Supplementary material

This supplementary section provides more detail on the methods used in the accompanying manuscript. This section also includes Supplemental Figures S1, S2 and S3 and the figure legends.

PPI testing

PPI was tested in the San Diego Instruments (San Diego, CA) SD Inst SR-Lab Startle Response System. The startle pulse was 120dB for 40msec, and pre-pulses were either 75 or 80dB for 20msec, with a continuous background of 65dB. Prepulse-startle latency was 100ms, with inter-trial time of 15-30 seconds. Trials were either pulse alone, pulse + pre-pulse (PP75, 80) or no stimulus (background alone). Testing sessions began with five pulse alone trials followed by 10 trials each of pulse alone, PP75, PP80, or no stimulus in a pseudorandom order, and finished with five pulse alone trials, with a total of 50 testing trials per session. Scores for each measure were averaged across the three sessions. PPI was calculated as: [(startle only – prepulse+startle)/(startle only)] × 100. The overall PPI score for each strain was the mean of the 75dB and 80dB trials. Mice were tested at the same time each day to reduce potential circadian effects. Mice with persistently low acoustic startle response (≤100) or negative PPI scores were excluded (2/36 mice).

Microarray analysis of mRNA transcript levels

Mice were sacrificed by cervical dislocation on PND 60. The striatum, hippocampus, and brainstem were dissected on ice and frozen at -80°C. RNA was extracted from brain with TRIzol® reagent (Invitrogen, Mississauga, ON), as previously described.(1) Samples were treated with DNAse1, and eluted twice using the RNeasy mini spin kit (#74104, Qiagen,

Toronto, ON). RNA was concentrated using a Microcon centrifugal filter device (Millipore YM-100 membrane), quantified by spectrophotometric analysis (OD260/280) and integrity was measured with an agarose-formaldehyde gel. RNA was amplified and biotin labelled with the Enzo IVT kit.

The Affymetrix (Cleveland, OH) mouse genome MOE 430 2.0 GeneChip® array was used to measure the mRNA levels of > 34,000 genes using 45,037 probe sets. Three arrays were run per strain, for each of the three tissues, for a total of 54 arrays (18 per brain region). Because we sought to determine mRNA expression differences between and not within strains, we pooled RNA samples for each strain and tissue group from three individual mice per array and combined samples from both left and right sides of the brain. Array hybridization and imaging was performed by The Centre for Applied Genomics (Toronto, Ontario, Canada).

Raw array data were loaded into the R statistical environment (v2.6.1) using the affy package (v1.16.0) of the BioConductor open-source project.(2, 3) Data were pre-processed with the GCRMA variant of the RMA algorithm,(4) as implemented in the gcrma package (v2.10.0) of BioConductor. To determine the relationship between mRNA expression levels and PPI scores, we employed two complementary statistical approaches: first, we performed a transcriptome-wide correlation analysis by calculating Spearman's correlation coefficient between the behavioural scores and the expression levels for each probe set across the six strains. This analysis generated 45,101 correlation values, that were assessed for statistical significance,(5) coupled with a false-discovery rate adjustment.(6) Second, we performed a linear modelling analysis in which the 18 arrays for each tissue were simultaneously fit to the linear-model: $y = b + (m)\ x\ (PPI)$, where y is the GCRMA pre-processed signal intensity, b the expression level, PPI levels for a given strain, and m the slope of the relationship between PPI

and mRNA levels. This analysis used the limma software package (v2.12.0), and was followed by a Bayesian moderation of standard-error(7) and a false-discovery rate adjustment for multiple-testing.(6)

Gene annotations for each probe set were obtained from Affymetrix NetAffx (v27).(8) For each probe set, we calculated an FDR-adjusted p-value (q-value) and slope from the linear-modelling analysis, and a correlation and a q-value from the correlation analysis. To facilitate candidate gene selection, a composite score combining the q-values from the three brain regions was calculated as: score = -log| q(H) x q(S) x q(B) |, where 'H' is hippocampus, 'S' is striatum and 'B' is brainstem.

RT-PCR verification of microarray results

Reverse transcription of mRNA to cDNA was performed with the Omniscript RT kit (Qiagen GmbH) with 5 μg of RNA, according to the manufacturers' instructions. cDNA was synthesized from the same set of RNA used for the microarrays. Pooled and individual samples of RNA were used to quantify RNA with real-time RT-PCR. There were a total of 24 samples per tissue (1 tested and 2 control pools per strain plus individual samples from 5-6 mice (PPI tested) and 6 mice with no PPI testing, for n=11-12 mice for each of the 6 strains, covering 3 tissues). The testing control group was used to determine if PPI testing altered gene expression.

Real-time RT-PCR experiments were conducted on an ABI Prism 7000 (Applied Biosystems Inc) following the ABI Gene Expression Assay kit instructions. The control genes were: GAPDH and β-actin ((#4308313 and #4352933E TaqMan® Gene Expression Assays) with DNase-free water used as negative control on each plate. Samples were run in quadruplicate. Gene expression assays selected for validation were: Dbndd2 (Mm10243779_m1), Rgs5 (Mm00501393_m1), Hist1H2bc (Mm_00517807_m1), Dnm3

(Mm_00554098_m1) and Pdxdc1 (Mm_00459752_m1) (all TaqMan® Gene Expression Assays, from ABI). PCR cycle parameters were: 2min at 50°C, 10min at 95°C, then 40 cycles of 15sec at 95°C followed by 1min at 60°C. Expression levels of each mRNA were calculated as a ratio of the candidate gene/geometric mean of GAPDH and β-actin C_T values, generating a relative (not absolute) expression level for each mRNA sample tested.(9) Regression coefficients were calculated using the relative Standard Curve Method.(10) Array and RT-PCR expression values were compared by Spearman correlation, and rho and p-values were calculated. Relative RT-PCR expression values were also correlated with behavioural scores.

PDXDC1 cloning, protein production and purification

A human PDXDC1 cDNA clone (GenBank: BC060871; Structural Genomics Consortium, Toronto, ON, the kind gift of Dr. A Iakounine), and a full length fragment (amino acids 1-788) was cloned into vector pMCSG53 using Ligation Independent Cloning.(11) Forward: TACTTCCAATCCAATGCCATGGACGCGTCCC and reverse: TTATCCACTTCCAATGTTATCTTAAGCTCTCCGGTCCTTC primers were purchased from Integrated DNA Technologies (Coralville, Iowa, USA) and inserts sequenced at ACGT Corporation (Toronto, Canada). Vector and insert were transformed into DH5α with In-Fusion® HD Cloning Plus pellet (Clonetech, 638909) for insert screening on LB/ampicillin plates with 5% sucrose, extracted with the QIAprep Spin Miniprep Kit (Qiagen, 27104), and inserted into *E.coli* BL21 DE3 for expression. Protein was grown and purified as previously described.(12) 6L cultures were grown in TB (BioShop, Burlington, ON. TER409) with glycerol, seeded with overnight culture and 100µg/mL ampicillin and grown at 37°C until the culture reached OD0.70. 100mg/mL IPTG (BioShop, IPT001) was added, protein grown 18°C overnight, then cells were pelleted and frozen. Cells were thawed and re-suspended in 45mL of binding buffer (BB,

250mM NaCl, 5% Glycerol, 50mM HEPES pH7.5, 5mM Imidazole), then sonicated and lysate centrifuged. 2.5ml of packed, BB-equilibriated Ni-NTA Superflow beads (Qiagen, 30450) were used per 1x25cm glass column during the purification process. Cleared lysate was placed on the column and allowed to flow through, then washed with 50mL of wash buffer (250mM NaCl, 5% Glycerol, 50mM HEPES pH7.5, 30mM Imidazole), and protein eluted with 13ml of elution buffer (250mM NaCl, 5% Glycerol, 50mM HEPES pH7.5, 250mM Imidazole). Flow through was processed through a second nickel bead column as above. Concentration was checked with Bradford reagent (Sigma-Aldrich, Oakville, ON, B6916).

Primary antibody validation and Western Blots

Tissue was homogenized in 4°C RIPA buffer (150mM NaCl, 1.0% Triton X-100 (Bioshop), 0.5% NA deoxycholate, 0.1% SDS, 50mM Tris pH7.4 and 2mM EDTA pH 8.0 in ddH2O). Protease inhibitor (Sigma-Aldrich, P8340-5ML) and 0.1% phenylmethanesulfonyl fluoride (PMSF, Sigma-Aldrich, 78830) were added at final concentration of 1mM, and lysate agitated for 1.5hr before centrifuging at 13.5xg for 10min, and supernatant retained. Protein was quantified with a Pierce BCA Protein Assay as per kit instructions (Pierce BCA Protein Assay #23228 and 23224, ThermoScientific) using a albumin standard curve (Thermo Scientific #23209). 50μg total protein for both PDXDC1 and mouse lysate samples from surgical and naïve tissue were boiled with 5x Laemelli buffer at 95°C for 5min, then loaded onto a SDS-PAGE gel (4% loading gel and 7.5% 1mm separating gel) and run in 1xTris-glycine SDS buffer (BioShop TGS222.4) at 90V for 30min, then at 120V until the dye front reached the end of the gel with PageRuler prestained (Thermo Scientific #26619) or Precision Plus Protein™Kaleidoscope™ (Bio-Rad, Mississauga, ON, #161-0375) ladders.

A wet transfer was run in 50mM boric acid (Bioshop), 2.55mM EDTA (Bioshop), pH8.9 onto nitrocellulose (Pall Life Sciences, Port Washington, NY, BioTraceTM NT #66485) at 390mA for 2hr on ice, rinsed in TBST and blocked for 2hr at room temperature (RT) with 5% skim milk powder (BioShop, SKI400). The blot was rinsed 2x in TBST for 5min then incubated overnight with 1° antibody in TBST with 1% BSA and 0.02% NaH_3 at: 1:300 (Rabbit anti-Pdxdc1 (polyclonal, Abcam,, Cambridge, MA, ab83209) and 1:5000 (Rabbit anti-beta actin (polyclonal, Abcam ab8227). Blots were washed 3x with TBST, then incubated 1hr at RT with 2° (1:5000 Goat anti-rabbit HRP IgG (Abcam, ab6721)). Blots were washed 3x in TBST and protein was detected with BioRad Clarity™ Western ECL Substrate (**170-5060)** on a BioRad ChemiDoc™ MP imaging system, quantity was analyzed using ImageLab™ (v.1.4) (BioRad, Mississauga, ON).

In Situ Hybridization and mRNA distribution in brain

12 week old C57BL6/J mice (Jackson Laboratories, Bar Harbour, Maine) were anaesthetized with Avertin® and were transcardially perfused with saline at 4°C at 5ml/min. Brains were removed, snap frozen on dry ice, then stored at -80°C. 20μM sections were cut on a Leica cryostat and thaw-mounted onto Fisher Superfrost™ slides (VWR, Mississauga, ON). ISH procedures followed those described in (13). Briefly: Probe primers were developed against regions of the mRNA sequence for transcript variant 1 (NM_053181.2) so that they would not align with any other known expressed mRNA sequence. Primers were designed to generate a 375 base pair pre-probe aligning with bases 1976 to 2350. Primers: *forward*: 5'atttaggtgacactatagaatgcagtcacagcccgggaaa3', aligning with bases 1976-1995, and *reverse:* 5'taatacgactcactataggacagcatccgagcctctcaggg3', aligning with 2329-2350 (ACGT Corporation, Toronto, ON), and included a (reverse) T7 primer sequence and (forward) SP6 primer sequence.

Mouse hippocampal cDNA prepared for real-time RT-PCR experiments was used as the template. Slides were dried and Kodak (Toronto, ON) BioMax film was exposed for 21 days along with calibrated radioactivity standards before film was processed. Probe specificity was confirmed by testing labeled sense probes which produced no measurable signal on film.

Densitometry analyses were performed with MCID 7.0 (InterFocus, Linton UK) with illumination adjusted for each film for consistent background readings. Raw optical density (ROD) values were converted to radioactivity levels in μCi/g via calibrated radioactivity standards. Regions defined according to a mouse brain atlas(14) were sampled in 4-5 sections per animal, and readings averaged across all sections to produce the density value per region.

Immunohistochemistry using DAB to visualize Pdcdx1 protein distribution

12 week old male C57BL6/J mice (Jackson Laboratories) were anaesthetized with Avertin® and transcardially perfused with saline then 4% PFA at 5ml/min. Brains were removed and stored overnight in 4% PFA, then went through increasing concentrations of sucrose (10-30%) in phosphate buffer (275μM thimerosal, 150mM $NaH_2PO_4.H_2O$, 120mM NaOH), snap frozen and stored at -80°C. Brains were mounted in Tissue-Tek® Optimal Cutting Temperature (OCT) compound (Fisher Scientific, 14-373-65) and 40μM sections cut on a Leica cryostat and retained in cryoprotectant solution (phosphate buffer, ethylene glycol and glycerol 1.5:1:0.8) at -20°C. Sections were rinsed with PBS + 0.02% NaN3, then TBS before being treated with TBS+0.25% Triton X-100 for 30min, rinsed in TBS and blocked for 1hr in 2% BSA and 5% goat serum in TBS. Rabbit anti-Pdxdc1 (Abcam, ab83209, 1:300) antibody was diluted in 2% BSA in TBS and sections were incubated overnight. Sections were rinsed 6x in TBS+0.05% Tween 20 (TBST) and incubated in 2° biotinylated anti-rabbit IgG (Vectastain Elite ABC kit, Vector Laboratories, Burlington, ON, PK-6101) for 2hr and rinsed 3x with TBST. ABC-HRP was made in TBS+0.1%

Tween 20, sections were incubated 1hr, then rinsed 2x with TBS and developed with the DAB Peroxidase (HRP) Substrate kit (SK-4100) with nickel. Tissue was washed and float mounted onto Fisher Superfrost™ slides (VWR, Mississauga, ON), dried and dehydrated in increasing concentrations of ethanol, cleared in xylenes and coverslipped. Negative control tissue was processed without 1° antibody. No endogenous peroxide blocking was required. ROD measures were taken using the same process as for ISH, without conversion to radioactivity levels.

Results for IHC and ISH stains were averaged for each region for all animals. Mean, standard deviation (SD) and standard error of the mean (SEM) were calculated and used to generate a set of criteria to assess differential expression from the tissue mean (a composite average from all 105 regions sampled), or from PPI regions (n=32). Mean+SEM were plotted for each region, and overlaid with tissue mean (thick dotted) and ±2x SEM (thinner dotted) lines. Relative comparisons were made between ISH and IHC expression values based on each region's position relative to the tissue mean and 2x SEM confidence interval. Linear regression and correlational analyses were performed between IHC and ISH methods in the following manner: all 105 regions, PPI regions, and non-PPI regions using GraphPad Prism 4.0.

Lentiviral experiments

9 week old male C57BL6/J mice (Jackson Laboratories) were housed at The Centre for Phenogenomics (TCP; Toronto, ON), 5 mice per cage with 12hr light-dark cycle (0700-1900), 50-60% humidity, at 21±1°C, with *ad libitum* food (Purina LabDiet® mouse chow) and water, with regular handling. Mice were acclimated for 1 week before pre-operative PPI testing. One day later, mice underwent viral injection surgery. Experiments were carried out as per Figure 4(A). Apparatuses were cleaned between animals with Clidox-S® (Fisher Scientific, 96118). Behavioural tests were run between 0900-1700h, and mice acclimated to each testing room 30

min before testing. All procedures were approved by the Animal Management Committee of TCP, the Biosafety Officer of Mt. Sinai Hospital (Toronto, ON), and the Animal Care Committee of CAMH (Toronto, ON) and were conducted in accordance with the requirements of the Province of Ontario Animals for Research Act 1971 and the Canadian Council on Animal Care.

shRNA construct preparation and packaging into lentivirus

GIPZ constructs for mouse Pdxdc1 shRNAmir #V2LMM_63165 (*V2LMM*- targets: NM_001039533 open reading frame (ORF) and NM_053181 ORF, mature anti-sense sequence: TTTGCAACAAGCAGCAAGG) and #V3LMM_453828 (*V3LMM*- targets: NM_001039533 ORF, and NM_053181 ORF, mature anti-sense sequence: TAACTTTTTCAGAAGTCCA), non-silencing GIPZ shRNAmir negative control #RHS4346 (-*shRNA* - mature antisense sequence: CTTACTCTCGCCCAAGCGAGAG)(all from Thermo Fisher Scientific, Ottawa, ON). Constructs were grown in the provided bacterial cells in Luria Broth with ampicillin at 37°C overnight to OD600 and maxi-prepped (GenElute HP Endotoxin-Free Plasmid Maxiprep Kit, NA0400, Sigma-Aldrich). 250µg DNA was concentrated to 1µg/µL with Microcon Ultracel 10-membrane 10kDa (Millipore, #MCRPRT010) then sent to the SIDNET facility (The Hospital for Sick Kids, Toronto, ON) for lentiviral packaging with the Open Biosystems TransLenti™ Viral GIPZ Packaging System (Thermo Fisher Scientific, USA). Virus production, concentration and titering were conducted as per GIPZ lentiviral packaging product manual (15) with titres of: $1.64X10^9$ TU/mL (V2LMM_631165), $1.02x10^9$ TU/mL (V3LMM_453828), and $2.1x10^8$ TU/mL (RHS4346).

Surgery and infusion

Mice were anaesthetized using Isoflurane (Abbott Animal Health, IL), and placed on a Stoeltling stereotaxic frame. The scalp was shaved and treated alternately with 70% ethanol (3x) and Betadine® (2x). A single incision was made and two 1mm diameter holes were drilled at -2mm from bregma, +/-2mm from midline. A gastight microsyringe (Model 1701 RN SYR 10µL Hamilton (Reno, NV) syringe, #80030) with a 26s gauge needle with a non-coring tip, mounted on a manual stereotaxic injector (Stoelting Co., Wood Dale, IL, 51639) was used to deliver the virus. The needle was lowered to -2.5mm from the surface of the brain, and 2.5µL of virus was delivered at 0.5µL/min. The needle was left in place for 3min after injection then withdrawn at 1mm/min. Mice were dosed 2x with 2mg/kg daily meloxicam (Boehringer Ingelheim, Burlington, ON), group housed after surgery and recovered 2 weeks before testing resumed.

Behaviour testing after shRNA injection into brain

Prepulse Inhibition

PPI testing in shRNA injected mice was modified from previous reports.(16) Briefly, prepulses of 81, 73, 69dB were used. Testing sessions consisted of the following: PA, pulse + prepulse (PP81, PP73, PP69), prepulse alone (P81, P73, P69) or no stimulus (NS) which consisted of background (65dB). Each session had 5 PA trials followed by 10-trials each of PA, PP81, PP73, PP69, P81, P73, P69, NS in a pseudo-randomized order and finished with 5 PA trials, resulting in a total of 90 testing trials per session. Mice were tested 7 times – once pre-operatively, and then post-operatively 6 times, once per week.

Locomotor Activity

Mice were tested for 1hr in dimly lit (120 lux) Plexiglas locomotor boxes with infrared sensors and automated beam break recording (Versamax 4.12-AFE, Accusan Instruments Inc, Columbus

OH). Horizontal, vertical, timecourse/habituation, time spent in centre of field and in periphery of field were analyzed, using Versadat v3.02-1AFE (Accusan Instruments Inc, Columbus OH).

Y-maze and spontaneous alternation

Spontaneous alternation on the Y-maze was measured in 5min periods as previously described.(17) A three-arm horizontal maze (40cm x 8cm x 15cm per arm, Noldus Information Technology, the Netherlands) with arms at 120° from one another was used. Maze floors were opaque grey polyvinyl plastic and walls were white, black, or patterned polyvinyl plastic. Arm entries needed all four paws inside the arm. Spontaneous alternation required visiting all three arms in sequence (e.g. ABC, BCA, but not CBC), and a % alternation was calculated as: #alternations/(total possible # alternations - 2)x100.(18, 19)

Morris Water Maze

MWM protocol was modified from previous reports.(20, 21) A 1.2m diameter, 0.5m deep circular pool filled with water at 23-24°C made opaque with Tempera non-toxic paint was used with a transparent platform 12cm in diameter, 0.35m high placed in the centre of the southeast (SE) quadrant of the tank. Peripheral optical markers were placed around the testing room at the four cardinal points, and an additional high-contrast visual marker was placed at the SE corner of the room, such that it would be a clear indicator of the platform location. A video camera and Water 2020 ™ tracker PLUS+ ™ (HVS Image Ltd, UK) were used to acquire and analyze the data. *Day 1* of testing consisted of training/assessment of swimming capability of mice to find the platform when it was visible with a high contrast marker. *Days 2-6 - Training days:* Mice were given 4 training trials per day, with semi-random starts from N, E, S, and W quadrants of the tank. Mice were allowed to swim until they found the platform and mounted it, or until 60sec had elapsed. If the mouse did not find the platform, it was guided to the platform by hand. The

mouse was allowed to remain on the platform for 10sec after it was found/had been guided there the first day, and 5sec each trial for every day thereafter. Mice were tested each day until the control groups exhibited escape latency of ~10sec, at which point a probe test was made. Between each trial, mice were removed from the tank, dried, and allowed to rest approximately 10min in the home cage before being retested from a new start position. *Day 7 - Probe sessions:* Mice underwent 2 probe trials on probe day, with starts from the centre of the tank, and swam for the full 60sec. *Measures taken during training and probe days*: Latency to find the platform (in sec), number of crossovers of the platform, time spent in the target quadrant, time spent in thigmotaxis, speed of swimming during the trial, time spent floating during the trial.

Statistical Analyses

One or two-way ANOVA (depending on parameters of the test) with *post hoc* analysis of Tukey HSD or Bonferroni correction for multiple testing, Student's paired or unpaired two-tailed t-tests with or without Welch's correction (as indicated) were conducted using SPSS 15 or 17 (SPSS Inc). Graphs and some analyses were made using GraphPad Prism 5.0 (La Jolla, CA).

Tissue analysis for knockdown

After behavioural testing, brain tissue was analyzed by IHC, Western blot, and real-time RT-PCR methods to assess infection range, protein and mRNA knockdown.

Immunohistochemistry to determine infection localization and protein knockdown

Mice recovered 24hrs after the last behavioural test, and brains were harvested, prepared, sectioned and stained as previously described. Tissue from each brain was divided (one section at a time) between rabbit anti-tGFP (polyclonal, Pierce Antibodies through Thermo-Fisher Scientific, PA5-22688, 1:5000 dilution) and rabbit anti-Pdxdc1 (polyclonal, Abcam, ab83209,

1:400 dilution). Sections were mounted and observed at 4x and 10x magnification for distribution of tGFP stain in the tissue as well as to quantify whether sections adjacent those staining with tGFP had less Pdxdc1 staining than in the regions staining for tGFP. Densitometric quantification was conducted as previously described.

Western blot and real-time RT-PCR to quantify protein and mRNA knockdown

Mice recovered 24hr after the last behavioural test, then were cervically dislocated, had the hippocampus dissected out, snap frozen and stored at -80°C until processing.

Western blot: One hippocampus hemisphere was used for Western blot, the other for real-time RT-PCR. After BCA quantification of whole lysate protein concentration, 25μg of total protein was loaded per well along with 6x Laemmeli loading buffer. Western blotting to quantify Pdxdc1 between treatment groups was conducted as previously described. Pdxdc1 and β-actin were probed. Protein quantity was analyzed using ImageLab™ (v.1.4): absolute protein levels were quantified from digital blot image, and Pdxdc1 normalized to β-actin in the same lane. Normalized values were averaged and compared between treatment groups. Real-time RT-PCR was conducted as above.

REFERENCES

1. Semeralul MO, Boutros PC, Likhodi O, Okey AB, Van Tol HH, Wong AH. Microarray analysis of the developing cortex. J Neurobiol. 2006;66(14):1646-58.

2. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 2004;5(10):R80.

3. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics. 2004;20(3):307-15.

4. Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res. 2003;31(4):e15.

5. Best DJ, Roberts DE. Algorithm AS 89: The upper tail probabilities of Spearman's rho. Applied Statistics. 1975;24:377-9.

6. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci USA. 2003;100(16):9440-5.

7. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol. 2004;3:Article3.

8. Cheng J, Sun S, Tracy A, Hubbell E, Morris J, Valmeekam V, et al. NetAffx Gene Ontology Mining Tool: a visual approach for microarray data analysis. Bioinformatics. 2004.

9. Freeman WM, Walker SJ, Vrana KE. Quantitative RT-PCR: pitfalls and potential. Biotechniques. 1999;26(1):112-22, 24-5.

10. Larionov A, Krause A, Miller W. A standard curve based method for relative real time PCR data processing. BMC Bioinformatics. 2005;6:62.

11. Eschenfeldt W, Stols L, Sanville MIllard C, Joachimiak A, Donnelly M. A family of LIC vectors for high-throughput cloning and purification of proteins. Methods Mol Biol. 2009;498:105-15.

12. Beloglazova N, Brown G, Zimmerman MD, Proudfoot M, Makarova KS, Kudritska M, et al. A novel family of sequence-specific endoribonucleases associated with the clustered regularly interspaced short palindromic repeats. J Biol Chem. 2008;283(29):20361-71.

13. Nona CN, Guirguis S, Nobrega JN. Susceptibility to ethanol sensitization is differentially associated with changes in pCREB, trkB and BDNF mRNA expression in the mouse brain. Behav Brain Res. 2013;242:25-33.

14. Franklin KB, Paxinos G. The Mouse Brain in Stereotaxic Coordinates. 3rd ed. Amsterdam: Elsevier/Academic Press; 2008. 360 p.

15. Scientific T. Thermo Scientific Trans-Lentiviral Packaging Kits. Thermo Scientific; 2013.

16. Lipina T, Labrie V, Weiner I, Roder J. Modulators of the glycine site on NMDA receptors, D-serine and ALX 5407, display similar beneficial effects to clozapine in mouse models of schizophrenia. Psychopharmacology (Berl). 2005;179(1):54-67.

17. Kim DH, Yoon BH, Kim YW, Lee S, Shin BY, Jung JW, et al. The seed extract of Cassia obtusifolia ameliorates learning and memory impairments induced by scopolamine or transient cerebral hypoperfusion in mice. J Pharmacol Sci. 2007;105(1):82-93.

18. Mandillo S, Tucci V, Holter SM, Meziane H, Banchaabouchi MA, Kallnik M, et al. Reliability, robustness, and reproducibility in mouse behavioral phenotyping: a cross-laboratory study. Physiol Genomics. 2008;34(3):243-55.

19. Naert A, Gantois I, Laeremans A, Vreysen S, Van den Bergh G, Arckens L, et al. Behavioural alterations relevant to developmental brain disorders in mice with neonatally induced ventral hippocampal lesions. Brain Res Bull. 2013;94:71-81.

20. Wenk GL. Assessment of spatial memory using the radial arm maze and Morris water maze. Curr Protoc Neurosci. 2004;Chapter 8:Unit 8 5A.

21. Yadav R, Gupta SC, Hillman BG, Bhatt JM, Stairs DJ, Dravid SM. Deletion of glutamate delta-1 receptor in mouse leads to aberrant emotional and social behaviors. PLoS One. 2012;7(3):e32969.