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ARTICLE

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Neonatal infection with neurotropic influenza A virus induces the kynurenine pathway in early life and disrupts sensorimotor gating in adult *Tap1*$^{-/-}$ mice

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Abstract

Epidemiological studies suggest that early life infections may contribute to the development of neuropsychiatric disorders later in life. Experimental studies employing infections during neonatal life support this notion by reporting persistent changes in the behaviour of adult animals, including deficits in sensorimotor gating. We have previously described an induction of the kynurenine pathway in neonatal wild-type (WT) mice following a systemic infection with neurotropic influenza A/WSN/33 virus. Here, we use the same model of infection in both WT and *Tap1*$^{-/-}$ mice (expressing reduced levels of MHC class I) and study long-term effects of the infection on sensorimotor gating, as determined by measuring prepulse inhibition (PPI). Moreover, transcription of genes encoding enzymes in the kynurenine pathway and levels of kynurenic acid (KYNA), in the brain of *Tap1*$^{-/-}$ mice were investigated. In mice infected on postnatal day (P)3 or P4, the levels of several transcripts in the kynurenine pathway were altered at P7, P13 and P24. Transcripts encoding indoleamine-pyrrole 2,3-dioxygenase (IDO), degrading tryptophan in the first step of the kynurenine pathway were consistently up-regulated at all time-points investigated. The changes in transcript levels were accompanied by a transient elevation of KYNA in the brain of infected mice at P13. At age 5–6 months, neonatally infected *Tap1*$^{-/-}$, but not WT, mice exhibited a reduction in PPI. The present data show that a neonatal infection targeting the brain can induce the kynurenine pathway and that such an infection can disrupt sensorimotor gating in adulthood in genetically vulnerable mice.

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Introduction

Early life exposure to herpes simplex virus type 2, cytomegalovirus, rubella and influenza viruses, as well as to *Toxoplasma gondii*, has been associated with an increased risk of developing neuropsychiatric disorders, e.g. schizophrenia or autism (Karlsson, 2003; Libbey *et al*. 2005; Yolken & Torrey, 2008). Moreover, family studies suggest that these disorders share genetic risk factors (Daniels *et al*. 2008; Larsson *et al*. 2005; Lichtenstein *et al*. 2009). Although several susceptibility genes have been proposed, including genes involved in the immune response (Costa e Silva, 2008; Harrison & Weinberger, 2005; Schwab *et al*. 2003; Shirts *et al*. 2007), major risk alleles remain to be identified.

Experimental models support the plausibility that early life infections can give rise to persistent effects on behaviour. Neonatal infections in rodents are used to model human infections during the late 2nd/early 3rd trimester of gestation. Such models employing infections with herpes virus (Crnic & Pizer, 1988), Borna disease virus (Lancaster *et al*. 2007; Pletnikov *et al*. 1999; Rubin *et al*. 1999) or lymphocytic choriomeningitis virus (de la Torre *et al*. 1996; Gold *et al*. 1994; Hotchin & Seegal, 1977) have reported behavioural

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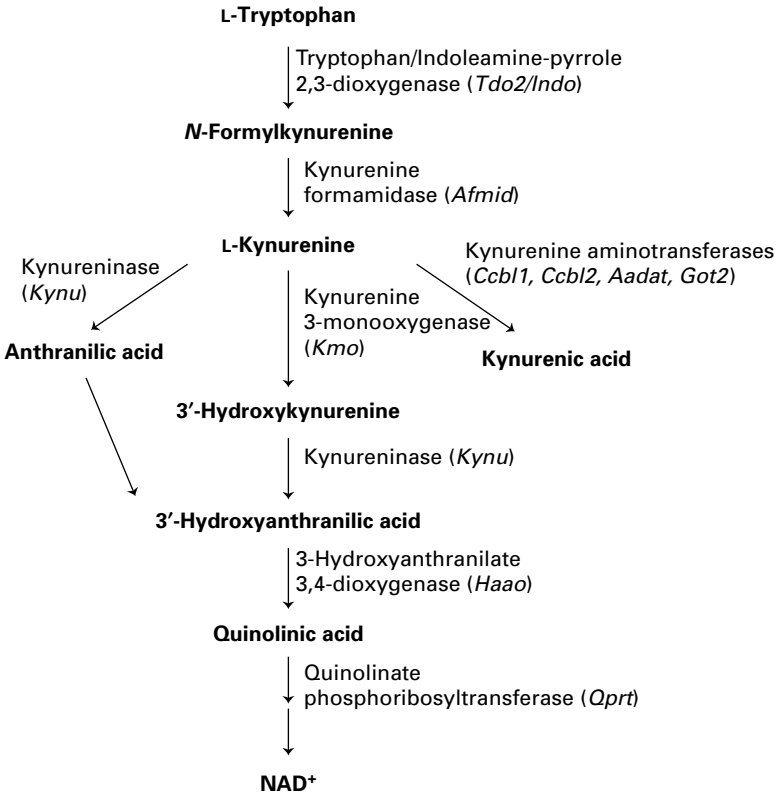

Fig. 1. Schematic diagram of the kynurenine pathway. Mouse gene symbols are given in parentheses.

disturbances related to both cognitive and emotional domains, as well as disrupted sensorimotor gating (Engel *et al*. 2000; Pletnikov *et al*. 2002) in adult animals. Sensorimotor gating disruptions are considered to reflect deficits in the ability to filter out extraneous stimuli that would interfere with attention and information processing (McGhie & Chapman, 1961). Prepulse inhibition (PPI) of a startle response is a cross-species operational measure of sensorimotor gating that refers to the ability of a non-startling 'prestimulus' to inhibit the response to a startling stimulus (Hoffman & Ison, 1980; Swerdlow *et al*. 2001). There are numerous studies reporting PPI deficits in patients with schizophrenia and other neuropsychiatric disorders (Geyer, 2006; Perry *et al*. 2007). Although the mechanism underlying the behavioural changes following the different experimental neonatal infections remains elusive, convergence on common inflammatory pathways has been proposed (Meyer & Feldon, 2008). This notion is supported by reports of altered behaviour related to anxiety, cognition and sensorimotor gating in rodents following experimental immune challenges, e.g. by interleukin (IL)-6, IL-1α, polyI:C or lipopolysaccharide, during fetal or neonatal life (Borrell *et al*. 2002; Ozawa *et al*. 2006; Shi *et al*. 2003; Smith *et al*. 2007; Spencer *et al*. 2005; Tohmi *et al*. 2004; Wolff & Bilkey, 2008).

Tryptophan degradation along the kynurenine pathway (Fig. 1) is induced by a number of infections (Atlas *et al*. 2007; Eastman *et al*. 1994; Fujigaki *et al*. 2002; Heyes *et al*. 1992*a*, *b*; Reinhard, 1998; Schwarcz & Hunter, 2007; Silva *et al*. 2002) and can be considered part of the host's innate immune response (King & Thomas, 2007). Indoleamine-pyrrole 2,3-dioxygenase (IDO) is the first and rate-limiting enzyme of the kynurenine pathway induced by pro-inflammatory cytokines such as interferon (IFN)-γ and IFN-β (Guillemin *et al*. 2001*a*; Takikawa *et al*. 1988). Such induction appears to inhibit viral replication *in vitro* (Adams *et al*. 2004*a*, *b*; Bodaghi *et al*. 1999; Suh *et al*. 2007). We previously reported a transient induction of the kynurenine pathway in the brain of wild-type (WT) (C57BL/6) mice during the acute stages of a neonatal infection with influenza A/WSN/33 virus (Holtze *et al*. 2008). Using this model of neonatal infection in both WT and *Tap1*$^{-/-}$ mice, we recently reported deficits in working memory and increased anxiety in adult *Tap1*$^{-/-}$, but not in adult WT mice (Asp *et al*. 2009). Due to a targeted disruption of the gene encoding 'transporter associated with antigen

processing 1', *Tap1*$^{-/-}$ mice express reduced levels of MHC class I and therefore lack functional CD8+ T cells (Van Kaer *et al*. 1992). Compared to WT mice, the absence of invading CD8+ T cells did not affect virus replication, distribution or clearance in *Tap1*$^{-/-}$ mice. However, a more pronounced innate immune response was observed in *Tap1*$^{-/-}$ mice than in WT mice, as indicated by increased expression of glial markers in the brain parenchyma 21 d after infection only in *Tap1*$^{-/-}$ mice (Asp *et al*. 2009). Based on these latter findings we here hypothesize a more persistent induction of the kynurenine pathway in neonatally influenza A/WSN/33 virus-infected *Tap1*$^{-/-}$ mice, than previously reported in WT mice (Holtze *et al*. 2008). Furthermore, we investigate the potential effects of the neonatal infection on sensorimotor gating in *Tap1*$^{-/-}$ and WT mice at age 5–6 months.

Material and methods

Experimental design

In accordance with our previous studies (Asp *et al*. 2009; Holtze *et al*. 2008), virus was intraperitoneally administered, mimicking a haematogenous spread to a human fetus following maternal infection. Mice were injected with neurotropic mouse-adapted influenza A/WSN/33 virus or phosphate-buffered saline (PBS) on postnatal day (P)3 or P4, i.e. when pups measured 3.5 cm in head–rump length. With regard to brain development, this time-point corresponds to ~25 wk of gestation in humans (i.e. late 2nd/early 3rd trimester; Rice & Barone, 2000). In this study, both WT and *Tap1*$^{-/-}$ mice were included and whole brains, including cerebellum, of the latter group were sampled 4 d (P7), 10 d (P13), and 21 d (P24) after injection in order to determine levels of transcripts encoding enzymes in the kynurenine pathway and kynurenic acid (KYNA). These time-points were selected based on our previous observations on the course of kynurenine pathway induction in WT mice (Holtze *et al*. 2008). At age 5–6 months, sensorimotor gating was determined in male WT and *Tap1*$^{-/-}$ mice by measuring PPI of the startle reflex. Mice were subsequently sacrificed and levels of KYNA were determined in their brains. All comparisons were made within each genotype group.

Animals

WT mice (C57BL/6) were obtained from Scanbur AB, Sweden, and mice with targeted disruption of the gene-encoding transporter associated with antigen processing 1 (*Tap1*$^{-/-}$; background strain C57BL/6), were obtained from the Jackson Laboratory (USA). Mice of both genotypes were bred at the Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden. Mice were kept under standard laboratory conditions with free access to food pellets and tap water. They were housed (2–5 per cage) in standard transparent type III Macrolon® cages (42 × 26 × 20 cm) in a light-controlled room (12-h light/dark cycle, lights on 06:00 hours) at 21 °C and 60% relative humidity. Experiments were conducted in accordance with permissions from the regional ethical committee for animal experimentation (N264/05, and supplements: N166/06, N101/07 and N225/07).

Influenza A/WSN/33 virus infection of mice

Mice were injected intraperitoneally with 2400 plaque-forming units of influenza A/WSN/33 virus obtained from Dr S. Nakajima (The Institute of Public Health, Tokyo, Japan) suspended in 30 μl PBS (Gibco, UK) or PBS alone, at P3 or P4 as previously described (Asp *et al*. 2009; Holtze *et al*. 2008).

Extraction of RNA and reverse transcription

Total RNA was extracted from whole brains using the RNeasy kit (Qiagen GmbH, Germany). One μg of RNA was subsequently DNaseI treated and reverse-transcribed into random hexamer-primed cDNA using reagents from Invitrogen (Carlsbad, USA), as previously described (Asp *et al*. 2007).

Real-time PCR and data analysis

Real-time PCR assays were performed as previously described (Asp *et al*. 2007) using reagents from Invitrogen and Applied Biosystems (Palo Alto, USA). Primers (Invitrogen) and probe (Applied Biosystems) sequences are provided in Table 1. Transcript levels were normalized to those encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH). From these values, relative levels of transcripts in the two groups were calculated according to the formula $2^{-\Delta\Delta Ct}$ (Livak & Schmittgen, 2001).

Analyses of KYNA

Whole brain tissues were homogenized in 0.4 M perchloric acid, 0.1% sodium hydrogen sulfite, and 0.05% EDTA. KYNA levels were measured, using an isocratic reversed-phase high-performance liquid chromatography system connected to a fluorescence detector with an excitation wavelength of 344 nm and an emission wavelength of 398 nm, as previously described (Holtze *et al*. 2008).

Table 1. Transcripts analysed by real-time PCR

Gene symbol	Gene product	Accession no. (positive strand)	Primer sequence, 5′ to 3′
Indo	Indoleamine-pyrrole 2,3-dioxygenase (IDO)	NM_008324	CGGACTGAGAGGACACAGGTTAC ACACATACGCCATGGTGATGTAC
Tdo2	Tryptophan 2,3-dioxygenase (TDO2)	NM_019911	CAGCATCAGGCTTCCAGAGTCT AGGGACTCTCAAGCTCTGAAGAAC
Ccbl1	Kynurenine aminotransferase 1 (KAT1)	NM_172404	GGAGATGGACCCACTCAAGAAT GCCTGAAAGGCTGTGAACAAG
Aadat	Kynurenine aminotransferase 2 (KAT2)	NM_011834	CCAGGAACCCTTTATGCTATGAA TGGAATAATCCCATGCTCATCA
Ccbl2	Kynurenine aminotransferase 3 (KAT3)	NM_173763	AACCCCGGCGACACCTA TGATCGTTCTCCTGGTTCCAA
Got2	Mitochondrial aspartate aminotransferase (mitAAT)	NM_010325	GATGGCCGAATCTCCGTG CCTGGTGAATGGCATGGG
Kmo	Kynurenine 3-monooxygenase (KMO)	NM_133809	CCTGTAGAGGACAATATAGGATCAACAA GCAAGCCCCATCTACTGCAT
Kynu	Kynureninase (KYNU)	NM_027552	GAGACTCGATCGCCGTGATC TGTTATGGCAGGAATGTTGAACA
Haao	3-hydroxyanthranilate 3,4-dioxygenase (HAAO)	NM_025325	GGATGTCCTCTTCGAGAAATGG AACTCTTGGATGATGGGTGCTAA
Qprt	Quinolinate phosphoribosyl-transferase (QPRT)	NM_133686	CACGCTCGCCGGTTCTA GCCCAACAAGCCGCAGTA
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	NM_008084	TGCACCACCAACTGCTTAGC CAGTCTTCTGAGTGGCAGTGATG Probe: TGGAAGGGCTCATGACCACAGTCCA

Behavioural testing

Startle response and PPI testing were performed on WT and *Tap1*$^{-/-}$ mice at age 5–6 months in commercial startle chambers (SR-LAB System, San Diego Instruments, USA) at the Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden. Within each chamber there was a Plexiglas cylinder (3.7 cm diameter) into which the mouse was placed. Sudden movements by the mouse were detected by a piezoelectric accelerometer attached below the cylinder. A loudspeaker provided the broadband background noise and acoustic stimuli, and the whole apparatus was housed within the ventilated, sound-attenuating chamber ($35 \times 33 \times 46$ cm). A standard computer controlled presentations of acoustic stimuli.

The experimental session consisted of a 5-min acclimatization period to a 65-dB background noise (continuous throughout the session), followed by a variable stimulus intensity block (i.e. to measure startle threshold), a variable prepulse intensity block, and a variable interstimulus interval (ISI) block. This session was designed to fully characterize the startle and PPI phenotype associated with neonatal infection similar to what we have reported previously in isolation-reared mice (Varty *et al*. 2006). Throughout the session, hidden NOSTIM trials (i.e. no acoustic stimulus) were presented in between each trial. During the variable stimulus intensity block, five trial types were presented: 40-ms startle pulses of 80, 90, 100, 110 and 120 dB. Each trial was presented four times. The varied prepulse intensity block consisted of four trial types: a 40-ms, 120-dB startle pulse (PULSE ALONE); and three 20-ms prepulse + pulse combinations (69, 73 or 81 dB prepulses followed 100 ms later by a 120 dB stimulus; PREPULSE + PULSE). There were 12 presentations of the PULSE ALONE trial and 10 presentations of each PREPULSE + PULSE combination. During the varied ISI block, six trial types were presented: a 40-ms, 120-dB startle pulse (PULSE ALONE); and five 20-ms prepulse + pulse combinations (73-dB prepulses followed 25, 50, 100, 200, 500 ms later by a P120 stimulus; PREPULSE + PULSE). There were eight presentations of the PULSE ALONE trial, and four presentations of each PREPULSE + PULSE trial. Throughout the session, trial types were presented in a pseudo-random order with an average inter-trial interval (ITI) of 15 s, not including the

hidden NOSTIM trials. In addition, five PULSE ALONE trials were presented at the beginning (Block 1: to assess startle reactivity before appreciable habituation) and the end of the acoustic test session (Block 5). Mean startle magnitude for each trial type presentation, the dependent measure, was determined by averaging 65 1-ms readings taken from the onset of the startle PULSE stimulus.

Statistical analysis

Relative transcript levels (i.e. ΔCt values) and levels of KYNA in *Tap1*$^{-/-}$ mice were compared between virus-infected and uninfected mice using the Mann–Whitney *U* test in GraphPad Prism (GraphPad Software Inc., USA). Startle and PPI data from *Tap1*$^{-/-}$ and WT mice were analysed separately. Startle reactivity data and mean startle magnitudes within the test session were analysed using one-factor ANOVAs with infection status as a between-subjects factor. Percent acoustic PPI from the ISI and prepulse intensity blocks of the test was calculated using the following formula:

$$100 - \frac{\text{average startle of prepulse} + \text{pulse trial}}{\text{average startle in pulse alone trial}} \times 100.$$

Percent acoustic PPI data were analysed using two-factor ANOVAs with prepulse intensity, or ISI, as a within-subject factor, and infection status as the between-subject factor. *Post-hoc* comparisons of means were performed with Tukey's test. Significance was set at $p < 0.05$.

Results

Following systemic influenza A/WSN/33 virus infection in WT and *Tap1*$^{-/-}$ mice, the virus targeted neurons in several different brain regions. Virus replication, distribution or rates of clearance did not differ between the two genotypes. At 6–12 d post-infection a number of mice developed signs of disease, such as reduced weight gain, and were therefore sacrificed and excluded from the study according to our institutional guidelines. The survival rates of virus-infected WT and *Tap1*$^{-/-}$ mice did not differ (~54%), as previously described (Asp *et al*. 2009; Holtze *et al*. 2008).

Gene expression in brains of Tap1$^{-/-}$ *mice*

Levels of transcripts encoding enzymes in the kynurenine pathway were investigated during 3 wk after the infection at P3 or P4. At P7, brains from virus-infected mice expressed elevated levels of transcripts encoding IDO ($p < 0.05$) as well as those encoding 3-hydroxyanthranilate 3,4-dioxygenase (HAAO) ($p < 0.01$) compared to brains from uninfected mice. Transcripts encoding kynurenine aminotransferase (KAT)2 were detected at lower levels in the virus-infected brains compared to uninfected brains ($p < 0.05$). All other transcripts investigated were detected at similar levels in the two groups (Fig. 2*a*).

At P13, levels of transcripts encoding IDO ($p < 0.001$) and those encoding HAAO ($p < 0.001$) remained elevated in virus-infected brains. While levels of transcripts encoding KAT2 remained reduced in virus-infected brains ($p < 0.05$), those encoding KAT3 ($p < 0.001$), kynurenine 3-monooxygenase (KMO) ($p < 0.01$) or kynureninase (KYNU) ($p < 0.001$) were now detected at increased levels in virus-infected brains compared to uninfected brains. All other transcripts investigated were detected at similar levels in the two groups (Fig. 2*b*).

Transcripts found to be differentially expressed at P13 were subsequently analysed at P24. At this time-point, the levels of transcript encoding IDO ($p < 0.01$), KYNU ($p < 0.05$) or HAAO ($p < 0.01$) all remained elevated in the virus-infected brains. Other transcripts analysed were detected at similar levels in the two groups of mice at this time-point (Fig. 2*c*).

KYNA in brains of Tap1$^{-/-}$ *mice at P7, P13 and P24*

In parallel to the gene expression analyses, we determined levels of KYNA; an end product of the kynurenine pathway (Stone, 1993) in the brains of *Tap1*$^{-/-}$ mice. No significant differences in the levels of KYNA were observed between virus-infected and uninfected mice at P7 or P24. However, at P13 levels of KYNA in virus-infected mice (4.6, range 2.3–6.9 nm) exceeded the levels observed in uninfected mice (1.9, range 1.1–3.5 nm, $p < 0.01$; Fig. 3).

Effects of neonatal influenza virus infection on PPI

At age 5–6 months, measurements of PPI were performed on WT and *Tap1*$^{-/-}$ mice following injection with PBS or influenza A/WSN/33 virus at P3 or P4. In the varied ISI block of the session, virus-infected *Tap1*$^{-/-}$ mice displayed impaired PPI as evidenced by a main effect of virus infection [$F(1,26) = 5.67$, $p < 0.05$; Fig. 4*a*] and no interaction between virus infection and ISI. The *post-hoc* test revealed a decrease in PPI in virus-infected mice at the 200-ms ISI ($p < 0.05$). Startle magnitude in the P120 trials in the varied ISI blocks did not differ between the groups [$F(1,26) < 1$, n.s.; Fig. 4*b*]. There was no effect of virus infection [$F(1,26) < 1$, n.s.] or any interaction between

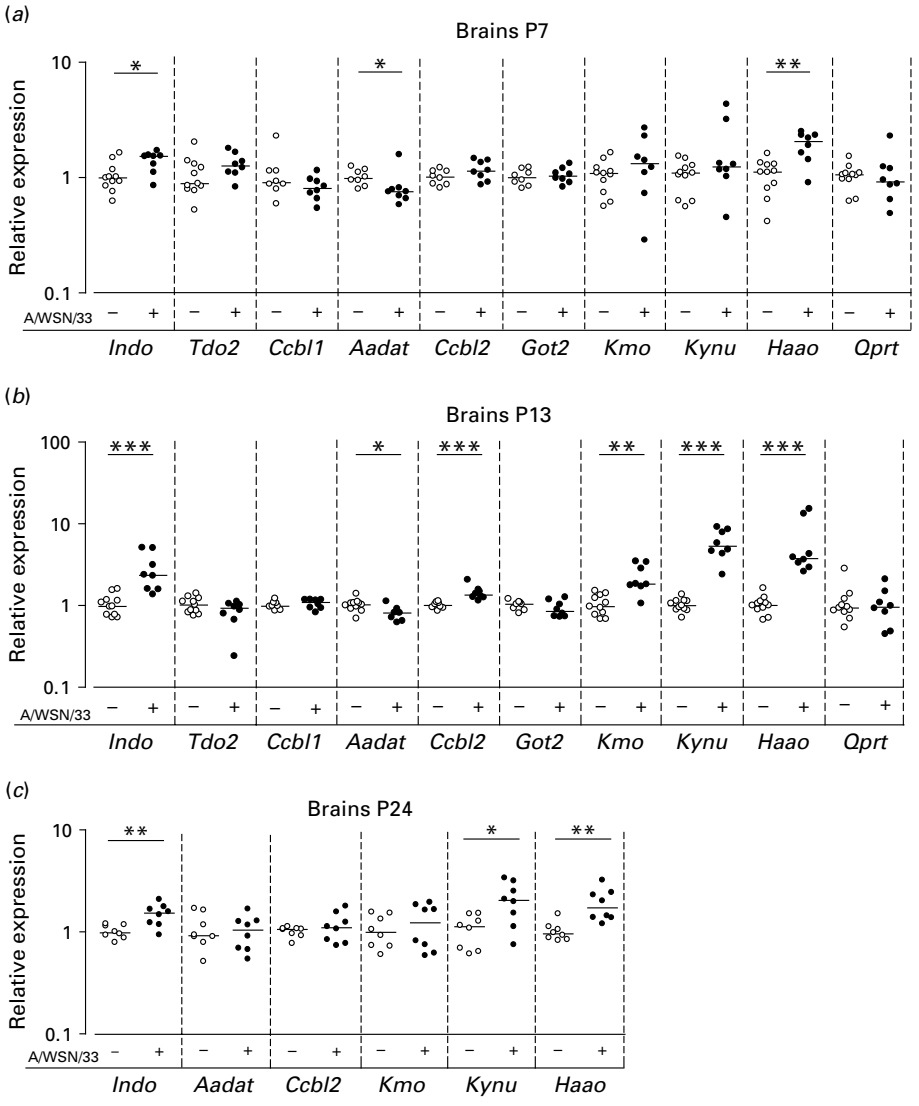

Fig. 2. Levels of transcripts in brains of *Tap1*$^{-/-}$ mice. Symbols (○, ●) represent individual mice. The levels of transcripts from the genes *Indo* (encoding IDO), *Tdo2* (encoding TDO), *Ccbl1* (encoding KAT1), *Aadat* (encoding KAT2), *Ccbl2* (encoding KAT3), *Got2* (encoding mitAAT), *Kmo* (encoding KMO), *Kynu* (encoding KYNU), *Haao* (encoding HAAO), and *Qprt* (encoding QPRT) in brains at (*a*) postnatal day (P)7, (*b*) P13, and (*c*) P24 following intraperitoneal injection with 2400 plaque-forming units of influenza A/WSN/33 virus (+) or phosphate-buffered saline (–) on P3 or P4. The levels of transcripts in virus-infected brains are relative to those observed in uninfected brains. The horizontal lines indicate median values ($n = 8$–11).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Mann–Whitney *U* test.

prepulse intensity and virus infection in the varied prepulse intensity block. In the startle threshold block, designed to measure any differences in startle threshold between uninfected and virus-infected *Tap1*$^{-/-}$ mice, there was a main effect of stimulus intensity [$F(4, 104) = 38.1$, $p < 0.001$] and a trend for higher startle in virus-infected compared to uninfected mice [values are mean (S.E.M.)] [uninfected: 42.3 (3.0); virus-infected: 58.8 (10.4); $F(1, 26) = 3.81$, $p = 0.062$]. However, there was no interaction between acoustic stimulus and virus infection suggesting that the groups did not differ in their threshold for startle response.

In contrast to *Tap1*$^{-/-}$ mice, neonatally infected WT mice did not differ from uninfected mice in PPI in the varied prepulse intensity block ($F < 1$, n.s.) or in the varied ISI block ($F < 1$, n.s.) when tested in adulthood. WT uninfected and virus-infected mice also did not differ in startle magnitude in the startle threshold block ($F < 1$, n.s.).

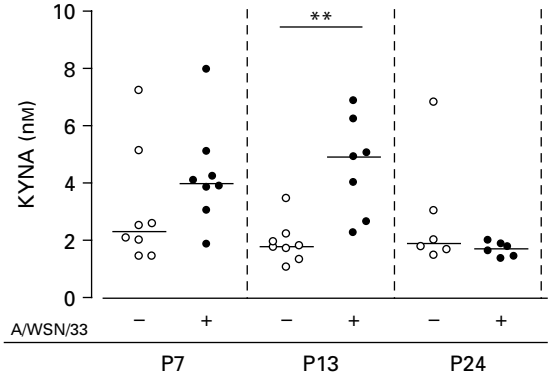

Fig. 3. Levels of kynurenic acid (KYNA) in brains of *Tap1*$^{-/-}$ mice at postnatal days (P)7, P13, P24 following intraperitoneal injection with 2400 plaque-forming units of influenza A/WSN/33 virus (+) or phosphate-buffered saline (–) on P3 or P4. Symbols (○,●) represent individual mice. The horizontal lines indicate median values ($n = 6$–8). ** $p < 0.01$. Mann–Whitney *U* test.

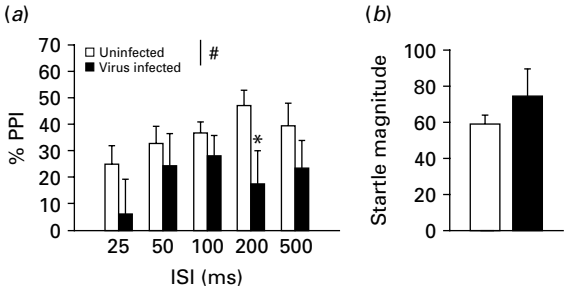

Fig. 4. (*a*) Prepulse inhibition (PPI) and (*b*) startle magnitude in uninfected ($n = 16$) and influenza A/WSN/33 virus-infected ($n = 12$) *Tap1*$^{-/-}$ mice at age 5–6 months, during the varied interstimulus interval (ISI) block of the startle session. # $p < 0.05$, main effect of virus infection on PPI during the varied ISI block (details of statistics in Results section). * $p < 0.05$, statistically different from respective uninfected mice, Tukey's *post-hoc* comparison. Data are presented as mean + S.E.M.

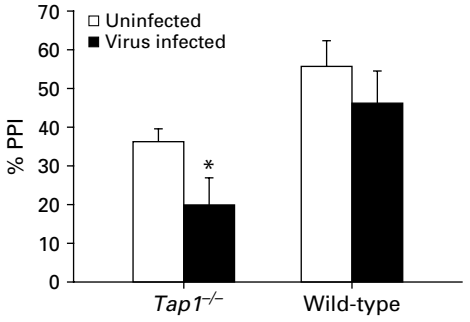

Fig. 5. Comparison of the effects of neonatal influenza in *Tap1*$^{-/-}$ mice (left) and wild-type (WT) mice (right) on average PPI during the ISI block. Data represent the mean (+S.E.M.). * $p < 0.05$, significantly different from respective control. Data were compared across experiments by computing the effect size (Cohen's *d*): *Tap1*$^{-/-}$ mice, $d = 1.23$; WT mice, $d = 0.61$. For details of statistics see Results section.

In order to evaluate the effect of the influenza virus infection on PPI in the two genotypes, we examined the average PPI during the ISI block in the two experiments. In the ISI block, virus-infected WT mice had slightly less average PPI than uninfected WT mice [values are mean (S.E.M.)] [uninfected: 55.6 (6.6); virus-infected: 46.3 (8.1); 19% reduction], but this decrease was much less than that observed in the *Tap1*$^{-/-}$ mice [uninfected: 36.2 (3.3); virus-infected: 19.9 (7.0); 45% reduction]. A comparison of the effect of the neonatal virus infection on PPI across the two experiments yielded a greater effect size (Cohen, 1988) in *Tap1*$^{-/-}$ mice ($d = 1.23$) than in WT mice ($d = 0.61$; Fig. 5).

KYNA in brains of adult mice

After completion of PPI, mice were sacrificed and the brains sampled. In these brains, KYNA was detected at similar levels in virus-infected and uninfected mice of both genotypes (data not shown).

Discussion

In the present study, we report that a neonatal infection with a neurotropic strain of influenza A virus reduced PPI in adult *Tap1*$^{-/-}$ mice. Such reduction in PPI was not observed in adult WT mice following neonatal infection. With regard to changes in gene expression and KYNA levels in brains of virus-infected *Tap1*$^{-/-}$ mice during the early stages of the infection, the present findings are in general agreement with our previously reported findings in WT mice (Holtze *et al*. 2008). However, a notable finding was the consistent elevation of transcripts encoding IDO throughout the time-points investigated.

The changes in gene expression were accompanied by a transient increase in the levels of KYNA in the brain parenchyma at P13. In agreement with a previous study, reporting a close relationship between transcripts encoding IDO and enzyme activity (Fujigaki *et al*. 2002), our findings support functionality of the induction of the kynurenine pathway observed at the gene expression level. In accordance, levels of transcript encoding IDO have been correlated with the production of quinolinic acid (QUIN; Smith

et al. 2001), another metabolite of tryptophan degradation (Stone, 1993). Thus, levels of QUIN and other neuroactive (Stone, 1993) and/or immunomodulatory (Moffett & Namboodiri, 2003) metabolites of the kynurenine pathway can also be altered in the brain parenchyma of infected *Tap1*$^{-/-}$ mice, although measurements of these metabolites were not performed in the present study.

In humans and rats, KAT2 is considered to play a major role in KYNA formation in the brain (Guidetti *et al*. 2007). However, mice lacking KAT2, exhibit reduced cerebral KYNA levels only during the first weeks of life (Yu *et al*. 2004). It has therefore been proposed that mitAAT accounts for most of the KYNA formation in the adult mouse brain (Guidetti *et al*. 2007). During P7–P24, levels of transcripts encoding mitAAT were not altered in virus-infected mice. In contrast, decreased levels of transcripts encoding KAT2 (P7 and P13), and increased levels of transcripts encoding KAT3 (P13) were observed. Thus, the present and previous findings (Holtze *et al*. 2008) suggest that, in addition to the availability of L-kynurenine, transcriptional regulation of genes encoding KAT activity might modulate KYNA formation during an infection.

In our previous study of infected WT mice, no evidence of an activated kynurenine pathway was observed at P24 (Holtze *et al*. 2008). The increased levels of transcripts encoding IDO, KYNU, and HAAO observed in the present study at this time-point, suggest a more persistent induction of the kynurenine pathway in the brains of *Tap1*$^{-/-}$ mice. Although the cellular origins of these transcripts were not further investigated, IDO, KYNU, and HAAO can all be expressed by resident cells in the brain parenchyma, as well as by invading cells (Alberati-Giani *et al*. 1996; Guillemin *et al*. 2001*b*, 2003, 2007; Holtze *et al*. 2008). These observations thus provide further support for a more pronounced innate immune response in the brains of *Tap1*$^{-/-}$ mice than in WT mice following a neonatal virus infection (Asp *et al*. 2009). This difference in innate immune response between the two genotypes may be an effect of the absence of functional CD8+ T cells in *Tap1*$^{-/-}$ mice. These and CD4+ T cells were recently reported to be critically important in down-regulating the systemic innate immune response in young mice (Zhao *et al*. 2008).

The kynurenine pathway has previously been implicated in PPI. For example, acute pharmacological elevation of the *N*-methyl D-aspartate (NMDA) receptor antagonist KYNA in the brain disrupts PPI, and is suggested to be an endogenous modulator of sensorimotor gating (Erhardt *et al*. 2004). However, it should be noted that levels of KYNA were not elevated in the brain of infected *Tap1*$^{-/-}$ mice at the time of PPI testing in the present study. The persistent effects of other neonatally administered NMDA receptor antagonists, e.g. phencyclidine or MK-801, on sensorimotor gating in rats (Harris *et al*. 2003; Wang *et al*. 2001) indicate that the transient elevation of KYNA contributed to the deficits in PPI observed in adulthood. The transient increase in KYNA concentration in *Tap1*$^{-/-}$ mice was similar to that reported previously in WT mice (Holtze *et al*. 2008). However, it is possible that *Tap1*$^{-/-}$ mice are more vulnerable than WT mice to perturbed synaptic functions due to their reduced expression of MHC class I molecules, which are suggested to be of importance for synaptic plasticity and regeneration (Goddard *et al*. 2007; Oliveira *et al*. 2004).

In conclusion, the present results show that a neonatal CNS infection can cause long-term deficits in sensorimotor gating in genetically vulnerable individuals. Although innate immunity appears to be an important mediator of these effects, a specific role for the kynurenine pathway in the establishment of PPI deficits remains to be determined.

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Statement of Interest

None.

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