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Behavioral disturbances in adult mice following neonatal virus infection or kynurenine treatment – role of brain kynurenic acid

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Abstract

Exposure to infections in early life is considered a risk-factor for developing schizophrenia. Recently we reported that a neonatal CNS infection with influenza A virus in mice resulted in a transient induction of the brain kynurenine pathway, and subsequent behavioral disturbances in immune-deficient adult mice. The aim of the present study was to investigate a potential role in this regard of kynurenic acid (KYNA), an endogenous antagonist at the glycine site of the N-methyl-D-aspartic acid (NMDA) receptor and at the cholinergic α7 nicotinic receptor. C57BL/6 mice were injected i.p. with neurotropic influenza A/WSN/33 virus (2400 plaque-forming units) at postnatal day (P) 3 or with L-kynurenine (2×200 mg/kg/day) at P7-16. In mice neonatally treated with L-kynurenine prepulse inhibition of the acoustic startle, anxiety, and learning and memory were also assessed. Neonatally infected mice showed enhanced sensitivity to d-amphetamine-induced (5 mg/kg i.p.) increase in locomotor activity as adults. Neonatally L-kynurenine treated mice showed enhanced sensitivity to d-amphetamine-induced (5 mg/kg i.p.) increase in locomotor activity as well as mild impairments in prepulse inhibition and memory. Also, d-amphetamine tended to potentiate dopamine release in the striatum in kynurenine-treated mice. These long-lasting behavioral and neurochemical alterations suggest that the kynurenine pathway can link early-life infection with the development of neuropsychiatric disturbances in adulthood.

Keywords

Neurodevelopment; postnatal; kynurenine; immune activation; schizophrenia; amphetamine; locomotor activity; prepulse inhibition; microdialysis

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Conflict of Interest Statement

All authors declare that there are no conflicts of interest.

1. Introduction

Schizophrenia is a mental disorder, usually emerging in adolescence or early adulthood (Andreasen, 1995) and is characterized by a large diversity of positive and negative symptoms as well as cognitive deficits. Aberrations in brain dopamine (DA) neurotransmission as part of the pathophysiology has for long been the predominant biological hypothesis of schizophrenia (Carlsson and Lindqvist, 1963; Carlsson and Carlsson, 2006). The DA hypothesis arises from the finding of amelioration in positive symptoms following blockade of DA D_2-receptors, as well as from observations of psychosis following frequent abuse of the indirect DA agonist damphetamine (Angrist and Gershon, 1970; Cruickshank and Dyer, 2009; Griffith et al., 1972). Further supporting the DA hypothesis, brain imaging studies reveal an enhanced DA release following administration of d-amphetamine in patients with schizophrenia (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle et al., 1996). Research during the last decade however, proposes that DA only plays an intermediary role in the pathophysiology and that deficits in brain glutamatergic systems are of major importance for the disease (Carlsson et al., 2001; Javitt, 2004; Jentsch and Roth, 1999). For example, administration of N-methyl-D-aspartic acid (NMDA) receptor antagonists (e.g. phencyclidine and ketamine) evokes behavior similar to schizophrenia symptoms in healthy individuals, and exacerbates symptoms in patients with schizophrenia (Adler et al., 1999; Javitt and Zukin, 1991; Luby et al., 1959). Providing strong support for a dysfunction of glutamatergic transmission in schizophrenia, the concentration of kynurenic acid (KYNA) is elevated in the cerebrospinal fluid (CSF) and in post mortem brain of patients with schizophrenia (Erhardt et al., 2001a; Linderholm et al., 2012; Nilsson et al., 2005; Sathyasaikumar et al., 2011; Schwarcz et al., 2001). KYNA is a tryptophan metabolite, synthesized in astrocytes via the kynurenine pathway. At nanomolar concentrations, KYNA antagonizes the glycine-site of the NMDA receptor as well as the cholinergic α7 nicotinic receptor (α7nAChR; Schwarzc et al., 2012,). At higher, micromolar concentrations, KYNA blocks the glutamate recognition-site of the NMDA receptor (Kessler et al., 1989). Notably, endogenous concentrations of KYNA tonically control DA transmission in the rat brain (Erhardt et al., 2009; Linderholm et al., 2007; Amori et al., 2009b; Schwieler et al., 2008), findings functionally linking the DA hypothesis to the glutamate deficiency theory of schizophrenia.

Synthesis of KYNA is induced following immune activation (Dantzer et al., 2008). Indeed, CSF KYNA is elevated in various infectious diseases (Heyes et al., 1992), such as those caused by human immunodeficiency virus-1 or tick-borne encephalitis (Atlas et al., 2007; Holtze et al., 2012) or during influenza A virus infections of neuron or glial cultures in vitro (Holtze et al., 2008). Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO), the initial and rate-limiting enzymes in the production of KYNA, are induced by interferon-γ or other pro-inflammatory cytokines (Schwarcz et al., 2012) and are important in controlling microbial growth as well as the host immune response (King and Thomas, 2007). Moreover, activation of the pro-inflammatory cytokine interleukin (IL)-1β in CSF from first-episode patients with schizophrenia was recently reported, strongly indicating immune dysregulation in the disease (Soderlund et al., 2009). Interestingly, exposure to infections, including influenza A virus, during early-life appear to increase the risk for the future development of schizophrenia and related psychotic disorders (Blomström et al., 2012; Brown and Derkits, 2010; Dalman et al., 2008; Ellman et al., 2009; Yolken and Torrey, 2008). While a causal link between early-life exposure to infections and the later development of psychoses is still missing, several animal studies have demonstrated deficits in prepulse inhibition (PPI), a cross-species measure of sensorimotor gating which is impaired in patients with schizophrenia (Braff et al., 2001), in adult animals following either prenatal or early-life exposure to immunostimulatory agents (Crnic and Pizer, 1988; Engel et al., 2000; Gold et al., 1994; Meyer and Feldon, 2010; Tohmi et al., 2004). We recently

reported that a neonatal infection with neurotropic influenza A/WSN/33 virus transiently increases brain KYNA concentrations in wild type mice as well as in immunodeficient mice with a targeted disruption of the gene-encoding transporter associated with antigen processing 1 (*Tap1*$^{-/-}$), leading to a lack of MHC class I expression and functional CD8+ T cells (Asp et al., 2010; Holtze et al., 2008). The neonatal infection was associated with deficits in PPI and working memory as well as with increased rearing and anxiety in adult immunodeficient mice, but not in adult wild type mice (Asp et al., 2009; Asp et al., 2010). A recent study from our laboratory also shows that mice with subchronically elevated levels of KYNA in adulthood have normal spontaneous locomotor activity but a potentiated locomotor response to d-amphetamine (Olsson et al., 2012a). Since our previous studies showed that wild type mice neonatally infected with influenza A virus do not show aberrant behavior under baseline conditions, in the present study we investigated the locomotor response to challenge with d-amphetamine. A second aim of the present study was to investigate whether neonatally elevated brain KYNA is associated with the disturbed behavior in adulthood seen after infection in early life.

2. Materials and methods

2.1. Animals

C57BL/6 mice were used for all experiments. Pregnant mice were obtained from Scanbur AB, Sweden and kept in a single cage until delivery. All pups (males and females) stayed with their mothers until weaning (5–8 mice per cage). After weaning at the age of four weeks, all mice were group-housed in standard transparent cages (2–5 mice per cage, as determined by the number of male mice in each litter) because isolation rearing (i.e. housing mice one per cage) can affect amount of PPI and locomotor response to amphetamine. All mice were handled according to institutional guidelines and environmental conditions and checked daily. The animals were maintained under standard laboratory conditions with free access to food and tap water in a light-controlled room (12 h light/dark cycle, light on at 6.00 a.m.), under constant temperature (22°C) and humidity (40–60%). To check for confounding factors regarding differences in litter size we plotted individual test responses with regard to PPI. The responses were found to be almost similar in all mice with no deviation with regard to litter sizes. Two separate sets of pups were neonatally treated with L-kynurenine or saline. In the first set, only male mice were used and locomotor activity and PPI assessed. In the second set, both male and female mice were neonatally treated with L-kynurenine or saline and assessed in the light dark box and the elevated plus maze. Thereafter female mice were assessed for trace fear conditioning and male mice were assessed biochemically utilizing in vivo microdialysis. In total, 79 male and 35 female mice were used in these experiments, including a smaller batch of mice used for verification of brain KYNA levels at P16. All experiments were approved by and performed in accordance with the guidelines of the Ethical Committee of Northern Stockholm, Sweden. All efforts were made to minimize the number of animals used and their suffering.

2.2. Neonatal influenza A/WSN/33 infection

The neonatal infection model has previously been described in detail inAsp et al. (2007) andAsp et al. (2009). Male C57BL/6 mice (n = 9 from a total of three litters) were infected intraperitoneally (i.p.; thus mimicking a hematogenous route of infection) with 2400 plaqueforming units of mouse adapted neurotropic influenza A/WSN/33 virus (obtained from Dr. S Nakajima, Institute of Public Health, Tokyo, Japan) suspended in 30 μl of phosphate buffered saline (PBS; Gibco), at 3.5 centimeters head-to-rump length, i.e. at postnatal day (P)3 or P4. Control mice were injected similarly with PBS (n = 10 from a total of four litters).

2.3. Neonatal kynurenine treatment

Two cohorts of male and female C57BL/6 mice were i.p. injected with L-kynurenine sulfate salt (2×200 mg/kg/day; Sigma Aldrich), adjusted to pH ~8.2, every 12th hour for ten days to mimic the increase in brain KYNA concentration following influenza infection (Holtze et al., 2008). Administration of L-kynurenine started at P7 (first cohort; n = 8 (only males) from a total of three litters, second cohort; n = 19 males and 18 females from a total of 15 litters). Similarly, control mice were injected with saline (0.9% NaCl; first cohort; n = 10 (only males) from a total of two litters, second cohort: n = 17 males and 17 females from a total of 15 litters).

2.4. Behavioral and neurochemical assessments in adult life

Locomotor activity of influenza A virus-infected male mice and uninfected male controls was assessed at the age of 5–6 months (Fig.1A). The L-kynurenine treated mice and saline controls were tested at the age of 3–4 months. In the first cohort of mice neonatally treated with L-kynurenine or saline and in the influenza infected mice, startle response and PPI tests were performed approximately one week prior to the locomotor activity recording. No difference in body weight was observed between influenza A virus-infected mice and uninfected controls nor between L-kynurenine treated mice and saline controls at the time of these behavior experiments. In the second cohort of mice (both males and females) neonatally treated with L-kynurenine or saline, anxiety-like behavior was measured in the elevated plus-maze (age 4 months) and the light dark box test (5–6 months). At 7–8 months of age, females were tested for the trace fear conditioning to measure contextual memory and male mice underwent microdialysis for investigations of dopaminergic neurotransmission and extracellular levels of KYNA and 3-hydroxykynurenine (Fig.1B). Before all behavioral assessments, mice were transferred to the laboratory at 07:30 a.m. on the day of the experiment. Behavioral assessment started at 08:30 a.m. and for each tests, the same experimenter performed all tests.

2.4.1. Prepulse inhibition—PPI of the startle response is used as an operational measure of sensorimotor gating and is analyzed by measuring the ability of a non-startling “prepulse” to inhibit the response to a startling stimulus (Hoffman and Ison, 1980). Startle response and PPI testing were performed in commercial startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA). For details see Asp et al., 2010.

2.4.2. Open field behavior—Locomotor behavior, as defined by horizontal activity was asseded in an open field apparatus for mice. For details, see Olsson et al 2012a. All mice were habituated during three sessions of 60 minutes, 24 hours apart. At the start of each session the mouse was placed in the front left corner of the apparatus. The last habituation session was immediately followed by a test session of 90 minutes. d-amphetamine (5mg/kg; Sigma Aldrich) or vehicle (0.9% NaCl) was injected i.p. approximately one minute prior to the test session. All habituation sessions and test sessions were performed in the dark during the animal’s light cycle, between 8 a.m. and 6 p.m. The d-amphetamine/vehicle administrations were performed in a crossover design for the influenza A virus-infected and uninfected mice with a seven-day washout period.

2.4.3. Elevated plus maze—The procedure of elevated plus maze was based on the method of Lister and colleagues (Lister et al., 1987). The plus maze was shaped like a “plus” sign and consisted of two open arms (30 × 5 cm) and two equal-sized closed (30 × 5 × 15 cm) arms opposite to each other. Open and closed arms extended from a central platform (5 × 5 cm). The maze was made of stainless steel and mounted on a base, raising it to a height of 50 cm above the floor. Mice were individually placed on the central platform facing an open arm, and allowed to freely explore the maze for 5 min. The number of entries into the

different arms and the time spent in each arm were recorded by a video camera and then analyzed using the top-view based behavior analysis software TopScan Lite (Clever Sys Inc, Reston, Virginia). The number of entries and percent time spent in different arms was measured as an index of exploratory behavior. After each test, the maze was cleaned with 70% ethanol solution and dried for 10 min. 19 neonatally L-kynurenine treated and 17 saline treated male and 18 neonatally L-kynurenine treated and 17 saline treated female mice were assessed. A total of 71 mice were tested (neonatal L-kynurenine, male=19, female=18; neonatal saline, male=17, female=17).

2.4.4. Light/dark box test—The mouse light/dark box (50 × 25 × 25 cm) consisted of two parts: a lit (800 lx) open white compartment and a darkened (0 lx) closed black compartment with a volume ratio of 1:1. The two compartments were separated by a black Plexiglas partition containing a 10 × 5 cm opening to allow animals to freely move from one compartment to the other. The light/dark box test was performed according to the method described by Costall and colleagues (Costall et al., 1988). Each mouse was placed in the center of the white area facing away from the dark chamber and allowed to explore both compartments freely for 5 min. After each test, the box was cleaned with a 70% ethanol solution and dried for 10 min. Videos were analyzed using the top-view based behavior analysis software TopScan Lite (Clever Sys Inc, Reston, Virginia). For each mouse, time spent in light (white) and dark (black) compartment, number of transitions between the two compartments as well as latency to first leave (escape) from the light compartment was measured. A total of 70 mice were tested (neonatal L-kynurenine, male=19, female=18; neonatal saline, male=17, female=16).

2.4.5. Trace fear conditioning—The trace fear conditioning test was carried out by means of a fear conditioning chamber (Med Associates Inc., St. Albans, VT, USA). Training (conditioning) consisted of 2 trials of tone and foot-shock pairings as previously described (Terrando et al., 2010). Briefly, during conditioning mice were allowed to explore the chamber for 100 s before a 20 s tone cue (90 dB). After 18 s interval a foot shock (2 s duration, 0.5 mA intensity) was delivered through the stainless steel rods on the floor of the apparatus. A second tone-shock pairing was repeated after 100 s inter trial interval and the following 5 s after the second shock was defined as working memory. Working memory is generally defined as cognitive entities (or “central executive” mechanisms) relating to temporary storage and operation of information in both humans and animals (Yoon et al., 2008). The mouse was removed 30 s after the last shock. Three days later, freezing was recorded in the same context, with no tone or shock exposures. Freezing was scored automatically by the Med Associate software and defined as the absence of movement except that required for respiration (Kim and Fanselow, 1992).

Approximately 3 hours after the contextual assessment, mice were again placed in the apparatus and freezing was recorded in a novel environment (a plastic floor covered the metal grid and a pyramidal shape was inserted in the rectangular box) and in response to the cue (tone). After 100 s exploration, the auditory cue was presented for 20 s followed by a 120 s inter trial interval, then another 20 s cue presented (Cibelli et al., 2010). The percentage of time spent freezing was used to score learning and memory. A decrease in percent freezing represents impairment of these abilities. 12 neonatally L-kynurenine treated and 12 saline treated female mice were assessed.

2.4.6. Microdialysis surgery—Male mice were anesthetized in a Plexiglas chamber continuously ventilated with 4.8% isoflurane in air using a vaporizer (Univentor 400 Anesthesia Unit; Univentor Ltd, Zejtun, Malta) and then mounted onto the ear bars of a conventional stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Anesthesia was maintained using a nose cone delivering 2.4% isoflurane. Body temperature was

maintained at 37 °C throughout the surgery, by a thermometer and a heating pad (Homeothermic Blanket Control Unit 50-7053-F, Harvard Apparatus, Holliston, MA, USA). An ocular lubricant was applied and 0.5 mL sterile saline was given s.c. to prevent dehydration. Before surgery, the scalp was swabbed with bupivacaine (5 mg/ml), to provide post-operative analgesia, incised (7 – 8 mm) and the skull was cleaned from adhesions. Next, a thin layer of quick-setting cyanoacrylate glue (BT AB, Stockholm, Sweden) was applied to the exposed skull, serving as an adhesion surface to the dental cement. A small hole was then drilled over striatum (AP: 0.5 mm anterior to bregma, L: 2 mm from midline), and, following careful removal of the dura, a guide cannula (AT4.7.IC, AgnTho's AB) containing a dummy probe (outer diameter: 0.2 mm) was implanted (V: 1.5 mm below the brain surface), and secured to the skull with acrylic dental cement (Dentalon® plus, Heraeus, Hanau, Germany). Before each guide cannula implantation the incisor bar was adjusted so that the skull was set in a horizontal flat plane. The wound was then sutured and mice were allowed to recover single-housed for 48 hours with food and water *ad libitum*.

2.4.7. In vivo microdialysis—On the day of experiment, microdialysis was performed in the home cage in unanaesthetized freely moving male mice. Mice were tethered to a swivel, the guide was removed and a microdialysis probe (AT4.7.2.PES, shaft length: 7 mm, membrane length: 2 mm, molecular cut-off: 6 kDa, AgnTho's AB) was inserted through the guide cannula. Probes were perfused with perfusion fluid (Ringer solution containing 148 mM NaCl, 4 mM KCl, 0.8 mM $MgCl_2$, 1.4 mM $CaCl_2$), delivered via polyethylene tubing from a microinfusion pump (Univentor 864, Univentor Ltd) at a flow rate of 1 µl/min. All samples were collected in plastic tubes attached to the tether in 30 min (for the analysis of DA) or 45 min (for the analysis of KYNA) intervals throughout the experiment and immediately manually injected (Rheodyne, Cotati, CA, USA) into a high performance liquid chromatography (HPLC) system. To minimize the inter-individual variation due to differences in probe recovery, the dialysate concentrations were transformed to percent of baseline before statistical analysis. A stable baseline, consisting of three consecutive samples with a maximal variation of 10 %, was usually obtained after 2–3 h and defined as 100%. These three samples were taken for determination of basal extracellular DA or KYNA concentrations. Results for subsequent samples were calculated as percentages of this average basal release. The mice then received an i.p. injection of d-amphetamine (2 mg/kg), and DA or KYNA concentrations were measured for up to 300 minutes. After the session, the mice were sedated with isoflurane and sacrificed with cervical dislocation and tissues were frozen for later analysis and histological verification of probe placement.

2.4.8. Brain sample preparation for analysis of KYNA—To verify that brain KYNA was elevated neonatally, a sub-set of mice was sacrificed at P16, six hours after the last L-kynurenine or saline injection (n = 3/group). Mice that underwent behavioral testing in adulthood were sacrificed within one week after the last test session. Brains were rapidly sampled for KYNA analysis. Brain tissue was diluted eightfold in 0.4M PCA and homogenized (Bullet Blender® Next Advance, Inc. Averill Park, NY, USA). The homogenate was then centrifuged at 14000 rpm for 5 min and 1ml of the supernatant was mixed with 100 µl 70% PCA. This solution was then re-centrifuged (14000 rpm, 5 min), and the supernatant transferred to a new eppendorf tube for analysis.

2.4.9. Analysis of kynurenic acid—Briefly, 30 µl of the perfusate, or 50 µl of brain-tissue homogenate, respectively, were subsequently analyzed for KYNA levels using an isocratic reversed-phase high performance liquid chromatograph (HPLC) system. For details, see Olsson et al., 2012a.

2.4.10. HPLC detection of 3-hydroxykynurenine in microdialysate—To determine 3-hydroxykynurenine concentration, 20 μl of the perfusate, was subjected to analysis utilizing an isocratic reversed-phase HPLC system, coupled to an electrochemical detector (Coulochem III; ESA Inc.). A mobile phase consisting of 20 mM sodium phosphate, 0.7mM octanesulfonic acid and 10% acetonitrile (pH set to 3.2 using acetic acid) was pumped through a ReproSil-Pur C18 column (4 × 150 mm, Dr. Maisch GmbH, Ammerbuch, Germany), at a flow rate of 0.6 ml/min, delivered by a LC-10AD VP (Shimadzu Corporation, Kyoto, Japan). Signals from the detector were transferred to a computer for analysis with Clarity (Data Apex Ltd, The Czech Republic)

2.4.11. HPLC detection of DA in microdialysate—Separation of DA was achieved by reversed-phase HPLC using a 55 mM sodium acetate buffer (pH 4.1, 10 % methanol) with 0.8 mM octanesulfonic acid and 0.01 mM Na2EDTA. The mobile phase was delivered by an HPLC pump (Bischoff Chromatography, Leonberg, Germany) through a ReproSil-Pur C18 column (4 × 150 mm, Dr Maisch GmbH, Ammerbuch, Germany) at a rate of 0.8 ml/min. Samples were then quantified by sequential oxidation and reduction in a high-sensitivity analytical cell (ESA 5011); ESA Inc., Chelmsford, MA, USA) controlled by a potentiostat (Coulochem III; ESA Inc.) with an applied potential of −450 mV for detection of DA. The signals from the detector were transferred to a computer for analysis (Datalys Azur, Grenoble, France). The retention time of DA was approximately 8 min.

2.5. Statistical analysis

The statistical software package GraphPad Prism® 6 (GraphPad Software Inc., San Diego, CA, USA) for Mac OS X was used. All data are expressed as mean ± S.E.M. In this study two experiments in separate sets of mice are included; influenza A virus-infected mice vs. uninfected controls and L-kynurenine treated mice vs. saline controls. No comparisons were performed between the two sets of mice.

PPI—Mean startle magnitudes within the test session were analyzed using one-factor ANOVAs with neonatal treatment 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 – ((average startle of prepulse + pulse trial) / (average startle in pulse alone trial))×100. Percent acoustic PPI data were analyzed using two-factor ANOVAs with prepulse intensity, or ISI, as a within-subject factor, and L-kynurenine treatment or infection status as the between-subject factors. *Post hoc* multiple comparisons of means were performed with Tukey's test.

Locomotor Behavior—For the locomotor parameter, horizontal activity, two-way repeated measure ANOVA (treatment×time) followed by Bonferroni's *post hoc* tests were used.

TFC—Percent freezing was analyzed by two-way repeated measures ANOVA with time block and treatment (L-kynurenine or Saline) as the two factors. When interaction between the factors was observed, the Bonferroni multiple comparison test was used.

Data obtained in the elevated plus maze and the light-dark box were analyzed with the student t-test.

Microdialysis data were analyzed via two-way repeated measures ANOVA (with time as the repeated measure and treatment as a between subject factor) followed by Bonferroni multiple comparison test.

Whole brain KYNA concentrations between groups were analyzed with the non-parametric Mann-Whitney U test.

A p value of < .05 was considered statistically significant throughout the study.

3. Results

3.1. Locomotor activity assessed in the open field following virus infection

Basal horizontal activity in adult animals did not differ between neonatally influenza A/WSN/33 virus infected mice (n = 16) and their uninfected controls (n = 17; Fig. 2). Acute administration of d-amphetamine (5 mg/kg, i.p.) in adult life increased horizontal activity in neonatally influenza A/WSN/33 virus-infected mice (n = 8) and in uninfected controls (n = 9). As compared to the uninfected control mice, neonatally influenza A virus-infected mice showed a more pronounced increase in d-amphetamine-induced response (Interaction: F (51, 493) = 3.629, $p < .001$; Time: F (17, 493) = 25.34, $p < .001$; Treatment: F (3, 29) = 16.29, $p < .001$). No d-amphetamine-induced signs of stereotypy were visible when mice were removed from the chambers.

3.2. Elevation of endogenous KYNA during early-life in mice treated with L-kynurenine

Neonatal administration of L-kynurenine (2×200 mg/kg/day, i.p., P7-16) increased brain KYNA concentrations measured six hours after the last injection at P16 (mean ± S.E.M: 4.61 ± 1.09 nM, n = 3 vs. 2.25 ± 0.46 nM in saline treated controls, n = 3).

3.3. Locomotor activity assessed in the open field following neonatal L-kynurenine treatment

Basal horizontal activity in adult life did not differ between neonatally L-kynurenine-treated mice (n = 8) and their saline-treated controls (n = 8; Fig. 3). Administration of d-amphetamine (5 mg/kg, i.p.) in adult life increased horizontal activity in mice neonatally treated with L-kynurenine (n = 4) and in saline treated controls (n = 4). In the neonatally L-kynurenine treated mice, d-amphetamine potentiated the increase in response in horizontal activity as compared to the saline treated control mice (Interaction: F (51, 204) = 2.631, $p < .001$; Time: F (17, 204) = 10.61, $p < .001$; Treatment: F (3, 12) = 8.788, $p < .01$). No d-amphetamine-induced signs of stereotypy were visible when mice were removed from the chambers.

3.4. Assessment of prepulse inhibition following neonatal L-kynurenine treatment

Measurements of PPI were performed in adult mice, neonatally treated with L-kynurenine (n = 8) or with saline (n = 10). In the varied ISI block, neonatally L-kynurenine treated mice displayed an ISI-dependent impairment in PPI as evidenced by an interaction between L-kynurenine treatment and ISI (F (4,64) = 3.37, $p < .05$). *Post hoc* analysis revealed a significant decrease in PPI with L-kynurenine treatment at the 500 ms ISI ($p < .01$; Fig. 4A). There was no main effect of neonatal L-kynurenine treatment on PPI in the varied ISI block ($F < 1$, NS), and no difference in startle magnitude to the P120 trials in this block between the groups ($F < 1$, NS; Fig. 4B). In the varied prepulse intensity block, there was no main effect on L-kynurenine treatment ($F < 1$, NS) or any interaction between L-kynurenine treatment and prepulse intensity (F (2,32) = 1.12, NS). In the startle threshold block, there was a main effect of stimulus intensity (F (4,64) = 38.89, $p < .001$), but no difference in startle threshold between the L-kynurenine treated mice and the saline control (F (1,16) = 1.22, NS).

3.5. Assessment of anxiety following neonatal L-kynurenine treatment

To assess anxiety, male and female mice were tested separately in the elevated plus maze and in the light/dark box tests. In these studies no differences with regard to gender was observed.

Elevated plus maze—Percent entries and percent time in the open arms did not differ between mice neonatally treated with L-kynurenine or saline (data not shown).

Light/dark box test—There were no differences in the number of light compartment entries, the percentage of time spent in the light compartment or the latency to first leave the light compartment between mice neonatally treated with L-kynurenine or saline (data not shown).

3.6. Learning and memory assessment following neonatal L-kynurenine treatment

To assess learning and memory in neonatally L-kynurenine and saline treated C57BL/6 mice, we tested animals with a trace fear conditioning paradigm. Percent freezing during each phase of the task was measured. In the training stage, a two-way repeated measures ANOVA revealed a significant main effect (Treatment F (1,22) = 24.82, p <.001; Time F (3, 66) = 15.80, p <.001; Interaction: F (3, 66) = 3.406, p <.01), Bonferroni multiple comparison test showed that the working memory after an unpleasant stimulus was significantly impaired in neonatally L-kynurenine-treated female mice compared to saline-treated female mice (mean ± S.E.M: L-kynurenine treatment: 9.89 ± 4.66 s, n = 12, controls: 40.22 ± 8.14 s, n = 12, p < .001, Fig. 5A). The neonatally L-kynurenine treated mice and the saline treated mice exhibited similar freezing times in the contextual assessment (Fig. 5B) and in the cued assessment (F (1,22) < 1, NS, Fig. 5C).

3.7. Measurement of dopamine release following neonatal L-kynurenine treatment

Mean basal concentration of DA in a perfusate collected over 30 min from striatum in awake, freely moving male mice did not differ between mice neonatally treated with L-kynurenine (4.22 nM ± 0.70; n = 5) or saline (4.80 nM ± 0.82; n = 6). Data were not corrected for in-vitro dialysis probe recovery. The d-amphetamine-induced release of striatal DA is summarized in Fig. 6. Systemic administration of d-amphetamine (2 mg/kg, i.p.) was associated with a marked increase in DA release in the striatum in mice neonatally treated with L-kynurenine or saline. This effect reached its maximum 30 min after the d-amphetamine injection (+ 733%, in kynurenine-treated mice, n = 5, p < .001). A tendency towards an enhanced d-amphetamine-induced DA release in kynurenine-treated mice compared to saline treated mice (+ 437%, n = 6, NS) was observed (Between-group comparison: Interaction: F (11,99) = 1.67, p = .09; Time: F (11,99) = 20.15, p < .001; Treatment: F (1,9) = 1.51, p = .25).

3.8. Brain KYNA concentration in adult animals

In adult life, brain KYNA concentrations did not differ between controls and L-kynurenine-treated mice (mean ± S.E.M: Controls: 4.06 ± 0.51 nM, n = 9, L-kynurenine treatment: 5.37 ± 1.10 nM, n = 8, p = 0.37) or between uninfected controls and influenza-treated mice (mean ± S.E.M: uninfected controls: 1.69 ± 0.16 nM, n = 10, influenza A virus-infected mice: 2.03 ± 0.20 nM, n = 8, p = 0.17). Extracellular KYNA levels in microdialysates, collected over 45 min from striatum in awake, freely moving male mice, did not differ between mice neonatally treated with L-kynurenine (2.27 nM ± 0.32; n = 3) and saline treated mice (2.02 nM ± 0.54; n = 4). Extracellular levels of 3-hydroxykynurenine were found to be below the lowest level of detection (data not shown).

DISCUSSION

The present study shows that a neonatal infection with a neurotropic mouse-adapted strain of influenza A virus is associated with enhanced responsiveness to d-amphetamine in adult animals, as measured in the open field. Similarly, neonatal administration of L-kynurenine, leading to elevated brain levels of endogenous KYNA during treatment, enhanced the locomotor responsiveness to d-amphetamine in adult mice. d-amphetamine administration also tended to potentiate DA release in the striatum of adult mice following neonatal treatment with L-kynurenine. In addition, mice neonatally treated with L-kynurenine displayed mild reductions of PPI as well as reduced working memory in the trace fear conditioning task as adults. Despite a reduction in working memory in the L-kynurenine treated mice, we did not observe significant changes in context fear memory, suggesting that the hippocampus and/or other related areas (prefrontal cortex and amygdala) might only be partly affected in this model (Han et al., 2003; Runyan et al., 2004). Consistent with Chess and colleagues, no changes were observed during the tone-cued assessment (Chess et al., 2009).

The neonatal L-kynurenine treatment was associated with subtle disruptions in PPI in adult mice. The decreased PPI at the 500 ms interstimulus interval suggests that processing time is reduced in L-kynurenine-exposed mice. If the active processing of the prepulse is not maintained for as long in these mice, the period of filtering is shorter which results in less prepulse inhibition. The data on PPI in the neonatal L-kynurenine treated mice should be interpreted with caution, however, since the only statistically significant decrease in PPI was observed at the 500 ms interstimulus interval. Previously we reported that PPI was not affected in wild-type mice neonatally infected with influenza A virus (Asp et al., 2010). This discrepancy may possibly be explained by a more robust and lasting increase in brain KYNA concentration in the L-kynurenine treated mice compared to the infected mice. This view is in line with the behavioral deficits observed in adult immunodeficient mice exposed to the same neonatal infection, as these mice exhibited a more persistent induction of the kynurenine pathway in early-life (Asp et al., 2010) (Asp et al., 2009).

In line with our previous study (Asp et al., 2009), we found no alterations in spontaneous locomotor activity in mice neonatally infected with influenza A virus. However, administration of d-amphetamine potentiated the locomotor response in the infected mice as well as in mice neonatally treated with L-kynurenine as compared to control mice. Generally, d-amphetamine produces a bimodal locomotor response in C57BL/6 mice, where increased locomotion predominates at lower doses (≤6 mg/kg) but is gradually displaced by stereotypic behavior at higher doses (Yates et al., 2007). Presently observed changes in behavioral response to 5 mg/kg of d-amphetamine should thus mostly reflect an increase in locomotor activity and not a reduction in stereotypic behavior. No stereotypic behavior was observed when mice were removed from the locomotor chambers. The closed chambers, however, prevented explicit measurements of stereotypic behavior in the mice during the experiments.

Our previous studies indicated that C57BL/6 mice neonatally infected with influenza A virus display normal behavior with regard to PPI, anxiety, and spatial working memory (Asp et al., 2009, 2010). However, when using the same model of neonatal infection in immunodeficient mice lacking functional CD8+ T cells, *i.e.* $Tap1^{-/-}$ mice, we observed deficits within these domains in adult animals (Asp et al., 2009, 2010). Thus, neonatal infection along with a genetic variation affecting immune function appears to more potently affect PPI, working memory and anxiety, tentatively related to the more robust elevation of brain KYNA as compared to environmental exposure only. Present results also indicate that neonatal treatment with L-kynurenine is sufficient to induce mild deficits in PPI and

working memory in adult animals. Taken together, these findings suggest that the more robust increase in brain KYNA concentration in the L-kynurenine treated mice (present study) and in the infected *Tap1*$^{-/-}$ mice (Asp et al., 2009, 2010) contributed to the behavioral deficits in adult animals.

The underlying mechanism behind the enhanced responsiveness to d-amphetamine in adult mice treated neonatally with influenza A virus or with L-kynurenine is intriguing. With regard to the development of schizophrenia, it has been suggested that the last months of gestation and the first years of life is the most vulnerable period of exposure to environmental insults, such as a CNS infection (Marenco and Weinberger, 2000). The time point of infection in the present study (i.e. P3/P4) corresponds to approximately late 2nd to early 3rd trimester of human gestation with regard to brain development (Rice and Barone, 2000). The present experimental protocol of systemic influenza infection is thus designed to mimic a hematogenous route of infection of the human fetus. During this period (the brain growth spurt), neurons are very sensitive to specific disturbances in their synaptic activity (Olney, 2002). As previously reported, the neonatal influenza infection is associated with increased transcription of the gene encoding IDO and increased levels of brain KYNA (Asp et al., 2010; Holtze et al., 2008). Interestingly, administration of other NMDA receptor antagonists, such as PCP during early neonatal life has been reported to result in apoptotic neurodegeneration in the developing brain (Ikonomidou, 2009; Rudin et al., 2005; Young et al., 2005) and persistent behavioral deficits (Fredriksson et al., 2007; Harris et al., 2003; Mouri et al., 2007) in rodents. NMDA receptor blockade in early postnatal life is also suggested to alter the maturation process of the parvalbumin interneuron circuitry tentatively increasing the risk of developing mental diseases in adulthood (Powell et al., 2012). It is thus possible that the behavioral alterations observed in adult mice in the present study are related to neonatal blockade of the NMDA receptor and consequent altered parvalbumin interneuron circuitry. In addition, the ability of KYNA to block the α7nAChR may also have contributed to the longterm developmental actions (Ross et al., 2010). In agreement, with our current findings, earlylife elevation of brain KYNA concentration, from gestational day 15 to postnatal day 21, has been reported to cause dysfunctions in cognitive flexibility, assessed by an attentional set-shifting task, as well as deficits in the passive avoidance test and the Morris water maze test in the adult rat (Pocivavsek et al., 2012; Alexander et al., 2013). As neonatal L-kynurenine-treatment was associated with a reduced PPI and working memory deficits as well as a potentiated d-amphetamine induced DA response, the long-term effects of the neonatal virus infection might be due to a transient increase of endogenous brain KYNA in early-life hereby affecting the development of neuronal circuits important for sensorimotor gating and cognitive functions. This view is in agreement with the neurodevelopmental theory of schizophrenia and other related psychotic disorders, suggesting the early-life as a particularly vulnerable period for environmental insults (Ikonomidou et al., 1999; Lu et al., 2010; Marenco and Weinberger, 2000; Mohn et al., 1999; Olney and Farber, 1995).

In contrast to a recent rat microdialysis study showing increased extracellular KYNA levels in adulthood following pre- and postnatal oral kynurenine treatment (Pocivavsek et al., 2012), we did not observe increased KYNA content in adulthood following neonatal influenza infection or neonatal L-kynurenine treatment. This discrepancy might be related to species differences as well as differences in administration routes of kynurenine and/or in exposure periods. Subchronic elevation of brain KYNA in adult rodents, possibly mimicking a pathophysiological condition in patients with psychiatric disorders (Erhardt et al., 2001b; Linderholm et al., 2012; Olsson et al., 2010, 2012b, Lavebratt et al., 2013), has previously been shown to potentiate the d-amphetamine-induced increase in brain dopamine efflux (Olsson et al., 2009) and in locomotor activity (Olsson et al., 2012a). Furthermore, acutely increased brain KYNA in rats is associated with disruptions in PPI (Erhardt et al., 2004).

Although the increase in brain KYNA concentration was restricted to the period of kynurenine treatment, the present data are in line with these observations as well as with the enhanced striatal dopamine release by d-amphetamine seen in brain imaging studies in patients with schizophrenia (c.f. Introduction), thus suggesting that elevation of KYNA during a critical period in neonatal life produces long-lasting effects with regard to behavior and neurotransmission related to schizophrenia.

Although KYNA is suggested to play a pathophysiological role in dopamine-related disorders, e.g. schizophrenia and bipolar disorder (Erhardt et al., 2001a; Nilsson et al., 2005; Erhardt et al., 2007; Erhardt et al., 2009; Olsson et al., 2010, 2012b, Linderholm et al., 2012, Lavebratt et al., 2013), the present results do not establish a causal relation between KYNA and the behavioral deficits since other metabolites of the kynurenine pathway, e.g. quinolinic acid, a NMDA receptor agonist, and 3-hydroxykynurenine, a free radical generator, may also increase following neonatal influenza virus infection or L-kynurenine treatment, respectively. With regard to infection it is well established that not only KYNA, but also quinolinic acid levels increase (Heyes et al., 1992). Expression of enzymes downstream kynurenine, i.e. kynurenine monooxygenase (KMO), kynureninase (KYNU) and 3-hydroxyanthranilate 3,4-dioxygenase (HAAO), were induced in the brain following the neonatal influenza virus infection (Holtze et al., 2008). Therefore, central levels of other kynurenine metabolites, including quinolinic acid may also have been transiently elevated in these mice. Induction of this arm of the kynurenine pathway has been linked to depression-like behavior in both clinical (Raison et al., 2010, Erhardt et al., 2013) and experimental studies (see Dantzer et al., 2008) and mice infected with influenza virus might experience both sickness behavior and depressive-like symptoms at the time of infection. However, with regard to the neonatal kynurenine treatment, it has been shown that a rise in kynurenine levels disproportionately favors the synthesis of KYNA over that of quinolinic acid (Amori et al., 2009a; Guidetti et al., 1995). Thus, the behavioral effects observed following neonatal administration of L-kynurenine may be specifically related to increased levels of brain KYNA rather than any other kynurenine pathway metabolite. Since similar aberrations in behavior were observed in mice neonatally treated with L-kynurenine or infected with influenza virus, these persistent effects might be due to a transient increase in KYNA during a critical period of neurodevelopment.

In conclusion, the results of the present study show that a neonatal CNS influenza virus infection potentiates d-amphetamine-induced increase in locomotor response in adult mice. The present results also suggest that neonatal NMDA-receptor and/or α7nAChR blockade, by elevated brain KYNA levels, induces mild impairments in PPI and working memory as well as exaggerates d-amphetamine-induced DA responses. Neonatally elevated brain KYNA levels, might thus link infections in early-life with disturbed behavior in adulthood in accordance with the developmental theory of neuropsychiatric disorders. In addition, the present results suggest that early-life elevation of KYNA in the brain might serve as a novel animal model of schizophrenia development.

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REFERENCES

Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M, van Dyck CH, Charney DS, Innis RB, Laruelle M. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am. J. Psychiatry. 1998; 155:761–767. [PubMed: 9619147]

Adler CM, Malhotra AK, Elman I, Goldberg T, Egan M, Pickar D, Breier A. Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia. Am. J. Psychiatry. 1999; 156:1646–1649. [PubMed: 10518181]

Alexander KS, Pocivavsek A, Wu HQ, Pershing ML, Schwarcz R, Bruno JP. Early developmental elevations of brain kynurenic acid impair cognitive flexibility in adults: reversal with galantamine. Neuroscience. 2013; 238:19–28. [PubMed: 23395862]

Amori L, Guidetti P, Pellicciari R, Kajii Y, Schwarcz R. On the relationship between the two branches of the kynurenine pathway in the rat brain in vivo. J. Neurochem. 2009a; 109:316–325. [PubMed: 19226371]

Amori L, Wu HQ, Marinozzi M, Pellicciari R, Guidetti P, Schwarcz R. Specific inhibition of kynurenate synthesis enhances extracellular dopamine levels in the rodent striatum. Neuroscience. 2009b; 159:196–203. [PubMed: 19138730]

Andreasen NC. Symptoms, signs, and diagnosis of schizophrenia. Lancet. 1995; 346:477–481. [PubMed: 7637483]

Angrist BM, Gershon S. The phenomenology of experimentally induced amphetamine psychosis--preliminary observations. Biol. Psychiatry. 1970; 2:95–107. [PubMed: 5459137]

Asp L, Nellaker C, Karlsson H. Influenza A virus transactivates the mouse envelope gene encoding syncytin B and its regulator, glial cells missing 1. J. Neurovirol. 2007; 13:29–37. [PubMed: 17454446]

Asp L, Beraki S, Kristensson K, Ogren SO, Karlsson H. Neonatal infection with neurotropic influenza A virus affects working memory and expression of type III Nrg1 in adult mice. Brain. Behav. Immun. 2009; 23:733–741. [PubMed: 19362585]

Asp L, Holtze M, Powell SB, Karlsson H, Erhardt S. Neonatal infection with neurotropic influenza A virus induces the kynurenine pathway in early life and disrupts sensorimotor gating in adult Tap1−/− mice. Int. J. Neuropsychopharmacol. 2010; 13:475–485. [PubMed: 19607757]

Atlas A, Gisslen M, Nordin C, Lindstrom L, Schwieler L. Acute psychotic symptoms in HIV-1 infected patients are associated with increased levels of kynurenic acid in cerebrospinal fluid. Brain. Behav. Immun. 2007; 21:86–91. [PubMed: 16603336]

Blomström A, Karlsson H, Wicks S, Yang S, Yolken RH, Dalman C. Maternal antibodies to infectious agents and risk for non-affective psychoses in the offspring--a matched case-control study. Schizophr. Res. 2012; 140:25–30. [PubMed: 22819777]

Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl). 2001; 156:234–258. [PubMed: 11549226]

Breier A, Su TP, Saunders R, Carson RE, Kolachana BS, de Bartolomeis A, Weinberger DR, Weisenfeld N, Malhotra AK, Eckelman WC, Pickar D. Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc. Natl. Acad. Sci. USA. 1997; 94:2569–2574. [PubMed: 9122236]

Brown AS, Derkits EJ. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am. J. Psychiatry. 2010; 167:261–280. [PubMed: 20123911]

Carlsson A, Lindqvist M. Effect of Chlorpromazine or Haloperidol on Formation of 3-Methoxytyramine and Normetanephrine in Mouse Brain. Acta. Pharmacol. Toxicol. (Copenh). 1963; 20:140–144. [PubMed: 14060771]

Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson ML. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. Annu. Rev. Pharmacol. Toxicol. 2001; 41:237–260. [PubMed: 11264457]

Carlsson A, Carlsson ML. A dopaminergic deficit hypothesis of schizophrenia: the path to discovery. Dialogues Clin. Neurosci. 2006; 8:137–142. [PubMed: 16640125]

Chess AC, Landers AM, Bucci DJ. L-kynurenine treatment alters contextual fear conditioning and context discrimination but not cue-specific fear conditioning. Behav. Brain Res. 2009; 201:325–331. [PubMed: 19428652]

Cibelli M, Fidalgo AR, Terrando N, Ma D, Monaco C, Feldmann M, Takata M, Lever IJ, Nanchahal J, Fanselow MS, Maze M. Role of interleukin-1beta in postoperative cognitive dysfunction. Ann. Neurol. 2010; 68:360–368. [PubMed: 20818791]

Costall B, Jones BJ, Kelly ME, Naylor RJ, Tomkinks DM. Exploration of mice in a black and white test box: Validation as a model of anxiety. Pharmacol. Biochem. Behav. 1988; 31:777–785.

Crnic LS, Pizer LI. Behavioral effects of neonatal herpes simplex type 1 infection of mice. Neurotoxicol. Teratol. 1988; 10:381–386. [PubMed: 3226382]

Cruickshank CC, Dyer KR. A review of the clinical pharmacology of methamphetamine. Addiction. 2009; 104:1085–1099. [PubMed: 19426289]

Dalman C, Allebeck P, Gunnell D, Harrison G, Kristensson K, Lewis G, Lofving S, Rasmussen F, Wicks S, Karlsson H. Infections in the CNS during childhood and the risk of subsequent psychotic illness: a cohort study of more than one million Swedish subjects. Am. J. Psychiatry. 2008; 165:59–65. [PubMed: 18056223]

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 2008; 9:46–56. [PubMed: 18073775]

Ellman LM, Yolken RH, Buka SL, Torrey EF, Cannon TD. Cognitive functioning prior to the onset of psychosis: the role of fetal exposure to serologically determined influenza infection. Biol. Psychiatry. 2009; 65:1040–1047. [PubMed: 19195645]

Engel JA, Zhang J, Bergstrom T, Conradi N, Forkstam C, Liljeroth A, Svensson L. Neonatal herpes simplex virus type 1 brain infection affects the development of sensorimotor gating in rats. Brain Res. 2000; 863:233–240. [PubMed: 10773211]

Erhardt S, Blennow K, Nordin C, Skogh E, Lindström LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. Neurosci. Lett. 2001a; 313:96–98. [PubMed: 11684348]

Erhardt S, öberg H, Mathe JM, Engberg G. Pharmacological elevation of endogenous kynurenic acid levels activates nigral dopamine neurons. Amino Acids. 2001b; 20:353–362. [PubMed: 11452979]

Erhardt S, Schwieler L, Emanuelsson C, Geyer M. Endogenous kynurenic acid disrupts prepulse inhibition. Biol. Psychiatry. 2004; 56:255–260. [PubMed: 15312813]

Erhardt S, Schwieler L, Nilsson L, Linderholm K, Engberg G. The kynurenic acid hypothesis of schizophrenia. Physiol. Behav. 2007; 92:203–209. [PubMed: 17573079]

Erhardt S, Olsson SK, Engberg G. Pharmacological manipulation of kynurenic acid: potential in the treatment of psychiatric disorders. CNS Drugs. 2009; 23:91–101. [PubMed: 19173370]

Erhardt S, Lim CK, Linderholm KR, Janelidze S, Lindqvist D, Samuelsson M, Lundberg K, Postolache TT, Tr&#xfffd;skman-Bendz L, Guillemin GJ, Brundin L. Connecting inflammation with glutamate agonism in suicidality. Neuropsychopharmacology. 2013; 38:743–752. [PubMed: 23299933]

Fredriksson A, Ponten E, Gordh T, Eriksson P. Neonatal exposure to a combination of N-methyl-D-aspartate and gamma-aminobutyric acid type A receptor anesthetic agents potentiates apoptotic neurodegeneration and persistent behavioral deficits. Anesthesiology. 2007; 107:427–436. [PubMed: 17721245]

Gold LH, Brot MD, Polis I, Schroeder R, Tishon A, de la Torre JC, Oldstone MB, Koob GF. Behavioral effects of persistent lymphocytic choriomeningitis virus infection in mice. Behav. Neural. Biol. 1994; 62:100–109. [PubMed: 7993301]

Griffith JD, Cavanaugh J, Held J, Oates JA. Dextroamphetamine. Evaluation of psychomimetic properties in man. Arch. Gen. Psychiatry. 1972; 26:97–100. [PubMed: 4551260]

Guidetti P, Eastman CL, Schwarcz R. Metabolism of [5-3H]kynurenine in the rat brain in vivo: evidence for the existence of a functional kynurenine pathway. J. Neurochem. 1995; 65:2621–2632. [PubMed: 7595559]

Han CJ, O'Tuathaigh CM, van, Trigt L, Quinn JJ, Fanselow MS, Mongeau R, Koch C, Anderson DJ. Trace but not delay fear conditioning requires attention and the anterior cingulate cortex. Proc. Natl. Acad. Sci. USA. 2003; 100:13087–13092. [PubMed: 14555761]

Harris LW, Sharp T, Gartlon J, Jones DN, Harrison PJ. Long-term behavioural, molecular and morphological effects of neonatal NMDA receptor antagonism. Eur. J. Neurosci. 2003; 18:1706–1710. [PubMed: 14511349]

Heyes MP, Saito K, Crowley JS, Davis LE, Demitrack MA, Der M, Dilling LA, Elia J, Kruesi MJ, Lackner A, et al. Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. Brain. 1992; 115:1249–1273. [PubMed: 1422788]

Hoffman HS, Ison JR. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. Psychol. Rev. 1980; 87:175–189. [PubMed: 7375610]

Holtze M, Asp L, Schwieler L, Engberg G, Karlsson H. Induction of the kynurenine pathway by neurotropic influenza A virus infection. J. Neurosci. Res. 2008; 86:3674–3683. [PubMed: 18655201]

Holtze M, Mickiené A, Atlas A, Lindquist L, Schwieler L. Elevated cerebrospinal fluid kynurenic acid levels in patients with tick-borne encephalitis. J. Intern. Med. 2012; 272:394–401. [PubMed: 22443218]

Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vockler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science. 1999; 283:70–74. [PubMed: 9872743]

Ikonomidou C. Triggers of apoptosis in the immature brain. Brain Dev. 2009; 31:488–492. [PubMed: 19307071]

Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry. 1991; 148:1301–1308. [PubMed: 1654746]

Javitt DC. Glutamate as a therapeutic target in psychiatric disorders. Mol. Psychiatry. 2004; 9:984–997. 979. [PubMed: 15278097]

Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology. 1999; 20:201–225. [PubMed: 10063482]

Kessler M, Terramani T, Lynch G, Baudry M. A glycine site associated with Nmethyl- D-aspartic acid receptors: characterization and identification of a new class of antagonists. J. Neurochem. 1989; 52:1319–1328. [PubMed: 2538568]

Kim JJ, Fanselow MS. Modality-specific retrograde amnesia of fear. Science. 1992; 256:675–677. [PubMed: 1585183]

King NJ, Thomas SR. Molecules in focus: indoleamine 2,3-dioxygenase. Int. J.Biochem. Cell Biol. 2007; 39:2167–2172. [PubMed: 17320464]

Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, D'Souza CD, Erdos J, McCance E, Rosenblatt W, Fingado C, Zoghbi SS, Baldwin RM, Seibyl JP, Krystal JH, Charney DS, Innis RB. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc. Natl. Acad. Sci. USA. 1996; 93:9235–9240. [PubMed: 8799184]

Lavebratt C, Olsson S, Backlund L, Frisén L, Sellgren C, Priebe L, Nikamo P, Tr&#xe;skman-Bendz L, Cichon S, Vawter MP, Osby U, Engberg G, Landén M, Erhardt S, Schalling M. The KMO allele encoding Arg452 is associated with psychotic features in bipolar disorder type 1, and with increased CSF KYNA level and reduced KMO expression. Mol. Psychiatry. 2013

Linderholm KR, Andersson A, Olsson S, Olsson E, Snodgrass R, Engberg G, Erhardt S. Activation of rat ventral tegmental area dopamine neurons by endogenous kynurenic acid: A pharmacological analysis. Neuropharmacology. 2007; 53:918–924. [PubMed: 17959203]

Linderholm KR, Skogh E, Olsson SK, Dahl ML, Holtze M, Engberg G, Samuelsson M, Erhardt S. Increased Levels of Kynurenine and Kynurenic Acid in the CSF of Patients With Schizophrenia. Schizophr. Bull. 2012; 38:426–432. [PubMed: 20729465]

Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology. 1987; 92:180–185. [PubMed: 3110839]

Lu L, Mamiya T, Lu P, Toriumi K, Mouri A, Hiramatsu M, Kim HC, Zou LB, Nagai T, Nabeshima T. Prenatal exposure to phencyclidine produces abnormal behaviour and NMDA receptor expression in postpubertal mice. Int J Neuropsychopharmacol. 2010; 13:877–889. [PubMed: 19835658]

Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R. Study of a new schizophrenomimetic drug; sernyl. AMA. Arch. Neurol. Psychiatry. 1959; 81:363–369.

Marenco S, Weinberger DR. The neurodevelopmental hypothesis of schizophrenia: following a trail of evidence from cradle to grave. Dev. Psychopathol. 2000; 12:501–527. [PubMed: 11014750]

Meyer U, Feldon J. Epidemiology-driven neurodevelopmental animal models of schizophrenia. Prog. Neurobiol. 2010; 90:285–326. [PubMed: 19857543]

Mohn AR, Gainetdinov RR, Caron MG, Koller BH. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell. 1999; 98:427–436. [PubMed: 10481908]

Mouri A, Noda Y, Enomoto T, Nabeshima T. Phencyclidine animal models of schizophrenia: approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. Neurochem. Int. 2007; 51:173–184. [PubMed: 17669558]

Nilsson LK, Linderholm KR, Engberg G, Paulson L, Blennow K, Lindström LH, Nordin C, Karanti A, Persson P, Erhardt S. Elevated levels of kynurenic acid in the cerebrospinal fluid of male patients with schizophrenia. Schizophr. Res. 2005; 80:315–322. [PubMed: 16125901]

Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. Arch. Gen. Psychiatry. 1995; 52:998–1007. [PubMed: 7492260]

Olney JW. New insights and new issues in developmental neurotoxicology. Neurotoxicology. 2002; 23:659–668. [PubMed: 12520755]

Olsson SK, Andersson AS, Linderholm KR, Holtze M, Nilsson-Todd LK, Schwieler L, Olsson E, Larsson K, Engberg G, Erhardt S. Elevated levels of kynurenic acid change the dopaminergic response to amphetamine: implications for schizophrenia. Int. J. Neuropsychopharmacol. 2009; 12:501–512. [PubMed: 18796185]

Olsson SK, Samuelsson M, Saetre P, Lindstrom L, Jonsson EG, Nordin C, Engberg G, Erhardt S, Landen M. Elevated levels of kynurenic acid in the cerebrospinal fluid of patients with bipolar disorder. J. Psychiatry Neurosci. 2010; 35:195–199. [PubMed: 20420770]

Olsson SK, Larsson MK, Erhardt S. Subchronic elevation of brain kynurenic acid augments amphetamine-induced locomotor response in mice. J. Neural Transm. 2012a; 119:155–163. [PubMed: 21904895]

Olsson SK, Sellgren C, Engberg G, Landén M, Erhardt S. Cerebrospinal fluid kynurenic acid is associated with manic and psychotic features in patients with bipolar I disorder. Bipolar Disord. 2012b; 14:719–726. [PubMed: 23030601]

Pocivavsek A, Wu HQ, Elmer GI, Bruno JP, Schwarcz R. Pre- and postnatal exposure to kynurenine causes cognitive deficits in adulthood. Eur. J. Neurosci. 2012; 35:1605–1612. [PubMed: 22515201]

Powell SB, Sejnowski TJ, Behrens MM. Behavioral and neurochemical consequences of cortical oxidative stress on parvalbumin-interneuron maturation in rodent models of schizophrenia. Neuropharmacology. 2012; 62:1322–1331. [PubMed: 21315745]

Raison CL, Dantzer R, Kelley KW, Lawson MA, Woolwine BJ, Vogt G, Spivey JR, Saito K, Miller AH. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. Mol. Psychiatry. 2010; 15:393–403. [PubMed: 19918244]

Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ. Health Perspect. 2000; 108(S3):511–533. [PubMed: 10852851]

Ross RG, Stevens KE, Proctor WR, Leonard S, Kisley MA, Hunter SK, Freedman R, Adams CE. Research review: Cholinergic mechanisms, early brain development, and risk for schizophrenia. J. Child PsycholPsychiatry. 2010; 51:535–549.

Rudin M, Ben-Abraham R, Gazit V, Tendler Y, Tashlykov V, Katz Y. Singledose ketamine administration induces apoptosis in neonatal mouse brain. J. Basic Clin. Physiol. Pharmacol. 2005; 16:231–243. [PubMed: 16438390]

Runyan JD, Moore AN, Dash PK. A role for prefrontal cortex in memory storage for trace fear conditioning. J. Neurosci. 2004; 24:1288–1295. [PubMed: 14960599]

Sathyasaikumar KV, Stachowski EK, Wonodi I, Roberts RC, Rassoulpour A, McMahon RP, Schwarcz R. Impaired Kynurenine Pathway Metabolism in The Prefrontal Cortex of Individuals With Schizophrenia. Schizophr. Bull. 2011; 37:1147–1156. [PubMed: 21036897]

Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. Biol. Psychiatry. 2001; 50:521–530. [PubMed: 11600105]

Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci. 2012; 13(7):465–477. [PubMed: 22678511]

Schwieler L, Linderholm KR, Nilsson-Todd LK, Erhardt S, Engberg G. Clozapine interacts with the glycine site of the NMDA receptor: electrophysiological studies of dopamine neurons in the rat ventral tegmental area. Life Sci. 2008; 83:170–175. [PubMed: 18590745]

Soderlund J, Schroder J, Nordin C, Samuelsson M, Walther-Jallow L, Karlsson H, Erhardt S, Engberg G. Activation of brain interleukin-1beta in schizophrenia. Mol. Psychiatry. 2009; 14:1069–1071. [PubMed: 19920835]

Terrando N, Monaco C, Ma D, Foxwell BM, Feldmann M, Maze M. Tumor necrosis factor-alpha triggers a cytokine cascade yielding postoperative cognitive decline. Proc. Natl. Acad. Sci. USA. 2010; 107:20518–20522. [PubMed: 21041647]

Tohmi M, Tsuda N, Watanabe Y, Kakita A, Nawa H. Perinatal inflammatory cytokine challenge results in distinct neurobehavioral alterations in rats: implication in psychiatric disorders of developmental origin. Neurosci. Res. 2004; 50:67–75. [PubMed: 15288500]

Yates JW, Meij JT, Sullivan JR, Richtand NM, Yu L. Bimodal effect of amphetamine on motor behaviors in C57BL/6 mice. Neurosci. Lett. 2007; 427:66–70. [PubMed: 17920769]

Yolken RH, Torrey EF. Are some cases of psychosis caused by microbial agents? A review of the evidence. Mol. Psychiatry. 2008; 13:470–479. [PubMed: 18268502]

Yoon T, Okada J, Jung MW, Kim JJ. Prefrontal cortex and hippocampus subserve different components of working memory in rats. Learn. Mem. 2008; 15:97–105. [PubMed: 18285468]

Young C, Jevtovic-Todorovic V, Qin YQ, Tenkova T, Wang H, Labruyere J, Olney JW. Potential of ketamine and midazolam, individually or in combination, to induce apoptotic neurodegeneration in the infant mouse brain. Br. J. Pharmacol. 2005; 146:189–197. [PubMed: 15997239]

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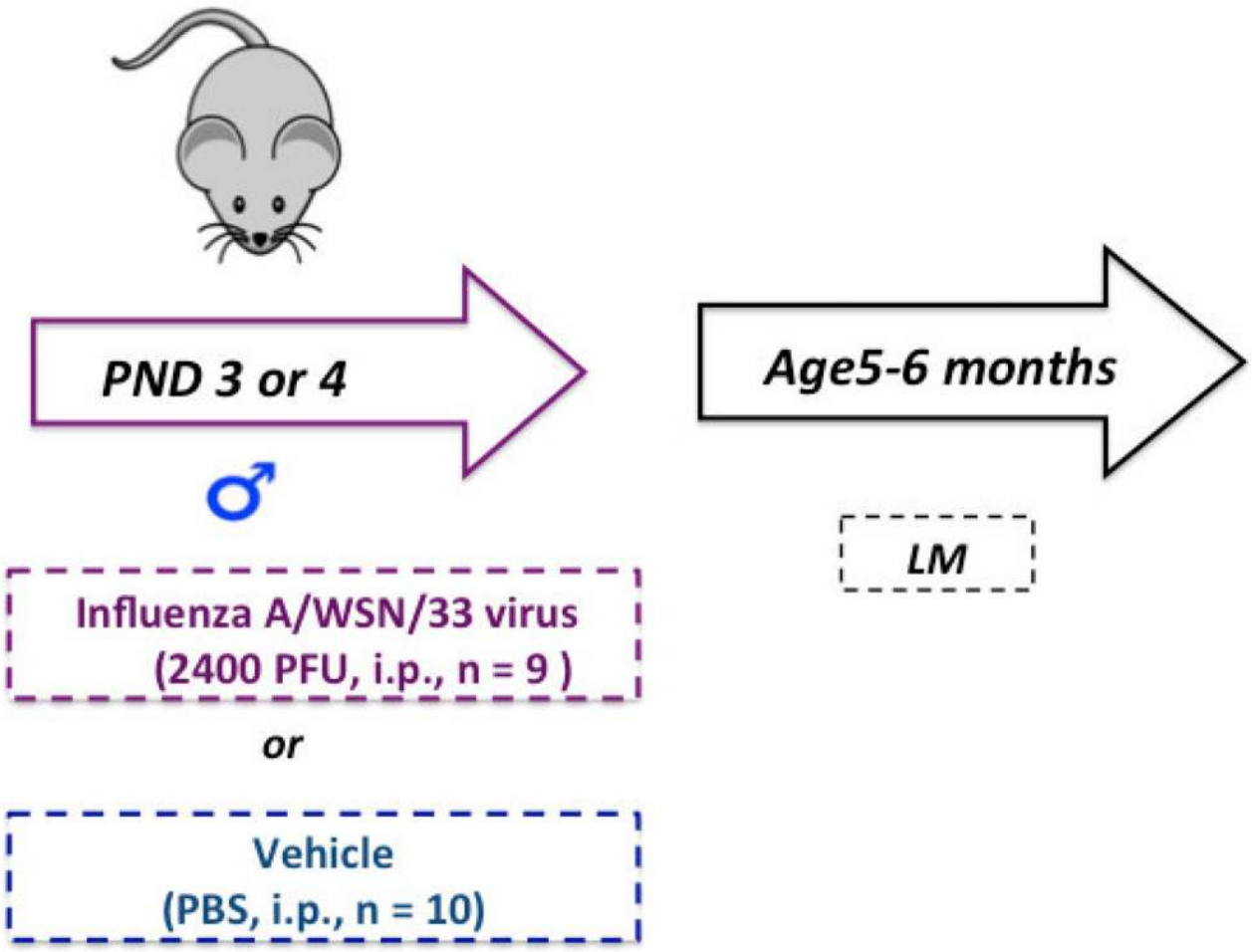

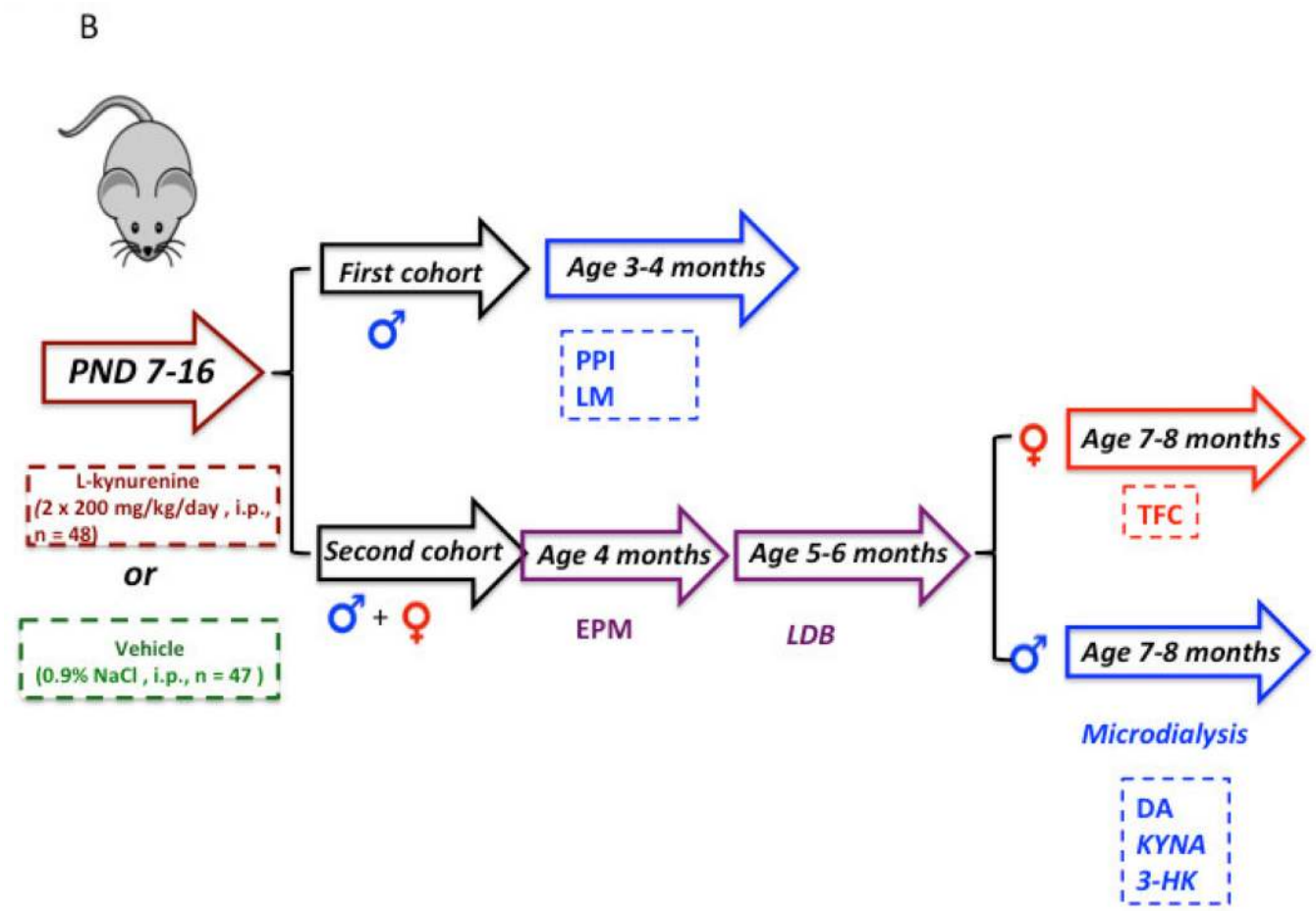

Fig. 1.

(A) Schematic representation of the neonatal influenza model. Male C57BL/6 mice were infected i.p. with 2400 plaque-forming units of mouse adapted neurotropic influenza A/WSN/33 virus at P3 or P4 (n = 9). Control mice were injected similarly with PBS (n = 10). At the age of 5–6 months, locomotor activity was assessed using an open field apparatus for mice.

(B) Schematic representation of the neonatal kynurenine model. Two cohorts of C57BL/6 mice were i.p. injected with L-kynurenine sulfate salt (2×200 mg/kg/day, every 12^{th} hour for ten days starting at P7 (first cohort; n = 11 (only males), second cohort; n = 37 (19 males and 18 females)). Similarly, control mice were injected with saline (0.9% NaCl; first cohort; n = 13 (only males), second cohort: n = 34 (17 males and 17 females). In the first cohort, PPI and locomotor activity (using an open field apparatus for mice) was assessed. In the second cohort of mice (both males and females) neonatally treated with L-kynurenine or saline, anxiety status by means of elevated plus-maze (age 4 months) and the light dark box test (5–6 months), respectively. Then the trace fear conditioning test (age 7–8 months) was performed to female mice in order to analyze contextual memory and male mice underwent microdialysis (age 7–8 months) for investigations of striatal dopamine release as well as of extracellular levels of KYNA and 3-hydroxykynurenine.

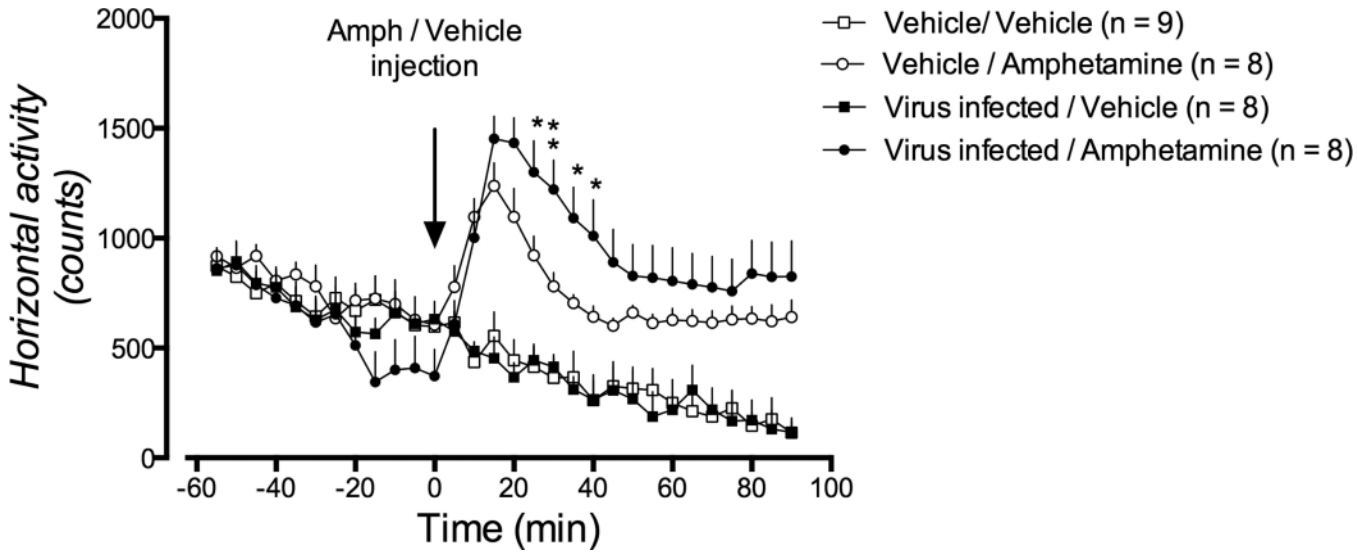

Fig. 2. Locomotor activity following neonatal treatment of influenza

Horizontal activity during habituation three and following acutely administered d-amphetamine (5 mg/kg) or vehicle to 5–6 months old mice, injected with influenza A virus (2400 plaque-forming units) or phosphate buffered saline (PBS) at postnatal day 3 or 4. Each point represents the mean ± S.E.M. of counts recorded during five-minute intervals. Statistical analysis was performed by a two-way ANOVA for repeated measurements (time × treatment) followed by Bonferroni's multiple comparison test (Interaction: $F(51, 493) = 3.629$, $p < .001$; Time: $F(17, 493) = 25.34$, $p < .001$; Treatment: $F(3, 29) = 16.29$, $p < .001$).

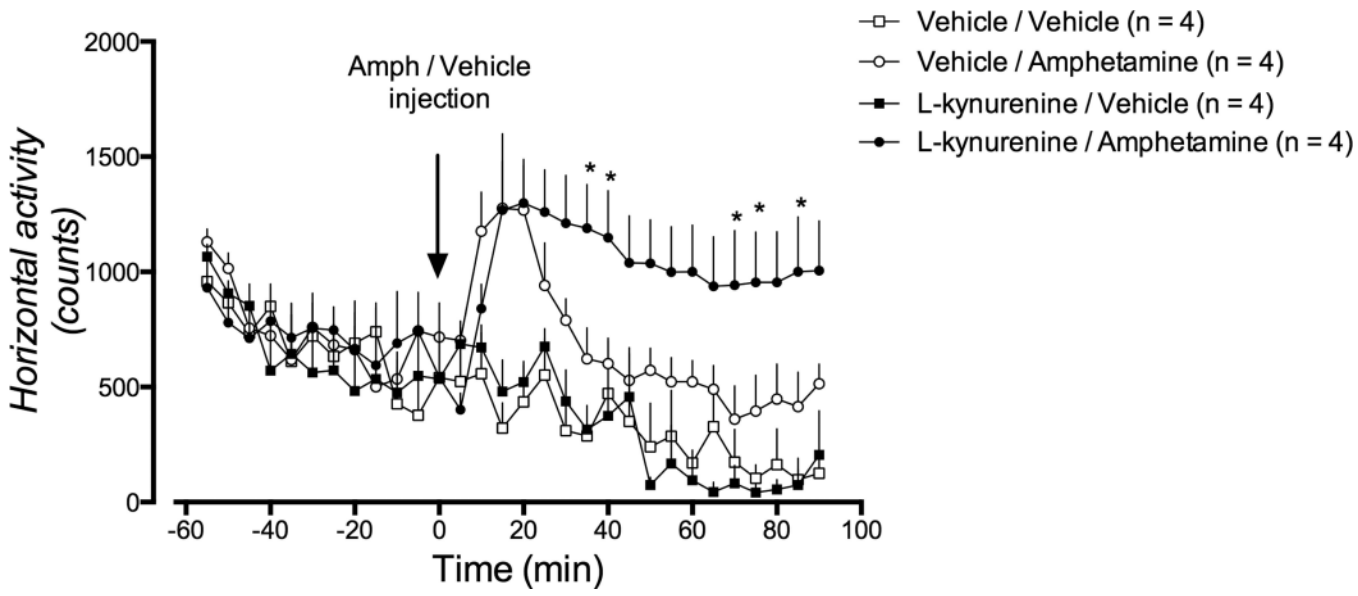

Fig. 3. Locomotor activity following neonatal treatment of kynurenine

Horizontal activity during habituation three and following acutely administered d-amphetamine (5 mg/kg) or vehicle to 3–4 months old mice, injected with L-kynurenine (2×200 mg/kg/day) or saline at postnatal day 7–16. Each point represents the mean ± S.E.M of counts recorded during five-minute intervals. Statistical analysis was performed by a two-way ANOVA for repeated measurements (time × treatment) followed by Bonferroni's multiple comparison test (Interaction: F (51, 204) = 2.631, $p < .001$; Time: F (17, 204) = 10.61, $p < .001$; Treatment: F (3, 12) = 8.788, $p < .01$).

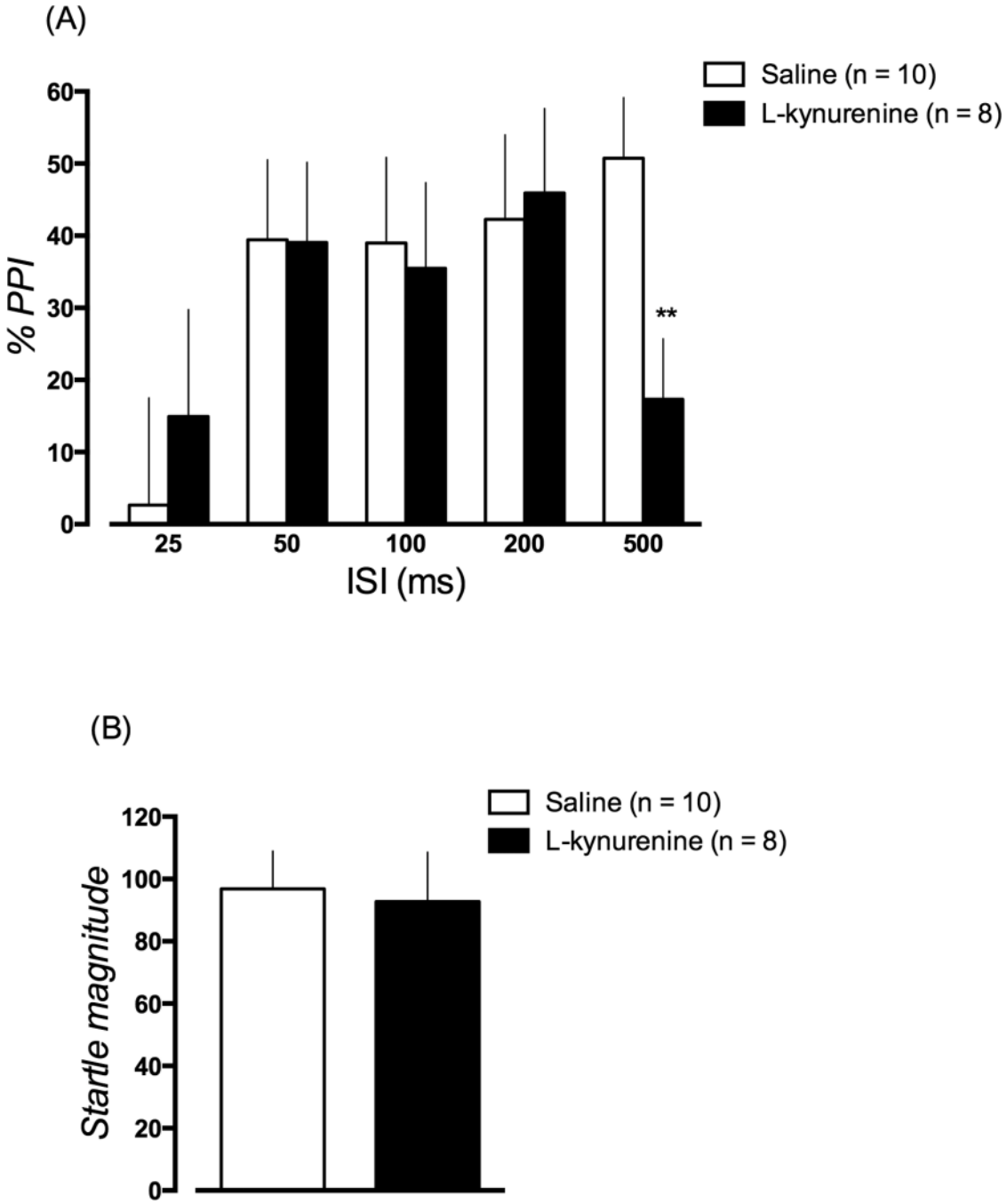

Fig. 4. Prepulse inhibition following neonatal treatment of kynurenine

(A) Prepulse inhibition (PPI) and (B) startle magnitude during the varied interstimulus interval (ISI) block of the startle session in 3–4 months old mice, neonatally injected with L-kynurenine (2×200 mg/kg/day, n = 8) or saline at postnatal day 7–16 (n = 10). Statistical analysis was performed using a one-factor ANOVA, followed by a Tukey's post hoc multiple comparison test. F (4,64) = 3.37, $p < .05$, interaction between L-kynurenine treatment and ISI. ** $p < .01$, statistically different from saline control mice. Data are presented as mean ± S.E.M.

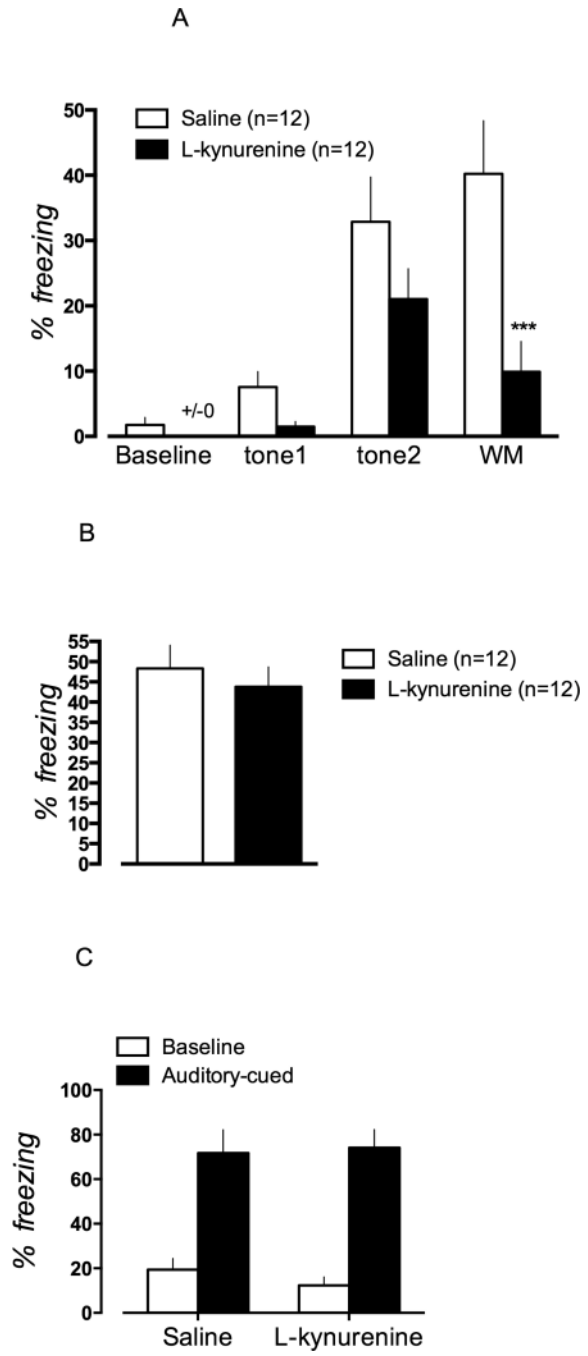

Fig. 5.

Trace fear conditioning of L-kynurenine treated and saline treated C57BL/6 female mice. (A) Percent component time freezing during the training trial was analyzed in the following time periods: Baseline, first tone cue (Tone1), Inter-trial interval (ITI), second tone cue (Tone2) and working memory (WM). Data were analyzed with two-way ANOVA for repeated measurements (time × pretreatment) followed by Bonferroni multiple comparison test, which revealed a significant impairment of working memory in L-kynurenine treated mice, as compared to saline treated controls, (Treatment: F (1, 22) = 24.82, ***p < .001). No changes in the contextual fear response (B) or the tone cued assessment (C) were observed.

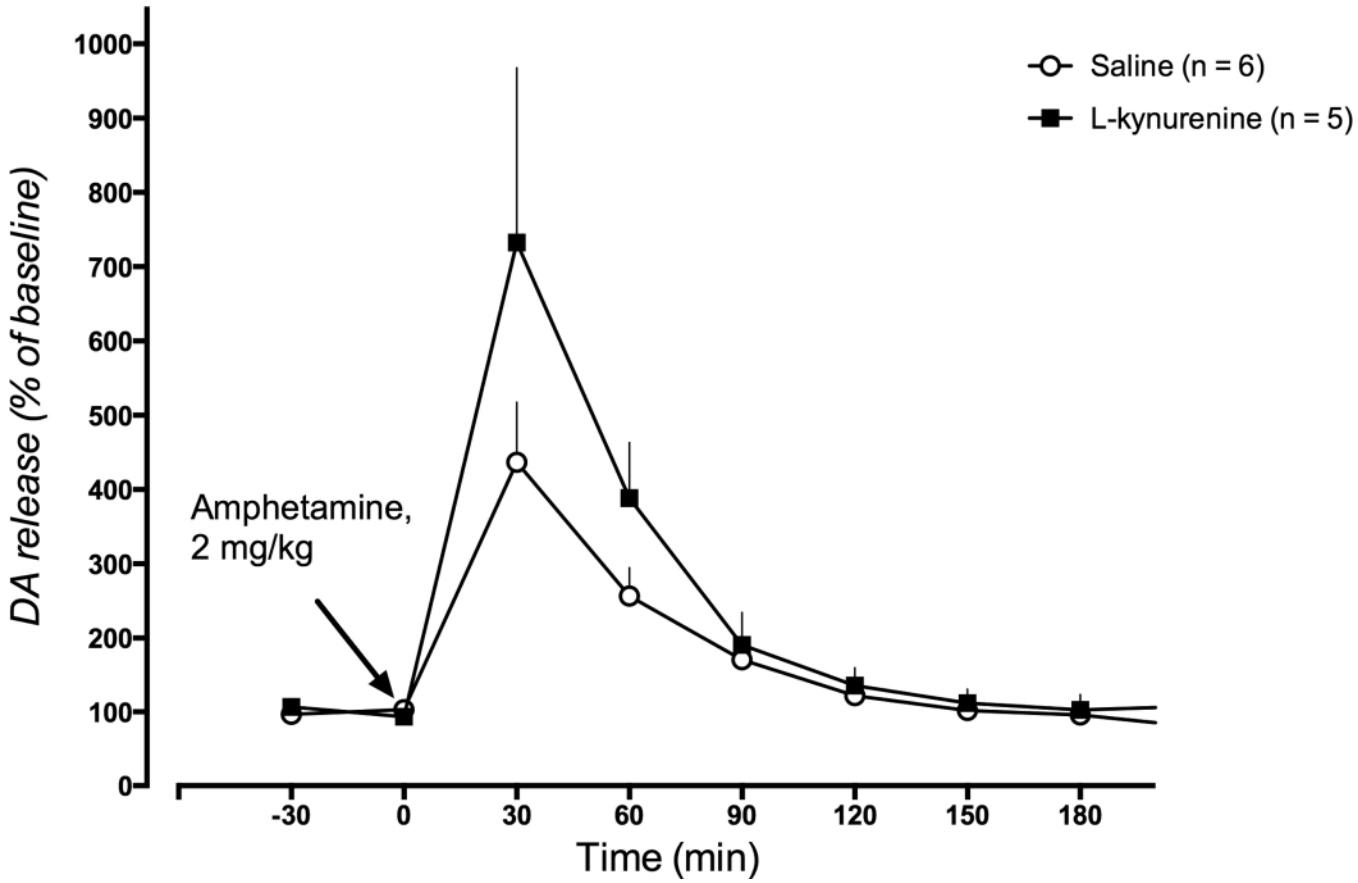

Fig. 6. Measurement of dopamine release following neonatal L-kynurenine treatment

Effects of d-amphetamine (2 mg/kg i.p.) administration on dopamine output in the nucleus accumbens in awake, freely moving mice. Each point represents the mean ± S.E.M. percent of baseline (n = 5–6). Between-group comparisons revealed a trend towards enhanced response in kynurenine-treated mice (two-way ANOVA for repeated measurements (time × treatment); Interaction: $F(11,99) = 1.67$, $p = .09$; Time: $F(11,99) = 20.15$, $p < .001$; Treatment: $F(1,9) = 1.51$, $p = .25$)).

Induction of the kynurenine pathway in early life is associated with long-lasting effects with regard to behavior and neurotransmission related to schizophrenia.