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Neonatal hepatitis B vaccination impaired the behavior and neurogenesis of mice transiently in early adulthood

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ABSTRACT

The immune system plays a vital role in brain development. The hepatitis B vaccine (HBV) is administered to more than 70% of neonates worldwide. Whether this neonatal vaccination affects brain development is unknown. Newborn C57BL/6 mice were injected intraperitoneally with HBV or phosphate-buffered saline. HBV induced impaired behavioral performances and hippocampal long-term potentiation at 8 weeks (w) of age without influence at 4 or 12 w. At 6 w, there was decreased neurogenesis, M1 microglial activation and a neurotoxic profile of neuroimmune molecule expression [increased tumor necrosis factor-α and reduced interferon (IFN)-γ, brain-derived neurotrophic factor and insulin-like growth factor-1] in the hippocampus of the HBV-vaccinated mice. In the serum, HBV induced significantly higher levels of interleukin (IL)-4, indicating a T helper (Th)-2 bias. Moreover, the serum IFN-γ/IL-4 ratio was positively correlated with the levels of neurotrophins and neurogenesis in the hippocampus at the individual level. These findings suggest that neonatal HBV vaccination of mice results in neurobehavioral impairments in early adulthood by inducing a proinflammatory and low neurotrophic milieu in the hippocampus, which follows the HBV-induced systemic Th2 bias.

1. Introduction

Hepatitis B vaccine (HBV) is enrolled in the global routine childhood vaccination schedule and is administered to more than 70% of neonates worldwide (Centers for Disease and Prevention, 2013). Perinatal immune activation has been demonstrated to influence brain development and behavior (French et al., 2013). The brain is still developing in the early postnatal time period and thus immune activation can impact the developmental programming of the brain (Bilbo and Schwarz, 2009, 2012; Xia et al., 2014a). Several animal models exhibited detrimental impacts of early postnatal immune activation, such as increased anxiety or vulnerability to later life cognition impairment (Bilbo et al., 2005; Spencer et al., 2005; Walker et al., 2004). Additionally, rats experiencing early life bacterial infections were more resistant to stressor-induced adult depression (Bilbo et al., 2008). Therefore, early vaccination with HBV, which induces strong immune activation, is suspected to influence brain development and behavior.

The T helper (Th)1/Th2 balance serves as an important mediator for the effects of immune activation on the central nervous system (CNS). Our recent studies showed that maternal influenza vaccination increased the levels of hippocampal neurogenesis and neurotrophin expression in both generations and that these neurobenefits were positively associated with a systemic Th1 bias (Xia et al., 2014a; Xia et al., 2014b). Moreover, Bacillus Calmette-Guerin (BCG) exerted a neuroprotective effect in several CNS disease conditions and improved brain development under normal conditions in rodents (Bourdette and Naismith, 2014; Laćan et al., 2013; Lee et al., 2008; Li et al., 2015a,b; Yang et al., 2016a; Yong et al., 2011). Conversely, a Th2 bias is regarded as neurodetrimental. A Th2 bias has been reported to be associated with cognitive deficits (Baruch et al., 2013; He et al., 2014; Palumbo et al., 2012) that are restored when the Th2 bias is reversed (Palumbo et al., 2012). This phenomenon was verified by our study, which demonstrated that neonatal HBV vaccination impaired the hippocampal synaptic plasticity of rats at early adulthood (Li et al., 2015a). However, whether neonatal HBV vaccination, which is a Th2 inducer, influences behavior and neu-

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rogenesis is not clear. We conducted the present study to address this question.

2. Material and methods

2.1. Animals and study design

This study was approved by the Sun Yat-Sen University (SYSU) Institute Research Ethics Committee and performed in strict accordance with the U.K. Animals (Scientific Procedures) Act, 1986. Litters of newborn C57BL/6 mice purchased from the SYSU Laboratory Animal Center (Guangzhou, China) were housed under specific pathogen-free conditions. The pups were weaned on postnatal day 21 (P21). As shown in Table S4 that provided with the study design, timeline and precise sample size of the main body of the study, a series of tests were performed in this study (Table S4). Each type of test consisted of two groups of mice: the immunized group (such as HBV-group) and the control (CON)-group. For the open field test (OFT), six litters of newborn C57BL/6 mice were divided into the two groups (HBV-group and CON-group). One male pup and one female pup selected from each litter were included in the HBV-group, and another male pup and another female pup selected from each litter were included in the CON-group. Thus, a total of 24 neonates were chosen and divided into the two groups (12 pups/group) with matched backgrounds and genders. The pairs of groups were set similarly for the other tests.

2.2. Immunization procedures

HBV was administered in a three-dose series (Mast et al., 1999). The time points for immunization of newborn mice imitated those for human infants, with P0-P7-P21 imitating P0-P30-P180. Although it is difficult to make the chosen ages of mice strictly correspond to those of humans, P0-P7-P21 was verified to be appropriate in a preliminary study (design: Table S1; results: Table S2 and Fig. S1). Mice in the HBV-group were injected intraperitoneally on P0 with a total volume of 50 μl/mouse of HBV (yeast-derived, Kangtai Biological Pharmaceutical Company, China) containing 1 μg of hepatitis B virus surface antigen (HBsAg) and 31 μg of Al^{3+}. We initially referred to previous studies (Weeratna et al., 2001) to select the HBV dosages that was approximately 1/5 of the dosage used in human infants in China. However, the specific dosage was eventually determined from the results of the preliminary study. The HBV-mice received two identical doses of HBV as the booster inoculations on P7 and P21. The control groups matching each immunized group were injected with phosphate-buffered saline (PBS) in the corresponding procedures.

2.3. Behavior tests

2.3.1. Selection of the time points for the behavior and electrophysiology tests

The ethological tests were performed at three ages: four, eight and twelve weeks (w). These age points span the vital development period when mice grow from juveniles into adults.

2.3.2. OFT

The OFT tasks were performed as described previously (Chandran et al., 2008). Each mouse was placed in a 40 cm × 40 cm × 38 cm Plexiglas cubicle. Locomotor activities, rearing, distances travelled and the time proportion spent in the center zone by each mouse during a 30 min task were calculated using the Flex-Field activity system (San Diego Instruments, CA, USA). After each trial, the apparatus was cleaned thoroughly with 70% ethanol.

2.3.3. Elevated plus maze (EPM)

Each mouse was allowed to explore the EPM apparatus freely for 5 min after being placed in the center facing a constant open arm. Their behavior was recorded with a video tracking system (Noldus EthoVision XT, the Netherlands). The time spent in and the numbers of entries into the open arms and the number of times the mice reached the ends of the open arms were analyzed. The EPM apparatus was cleaned as described for the OFT apparatus.

2.3.4. Morris water maze (MWM)

The MWM task was performed as previously described (Yang et al., 2016a). There is a circular pool with 100 cm in diameter and 60 cm in height in the apparatus. The pool was filled with water with a depth of 30 cm and with a temperature at (22 ± 1)°C, which was colored with non-toxic white dye. Four imaginary quadrants were set in the pool and a white circular platform of 9 cm in diameter and 29 cm in height was placed in a constant quadrant. An MT-200 Morris image motion system (Chengdu Technology & Market Corp., China) was used to record the latency (time spent by animals) and the distance covered by each to find the platform. Briefly, each animal was subjected to four successive trials a day for 4 consecutive days. In each trial, the mouse was given a ceiling time of 60 s to find the hidden platform. On the 5th day, the mouse was given a single probe trial including a 60 s free swim with the platform removed. Mice were dried with a towel and blown by a warming fan for 20 min after every trial.

2.4. Electrophysiology

The mice were anaesthetized with urethane (1.5–1.7 g/kg) (Scott et al., 2012). The skull co-ordinates of the mice used for the in vivo long-term potentiation (LTP) tests were the same as those described by Kameda et al. A unipolar-recording electrode was located 2 mm posterior to the bregma, 1.4 mm lateral to the midline and deepened into the hilus of the DG; a bipolar-stimulating electrode was located ipsilaterally 2.5 mm lateral to lambda and deepened by 1.5 mm into the perforant pathway (Kameda et al., 2012). After setting the stimulation strength to produce half of the maximal population spike (PS) amplitude, we began recording for a 20 min baseline period followed by a 60 min post-LTP period. LTP was induced using a 400 Hz high-frequency stimulation (HFS) protocol consisting of six trains of six pulses (pulse width 50 μs, inter-train interval 100 ms) repeated six times (15 s between repetitions) (Kameda et al., 2012). To describe the LTP, the data were averaged into two-minute bins and normalized to the average excitatory postsynaptic potential (fEPSP) slope in the baseline period. The magnitude of the LTP was calculated between 58 and 60 min in the last bin of the recording session and was expressed as the percentage change from the fEPSP slope baseline. Values from the final 2 min bin were compared within each pair of groups using Student's *t*-test.

2.5. Selection of the time point for the cellular and molecular tests

Cellular and molecular mechanisms underlying behavior, including neurogenesis, microglial activation and neurochemicals, should be affected by systemic immune activation theoretically earlier than behavior. Therefore, neurogenesis, microglial activation and certain neuroimmune molecules were examined at 6 w since significant behavior effects were found at 8 w. In terms of neurogenesis, the test time point, that is 2 w earlier than the age when behavior was significantly influenced, is consistent with the timeline for the maturing of newborn neurons in the hippocampus (Zhao et al., 2008).

2.6. Immunofluorescence and cell quantification

2.6.1. 5-bromo-2-deoxyuridine (BrdU) labeling and tissue preparation

At 6 w of age, the mice were injected with BrdU (Sigma-Aldrich, 50 mg/kg body weight, dissolved in 0.9% NaCl and filtered, intraperitoneally) twice a day to label dividing cells. The animals were deeply anaesthetized and transcardially perfused with 4% paraformaldehyde (PFA) at different time points (2, 7 and 21 days after the first injection). Their brains were excised two days after the first injection and prepared for the examination of $BrdU^+$ cells, 7 days after the first injection for $BrdU^+$/doublecortin $(Dcx)^+$ cells and 21 days after the first injection for $BrdU^+$/neuronal nuclei $(NeuN)^+$ cells. After excision, the brains were immediately fixed overnight in 4% PFA at 4 °C and dehydrated with 10% sucrose followed by 20% and 30% sucrose for 24 h each at 4 °C. Serial coronal sections (40 μm) obtained using a freezing microtome (Leica SM2000R) were stored in PBS at 4 °C prior to immunostaining.

2.6.2. Immunofluorescence staining and cell quantification

Free-floating sections were washed in 0.1 m PBS and then incubated in 2 N HCl for 30 min at 37 °C. Then the sections were blocked in PBS containing 1% bovine serum albumin, and 0.25% Triton X-100 (Sigma-Aldrich) for 1 h. For single labeling, the specimens were then incubated overnight at 4 °C with either rat anti-BrdU (1:500; Oxford Biotechnology) or rabbit anti-Iba-1 (1:1000; Wako Chemicals). For the double labeling, the sections were stained with specified combinations of primary antibodies as follow: rat anti-BrdU (1:500; Oxford Biotechnology)/goat anti-DCX (1:100; Santa Cruz Biotechnology), or rat anti-BrdU (1:500; Oxford Biotechnology)/mouse anti-NeuN (1:1000; sigma) or rabbit anti-Iba-1 (1:1000; Wako Chemicals)/rat anti-MHC-2 (1:100; IQ Products). Specimens were washed three times before incubated at 37 °C for 2 h with secondary antibodies, including Alexa594-conjugated donkey anti-rat, Alexa Fluor 488-conjugated goat anti-rabbit, Alexa594-conjugated donkey anti-rat/Alexa488-conjugated donkey anti-goat or Alexa594-conjugated donkey anti-rat/Alexa488-conjugated goat anti-mouse, Alexa488-conjugated donkey anti-rat/Alexa Fluor 555-conjugated goat anti-rabbit. All these secondary antibodies (Invitrogen) were diluted to 1:400.

The labeled cells were quantified as previously described (Hong et al., 2007). The unilateral DG of each animal were estimated using a stereology system, Stereo Investigator (MicroBrightField, Williston, USA). For each DG, a total of six coronal sections equidistantly spanning the entire rostrocaudal extent of the DG were used. After measuring the actual section thickness, the appropriate guard zones both at the top and the bottom of each of the sections were defined to avoid oversampling. Counting frames of $15 \times 15 \times 20$ μm were set in a 40×40 μm matrix (for estimating the $BrdU^+$ and $BrdU^+/Dcx^+$ cells) that were randomly superimposed onto the regions of interest. The coefficient of error in all estimation were less than 0.10. The numbers of $BrdU^+/NeuN^+$ and Iba-1^+ cells in all six selected sections were straightly quantified, without using a grid or counting frames. All the four types of cells, mentioned above were quantified in the granular cell layer and the hilus of the DG. Zeiss LSM 780 confocal laser-scanning microscope was used to capture the representative confocal micrographs of labeled cells.

2.7. Molecular tests

2.7.1. Multiplex assay

The mouse cytokine/chemokine panel (MCYTOMAG-70K-04; Millipore, Billerica, MA, USA) and bead-based Luminex system were used to detect the concentrations of interferon (IFN)-γ, interleukin (IL)-4, TNF-α and IL-1β in the sera and hippocampi. Hippocampus homogenates were also assayed to determine the concentration of brain-derived neurotrophic factor (BDNF) and insulin-like growth factor (IGF)-1 using the MPTMAG-49K-01 and the RMIGF187K kits, respectively (Millipore, Billerica, MA, USA). The serum samples were diluted 1:2 with assay buffer. The hippocampi were homogenized in radioimmunoprecipitation assay buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, protease inhibitor cocktail and phosphatase inhibitor cocktail I and II; Sigma) according to the manufacturer's instructions (Milliplex MAP kit, Millipore). The total protein concentration of each sample was adjusted to 4.5 mg/ml using the BCA protein assay kit (Biotime). Then the prepared serum and hippocampi samples were used for the multiplex assays, strictly according to the manufacturer's protocols. The assays were run in triplicate to confirm the results. The data were collected on a Bio-Plex-200 system (Bio-Rad, Hercules, CA, USA) and analyzed using a professional software (Bio-Plex Manager).

2.7.2. RT-PCR analyses

After the total RNA was isolated using TRIzol reagent (Sangon Biotech, Shanghai, China), the mRNAs (2 μg) were converted to cDNAs by means of a GoScript TM cDNA Reverse Transcription Kit (Promega, Madison, USA). The expression of several interested mRNAs was assayed using real-time quantitative PCR (RT-PCR). The quantitative PCR reactions were conducted in triplicate for each sample using the TransStart Tip Green qPCR SuperMix (TransGen Biotech, Beijing, China). The reference gene was β-actin according to its stability in the hippocampus. The amplification cycles were set as 94 °C for 5 s and 60 °C for 30 s. A melting curve was constructed to evaluate the specificity of the reaction. All quantitative real-time PCR reactions were determined and analyzed using a Bio-Rad IQ5 Real-Time PCR System with the comparative Ct method. The primers used in this study were: IL-6, Forward 5′-TCTGGGAAATCGGGAAATGAG and Reverse 5′-TCTCTGAAGGACTCTGGCTTTGTC; IL-1β, Forward 5′-TGTCTTTCCCGTGGACCTTC and Reverse 5′-CTAATGGGAACGTCACACACC; Ym1, Forward 5′-GGCATACCTTTATCCTGAG and Reverse 5′CCACTGAAGTCATCCATGTC; ARG, Forward 5′-AGCCAATGAAGAGCTGGCTGGT and Reverse 5′AACTGCCAGACTGTGGTCTCCA; and β-action, Forward 5′-GGTACCACCATGTACCCAGG and Reverse 5′-ACATCTGCTGGAAGGTGGAC.

2.8. Corticosterone measurements

At 6 w, serum was prepared and was assayed for corticosterone levels using ELISA kits (from EIAab Science Co, Wuhan, China) according to the manufacturer's specifications.

2.9. Statistical analyses

The data were statistically analyzed using the Statistical Package for the Social Sciences 17.0. Because no significant differences were found between genders in the immune responses to HBV (Tables S2–S3), gender has been included as a factor only in the analyses for data from behavior, LTP and neurogenesis experiments in the formal study that were not immune indices (Supplementary material 2). The results (Supplementary material 2) show that there is not significant effects of gender on any indices in all the experiments. Therefore, gender was not been included in the analyses that were described and shown in the Results and Figures of the main text. Data from the place navigation task (part of the data from the MWM test) were analyzed using two-way (group × time) repeated measures analysis of variance (RM-ANOVA) followed by a post-hoc test (Bonferroni). The remaining data were analyzed using Student's *t*-test or a nonparametric test for comparisons between

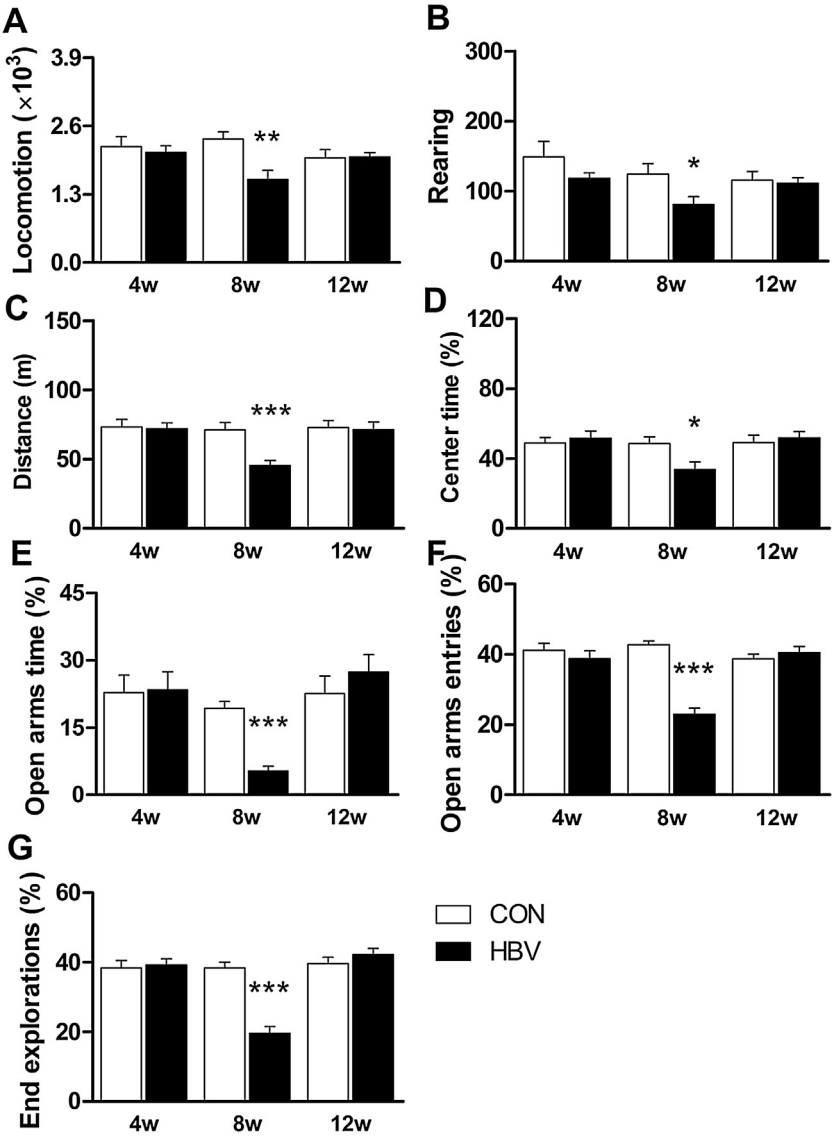

Fig. 1. The HBV-group showed increased anxiety-like behavior at 8 w. Data represent the average numbers of locomotion activities (A), rearing activities (B), distances (C) and center time proportion (D) of each group in the OFT. Data represent the average time proportion spent in the open arms (E), the proportion of the numbers of entries into the open arms in that into both open and closed arms (F) and the proportion of open arm end explorations in the total end explorations (arriving at both open arm ends and closed arm ends). (G) for each group of mice. Data represent the mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; $n = 9$–10 mice/group; Student's *t*-test or Mann-Whitney *U* test. The experiment was repeated three times with similar results and a representative experiment is shown.

groups. Correlations between the serum IFN-γ/IL-4 ratio and the levels of neurogenesis and neurotrophin expression were assessed by correlation analysis (Pearson). The significance level was set to $\alpha = 0.05$.

3. Results

3.1. HBV decreased locomotor activity and increased the anxiety of mice at 8 w

In the OFT, the HBV-group showed a significant decrease in locomotion (*t*-test, $t = -3.513$, $df = 18$, $p = 0.002$), rearing (*t*-test, $t = -2.276$, $df = 18$, $p = 0.035$), distance (*t*-test, $t = -4.078$, $df = 18$, $p < 0.001$) and center time proportion (*t*-test, $t = -2.504$, $df = 18$, $p = 0.022$) compared to the matched CON-group at 8 w but no significant alterations were detected at 4 or 12 w (Fig. 1A–D).

In the EPM, the HBV-group spent less time in the open arms (*t*-test, $t = -4.104$, $df = 17$, $p < 0.001$) and had less open arms entries (*t*-test, $t = -9.554$, $p < 0.001$) and end explorations (arriving at the ends of the open arms) (*t*-test, $t = -7.444$, $df = 17$, $p < 0.001$) than the CON-group at 8 w but had no significant alterations at 4 or 12 w (Fig. 1E–G).

3.2. HBV impaired spatial cognition and hippocampal LTP of mice at 8 w

The HBV-group showed a longer mean escape latency (RM-ANOVA; group: $F_{1,18} = 6.44$, $p = 0.021$; time: $F_{3,54} = 22.32$, $p < 0.001$; group × time: $F_{3,54} = 0.98$, $p = 0.409$) and a longer mean swimming path (RM-ANOVA; group: $F_{1,18} = 6.11$, $p = 0.024$; time: $F_{3,54} = 27.47$, $p < 0.001$; group × time: $F_{3,54} = 0.84$, $p = 0.477$) without different velocity (RM-ANOVA; group: $F_{1,18} = 0.863$, $p = 0.367$; time: $F_{3,54} = 1.855$, $p = 0.150$; group × time: $F_{3,54} = 0.367$, $p = 0.777$) and had less platform area crossings (In the probe test, the numbers of crossing the area where the platform had been put during the first four days) (*t*-test, $t = -2.226$, $df = 18$, $p = 0.039$) and less time spent in the target quadrant (*t*-test, $t = -2.489$, $df = 18$, $p = 0.023$)

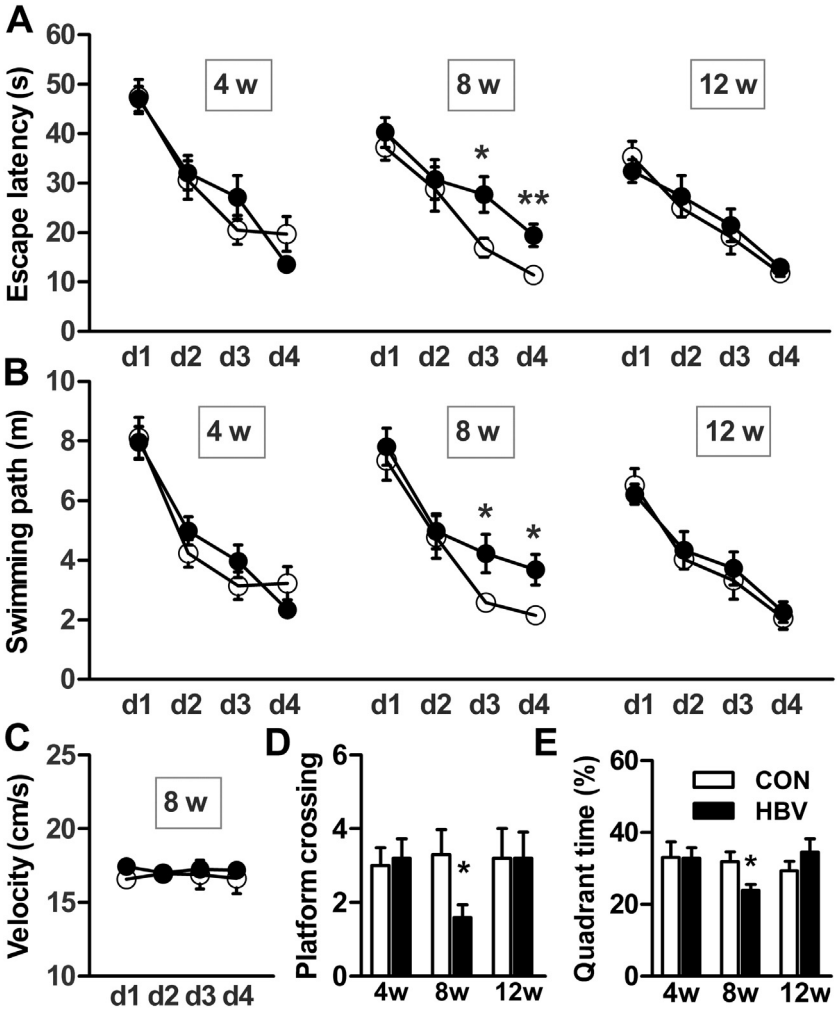

Fig. 2. The HBV-group showed impaired spatial cognition at 8 w. (A-B) Learning-memory lines show the average latency (A) or average swimming path (B) to reach the platform. (C) Data show the average swimming velocity of the 8 w mice. Two-way RM-ANOVA followed by the Bonferroni post-hoc test; $^{*}p < 0.05$, $^{**}p < 0.01$: significant post-hoc differences. (D) Data show the average numbers of platform area crossings. (E) Data show the average time proportion spent in the target quadrant by each group of mice. Student's *t*-test or Mann-Whitney *U* test; $^{*}p < 0.05$: significant differences. Data represent the mean ± SEM of 10 animals per group. The experiment was repeated three times with similar results and a representative experiment is shown.

than the controls at 8 w but had no significant alterations at 4 or 12 w (Fig. 2).

The hippocampal LTP is involved in cognitive processes in the MWM task (Bannerman et al., 1995; Femenia et al., 2012); thus, we recorded the hippocampal LTP of mice at 8 w when learning and memory were significantly impaired. HBV inhibited the induction of LTP (*t*-test, $t = -2.410$, $df = 18$, $p = 0.027$) (Fig. 3) compared with the controls.

3.3. HBV impaired hippocampal neurogenesis

Because behavior alterations were significant at 8 w, neurogenesis and several neuroimmune molecules were examined at 6 w, which is two weeks earlier than described previously (Zhao et al., 2008). The neurogenesis of mice was examined in the dentate gyrus (DG) at 6 w after the mice were administered BrdU to label the proliferating cells. There were significantly smaller numbers of proliferating cells ($BrdU^+$) (*t*-test, $t = -2.553$, $df = 10$, $p = 0.029$), new progenitors ($BrdU^+/Dcx^+$) (*t*-test, $t = -2.418$, $df = 10$, $p = 0.036$) and new neurons ($BrdU^+/NeuN^+$) (*t*-test, $t = -2.488$, $df = 10$, $p = 0.032$) in the DG of the HBV-group than in the controls (Fig. 4).

3.4. HBV induced M1 microglial activation without altering the microglia numbers in the hippocampi

Microglia are important immune cells that mediate the influences of peripheral immune signals on the CNS (Schwartz and Shechter, 2010). Therefore, we examined whether neonatal HBV vaccination altered their numbers and phenotypes in the hippocampi. The HBV-mice showed significantly increased hippocampal mRNA levels of IL-1β (*t*-test, $t = 6.579$, $p < 0.001$) and IL-6 (*t*-test, $t = 8.046$, $p < 0.001$) (M1-type genes) compared to the controls (Fig. 5A). Additionally, a few microglia ($Iba\text{-}1^+$ cells) in the HBV-mice co-expressed MHC-2 (Fig. 5B–F), which is one marker for proinflammatory microglial activation (Shi et al., 2011). However, the microglial numbers were not significantly different between the two groups (*t*-test, $p > 0.05$) (Fig. 5G–I).

3.5. HBV induced a neurodetrimental hippocampal neuroimmune molecular profile and a serum th2 bias

In the hippocampus, the HBV-group showed significantly lower IFN-γ, BDNF and IGF-1 and higher TNF-α levels at 6 w (*t*-test, $p < 0.05$) (Fig. 6A, B). Tests for serum cytokine levels showed that

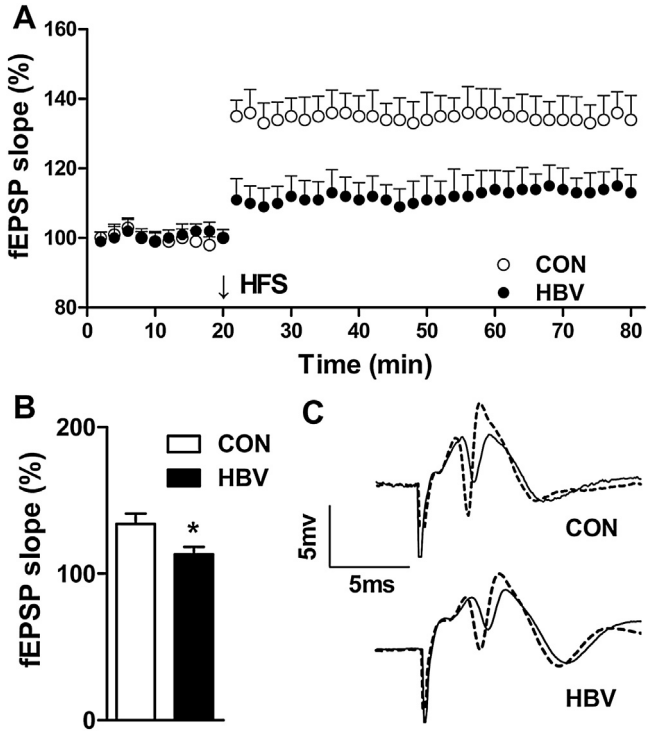

Fig. 3. The HBV-group showed impaired hippocampal LTP at 8 w. (A, B) Data in (B) are expressed as the percent change from the fEPSP slope baseline of the last 2 min bin in (A). Traces in (C) represent the typical PS recorded before (full line) and after (dotted line) HFS. Data represent the mean ± SEM. $^*p < 0.05$; $n = 10$/group; Student's *t*-test. The experiment was repeated twice with similar results and a representative experiment is shown.

HBV induced a Th2 bias (lower IFN-γ/IL-4 ratio and less TNF-α) (*t*-test or nonparametric test, $p < 0.05$) (Fig. 6C, D).

3.6. The serum IFN-γ/IL-4 ratio positively correlated to the levels of the hippocampal neurotrophins and neurogenesis at the individual level

A total of 30 neonatal mice were vaccinated with HBV. At 6 w, the levels of the hippocampal neurotrophins and neurogenesis and the serum IFN-γ/IL-4 ratio were examined. Positive correlations were found between the serum IFN-γ/IL-4 ratio and the hippocampal BDNF levels (Pearson's correlation coefficient, $r = 0.419$, $p < 0.05$) and the hippocampal IGF-1 levels (Pearson's correlation coefficient, $r = 0.467$, $p < 0.01$) (Fig. 7A, B). Positive correlations were also found between the serum IFN-γ/IL-4 ratio and hippocampal neurogenesis (proliferating cells: Pearson's correlation coefficient, $r = 0.467$, $p < 0.01$; new progenitors: Pearson's correlation coefficient, $r = 0.397$, $p < 0.05$) (Fig. 7C, D).

3.7. Immunization of hen egg lysozyme (HEL) with alum induced effects on Th bias and behavior similar to HBV's effects

The HBV that we used contained HBsAg and an aluminum adjuvant. Immunization with a protein antigen with an aluminum adjuvant, such as the HEL in alum, primarily drives the Th2 response (Yip et al., 1999). Therefore, we investigated whether immunization with HEL/alum had effects similar to those observed for HBV using a CON-group and a HEL-group (details in the Supplementary material). In agreement with the HBV results, neonatal HEL/alum injection decreased the serum IFN-γ/IL-4 ratio at 6 w, impaired spatial cognition and increased anxiety-related behavior at 8 w (Table S5).

3.8. No effects of aluminum hydroxide (ALH) alone on Th bias and neurobehavior

The ALH contained in HBV may impair neurobehavior (Dórea and Marques, 2010). Therefore, a CON-group and an ALH-group were set (details in the Supplementary material). No significant alterations in the serum IFN-γ/IL-4 ratio, spatial cognition and anxiety-related behaviors were detected between the two groups (Table S6).

3.9. No alteration in the activity of the hypothalamic–pituitary–adrenal (HPA) axis in HBV-mice

Activity of the HPA axis is another important aspect involved in the complicated pathophysiology of behavior (Idayu et al., 2011; Pariante and Lightman, 2008). Serum level of corticosterone is the most observed hallmark of activation of the HPA axis in rodents (Antidepressant-like effects of mixture of honokiol and magnolol from the barks of Magnolia officinalis in stressed rodents). To determine whether administration of HBV affect the production of glucocorticoids, we measured serum corticosterone levels of animals three days after immunization procedure. There is no significant difference in the level of corticosterone between HBV-mice and CON-mice (Fig. S2, $t = 0.843$, $p = 0.843$, $n = 6$).

3.10. HBV also induced a serum th2 bias and reduced mood and cognition-related behavior in mice reared in a conventional animal room

Human neonates and wild mice are subjected to the normal immune stimuli. These multiple immune stimuli may have complex influences on immune system as well as the CNS and therefore might modify the neurobehavior effects of the neonatal HBV vaccination. To address this issue, another experiment were conducted where CON-mice and HBV-mice were housed in a conventional animal room (section 4 of the Supplementary materials and Methods). This experiment showed similar effects to that housed in SPF condition (Table S8). To be specific, HBV also induced a serum Th2 bias and reduced mood and cognition-related behavior in mice reared in a conventional animal room.

4. Discussion

This study demonstrates that early HBV vaccination impairs the behavior, hippocampal LTP and neurogenesis of mice in early adulthood, which has not been previously reported. One possible mechanism underlying these effects is the alterations in the brain neuroimmune milieu following the systemic Th2 bias.

Cytokines and neurotrophins can mediate the effects of peripheral immune activation on the CNS. (Fidel et al., 1994; Shi et al., 2003; Urakubo et al., 2001; Xia et al., 2014a). Under physiological conditions IFN-γ exerts a neurobenefitial role, including elevating levels of neurogenesis (Baron et al., 2008) and inducing neuronal differentiation (Butovsky et al., 2006), whereas TNF-α has a detrimental effect on many neurobehavior processes (Brennan et al., 2004; Gerber et al., 2004). In this study, we found that there were reduced levels of IFN-γ and increased levels of TNF-α in the hippocampi of HBV-mice than that in the CON-mice. These findings suggest that HBV induced a profile of cytokine expression in the hippocampus, which may be neurodetrimental. Moreover, it was also found that HBV reduced the hippocampal levels of BDNF and IGF-1. BDNF and IGF-1 are neurotrophic and the decreased expression of them could result in impairments in cellular and behavior activities and functions of the CNS (Hoshaw et al., 2005). Therefore, such alterations in the expression of these cytokines and neurotrophins may be, at least part of, the mechanism by which

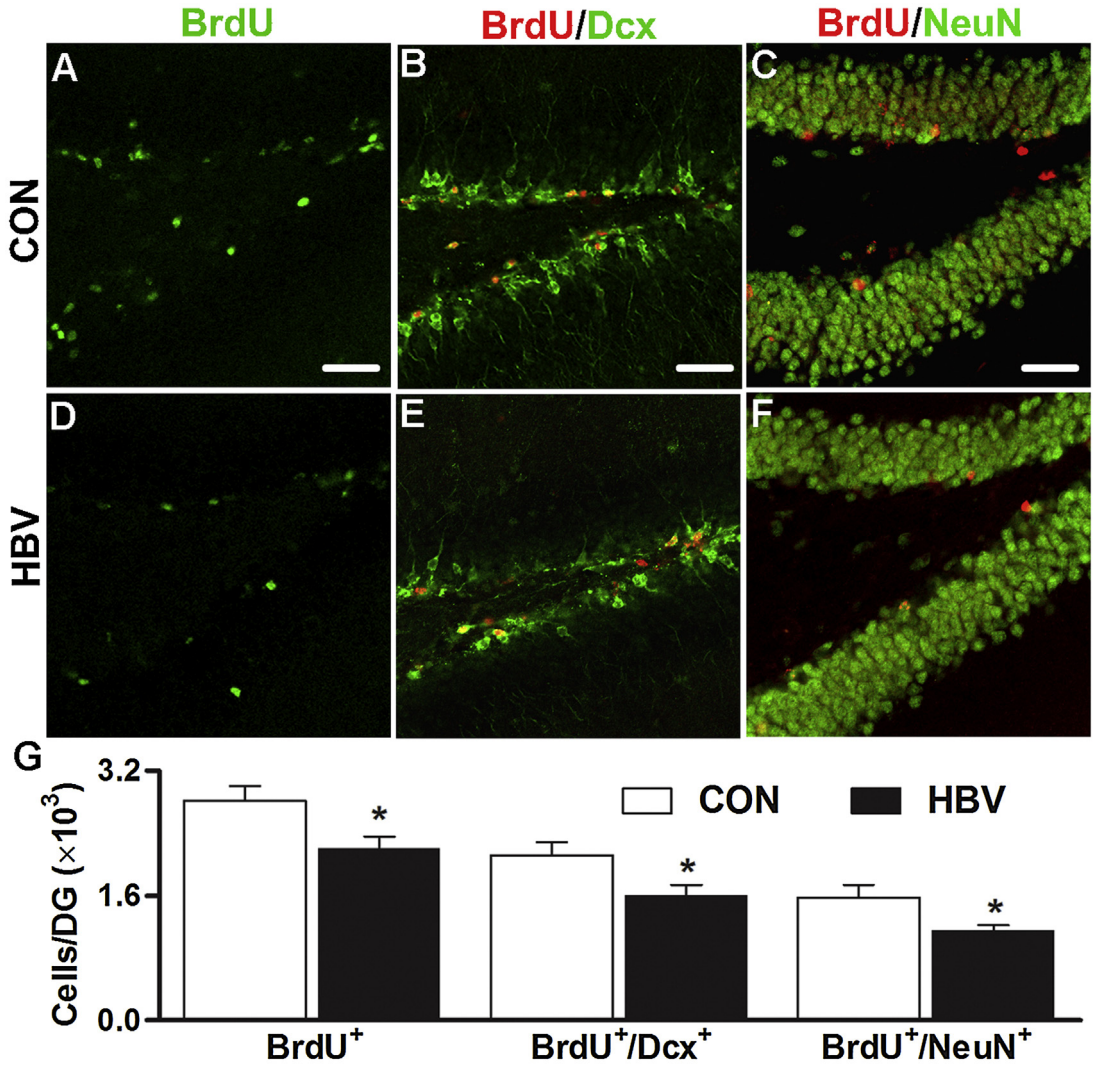

Fig. 4. HBV decreased hippocampal neurogenesis. (A-F) Representative confocal micrographs of labeled cells: $BrdU^+$ (A and D), $BrdU^+/Dcx^+$ (B and E) and $BrdU^+/NeuN^+$ cells (C and F) in DG. (I) Bars represent the average numbers of the $BrdU^+$, $BrdU^+/Dcx^+$ and $BrdU^+/NeuN^+$ cells in DG. Data represent the means ± SEM. $^*p < 0.05$; $n = 6$ mice/group; Student's *t*-test. Scale bar: 50 μm. The experiment was repeated twice with similar results and a representative experiment is shown.

the peripheral immune activation by HBV vaccination impaired behavior, hippocampal LTP and neurogenesis. To speculate the mechanism, the altered expression of IFN-γ and TNF-α may contribute to the mediation of the neurobehavior effects of HBV, either directly influencing the neurobehavior function or influencing the neurobehavior function through altering the BDNF and IGF-1 expression (Butovsky et al., 2005; Raison et al., 2006; Russo et al., 2012; Terrando et al., 2010).

What is the pathway by which peripheral immune activation affects the CNS? Cytokines derived peripherally may directly affect the CNS by permeating into it (Banks, 2005). Moreover, cytokines in the brain may exhibit crosstalk with the microglia and alter their phenotypes by regulating their secretion of neuroimmune molecules, including cytokines and neurotrophins (Schwartz and Shechter, 2010). In the present study, a few microglia in the hippocampi of HBV-mice were found to express CD11b. There were inconsistences between the directionality and magnitude of the alterations of cytokine expression in the periphery and those in the hippocampi. Thus, the accurate mechanism remains unclear. However, one possible mechanism may be differential cytokine production by the resident immune cells (Yirmiya and Goshen, 2011) and the differential transport of cytokines across the BBB (Banks, 2005).

Microglia can be activated into two different phenotypes: the M1 and M2 phenotypes (Colton, 2009). Classically activated M1 microglia produce proinflammatory cytokines and affect neurogenesis and neuronal activities, whereas alternatively activated M2 microglia exert neuroprotective effects primarily through anti-inflammatory molecules and neurotrophins. This study verified that neonatal HBV vaccination skewed the microglia towards the M1 phenotype (Fig. 4). This result may explain the hippocampal neurodetrimental expression profile of neuroimmune molecules. Despite the M1 phenotype shift, HBV did not alter the numbers of microglia in the hippocampi (Fig. 4). In agreement with the report by Chen et al. (Chen et al., 2012), these results suggested that the neurobehavioral impairments were associated with microglial activation without affecting their numbers.

The HBV-induced systemic Th2 bias may have mediated the neurodetrimental neuroimmune milieu and thereby resulted in neurobehavioral impairment. The peripheral Th1/Th2 balance is associated with the effects of peripheral immune activation on the CNS. For instance, maternal influenza vaccination was shown to increase hippocampal neurogenesis and neurotrophin expression in both generations; these neurobenefits were positively associated with a systemic Th1 bias (Xia et al., 2014a; Xia et al., 2014b). Moreover, BCG is a well-known Th1 bias inducer that has been

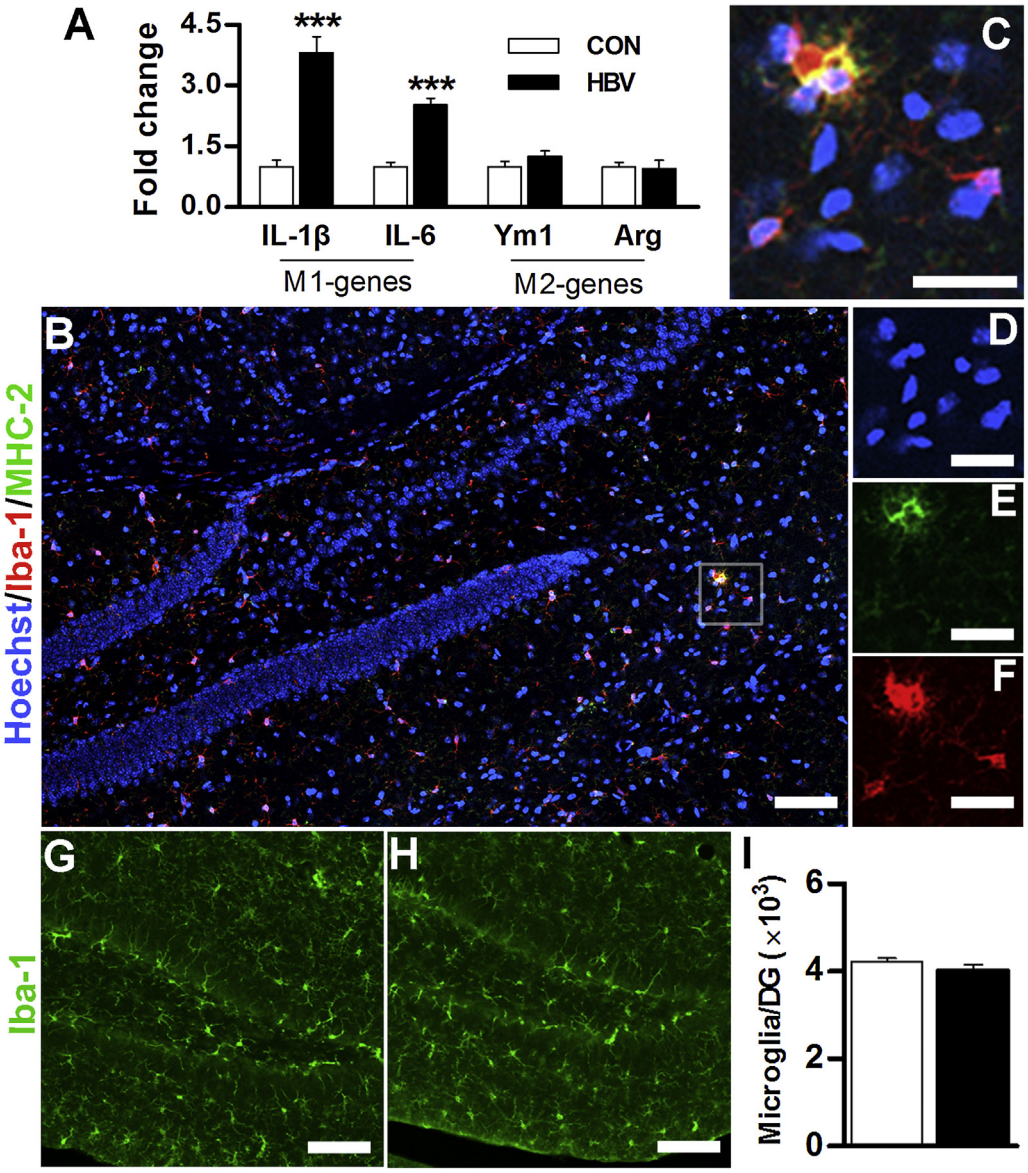

Fig. 5. HBV induced M1-activated microglia in the hippocampi but did not alter the microglial numbers. (A) Data represent the relative mRNA expression of the M1 and M2-type genes in the hippocampi compared to the CON-mice. (B–F) Representative confocal micrographs of the M1-activated microglia (Iba-1$^+$/MHC-2$^+$) in 6-w-old HBV-mice. Scale bar, 50 μm in (B), 20 μm in (C–F). (G–H) Representative micrographs showing the microglia (Iba-1$^+$) in the DG in the HBV-mice (G) and CON-mice (H) at 6 w. Scale bar, 100 μm. (I) Data represent the average numbers of microglia in the DG in each group of mice. The data in (A) and (I) represent the means ± SEM. *** $p < 0.001$; $n = 6$ mice/group; Student's *t*-test. The experiment was repeated twice with similar results and a representative experiment is shown.

found to be neuroprotective (Bourdette and Naismith, 2014; Laćan et al., 2013; Lee et al., 2008; Li et al., 2015a,b; Yang et al., 2016a,b; Yong et al., 2011). In contrast, a Th2 bias has been verified to be associated with cognitive deficits that are restored when the Th2 bias is reversed (Palumbo et al., 2012). Our previous study showed that neonatal HBV vaccination impaired the hippocampal synaptic plasticity of rats at early adulthood (Li et al., 2015a). Moreover, other mechanisms might underlie HBV's neurobehavioral effects. This topic requires further study in the future, such as an investigation of the regulation of the hypothalamic-pituitary-adrenal axis (Spencer et al., 2005).

The current study provides more evidence supporting this hypothesis. First, HEL/alum induced a Th2 bias and behavioral impairments that were similar to HBV. Second, a more powerful supporting finding is that the serum IFN-γ/IL-4 ratio positively correlated with the levels of hippocampal neurotrophins and neurogenesis at the individual level. Third, it has also been found that HBV induced a serum Th2 bias and neurobehavior impairments in mice even reared in a conventional animal room where the mice were subjected to the normal and complex immune stimuli. These findings suggest that the serum Th2 bias induced by HBV may be the critical bridge connecting the vaccination and the neurobehavior effects, instead of other aspects induced or associated by vaccination.

Along this line, similar neurobehavior effects of the HBV and the HEL/alum may be seen when vaccinated with other vaccine that consists of a protein and the aluminum hydroxide adjuvant and induces a systemic Th2 bias. But there are not many such vaccines. In fact, only pertussis-diphtheria-tetanus triple vaccine, one of these vaccines, is given to human infants (at three month old). We will observe whether the pertussis-diphtheria-tetanus triple vaccine can induce neurobehavior effects in the future work.

HBV's behavioral effects are only detectable at the age of early adulthood (at 8 w but not at 4 or 12 w). These findings suggest that

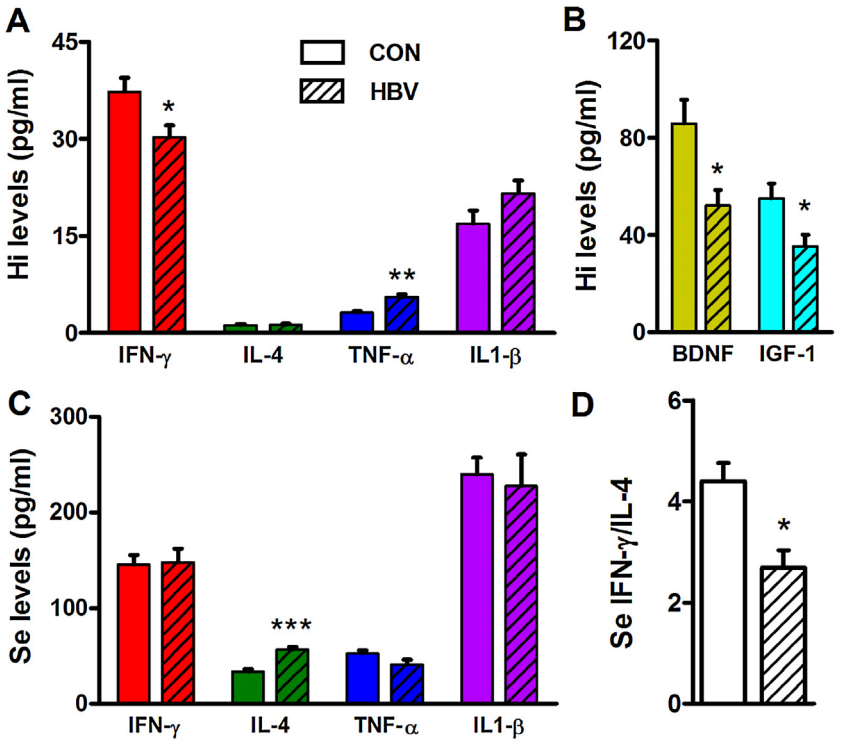

Fig. 6. HBV altered the hippocampal levels of cytokines and neurotrophins and the serum Th2 cytokine bias. (A–C) The bars represent the average levels of these molecules in the hippocampi (A, B) and serum (C) in each group. (D) The bars represent the Th1/Th2 cytokine balance in the serum of each group. The data represent the means ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $n = 6$/group; Student's *t*-test, Welch's *t*-test or nonparametric test. Hi: hippocampus; Se: serum. The experiment was repeated twice with similar results and a representative experiment is shown.

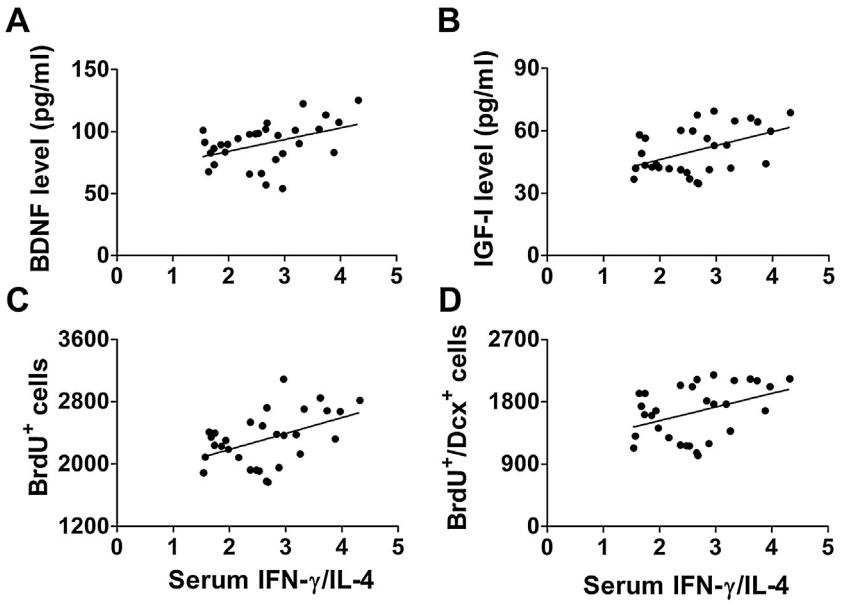

Fig. 7. Positive correlation of the serum IFN-γ/IL-4 ratio with neurotrophic factors and neurogenesis in the HBV-mice. (A–B) Correlation analyses between the serum IFN-γ/IL-4 ratio and the hippocampal levels of BDNF (A: $r = 0.419$, $p < 0.05$) and IGF-I (B: $r = 0.467$, $p < 0.01$). (C–D) Correlation analyses between the serum IFN-γ/IL-4 ratio and the numbers of proliferating cells (C: $r = 0.467$, $p < 0.01$) and new progenitors (D: $r = 0.397$, $p < 0.05$). $n = 30$, Pearson's correlation analysis. The experiment was repeated twice with similar results and a representative experiment is shown.

the vaccine's influence on behavior has a latency period prior to emergence and that the vaccine's influences are transient. Mice could be considered in the pubertal stage at 8 weeks, and this pubertal stage might alter behavior in ways that may not be evident at 4 or 12 weeks. However, the significant difference found in the present study is between the immunized mice and the control mice, rather than between the mice of 8-week-old and the mice of another age. Therefore, this difference does reflect the effects of the neonatal vaccination. The mechanism underlying the latency and transient phenomenon is very complex and needs further studies for well understanding, because such latency involves many aspects of the

immune responses in the periphery and CNS as well as neural plasticity.

This work reveals for the first time that early HBV vaccination induces impairments in behavior and hippocampal neurogenesis. This work provides innovative data supporting the long suspected potential association of HBV with certain neuropsychiatric disorders such as autism and multiple sclerosis (Gallagher and Goodman, 2010; Stubgen, 2012). This study used the same vaccine and a similar time schedule to those used for human infant vaccination in China. Therefore, these findings suggest that there may be similar effects of neonatal HBV vaccination on brain development and behavior in humans. Given the huge welfare brought by the HBV vaccination in the protection against hepatitis B, the proper implications of findings in the present study should be that HBV vaccination of human infants may be attempted to be performed with non-Th2 bias-inducing vaccine (eg, DNA vaccine) or even at different ages.

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Contributors

Junhua Yang, Fangfang Qi and Zhibin Yao participated in the study design. Zhibin Yao managed and supervised the experiments. Junhua Yang, Fangfang Qi, Juntao Zou and Kaihua Guo performed all of the experiments except the electrophysiology test, which was finished by Yang Yang. Junhua Yang, Fangfang Qi and Qunfang Yuan undertook the statistical analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

We declare that we have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2016.08.002.

References

Banks, W.A., 2005. Blood-brain barrier transport of cytokines: a mechanism for neuropathology. Curr. Pharm. Des. 11, 973–984.

Bannerman, D.M., Good, M.A., Butcher, S.P., Ramsay, M., Morris, R.G., 1995. Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. Nature 378, 182–186.

Baron, R., Nemirovsky, A., Harpaz, I., Cohen, H., Owens, T., Monsonego, A., 2008. IFN-gamma enhances neurogenesis in wild-type mice and in a mouse model of Alzheimer's disease. FASEB J. 22, 2843–2852.

Baruch, K., Ron-Harel, N., Gal, H., Deczkowska, A., Shifrut, E., Ndifon, W., Mirlas-Neisberg, N., Cardon, M., Vaknin, I., Cahalon, L., Berkutzki, T., Mattson, M.P., Gomez-Pinilla, F., Friedman, N., Schwartz, M., 2013. CNS- specific immunity at the choroid plexus shifts toward destructive Th2 inflammation in brain aging. Proc. Natl. Acad. Sci. U. S. A. 110, 2264–2269.

Bilbo, S.D., Schwarz, J.M., 2009. Early-life programming of later-life brain and behavior: a critical role for the immune system. Front. Behav. Neurosci. 3, 14.

Bilbo, S.D., Schwarz, J.M., 2012. The immune system and developmental programming of brain and behavior. Front. Neuroendocrinol. 33, 267–286.

Bilbo, S.D., Levkoff, L.H., Mahoney, J.H., Watkins, L.R., Rudy, J.W., Maier, S.F., 2005. Neonatal infection induces memory impairments following an immune challenge in adulthood. Behav. Neurosci. 119, 293–301.

Bilbo, S.D., Yirmiya, R., Amat, J., Paul, E.D., Watkins, L.R., Maier, S.F., 2008. Bacterial infection early in life protects against stressor-induced depressive-like symptoms in adult rats. Psychoneuroendocrinology 33, 261–269.

Bourdette, D., Naismith, R.T., 2014. BCG vaccine for clinically isolated syndrome and MS: infections and protective immunity. Neurology 82, 15–16.

Brennan, F.X., Beck, K.D., Servatius, R.J., 2004. Proinflammatory cytokines differentially affect leverpress avoidance acquisition in rats. Behav. Brain Res. 153, 351–355.

Butovsky, O., Talpalar, A.E., Ben-Yaakov, K., Schwartz, M., 2005. Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. Mol. Cell. Neurosci. 29, 381–393.

Butovsky, O., Ziv, Y., Schwartz, A., Landa, G., Talpalar, A.E., Pluchino, S., Martino, G., Schwartz, M., 2006. Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol. Cell. Neurosci. 31, 149–160.

Centers for Disease and Prevention, 2013. Global routine vaccination coverage-2012. MMWR Morb. Mortal. Wkly. Rep. 62, 858–861.

Chandran, J.S., Lin, X., Zapata, A., Hoke, A., Shimoji, M., Moore, S.O., Galloway, M.P., Laird, F.M., Wong, P.C., Price, D.L., Bailey, K.R., Crawley, J.N., Shippenberg, T., Cai, H., 2008. Progressive behavioral deficits in DJ-1-deficient mice are associated with normal nigrostriatal function. Neurobiol. Dis. 29, 505–514.

Chen, Z., Jalabi, W., Shpargel, K.B., Farabaugh, K.T., Dutta, R., Yin, X., Kidd, G.J., Bergmann, C.C., Stohlman, S.A., Trapp, B.D., 2012. Lipopolysaccharide-induced microglial activation and neuroprotection against experimental brain injury is independent of hematogenous TLR4. J. Neurosci. 32, 11706–11715.

Colton, C.A., 2009. Heterogeneity of microglial activation in the innate immune response in the brain. J. Neuroimm. Pharmacol. 4, 399–418.

Dórea, J.G., Marques, R.C., 2010. Infants' exposure to aluminum from vaccines and breast milk during the first 6 months. J. Exp. Sci. Environ. Epidemiol. 20, 598–601.

Femenia, T., Gomez-Galan, M., Lindskog, M., Magara, S., 2012. Dysfunctional hippocampal activity affects emotion and cognition in mood disorders. Brain Res. 1476, 58–70.

Fidel Jr., P.L., Romero, R., Wolf, N., Cutright, J., Ramirez, M., Araneda, H., Cotton, D.B., 1994. Systemic and local cytokine profiles in endotoxin-induced preterm parturition in mice. Am. J. Obstet. Gynecol. 170, 1467–1475.

French, S.S., Chester, E.M., Demas, G.E., 2013. Maternal immune activation affects litter success, size and neuroendocrine responses related to behavior in adult offspring. Physiol. Behav. 119, 175–184.

Gallagher, C.M., Goodman, M.S., 2010. Hepatitis B vaccination of male neonates and autism diagnosis, NHIS 1997–2002. J. Toxicol. Environ. Health A 73, 1665–1677.

Gerber, J., Bottcher, T., Hahn, M., Siemer, A., Bunkowski, S., Nau, R., 2004. Increased mortality and spatial memory deficits in TNF-alpha-deficient mice in ceftriaxone-treated experimental pneumococcal meningitis. Neurobiol. Dis. 16, 133–138.

He, F., Zou, J.T., Zhou, Q.F., Niu, D.L., Jia, W.H., 2014. Glatiramer acetate reverses cognitive deficits from cranial-irradiated rat by inducing hippocampal neurogenesis. J. Neuroimmunol. 271, 1–7.

Hong, S.M., Liu, Z., Fan, Y., Neumann, M., Won, S.J., Lac, D., Lum, X., Weinstein, P.R., Liu, J., 2007. Reduced hippocampal neurogenesis and skill reaching performance in adult Emx1 mutant mice. Exp. Neurol. 206, 24–32.

Hoshaw, B.A., Malberg, J.E., Lucki, I., 2005. Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. Brain Res. 1037, 204–208.

Idayu, N.F., Hidayat, M.T., Moklas, M.A., Sharida, F., Raudzah, A.R., Shamima, A.R., Apryani, E., 2011. Antidepressant-like effect of mitragynine isolated from Mitragyna speciosa Korth in mice model of depression. Phytomedicine 18, 402–407.

Kameda, M., Taylor, C.J., Walker, T.L., Black, D.M., Abraham, W.C., Bartlett, P.F., 2012. Activation of latent precursors in the hippocampus is dependent on long-term potentiation. Transl. Psychiatry 2, e72.

Laćan, G., Dang, H., Middleton, B., Horwitz, M.A., Tian, J., Melega, W.P., Kaufman, D.L., 2013. Bacillus Calmette-Guerin vaccine-mediated neuroprotection is associated with regulatory T-cell induction in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. J. Neurosci. Res. 91, 1292–1302.

Lee, J., Reinke, E.K., Zozulya, A.L., Sandor, M., Fabry, Z., 2008. Mycobacterium bovis bacille Calmette-Guerin infection in the CNS suppresses experimental autoimmune encephalomyelitis and Th17 responses in an IFN-gamma-independent manner. J. Immunol. 181, 6201–6212.

Li, Q., Qi, F., Yang, J., Zhang, L., Gu, H., Zou, J., Yuan, Q., Yao, Z., 2015a. Neonatal vaccination with bacillus Calmette-Guerin and hepatitis B vaccines modulates hippocampal synaptic plasticity in rats. J. Neuroimmunol. 288, 1–12.

Li, Q., Zhang, Y., Zou, J., Qi, F., Yang, J., Yuan, Q., Yao, Z., 2015b. Neonatal vaccination with bacille Calmette-Guerin promotes the dendritic development of hippocampal neurons. Hum. Vacc. Immunother., 1–10.
Mast, E.E., Alter, M.J., Margolis, H.S., 1999. Strategies to prevent and control hepatitis B and C virus infections: a global perspective. Vaccine 17, 1730–1733.
Palumbo, M.L., Trinchero, M.F., Zorrilla-Zubilete, M.A., Schinder, A.F., Genaro, A.M., 2012. Glatiramer acetate reverts stress-induced alterations on adult neurogenesis and behavior Involvement of Th1/Th2 balance. Brain Behav. Immun. 26, 429–438.
Pariante, C.M., Lightman, S.L., 2008. The HPA axis in major depression: classical theories and new developments. Trends Neurosci. 31, 464–468.
Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 27, 24–31.
Russo, I., Caracciolo, L., Tweedie, D., Choi, S.H., Greig, N.H., Barlati, S., Bosetti, F., 2012. 3,6'-Dithiothalidomide, a new TNF-alpha synthesis inhibitor, attenuates the effect of Abeta1-42 intracerebroventricular injection on hippocampal neurogenesis and memory deficit. J. Neurochem. 122, 1181–1192.
Schwartz, M., Shechter, R., 2010. Protective autoimmunity functions by intracranial immunosurveillance to support the mind: the missing link between health and disease. Mol. Psychiatry 15, 342–354.
Scott, L., Feng, J., Kiss, T., Needle, E., Atchison, K., Kawabe, T.T., Milici, A.J., Hajos-Korcsok, E., Riddell, D., Hajos, M., 2012. Age-dependent disruption in hippocampal theta oscillation in amyloid-beta overproducing transgenic mice. Neurobiol. Aging 33 (148), e1413–1423.
Shi, L., Fatemi, S.H., Sidwell, R.W., Patterson, P.H., 2003. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. J. Neurosci. 23, 297–302.
Shi, Y., Chanana, V., Watters, J.J., Ferrazzano, P., Sun, D., 2011. Role of sodium/hydrogen exchanger isoform 1 in microglial activation and proinflammatory responses in ischemic brains. J. Neurochem. 119, 124–135.
Spencer, S.J., Heida, J.G., Pittman, Q.J., 2005. Early life immune challenge–effects on behavioural indices of adult rat fear and anxiety. Behav. Brain Res. 164, 231–238.
Stubgen, J.P., 2012. Immune-mediated myelitis following hepatitis B vaccination. Autoimmun. Rev. 12, 144–149.
Terrando, N., Monaco, C., Ma, D., Foxwell, B.M., Feldmann, M., Maze, M., 2010. Tumor necrosis factor-alpha triggers a cytokine cascade yielding postoperative cognitive decline. Proc. Natl. Acad. Sci. U. S. A. 107, 20518–20522.
Urakubo, A., Jarskog, L.F., Lieberman, J.A., Gilmore, J.H., 2001. Prenatal exposure to maternal infection alters cytokine expression in the placenta, amniotic fluid, and fetal brain. Schizophr. Res. 47, 27–36.
Walker, F.R., March, J., Hodgson, D.M., 2004. Endotoxin exposure in early life alters the development of anxiety-like behaviour in the Fischer 344 rat. Behav. Brain Res. 154, 63–69.
Weeratna, R.D., Brazolot Millan, C.L., McCluskie, M.J., Davis, H.L., 2001. CpG ODN can re-direct the Th bias of established Th2 immune responses in adult and young mice. FEMS Immunol. Med. Microbiol. 32, 65–71.
Xia, Y., Qi, F., Zou, J., Yang, J., Yao, Z., 2014a. Influenza vaccination during early pregnancy contributes to neurogenesis and behavioral function in offspring. Brain Behav. Immun. 42, 212–221.
Xia, Y., Qi, F., Zou, J., Yao, Z., 2014b. Influenza A(H1N1) vaccination during early pregnancy transiently promotes hippocampal neurogenesis and working memory Involvement of Th1/Th2 balance. Brain Res. 1592, 34–43.
Yang, J., Qi, F., Gu, H., Zou, J., Yang, Y., Yuan, Q., Yao, Z., 2016a. Neonatal BCG vaccination of mice improves neurogenesis and behavior in early life. Brain Res. Bull. 120, 25–33.
Yang, J., Qi, F., Yao, Z., 2016b. Neonatal Bacillus Calmette-Guerin vaccination alleviates lipopolysaccharide-induced neurobehavioral impairments and neuroinflammation in adult mice. Mol. Med. Rep.
Yip, H.C., Karulin, A.Y., Tary-Lehmann, M., Hesse, M.D., Radeke, H., Heeger, P.S., Trezza, R.P., Heinzel, F.P., Forsthuber, T., Lehmann, P.V., 1999. Adjuvant-guided type-1 and type-2 immunity: infectious/noninfectious dichotomy defines the class of response. J. Immunol. 162, 3942–3949.
Yirmiya, R., Goshen, I., 2011. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav. Immun. 25, 181–213.
Yong, J., Laćan, G., Dang, H., Hsieh, T., Middleton, B., Wasserfall, C., Tian, J., Melega, W.P., Kaufman, D.L., 2011. BCG vaccine-induced neuroprotection in a mouse model of Parkinson's disease. PLoS One 6, e16610.
Zhao, C., Deng, W., Gage, F.H., 2008. Mechanisms and functional implications of adult neurogenesis. Cell 132, 645–660.