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ORIGINAL ARTICLE

Dysregulation of kisspeptin and neurogenesis at adolescence link inborn immune deficits to the late onset of abnormal sensorimotor gating in congenital psychological disorders

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Neuropsychological syndromes including schizophrenia often do not manifest until late adolescence or early adulthood. Studies attributing a role in brain maintenance to the immune system led us to propose that malfunction of immune-dependent regulation of brain functions at adolescence underlies the late onset of such diseases/syndromes. One such function is sensorimotor gating, the ability to segregate a continuous stream of sensory and cognitive information, and to selectively allocate attention to a significant event by silencing the background (measured by prepulse inhibition; PPI). This activity is impaired in schizophrenia, as well as in several other neuropsychological diseases. Using a model of prenatal immune activation (maternal polyriboinosinic–polyribocytidylic acid (poly I:C) injection), often used as a model for schizophrenia, and in which abnormal PPI has a delayed appearance, we demonstrated a form of immune deficit in the adult offspring. Similar abnormal PPI with a delayed appearance was found in congenitally immune-deficient mice (severe combined immune deficient, SCID), and could be reversed by immune reconstitution. This functional deficit correlated with impairment of both hippocampal neurogenesis and expression of the gene encoding kisspeptin (*Kiss1*) that manifested at adulthood. Moreover, exogenous administration of a kisspeptin-derived peptide partially reversed the gating deficits in the SCID mice. Our results suggest that a form of congenital immune deficiency may be a key factor that determines manifestation of developmental neuropsychological disorders with onset only at early adulthood.
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Introduction

Schizophrenia is a complex and severe brain disorder with poorly defined etiology and pathophysiology, which affects approximately 1% of the world population.[1] Although the risk factors that correlate with the disease are mostly congenital (that is, genetic aberrations,[2,3] prenatal infections[4,5] and complications of birth[6]), the formal diagnostic symptoms and signs of schizophrenia are not typically manifested until late adolescence or early adulthood.[7]

During late adolescence and early adulthood, the brain undergoes extensive synaptic remodeling, including reduction in dendritic arborization,[8] axon myelination[9,10] and synaptic pruning.[11,12] The integrity of these processes may be crucial for maturation of a wide range of sensory and cognitive functions that are impaired in schizophrenia. Recently, we and others showed that the adaptive immune system, and specifically the population of T cells recognizing central nervous system (CNS) antigens, has a key function in CNS maintenance under physiological conditions.[13–16] Furthermore, we demonstrated that vaccination with a CNS-derived peptide can counteract the schizophrenia-like behavioral malfunctions induced in mice by MK-801 or amphetamine.[13] Taken together with the decreased immune response to brain antigens,[17] and the vast evidence of immune dysfunction in schizophrenic patients,[18] these

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findings led us to propose a working hypothesis suggesting that the congenital risk factors that correlate with schizophrenia result in immunological malfunction later in life. Such immunological malfunction could lead to the onset of various abnormal behaviors such as sensorimotor gating deficit, at the critical ages of adolescence and early adulthood.

In this study, we show that in rat and mouse models of congenital schizophrenia, induced by prenatal immune challenge with the viral mimetic, polyriboinosinic–polyribocytidylic acid (poly I:C),[19,20] a specific reduction in the T-cell response to CNS antigens is observed, despite an elevation in nonspecific T-cell proliferation. In addition, congenitally immune-deficient mice exhibit abnormal prepulse inhibition (PPI), which appeared only at adulthood, and could be reversed on immune reconstitution. We further demonstrate that immune-dependent regulation of hippocampal neurogenesis is manifested mostly at early adulthood. Furthermore, we found that hippocampal expression of *Kiss1*, a gene that is considered the gatekeeper for the onset of puberty[21,22] and regulates hippocampal synaptic transmission,[23–25] is elevated at adolescence, and that this is an immune-dependent event. Finally, we show that kisspeptin is involved in regulating sensorimotor gating. Together, hippocampal neurogenesis and Kiss1 mRNA expression suggest an immune-related mechanism that may underlie the late onset of behavioral malfunctions characteristic of schizophrenia.

Materials and methods

Animals
Inbred wild-type and Prkdc^{scid} (severe combined immune deficient, SCID) mice on BALB/c/OLA, or C57Bl/6 backgrounds, and inbred Lewis rats were supplied by the Animal Breeding Center of the Weizmann Institute of Science. SCID mice lack both T- and B-cell populations due to a mutation on chromosome 16 responsible for deficient Prkdc activity (protein kinase, DNA-activated, catalytic polypeptide). This mutation leads to defect in the rearrangement of genes that code for antigen-specific receptors on lymphocytes. However, they have a normal hematopoietic microenvironment, and normal myeloid and natural killer cell functions. Animals were matched for age in each experiment: adult animals (12- to 18-week old) were used in all the experiments, unless described otherwise. Male mice and female rats were used in all experiments. All animals were handled according to the regulations formulated by the Weizmann Institute's animal care and use committee and were maintained in a pathogen-free environment.

Poly I:C administration
Rats or mice were bred, and the first day after copulation was defined as day 1 of pregnancy. On gestation day 15, pregnant animals were injected intravenously with 4.0 mg kg^{-1} poly I:C (Sigma-Aldrich, Rehovot, Israel) dissolved in saline, or an equivalent volume of saline as a control. Both rats and mice received the same protocol of poly I:C administration.

Prepulse inhibition
Prepulse inhibition testing was performed within startle chambers purchased from Med Associates Inc. (St Albans, VT, USA). PPI was analyzed as described previously.[13] Briefly, during a period of acclimation, a 65 dB background noise was applied for 5 min, and continued throughout the test session. All sessions for testing of PPI consisted of startle trials (pulse alone, 40 ms, 120 dB), prepulse trials (prepulse 20 ms, 69, 73, 78 or 81 dB followed by a (100 ms delay) pulse), and no-stimulus trials. All sessions were presented in pseudorandom order. The average time between trials was 15 s (range 12–30 s). PPI was calculated as %PPI = 100−(((startle response for prepulse + pulse)/(startle response for pulse alone)) × 100). In all the experiments, there were no significant differences between groups in trails of startle reactivity to pulse alone, and in no-stimulation trails. Data from no-stimulation trails are not included in the results because the values obtained were negligible relative to values from trials containing startle stimuli. Reduced PPI is associated with schizophrenia-related behavior.[26] The same protocol was used in mice and rats.

Vaccination
Adult rats were immunized at the base of the tail with 75 μg of the peptide $MBP_{68–86}$ (YGSLPQKSQRSQDENPV)[27] (synthesized at the Weizmann Institute of Science), emulsified in an equal volume of complete Freund's adjuvant (CFA; DIFCO LABORATORIES, Detroit, MI, USA) containing 5 mg ml^{-1} *Mycobacterium tuberculosis* (strain H37Ra; DIFCO LABORATORIES).

Proliferation assay
Peripheral lymph nodes were harvested and mashed. The proliferation assay was carried out as described previously.[28] Briefly, lymphocytes were cultured in 200 μl of medium (1.5×10^6 cells per ml) with ovalbumin (Ova, 10 μg ml^{-1}; Sigma-Aldrich), $MBP_{68–86}$ (10 μg ml^{-1}; the Weizmann Institute of Science), concanavalin A (Con-A; 1.25 μg ml^{-1}; Sigma-Aldrich) or without antigen, for 72 h. [^{3}H]Thymidine (0.2 μCi per well) was added during the last 18 h of culture. Cells were harvested, and incorporation of [^{3}H]thymidine was measured using a direct γ-counter. The proliferation index (PI) was calculated as the ratio between the proliferation in the presence of antigen and proliferation in the absence of antigen.

Experimental autoimmune encephalomyelitis
To induce experimental autoimmune encephalomyelitis (EAE), adult female Lewis rats were immunized s.c. in the hind footpads and in the base of the tail with 25 μg $MBP_{68–86}$, emulsified (1:1 dilution) in

100 µl of CFA containing 2 mg ml^{-1} *M. tuberculosis* (strain H37Ra; DIFCO LABORATORIES).[29] Clinical signs were evaluated in a blinded manner by at least two investigators. Body weight and clinical score were recorded daily (0, healthy; 1, tail paralysis; 2, ataxia and/or paresis of hindlimb; 3, paralysis of both of the hindlimbs; 4, tetra paralysis; 5, moribund state or death).

RNA purification, cDNA synthesis, reverse transcription PCR and real-time quantitative PCR analysis
RNA expression was analyzed as described previously.[16] Briefly, cells from whole hippocampi were extracted with TRI Reagent (MRC, Cincinnati, OH, USA), and total cellular RNA was purified from the lysates using the RNeasy kit (Qiagen, Hilden, Germany). RNA (1 µg) was converted to cDNA using SuperScript II (Promega, Madison, WI, USA). The amplification cycle was 95 °C for 5 s, 60 °C for 20 s and 72 °C for 15 s. The following primers were used: GAPDH, 5′-AATG TGTCCGTCGTGGATCTGA and 5′-GATGCCTGCTTCA CCACCTTCT; Kiss1, 5′-AGCTGCTGCTTCTCCTCTGT and 5′-GCATACCGCGATTCCTTTT.[30]

Kisspeptin treatment
Mice were injected i.p with 100 µl of 10 µM kisspeptin-10 peptide (Kp-10, human metastin 45–54, YNWNSFGLRF-NH2, synthesized at the Weizmann Institute of Science) dissolved in phosphate-buffered saline (PBS), 30 min before the PPI analysis.

Lymphocyte replenishment
Peripheral lymph nodes from adult wild-type or from offspring of poly-I:C-treated C57Bl/6 mice were harvested and mashed. Lymphocytes were resuspended in PBS and injected intravenously to male C57Bl/6 SCID mice. Each SCID recipient received cells from one donor mouse. Behavioral assessment was performed 4 weeks after reconstitution, or as indicated in the relevant experiment.

Administration of 5-bromo-2′-deoxyuridine and tissue preparation
Neurogenesis was analyzed as described previously.[14] Briefly, mice were injected i.p. with BrdU (5-bromo-2′-deoxyuridine; Sigma-Aldrich; 50 mg per kg body weight), twice a day for 2 days. They were killed 7 days after the first injection and perfused transcardially. Their brains were removed and postfixed. Free-floating, 30-micron-thick coronal hippocampal sections were collected on a freezing microtome (Leica SM2000R, myNeuroLab, St Louis, MO, USA) and stored at 4 °C until immunohistochemical analysis.

Antibodies and reagents for immunohistochemistry
To quantify neurogenesis, tissue sections were washed with PBS, incubated in 2 N HCl at 37 °C for 30 min, and then blocked for 1 h with blocking solution (PBS containing 20% normal horse serum and 0.5% Triton X-100). The tissue sections were stained overnight with the following primary antibodies: rat anti-BrdU (1:200; Oxford Biotechnology, Kidlington, Oxfordshire, UK) and goat anti-DCX (1:300; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Secondary antibodies used were fluorescein-isothiocyanate-conjugated donkey anti-goat and Cy-3-conjugated donkey anti-rat.

Quantification
For microscopic analysis, a Nikon E800 microscope was used. The numbers of labeled cells were counted, by an observer blinded to the identity of the samples, in six coronal sections (370 micron apart) per mouse brain. To obtain an estimate of the total number of labeled cells per dentate gyrus, the total number of cells counted in the selected coronal sections from each brain was multiplied by the volume index (the ratio between the volume of the dentate gyrus and the total combined volume of the selected sections).

Statistical analysis
The StatView (SAS Institute Inc., Cary, NC, USA) statistics package was used to perform statistical analysis of all data. PPI experiments were analyzed using two-way analysis of variance (ANOVA) with group treatment and prepulse intensity as the between-subject factors. If no significant interaction was found between the tested groups and the intensities, whereas significant effects were found with respect to the tested factors themselves, a one-way ANOVA was performed for the single intensities. Only significant main effects were further evaluated using Fisher's least significant difference (LSD) or two-tailed Student's *t*-test *post hoc* analysis. Data from other experiments were analyzed using Student's *t*-test (for experiments that included two groups) or one-way ANOVA (for experiments that included three groups), with group treatment or immune background as the between-subject factor.

Results

Alterations in the immune response in a neurodevelopmental animal model for schizophrenia
Poly I:C injection into pregnant females is known to cause the emergence of psychopathological behavior in the offspring at early adulthood.[19,31] We first used this animal model[19,31,32] to test whether prenatal infection causes a reduced immune response to self-antigens, as previously observed in schizophrenic patients.[17] Pregnant Lewis rats were treated with 4 mg kg^{-1} of poly I:C or saline at gestational day 15. Female offspring were tested for PPI at adulthood, to verify the successful induction of schizophrenia-like behavior. As expected, adult offspring of the poly-I:C-treated rats showed a reduction in PPI compared to the progeny of saline-treated animals (Supplementary Figure 1). To measure alterations in immunity to brain-specific antigens in the poly-I:C-affected offspring, we vaccinated female and male offspring at adulthood with the myelin basic protein (MBP)-derived peptide, $MBP_{68–86}$, a CNS-associated

Table 1 Immune activation with poly I:C during pregnancy causes immune alterations in the offspring

(a) *Prenatal treatment*	*Saline (PI ± s.d.)*	*Poly I:C (PI ± s.d.)*	P-*value* (t-*test*)
Antigen			
MBP_{68-86}	1.3 ± 0.11	1.0 ± 0.11	0.01
Ova	1.0 ± 0.14	1.2 ± 0.09	0.13
Con-A	71 ± 49	126 ± 67	0.15

(b) *Prenatal treatment*	*Saline (day ± s.e.m.)*	*Poly I:C (day ± s.e.m.)*	P-*value* (t-*test*)
Onset	10.18 ± 0.2	11.17 ± 0.2	0.005
Duration	6.27 ± 0.3	5.08 ± 0.3	0.004

Abbreviations: Con-A, concanavalin A; EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; Ova, ovalbumin; PI, proliferation index.
(a) PI of lymphocytes from adult offspring of saline- or poly-I:C-treated dams in response to incubation with MBP_{68-86}, Ova or the lymphocyte mitogen Con-A, 5 days after vaccination with MBP_{68-86}. Values represent means ± s.d. of four replicates from each animal (MBP, $t_9 = 3.3$; Ova, $t_9 = -1.8$; Con-A, $t_9 = -1.5$; $n = 6$, 3 males and 3 females). (b) Clinical EAE score in adult female offspring of saline- or poly-I:C-treated dams (Onset, $t_{21} = -3.21$, Duration, $t_{21} = 3.15$; $n = 11$, 12 for Poly I:C and saline groups, respectively).

self-antigen known to be the immune-dominant epitope in this strain,[33] and examined lymphocyte proliferation in response to the injected antigen, MBP_{68-86}, as well as to Con-A, a lymphocyte mitogen, or to Ova (an irrelevant antigen). We found, in lymphocytes of the offspring of the poly-I:C-treated animals, a significant reduction in the proliferative response to MBP (Table 1a). Furthermore, in the poly-I:C-affected offspring, there was a slightly elevated response to Ova and to Con-A compared to the control, although this difference was not statistically significant (Table 1a).

To further examine the CNS-specific immune response in the poly I:C offspring, we tested their susceptibility to EAE, the animal model for multiple sclerosis. We expected that if there was indeed a reduction in CNS-specific T-cell response in the offspring of poly-I:C-treated animals, they would be less susceptible to development of EAE on challenge. In this experiment, we tested only the female offspring, as females are known to be more susceptible to the induction of EAE,[34] and thus any observed effect would be more pronounced. EAE was induced in adult offspring of control or poly-I:C-treated rats by immunization with MBP_{68-86} emulsified in CFA,[29] and clinical symptoms of encephalomyelitis were monitored daily. The offspring of poly-I:C-treated rats showed a delayed onset and a shorter duration of EAE symptoms following MBP_{68-86} vaccination, compared to the controls (Table 1b). These results supported our contention that immune activation during pregnancy is associated with reduced immune response to brain antigens in the offspring.

Congenital immune deficiency causes reduced PPI with onset at early adulthood

The correlation between the behavioral malfunction and the immune changes induced in the progeny of poly-I:C-treated animals prompted us to explore the more general question of whether a congenital immune deficit could cause mental dysfunction that emerges only at adulthood. To this end, we used SCID mice, which lack both functional T- and B-lymphocyte populations. SCID mice were previously shown to develop various cognitive abnormalities including impaired hippocampal-dependent spatial memory[13–16] and increased anxiety levels in response to acute stress.[35] Those abnormal behaviors were attributed to their immune deficiency. Moreover, these mice have reduced hippocampal brain-derived neurotrophic factor (BDNF) levels and reduced neurogenesis. Here, we tested whether PPI in SCID is impaired, and if so, whether its manifestation is age dependent. Adolescent (8-week old) SCID mice showed normal PPI behavior: ANOVA of percent PPI indicated a significant main effect of prepulse intensities, but not of immunological background (Figures 1ai and b). However, when the same mice were tested again at early adulthood (12 weeks of age), they showed reduced PPI relative to the age-matched controls: ANOVA of percent PPI indicated a significant main effect of the immunological background, but not of the prepulse intensities. To identify the prepulse intensities at which percent PPI significantly differed between the groups, we examined the performance of the groups at single intensities and found that the differences between SCID and wild-type mice reached statistical significance at prepulse intensities of 73 and 81 dB (Figure 1c). Importantly, the SCID and wild-type mice did not differ in their startle reactivity to pulse-alone trials, nor in their reaction to the prepulse alone trials (Supplementary Figure 2). To further demonstrate a causal relationship between the immune deficiency and the observed impairment in the PPI response, we tested whether repopulation with normal lymphocytes could prevent and/or

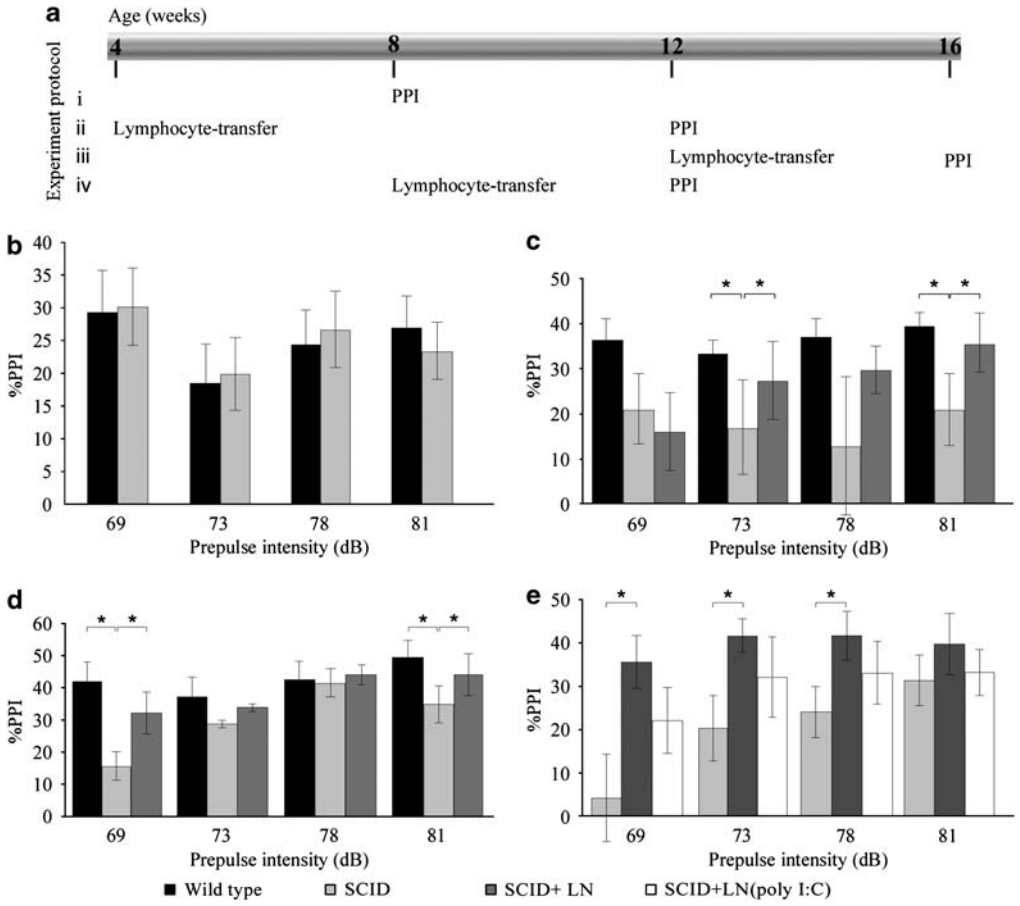

Figure 1 Immune deficiency causes abnormal prepulse inhibition (PPI) that is reversible by lymphocyte transfer. PPI in male C57Bl/6 wild-type and severe combined immune-deficient (SCID) mice was tested at different ages, according to the experimental design shown in (**a**). (**b**) PPI in adolescent (8-week old) C57Bl/6 wild-type ($n=18$) and SCID ($n=16$) mice (repeated-measure analysis of variance (ANOVA)—groups: $F(1,32)=0.07$, $P=0.79$; prepulse intensities: $F(3,96)=3.49$, $P=0.0186$; groups × prepulse intensities: $F(3,96)=0.04$, $P=0.99$). (**c**) PPI in adult (12-week old) C57Bl/6 wild-type ($n=8$), SCID ($n=7$) and SCID mice that were reconstituted with lymphocytes as juveniles (4 weeks, $n=8$) (repeated-measure ANOVA—groups: $F(2,20)=3.59$, $P=0.04$; prepulse intensities: $F(3,60)=1.74$, $P=0.17$; groups × prepulse intensities: $F(6,60)=1.43$, $P=0.22$. One-way ANOVA—69 dB: $F(2,20)=2.32$, $P=0.12$; 73 dB: $F(2,20)=4.11$, $P=0.03$; 78 dB: $F(2,20)=3.21$, $P=0.06$; 81 dB: $F(2,20)=3.70$, $P=0.04$; $^*P<0.05$, Fisher's LSD *post hoc* analysis). (**d**) PPI in adult (16-week old) C57Bl/6 wild-type ($n=8$), SCID ($n=7$) and SCID mice that were reconstituted with lymphocytes as adults (12 weeks, $n=7$) (repeated-measure ANOVA—groups: $F(2,19)=3.52$, $P=0.05$; prepulse intensities: $F(3,57)=8.92$, $P=0.0001$; groups × prepulse intensities: $F(6,57)=2.2$, $P=0.06$. One-way ANOVA—69 dB: $F(2,19)=6.05$, $P=0.01$; 73 dB: $F(2,19)=1.95$, $P=0.17$; 78 dB: $F(2,19)=0.26$, $P=0.77$; 81 dB: $F(2,19)=3.54$, $P=0.04$; $^*P<0.05$, Fisher's LSD *post hoc* analysis). (**e**) PPI in adult (12-week old) C57Bl/6 SCID mice ($n=8$) compared to SCID mice that were reconstituted with lymphocytes derived from offspring of poly-I:C- ($n=8$) or saline-treated dams ($n=8$) at the age of 8 weeks (repeated-measure ANOVA—groups: $F(2,21)=3.89$, $P=0.04$; prepulse intensities: $F(3,63)=6.27$, $P=0.001$; groups × prepulse intensities: $F(6,63)=1.82$, $P=0.11$. One-way ANOVA—69 dB: $F(2,21)=5.56$, $P=0.01$; 73 dB: $F(2,21)=3.46$, $P=0.05$; 78 dB: $F(2,21)=3.52$, $P=0.04$; 81 dB: $F(2,21)=1.11$, $P=0.35$; $^*P<0.05$, Fisher's LSD *post hoc* analysis). Values represent means ± s.e.m.

restore the impairment in the PPI. We therefore transferred lymphocytes from wild-type mice to SCID mice either at infancy (4 weeks; Figure 1aii) or in adulthood (12 weeks; Figure 1aiii). SCID mice that were reconstituted at 4 weeks of age exhibited normal percent PPI levels at both prepulse intensities (73 and 81 dB) in which significant differences were found between SCID and wild-type mice (Figure 1c). Reconstitution at adulthood (12 weeks) yielded similar results when PPI was examined 4 weeks later: ANOVA of percent PPI indicated a significant main effect of the immunological background, but not of the prepulse intensities. To identify the prepulse intensities at which percent PPI significantly differed between the groups, we examined the performance of the groups at single intensities and found that the differences between SCID and wild-type mice reached statistical significance at prepulse intensities of 69 and 81 dB. Immune reconstitution restored percent PPI to normal at those intensities (Figure 1d). These results demonstrate that a congenital immune deficit caused late manifestation of a behavioral abnormality, which could be reversed by immune reconstitution, either at infancy or in adulthood.

The ability of a normal lymphocyte population to reverse abnormal PPI behavior in immune-deficient mice encouraged us to test whether immune cells derived from the offspring of poly-I:C-treated dams would reverse the abnormal behavior of SCID mice in the PPI test. We therefore injected 4 mg kg^{-1} of poly I:C or saline to pregnant C57Bl/6 mice at gestational day 15; after validating that their adult offspring manifested reduced PPI (Supplementary Figure 3), we isolated lymphocytes from these adult offspring and transferred them to adolescent (8-week old) naive SCID mice (Figure 1aiv). As controls, we transferred lymphocytes from offspring of saline-treated dams to another group of SCID mice. At 12 weeks, SCID mice that received lymphocytes from the offspring of poly-I:C-treated dams showed similar PPI compared to nonreconstituted SCID mice (Figure 1e); in contrast, the SCID mice that received lymphocytes from offspring of saline-treated dams showed improved PPI: ANOVA of percent PPI yielded a significant main effect for the immune background, and the prepulse intensities. However, the interaction between prepulse intensities and immunological background did not reach statistical significance. To identify the prepulse intensities at which percent PPI significantly differed between the groups, we examined the performance of the groups at single intensities and found that immune reconstitution with lymphocytes from the offspring of saline-treated dams significantly improved percent PPI compared to SCID mice at prepulse intensities of 69, 73 and 78 dB. On the other hand, SCID mice reconstituted with lymphocytes derived from the offspring of poly-I:C-treated dams did not differ significantly at any of the intensities tested (Figure 1e). These results further support the observation of abnormal adaptive immunity in the offspring of poly-I:C-treated dams (Table 1), and strengthen our proposed linkage between this defect and the observed behavioral abnormalities.

Age-related requirements for immune cell support of hippocampal neurogenesis

Our previous studies showed that adult SCID mice suffer from impaired neurogenesis,[14] another aspect of brain plasticity that is compromised in schizophrenic patients,[36,37] and in the offspring of poly-I:C-treated dams.[38] The question that emerged, however, is whether this aspect of brain plasticity in these congenitally immune-deficient mice is also mainly manifested at adulthood. Neurogenesis was measured at infancy (4 week old) and early adulthood (12-week old) in the dentate gyrus of wild-type and SCID mice. All mice received four injections of the cell proliferation marker, BrdU (every 12 h, i.p.), and labeled cells were analyzed 7 days after the first injection. Their brains were excised and hippocampi were examined immunohistochemically for both BrdU and the early neuronal differentiation marker, doublecortin (DCX). At 4 weeks, a small (18%) reduction in progenitor cell proliferation (BrdU$^+$ cells) was observed in the SCID mice, compared to their wild-type controls (Figure 2a, left). It was noted, however, that SCID mice had a higher neuronal differentiation percentage than wild-type mice (81.3 ± 1.8 and 73.5 ± 1.2%, respectively), and this might account for the apparently similar numbers of newly formed neurons (BrdU$^+$/DCX$^+$ cells) in the two groups. Consistent with previous findings,[39] at 12 weeks, in both mouse groups, there was a reduction in proliferation relative to 4 weeks (Figure 2a, right versus left). Yet, at 12 weeks, cell proliferation diverged between the SCID mice and their wild-type controls; when cell proliferation was compared at this age, the difference was twice as great as that seen in the infant mice (Figure 2b). Moreover,

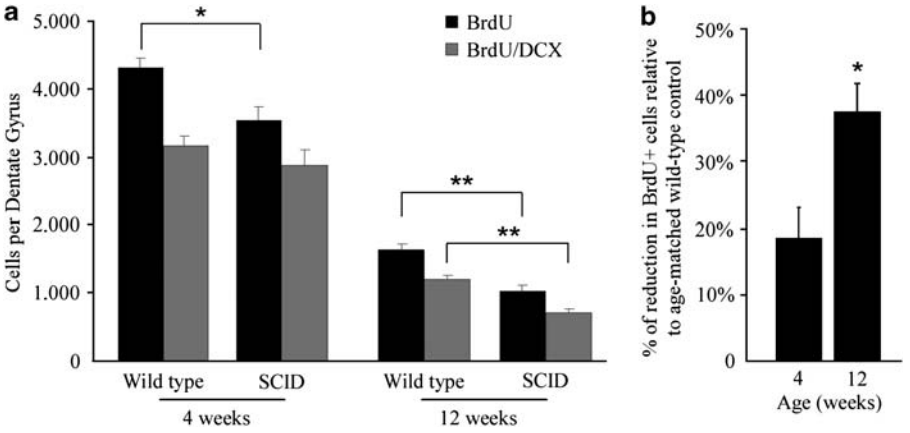

Figure 2 Age-dependent differences in neurogenesis between wild-type and immune-deficient mice. (**a**) BALB/c mice (severe combined immune-deficient (SCID) and wild type) at 4 and 12 weeks of age were injected with BrdU (5-bromo-2′-deoxyuridine, see Materials and methods), killed 7 days after the first BrdU injection and their dentate gyrus was analyzed for BrdU$^+$ and for BrdU$^+$/DCX$^+$ cells. For 4-week-old mice, for BrdU$^+$ cells: $t_8 = -3.1$, $^*P = 0.015$; for BrdU$^+$/DCX$^+$ cells: $t8 = 1.13$, $P = 0.29$; $n = 6$ and $n = 4$ for wild-type and SCID, respectively; for 12-week-old mice, for BrdU$^+$ cells: $t_6 = -5.70$, $^{**}P = 0.0011$; for BrdU$^+$/DCX$^+$ cells: $t_6 = -5.74$, $^{**}P = 0.0012$; $n = 4$. (**b**) Percentage reduction in the number of BrdU$^+$ cells in SCID compared to wild-type mice at 4 and 12 weeks. Calculation: 100% −(percentage of BrdU$^+$ cells in individual SCID from the number of BrdU$^+$ cells in wild type), ($t_6 = 3.01$, $^*P = 0.024$; same groups as in **a**). Values represent means ± s.e.m.

at 12 weeks of age, the total number of newly formed neurons ($BrdU^+/DCX^+$) was also significantly lower in SCID relative to wild-type mice (Figure 2a, right panel). These results suggest that impaired neurogenesis in congenitally immune-deficient mice is manifested maximally at early adulthood.

Kisspeptin expression links the congenital immune deficit and late onset of schizophrenia-like behavior

Changes in sex hormone expression can modulate the brain maturation process during adolescence,[40] and sex hormone expression was found to be abnormal in schizophrenic patients.[41–43] In addition, treatment with sex hormones was proposed as a treatment for schizophrenia.[44–46] Based on the known effect of the immune system on sex hormones,[47–49] we envisioned that the abnormal immunity in schizophrenia might affect brain maturation in part through the impaired regulation of sex hormones. We proposed that kisspeptin, a protein that is considered the gatekeeper for the onset of puberty[21,22] and regulates hippocampal synaptic transmission,[23–25] might be a potential candidate for the interface between the immune system and brain regulation at this critical period of puberty. To identify potential immune-dependent regulation of *Kiss1* expression in the hippocampus, we analyzed mRNA levels of *Kiss1* by quantitative real-time PCR (qPCR) in wild-type and SCID mice of different ages. We found that in the hippocampus at 8 weeks of age (puberty), there was a peak in *Kiss1* levels in the wild-type mice, which was absent in the SCID mice (Figure 3a). Moreover, when SCID mice were replenished with lymphocytes as juveniles (4 weeks of age), their hippocampal *Kiss1* expression level at puberty was restored to normal (Figure 3b). To further examine whether changes in *Kiss1* may be relevant to schizophrenia, we analyzed *Kiss1* mRNA expression levels in the hippocampus of poly-I:C-affected offspring, and offspring of control mice. qPCR analysis revealed a reduction in *Kiss1* mRNA levels in adult poly-I:C-affected mice compared to age-matched controls (Figure 3c). These results suggested that the immune system regulates *Kiss1* mRNA levels in the hippocampus in adolescence. Moreover, our data suggested that the lack of elevation of *Kiss1* expression in immune-deficient adolescent mice was relevant to the late manifestation of behavioral abnormalities in congenitally immune-

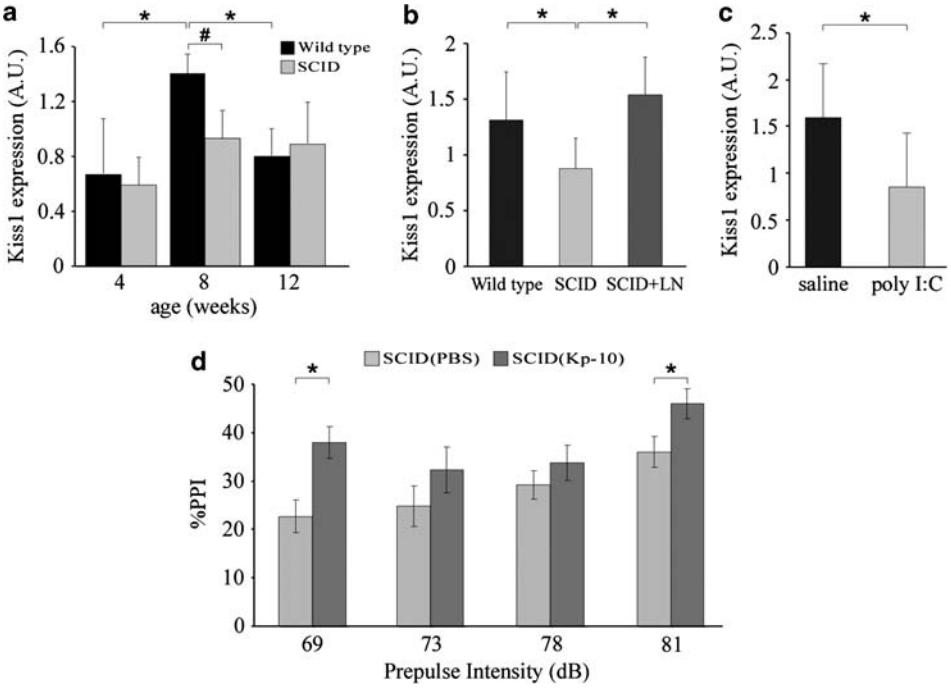

Figure 3 Immune regulation of kisspeptin expression in the hippocampus and its relevance to performance in the prepulse inhibition (PPI) test. (**a–c**) qPCR analysis of *Kiss1* expression in the hippocampus of (**a**) wild-type and severe combined immune-deficient (SCID) mice at different ages (one-way analysis of variance (ANOVA)—wild-type: $F(2,13)=4.11$, $P=0.04$, $^*P<0.05$; SCID: $F(2,11)=1.47$, $P=0.27$. Student's *t*-test—4 weeks: $t_7=-0.29$, $P=0.78$ ($n=5$, 4); 8 weeks: $t_{6.6}=-2.46$, $^\#P=0.04$ ($n=6$, 5); 12 weeks: $t_8=0.46$, $P=0.66$ ($n=5$)); (**b**) 8-week-old wild-type ($n=11$), SCID ($n=8$) and SCID mice that were reconstituted with lymphocytes at 4 weeks of age ($n=4$) ((one-way analysis of variance (ANOVA): $F(2,20)=5.25$, $P=0.01$; $^*P=0.05$, Fisher's LSD *post hoc* analysis) and (**c**) adult male offspring of poly-I:C- ($n=6$) or saline-treated dams ($n=6$) ($t_{10}=2.24$, $^*P=0.04$). Values represent means ± s.d. (**d**) Treatment with Kp-10 restores the abnormal PPI caused by congenital immune deficiency. PPI was tested in adult (12-week) male C57Bl/6J SCID that were injected i.p. with Kp-10 ($n=15$) or with phosphate-buffered saline (PBS; $n=16$) (repeated-measure ANOVA—groups: $F(1,29)=6.55$, $P=0.016$; prepulse intensities: $F(3,87)=7.74$, $P=0.0001$; groups × prepulse intensities: $F(3,87)=1.11$, $P=0.35$. One-way ANOVA—69 dB: $F(1,29)=10.75$, $P=0.003$; 73 dB: $F(1,29)=1.47$, $P=0.23$; 78 dB: $F(1,29)=0.97$, $P=0.33$; 81 dB: $F(1,29)=4.39$, $P=0.04$; $^*P<0.05$, Fisher's LSD *post hoc* analysis). Values represent means ± s.e.m.

compromised mice. To confirm this possibility, we examined whether injection of kisspeptin would reverse the abnormal PPI in adult (12-week old) SCID mice. We therefore injected SCID mice with the kisspeptin-10 (Kp-10) peptide or with PBS (control) 30 min before PPI testing. Indeed, we found that injection of Kp-10 improved the PPI of SCID mice: ANOVA of percent PPI revealed a significant main effect of the treatment, and of the prepulse intensities. However, the interaction between the prepulse intensities and the treatment did not reach statistical significance. To identify the prepulse intensities in which percent PPI significantly differed between the groups, we examined the performance of the groups at single intensities and found that the differences in percent PPI between SCID mice and SCID mice injected with Kp-10 reached statistical significance at prepulse intensities of 69 and 81 dB (Figure 3d). These results suggest that Kp-10 is a missing link between immunity and brain plasticity in adolescence with regard to regulation of sensorimotor gating.

Discussion

In this study, we demonstrated in a neurodevelopmental animal model for schizophrenia (maternal poly I:C treatment) that immune activation during pregnancy causes a form of immune deficit in the offspring. We further showed that similar to the poly I:C mice and rats, in congenitally immune-deficient mice, as well, abnormal PPI developed only at adulthood and could be reversed by transfer of lymphocytes from wild-type mice. In addition, we showed that adolescence and early adulthood is a period during which the adaptive immune system modulates developmental processes in the brain, such as hippocampal neurogenesis and *Kiss1* expression. Finally, administration of kisspeptin to congenitally immune-deficient mice overcame the behavioral deficit as measured by PPI.

Among the critical environmental factors that have been linked to an increased risk of developing schizophrenia later in life are maternal infections during pregnancy.[50,51] Because the specificity of the viral infection appears irrelevant to schizophrenia development,[52] poly I:C administration during pregnancy, causing nonspecific immune activation, is accepted as a valid animal model for inducing this disease. In addition, some of the symptoms observed in human patients were also seen in this animal model, including a sensorimotor gating deficit,[31,32] one of the hallmark symptoms of schizophrenia,[26] and alterations in limbic morphology.[19] Moreover, in this model, similar to the human disease, the psychopathological behavioral symptoms such as abnormal PPI develop only at adulthood.[19,31] The exact mechanism through which poly I:C acts to increase the risk for these neurodevelopmental pathologies is not fully understood. However, the maternal cytokine response to infections is suspected to have a crucial function in this association.[53,54] One possible outcome of such a cytokine response is the abnormal development of the immune system of the progeny. Indeed, in schizophrenic patients, several immune abnormalities have been described, including a shift in the cytokine response.[55–57] In addition, in some schizophrenic patients, a reduced immune response to brain antigens was observed.[17]

Here, we describe a novel functional linkage between maternal infection, abnormal cellular immunity and delayed onset of schizophrenia-like behavior. The fact that EAE could be induced in the progeny of poly-I:C-treated rats, though the disease was milder, indicates that maternal infection does not eliminate CNS-specific lymphocytes, but alters their regulation. The mechanism by which immune activation during pregnancy can cause reduced immune response to self-antigens is not clear. One possibility is that the increase in blood–brain barrier permeability that occurs during viral infection results in exposure to maternal brain antigens, thus leading to immune tolerance to those antigens in the offspring. Similarly, maternal immune activation to a specific antigen was shown to cause immune tolerance to the same antigen in the offspring.[58,59] Smith *et al*.[53] showed that behavioral abnormalities caused by maternal immune activation are mediated by the cytokine, interleukin-6 (IL-6). IL-6 promotes the induction of immune tolerance by regulating antigen-presenting cell maturation.[60] As a consequence, exposure to maternal brain antigens during development in a tolerogenic environment could lead to unresponsiveness to the corresponding autoantigens in the offspring.[17]

Whether the decreased immune response to brain antigens is the cause of the disease or its outcome is not yet known. Here, we show that congenital immune deficits resulting in abnormal PPI are manifested only in adulthood, and that transfer of lymphocytes from wild-type mice can prevent and/or restore this dysfunction. These data suggest that the abnormal sensorimotor gating, as tested by PPI, could be attributed, at least in part, to a defect in adaptive immunity. The fact that transfer of lymphocytes to adult SCID mice was sufficient to reverse the abnormal PPI suggests that such behavioral abnormalities are not solely the result of abnormal brain development or pathology, but rather reflect functional deficits in the continuous contribution of peripheral immunity to brain maintenance. Although SCID mice were reconstituted with the total lymph node cell population, we attribute the beneficial effect of immune reconstitution on behavior to the adaptive immune cells, and specifically to T lymphocytes; the other immune cell populations in SCID mice are normal. Nevertheless, it is possible that the other populations of transferred cells, that is, antigen-presenting cells, take part in regulating neuronal activity, for example by augmenting secretion of cytokines. Changes in cytokine balance can affect behavior.[61]

Previous reports by our laboratory and others have shown that SCID mice suffer from cognitive deficits[13–16] and impaired ability to cope with mental stress.[35]

Taken together with the results of the current study, these data further argue in favor of the function of the adaptive immune system in maintaining normal brain function. It is important to note that this study does not suggest that SCID mice should be viewed as a model for schizophrenia; rather these mice were used to support the connection between congenital immune deficiency and the late onset of behavioral abnormalities.

Prepulse inhibition, the phenomenon by which a low-intensity prepulse stimulus attenuates the response to a subsequent startle-eliciting noise, is used as a measurement of the sensory gating function.[62] Sensorimotor gating is an active process that contributes to the ability to segregate a continuous stream of sensory and cognitive information, and to selectively allocate attention to a significant event by silencing the background. The specific features of an individual's gating processes are considered to be plastic, and governed by genetic and developmental processes, but also by the environmental milieu, and the neurochemical and hormonal state of the CNS.[63] Impairment of PPI has been reported in several diseases including schizophrenia,[26] Huntington's chorea,[64] obsessive-compulsive disorder,[65] attention-deficit hyperactivity disorder[66] and Tourette's syndrome.[67] Thus, PPI deficits are not unique to a single form of psychopathology.

The hippocampus has an important function in modulation of sensorimotor gating.[68] Abnormalities in the hippocampal-related activities in schizophrenia are one of the most consistent findings in this disease.[69,70] Specifically, a reduction in hippocampal neurogenesis was reported in schizophrenic patients,[36,37] and was suggested to have a role in the disease pathophysiology.[71] We have previously shown that CNS-specific lymphocytes maintain normal levels of adult hippocampal neurogenesis.[14] Here, we show that the contribution of the adaptive immune system to neurogenesis is most significant at the critical periods of adolescence and early adulthood, at an age that correlates with schizophrenia onset. Importantly, however, additional study is required to determine whether the same immune-dependent pathways regulate neurogenesis and PPI. Lymphocytes contribute to several additional aspects of hippocampal plasticity, including modulation of BDNF production, under both normal conditions[14] and mental stress,[72,73] and to the regulation of GABA levels,[74] both processes that are important for normal PPI.[75–77]

In the present study, we identified kisspeptin as a novel immune-regulated player in hippocampal plasticity. *Kiss1* and its receptor are expressed in the dentate gyrus of the hippocampus and can regulate synaptic plasticity[23,25] and BDNF levels.[24] Kisspeptin was originally identified as a key signaling factor in the neural control of fertility.[21,78] It was shown to elicit the release of gonadotropin, through the stimulation of gonadotropin-releasing hormone (GnRH) secretion,[79] by direct activation of preoptic GnRH neurons.[80] During the transition from juvenile to adulthood, there is an increase in the expression of *Kiss1* mRNA in the anteroventral periventricular nucleus of the hypothalamus, which is believed to regulate GnRH neurons.[80] Here, we describe a similar increase in expression of *Kiss1* mRNA in the hippocampus of wild-type mice in adolescence, suggesting its involvement in regulating hippocampal plasticity at this critical age. Such an elevation in *Kiss1* expression was absent in SCID mice, but was reestablished following immune reconstitution. In addition, we found reduced levels of *Kiss1* mRNA in the hippocampus of adult poly-I:C-affected offspring. It is important to note that to reduce the effect of sex hormonal fluctuations[81] these experiments were carried out using male mice. Nevertheless, the present findings might suggest a novel approach for uncovering the mystery of gender differences in psychotic diseases.[82]

Finally, we demonstrated the causal relation between reduced *Kiss1* expression and the abnormal behavior in SCID mice. Injection of Kp-10, a polypeptide derived from *Kiss1*, previously shown to activate GPR54 *in vivo*,[78,79] restored PPI in SCID mice to normal. Taken together, these results show that an absent or abnormal immune response to CNS antigens can lead to the late onset of schizophrenia-like symptoms, though the precipitating event occurred prenatally.

This study extends the function of the adaptive immune system in brain maintenance, and emphasizes its importance in supporting the brain during the vulnerable period of adolescence. Therefore, congenital insults that lead to the development of an abnormal immune response might lead to late development of neuropathology in susceptible individuals. In addition, the association of kisspeptin with schizophrenia-like symptoms may offer novel possibilities for treatment and diagnosis of schizophrenia, and other puberty-associated psychoses. Moreover, this study paves the way to understanding a possible linkage between immune deficits and many behavioral abnormalities possibly including attention disorders and autism.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)