043> PMID: 22446566
5. Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: from animals to humans. *Int J Parasitol.* 2000; 30: 1217–1258. PMID: 11113252
6. Flegr J. Effects of toxoplasma on human behavior. *Schizophr Bull.* 2007; 33: 757–760. PMID: 17218612
7. Flegr J. How and why Toxoplasma makes us crazy. *Trends Parasitol.* 2013; 29: 156–163. <https://doi.org/10.1016/j.pt.2013.01.007> PMID: 23433494



8. Flegr J. Influence of latent Toxoplasma infection on human personality, physiology and morphology: pros and cons of the Toxoplasma-human model in studying the manipulation hypothesis. *J Exp Biol.* 2013; 216: 127â133. <https://doi.org/10.1242/jeb.073635> PMID: 23225875
9. Holub D, Flegr J, Dragomirecka E, Rodriguez M, Preiss M, Novak T, et al. Differences in onset of disease and severity of psychopathology between toxoplasmosis-related and toxoplasmosis-unrelated schizophrenia. *Acta Psychiatr Scand.* 2013; 127: 227â238. <https://doi.org/10.1111/acps.12031> PMID: 23126494
10. McConkey GA, Martin HL, Bristow GC, Webster JP. Toxoplasma gondii infection and behaviourâlocation, location, location? *J Exp Biol.* 2013; 216: 113â119. <https://doi.org/10.1242/jeb.074153> PMID: 23225873
11. Torrey EF, Yolken RH. Toxoplasma gondii and schizophrenia. *Emerg Infect Dis.* 2003; 9: 1375â1380. PMID: 14725265
12. Torrey EF, Yolken RH. Schizophrenia and toxoplasmosis. *Schizophr Bull.* 2007; 33: 727â728. PMID: 17426051
13. Yolken RH, Dickerson FB, Fuller Torrey E. Toxoplasma and schizophrenia. *Parasite Immunol.* 2009; 31: 706â715. <https://doi.org/10.1111/j.1365-3024.2009.01131.x> PMID: 19825110
14. Horacek J, Flegr J, Tintera J, Verebova K, Spaniel F, Novak T, et al. Latent toxoplasmosis reduces gray matter density in schizophrenia but not in controls: voxel-based-morphometry (VBM) study. *World J Biol Psychiatry.* 2012; 13: 501â509. <https://doi.org/10.3109/15622975.2011.573809> PMID: 21599563
15. Blomstr m A, Karlsson H, Wicks S, Yang S, Yolken RH, Dalman C. Maternal antibodies to infectious agents and risk for non-affective psychoses in the offspringâa matched case-control study. *Schizophr Res.* 2012; 140: 25â30. <https://doi.org/10.1016/j.schres.2012.06.035> PMID: 22819777
16. Brown AS, Schaefer CA, Quesenberry CP Jr., Liu L, Babulas VP, Susser ES. Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. *Am J Psychiatry.* 2005; 162: 767â773. PMID: 15800151
17. Mortensen PB, Norgaard-Pedersen B, Waltoft BL, Sorensen TL, Hougaard D, Yolken RH. Early infections of Toxoplasma gondii and the later development of schizophrenia. *Schizophr Bull.* 2007; 33: 741â744. PMID: 17329231
18. Brown AS, Derkits EJ. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *Am J Psychiatry.* 2010; 167: 261â280. <https://doi.org/10.1176/appi.ajp.2009.09030361> PMID: 20123911
19. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. Toxoplasma gondii infection in the United States, 1999 2004, decline from the prior decade. *Am J Trop Med Hyg.* 2007; 77: 405â410. PMID: 17827351
20. Stibbs HH. Changes in brain concentrations of catecholamines and indoleamines in Toxoplasma gondii infected mice. *Ann Trop Med Parasitol.* 1985; 79: 153â157. PMID: 2420295
21. Gaskell EA, Smith JE, Pinney JW, Westhead DR, McConkey GA. A unique dual activity amino acid hydroxylase in Toxoplasma gondii. *PLoS One.* 2009; 4: e4801. <https://doi.org/10.1371/journal.pone.0004801> PMID: 19277211
22. Prandovszky E, Gaskell E, Martin H, Dubey JP, Webster JP, McConkey GA. The neurotropic parasite Toxoplasma gondii increases dopamine metabolism. *PLoS One.* 2011; 6: e23866. <https://doi.org/10.1371/journal.pone.0023866> PMID: 21957440
23. Hay J, Aitken PP, Graham DI. Toxoplasma infection and response to novelty in mice. *Z Parasitenkd* 1984; 70: 575â588. PMID: 6506841
24. Hay J, Hutchison WM, Aitken PP, Graham DI. The effect of congenital and adult-acquired Toxoplasma infections on activity and responsiveness to novel stimulation in mice. *Ann Trop Med Parasitol* 1983; 77: 483â495. PMID: 6660954
25. Webster JP. The effect of Toxoplasma gondii on animal behavior: playing cat and mouse. *Schizophr Bull.* 2007; 33: 752â756. PMID: 17218613
26. Xiao J, Kannan G, Jones-Brando L, Brannock C, Krasnova IN, Cadet JL, et al. Sex-specific changes in gene expression and behavior induced by chronic Toxoplasma infection in mice. *Neuroscience* 2012; 206: 39â48. <https://doi.org/10.1016/j.neuroscience.2011.12.051> PMID: 22240252
27. Lau CI, Wang HC, Hsu JL, Liu ME. Does the dopamine hypothesis explain schizophrenia? *Rev Neurosci* 2013; 24: 389â400. <https://doi.org/10.1515/revneuro-2013-0011> PMID: 23843581
28. Miyake N, Thompson J, Skinbjerg M, Abi-Dargham A. Presynaptic dopamine in schizophrenia. *CNS Neurosci Ther.* 2011; 17: 104â109. <https://doi.org/10.1111/j.1755-5949.2010.00230.x> PMID: 21199451

29. Seeman P. Schizophrenia and dopamine receptors. *Eur Neuropsychopharmacol.* 2013; 23: 999â–1009. <https://doi.org/10.1016/j.euroneuro.2013.06.005> PMID: 23860356
30. Castillo SO, Baffi JS, Palkovits M, Goldstein DS, Kopin IJ, Witta J, et al. Dopamine biosynthesis is selectively abolished in substantia nigra/ventral tegmental area but not in hypothalamic neurons in mice with targeted disruption of the Nurr1 gene. *Mol Cell Neurosci.* 1998; 11: 36â–46. PMID: 9608532
31. Kadkhodaei B, Ito T, Joodmardi E, Mattsson B, Rouillard C, Carta M, et al. Nurr1 is required for maintenance of maturing and adult midbrain dopamine neurons. *J Neurosci.* 2009; 29: 15923â–15932. <https://doi.org/10.1523/JNEUROSCI.3910-09.2009> PMID: 20016108
32. Saucedo-Cardenas O, Quintana-Hau JD, Le WD, Smidt MP, Cox JJ, De Mayo F, et al. Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. *Proc Natl Acad Sci U S A.* 1998; 95: 4013â–4018. PMID: 9520484
33. ZetterstrÃ¶m RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. Dopamine neuron agenesis in Nurr1-deficient mice. *Science.* 1997; 276: 248â–250. PMID: 9092472
34. Buerenich S, Carmine A, Arvidsson M, Xiang F, Zhang Z, Sydow O, et al. NURR1 mutations in cases of schizophrenia and manic-depressive disorder. *Am J Med Genet.* 2000; 96: 808â–813. PMID: 11121187
35. Chen YH, Tsai MT, Shaw CK, Chen CH. Mutation analysis of the human NR4A2 gene, an essential gene for midbrain dopaminergic neurogenesis, in schizophrenic patients. *Am J Med Genet.* 2001; 105: 753â–757. PMID: 11803525
36. Ancin I, Cabranes JA, Vazquez-Alvarez B, Santos JL, Sanchez-Morla E, et al. NR4A2: effects of an "orphan" receptor on sustained attention in a schizophrenic population. *Schizophr Bull.* 2013; 39: 555â–563. <https://doi.org/10.1093/schbul/sbr176> PMID: 22294735
37. Eells JB, Yeung SK, Nikodem VM. Dopamine neurons heterozygous for the Nurr1-Null allele have reduced survival in vitro. *Neurosci Res Comm.* 2002; 30: 173â–183.
38. Eells JB, Mislner JA, Nikodem VM. Early postnatal isolation reduces dopamine levels, elevates dopamine turnover and specifically disrupts prepulse inhibition in Nurr1-null heterozygous mice. *Neuroscience.* 2006; 140: 1117â–1126. PMID: 16690213
39. Rojas P, Joodmardi E, Hong Y, Perlmann T, Ogren SO. Adult mice with reduced Nurr1 expression: an animal model for schizophrenia. *Mol Psychiatry.* 2007; 12: 756â–766. PMID: 17457314
40. Vuillermot S, Joodmardi E, Perlmann T, Ogren SO, Feldon J, Meyer U. Schizophrenia-relevant behaviors in a genetic mouse model of constitutive Nurr1 deficiency. *Genes Brain Behav.* 2011; 10: 589â–603. <https://doi.org/10.1111/j.1601-183X.2011.00698.x> PMID: 21545404
41. Braff DL, Swerdlow NR, Geyer MA. Gating and habituation deficits in the schizophrenia disorders. *Clin Neurosci.* 1995; 3: 131â–139. PMID: 7583619
42. Braff DL, Swerdlow NR, Geyer MA. Symptom correlates of prepulse inhibition deficits in male schizophrenic patients. *Am J Psychiatry.* 1999; 156: 596â–602. PMID: 10200740
43. Duncan EJ, Szilagyi S, Efferen TR, Schwartz MP, Parwani A, Chakravorty S, et al. Effect of treatment status on prepulse inhibition of acoustic startle in schizophrenia. *Psychopharmacology (Berl).* 2003; 167: 63â–71. PMID: 12632245
44. Light GA, Braff DL. Human and animal studies of schizophrenia-related gating deficits. *Curr Psychiatry Rep.* 1999; 1: 31â–40. PMID: 11122903
45. Ludewig K, Geyer MA, Etzensberger M, Vollenweider FX. Stability of the acoustic startle reflex, prepulse inhibition, and habituation in schizophrenia. *Schizophr Res.* 2002; 55: 129â–137. PMID: 11955972
46. Ludewig K, Geyer MA, Vollenweider FX. Deficits in prepulse inhibition and habituation in never-medicated, first-episode schizophrenia. *Biol Psychiatry.* 2003; 54: 121â–128. PMID: 12873801
47. Mackeprang T, Kristiansen KT, Glenthøj BY. Effects of antipsychotics on prepulse inhibition of the startle response in drug-naïve schizophrenic patients. *Biol Psychiatry.* 2002; 52: 863â–873. PMID: 12399139
48. Moore TM, Brown T, Cade M, Eells JB. Alterations in amphetamine-stimulated dopamine overflow due to the Nurr1-null heterozygous genotype and postweaning isolation. *Synapse.* 2008; 62: 764â–774. <https://doi.org/10.1002/syn.20550> PMID: 18655117
49. Dulawa SC, Grandy DK, Low MJ, Paulus MP, Geyer MA. Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. *J Neurosci.* 1999; 19: 9550â–9556. PMID: 10531457
50. Eells JB, Lipska BK, Yeung SK, Mislner JA, Nikodem VM. Nurr1-null heterozygous mice have reduced mesolimbic and mesocortical dopamine levels and increased stress-induced locomotor activity. *Behav Brain Res.* 2002; 136: 267â–275. PMID: 12385813

51. Vuillermot S, Feldon J, Meyer U. Nurr1 is not essential for the development of prepulse inhibition deficits induced by prenatal immune activation. *Brain Behav Immun*. 2011; 25: 1316â–1321. <https://doi.org/10.1016/j.bbi.2011.06.012> PMID: 21723940
52. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature*. 1996; 379: 606â–612. PMID: 8628395
53. Lacroix L, Broersen LM, Feldon J, Weiner I. Effects of local infusions of dopaminergic drugs into the medial prefrontal cortex of rats on latent inhibition, prepulse inhibition and amphetamine induced activity. *Behav Brain Res*. 2000; 107: 111â–121. PMID: 10628735
54. Ralph RJ, Paulus MP, Geyer MA. Strain-specific effects of amphetamine on prepulse inhibition and patterns of locomotor behavior in mice. *J Pharmacol Exp Ther*. 2001; 298: 148â–155. PMID: 11408536
55. Fernagut PO, Diguët E, Jaber M, Bioulac B, Tison F. Dopamine transporter knock-out mice are hypersensitive to 3-nitropropionic acid-induced striatal damage. *Eur J Neurosci*. 2002; 15: 2053â–2056. PMID: 12099912
56. Spieleswoy C, Roubert C, Hamon M, Nosten-Bertrand M, Betancur C, Giros B. Behavioural disturbances associated with hyperdopaminergia in dopamine-transporter knockout mice. *Behav Pharmacol*. 2000; 11: 279â–290. PMID: 11103882
57. Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V, et al. VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. *Proc Natl Acad Sci U S A*. 1997; 94: 9938â–9943. PMID: 9275230
58. Zhuang X, Oosting RS, Jones SR, Gainetdinov RR, Miller GW, Caron MG, et al. Hyperactivity and impaired response habituation in hyperdopaminergic mice. *Proc Natl Acad Sci U S A*. 2001; 98: 1982â–1987. PMID: 11172062
59. Kadkhodaei B, Alvarsson A, Schintu N, Ramskold D, Volakakis N, Joodmardi E, et al. Transcription factor Nurr1 maintains fiber integrity and nuclear-encoded mitochondrial gene expression in dopamine neurons. *Proc Natl Acad Sci U S A*. 2013; 110: 2360â–2365. <https://doi.org/10.1073/pnas.1221077110> PMID: 23341612
60. Eells JB, Misler JA, Nikodem VM. Reduced tyrosine hydroxylase and GTP cyclohydrolase mRNA expression, tyrosine hydroxylase activity, and associated neurochemical alterations in Nurr1-null heterozygous mice. *Brain Res Bull*. 2006; 70: 186â–195. PMID: 16782508
61. Hay J, Aitken PP, Arnott MA. The influence of congenital *Toxoplasma* infection on the spontaneous running activity of mice. *Z Parasitenkd*. 1985; 71: 459â–462. PMID: 4040684
62. Hutchinson WM, Bradley M, Cheyne WM, Wells BW, Hay J. Behavioural abnormalities in *Toxoplasma*-infected mice. *Ann Trop Med Parasitol*. 1980; 74: 337â–345. PMID: 7396566
63. Gulinello M, Acquarone M, Kim JH, Spray DC, Barbosa HS, Sellers R, et al. Acquired infection with *Toxoplasma gondii* in adult mice results in sensorimotor deficits but normal cognitive behavior despite widespread brain pathology. *Microbes Infect*. 2010; 12: 528â–537. <https://doi.org/10.1016/j.micinf.2010.03.009> PMID: 20348009
64. Skalova A, Kodym P, Frynta D, Flegr J. The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethological and ethopharmacological study. *Parasitology*. 2006; 133: 525â–535. PMID: 16882355
65. Vyas A, Kim SK, Giacomini N, Boothroyd JC, Sapolsky RM. Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc Natl Acad Sci U S A*. 2007; 104: 6442â–6447. PMID: 17404235
66. Vyas A, Kim SK, Sapolsky RM. The effects of *toxoplasma* infection on rodent behavior are dependent on dose of the stimulus. *Neuroscience*. 2007; 148: 342â–348. PMID: 17683872
67. Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)*. 2001; 156: 234â–258. PMID: 11549226
68. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology (Berl)*. 2001; 156: 117â–154. PMID: 11549216
69. Ralph RJ, Paulus MP, Fumagalli F, Caron MG, Geyer MA. Prepulse inhibition deficits and perseverative motor patterns in dopamine transporter knock-out mice: differential effects of D1 and D2 receptor antagonists. *J Neurosci*. 2001; 21: 305â–313. PMID: 11150348
70. Swerdlow NR, Braff DL, Masten VL, Geyer MA. Schizophrenic-like sensorimotor gating abnormalities in rats following dopamine infusion into the nucleus accumbens. *Psychopharmacology (Berl)*. 1990; 101: 414â–420. PMID: 2114026

71. Arias I, Sorlozano A, Villegas E, de Dios Luna J, McKenney K, Cervilla J, et al. Infectious agents associated with schizophrenia: a meta-analysis. *Schizophr Res*. 2012; 136: 128â–136. <https://doi.org/10.1016/j.schres.2011.10.026> PMID: 22104141
72. Kuepper R, Skinbjerg M, Abi-Dargham A. The dopamine dysfunction in schizophrenia revisited: new insights into topography and course. *Handb Exp Pharmacol*. 2012: 1â–26.
73. Lieberman JA, Koreen AR. Neurochemistry and neuroendocrinology of schizophrenia: a selective review. *Schizophr Bull*. 1993; 19: 371â–429. PMID: 8100644
74. Lodge DJ, Grace AA. Hippocampal dysregulation of dopamine system function and the pathophysiology of schizophrenia. *Trends Pharmacol Sci*. 2011; 32: 507â–513. <https://doi.org/10.1016/j.tips.2011.05.001> PMID: 21700346
75. Seeman P. All roads to schizophrenia lead to dopamine supersensitivity and elevated dopamine D2 (high) receptors. *CNS Neurosci Ther*. 2011; 17: 118â–132. <https://doi.org/10.1111/j.1755-5949.2010.00162.x> PMID: 20560996
76. Torrey EF, Bartko JJ, Lun ZR, Yolken RH. Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. *Schizophr Bull*. 2007; 33: 729â–736. PMID: 17085743
77. Abi-Dargham A. Do we still believe in the dopamine hypothesis? New data bring new evidence. *Int J Neuropsychopharmacol*. 2004; 7 Suppl 1: S1â–5. PMID: 14972078
78. Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry*. 2012; 69: 776â–786. PMID: 22474070
79. Carruthers VB, Suzuki Y. Effects of *Toxoplasma gondii* infection on the brain. *Schizophr Bull*. 2007; 33: 745â–751. PMID: 17322557
80. Sims TA, Hay J, Talbot IC. Ultrastructural immunocytochemistry of the intact tissue cyst of *Toxoplasma* in the brains of mice with congenital toxoplasmosis. *Ann Trop Med Parasitol*. 1990; 84: 141â–147. PMID: 2383094
81. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci*. 2012; 13: 465â–477. <https://doi.org/10.1038/nrn3257> PMID: 22678511
82. Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. *J Neurosci*. 2001; 21: 7463â–7473. PMID: 11567036
83. Parsons CG, Danysz W, Quack G, Hartmann S, Lorenz B, Wollenburg C, et al. Novel systemically active antagonists of the glycine site of the N-methyl-D-aspartate receptor: electrophysiological, biochemical and behavioral characterization. *J Pharmacol Exp Ther*. 1997; 283: 1264â–1275. PMID: 9400002
84. Perkins MN, Stone TW. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Res*. 1982; 247: 184â–187. PMID: 6215086
85. Stone TW, Stoy N, Darlington LG. An expanding range of targets for kynurenine metabolites of tryptophan. *Trends Pharmacol Sci*. 2013; 34: 136â–143. <https://doi.org/10.1016/j.tips.2012.09.006> PMID: 23123095
86. Eastwood SL, Heffernan J, Harrison PJ. Chronic haloperidol treatment differentially affects the expression of synaptic and neuronal plasticity-associated genes. *Mol Psychiatry*. 1997; 2: 322â–329. PMID: 9246673
87. Erhardt S, Schwieler L, Emanuelsson C, Geyer M. Endogenous kynurenic acid disrupts prepulse inhibition. *Biol Psychiatry*. 2004; 56: 255â–260. PMID: 15312813
88. Olsson SK, Andersson AS, Linderholm KR, Holtze M, Nilsson-Todd LK, Schwieler L, et al. Elevated levels of kynurenic acid change the dopaminergic response to amphetamine: implications for schizophrenia. *Int J Neuropsychopharmacol*. 2009; 12: 501â–512. <https://doi.org/10.1017/S1461145708009383> PMID: 18796185
89. Linderholm KR, Andersson A, Olsson S, Olsson E, Snodgrass R, Engberg G, et al. Activation of rat ventral tegmental area dopamine neurons by endogenous kynurenic acid: a pharmacological analysis. *Neuropharmacology*. 2007; 53: 918â–924. PMID: 17959203
90. Notarangelo FM, Wilson EH, Horning KJ, Thomas MA, Harris TH, Fang Q, et al. Evaluation of kynurenine pathway metabolism in *Toxoplasma gondii*-infected mice: implications for schizophrenia. *Schizophr Res*. 2014; 152: 261â–267. <https://doi.org/10.1016/j.schres.2013.11.011> PMID: 24345671
91. Schwarcz R, Hunter CA. *Toxoplasma gondii* and schizophrenia: linkage through astrocyte-derived kynurenic acid? *Schizophr Bull*. 2007; 33: 652â–653. PMID: 17434932

92. Erhardt S, Blennow K, Nordin C, Skogh E, Lindstrom LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neurosci Lett*. 2006; 313: 96â98. PMID: [11684348](#)
93. Linderholm KR, Skogh E, Olsson SK, Dahl ML, Holtze M, Engberg G, et al. Increased levels of kynurenic acid and kynurenic acid in the CSF of patients with schizophrenia. *Schizophr Bull*. 2012; 38: 426â432. <https://doi.org/10.1093/schbul/sbq086> PMID: [20729465](#)
94. Miller CL, Llenos IC, Dulay JR, Weis S. Upregulation of the initiating step of the kynurenic acid pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. *Brain Res*. 2006; 1073â1074: 25â37.
95. Muller N, Schwarz M. Schizophrenia as an inflammation-mediated dysbalance of glutamatergic neurotransmission. *Neurotox Res*. 2006; 10: 131â148. PMID: [17062375](#)
96. Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenic acid content in schizophrenia. *Biol Psychiatry*. 2001; 50: 521â530. PMID: [11600105](#)
97. Wonodi I, Stine OC, Sathyaikumar KV, Roberts RC, Mitchell BD, Hong LE, et al. Downregulated kynurenic acid 3-monooxygenase gene expression and enzyme activity in schizophrenia and genetic association with schizophrenia endophenotypes. *Arch Gen Psychiatry*. 2011; 68: 665â674. <https://doi.org/10.1001/archgenpsychiatry.2011.71> PMID: [21727251](#)
98. Keller WR, Kum LM, Wehring HJ, Koola MM, Buchanan RW, Kelly DL. A review of anti-inflammatory agents for symptoms of schizophrenia. *J Psychopharmacol*. 2013; 27: 337â342. <https://doi.org/10.1177/0269881112467089> PMID: [23151612](#)
99. Muller N. The role of anti-inflammatory treatment in psychiatric disorders. *Psychiatr Danub*. 2013; 25: 292â298. PMID: [24048400](#)
100. Sesack SR, Carr DB. Selective prefrontal cortex inputs to dopamine cells: implications for schizophrenia. *Physiol Behav*. 2002; 77: 513â517. PMID: [12526992](#)
101. Felger JC, Miller AH. Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise. *Front Neuroendocrinol*. 2012; 33: 315â327. <https://doi.org/10.1016/j.yfrne.2012.09.003> PMID: [23000204](#)
102. Seeman MV, Seeman P. Is schizophrenia a dopamine supersensitivity psychotic reaction? *Prog Neuropsychopharmacol Biol Psychiatry*. 2014; 48: 155â160. <https://doi.org/10.1016/j.pnpbp.2013.10.003> PMID: [24128684](#)
103. Ayhan Y, Sawa A, Ross CA, Pletnikov MV. Animal models of gene-environment interactions in schizophrenia. *Behav Brain Res*. 2009; 204: 274â281. <https://doi.org/10.1016/j.bbr.2009.04.010> PMID: [19379776](#)
104. Mouri A, Nagai T, Ibi D, Yamada K. Animal models of schizophrenia for molecular and pharmacological intervention and potential candidate molecules. *Neurobiol Dis*. 2013; 53: 61â74. <https://doi.org/10.1016/j.nbd.2012.10.025> PMID: [23142533](#)
105. Nagai T, Ibi D, Yamada K. Animal model for schizophrenia that reflects gene-environment interactions. *Biol Pharm Bull*. 2011; 34: 1364â1368. PMID: [21881218](#)
106. Eells JB, Wilcots J, Sisk S, Guo-Ross SX. NR4A gene expression is dynamically regulated in the ventral tegmental area dopamine neurons and is related to expression of dopamine neurotransmission genes. *J Mol Neurosci*. 2012; 46: 545â553. <https://doi.org/10.1007/s12031-011-9642-z> PMID: [21932041](#)
107. Hermanson E, Joseph B, Castro D, Lindqvist E, Aarnisalo P, Wallen A, et al. Nurr1 regulates dopamine synthesis and storage in MN9D dopamine cells. *Exp Cell Res*. 2003; 288: 324â334. PMID: [12915123](#)
108. Sacchetti P, Mitchell TR, Granneman JG, Bannon MJ. Nurr1 enhances transcription of the human dopamine transporter gene through a novel mechanism. *J Neurochem*. 2001; 76: 1565â1572. PMID: [11238740](#)
109. Xiao J, Li Y, Prandovszky E, Karuppagounder SS, Talbot CC Jr., Dawson VL, et al. MicroRNA-132 dysregulation in *Toxoplasma gondii* infection has implications for dopamine signaling pathway. *Neuroscience*. 2014; 268: 128â138. <https://doi.org/10.1016/j.neuroscience.2014.03.015> PMID: [24657774](#)
110. Yang D, Li T, Wang Y, Tang Y, Cui H, Tang Y, et al. miR-132 regulates the differentiation of dopamine neurons by directly targeting Nurr1 expression. *J Cell Sci*. 2012; 125: 1673â1682. <https://doi.org/10.1242/jcs.086421> PMID: [22328530](#)
111. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, et al. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell*. 2009; 137: 47â59. <https://doi.org/10.1016/j.cell.2009.01.038> PMID: [19345186](#)
112. Hermes G, Ajioka JW, Kelly KA, Mui E, Roberts F, Kasza K, et al. Neurological and behavioral abnormalities, ventricular dilatation, altered cellular functions, inflammation, and neuronal injury in brains of

mice due to common, persistent, parasitic infection. J Neuroinflammation. 2008; 5: 48. <https://doi.org/10.1186/1742-2094-5-48> PMID: 18947414

113. Hinze-Selch D, Daubener W, Eggert L, Erdag S, Stoltenberg R, Wilms S. A controlled prospective study of toxoplasma gondii infection in individuals with schizophrenia: beyond seroprevalence. Schizophr Bull. 2007; 33: 782â 788. PMID: 17387159
114. Ramanathan S, Mohammad SS, Brilot F, Dale RC. Autoimmune encephalitis: Recent updates and emerging challenges. J Clin Neurosci. 2013; 21: 722â 730. <https://doi.org/10.1016/j.jocn.2013.07.017> PMID: 24246947