Transcriptomic Analysis in the Striatum Reveals an Involvement of Nurr1 in Social Behavior of Prenatally Valproic Acid-Exposed Mice

Hyunju Kim
Seoul National University College of Medicine

Eun-Jeong Yang
Seoul National University College of Medicine

Han-Byeol Kim
Eulji University

Eun hwa Jo
Seoul National University College of Medicine

Ran-Sook Woo
Eulji University

Seonghan Kim
Inje University - Gimhae Campus: Inje University

Kim Hye-Sun
Seoul National University  https://orcid.org/0000-0001-6321-061X

Research

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Abstract

Background

The striatum of the basal ganglia is the major subcortical component of the mammalian forebrain. Magnetic resonance imaging (MRI) studies have implicated surface deformation of the striatum in the brains of patients with autism spectrum disorders (ASD) and their correlation with behavioral phenotypes.

Methods

Using RNA sequencing (RNA-Seq), we analyzed transcriptome alteration in striatal tissues from 10-week-old prenatally valproic acid (VPA)-exposed BALB/c male mice. To investigate the relationship of Nurr1 with synaptic and social deficit, we activated the expression level of nuclear receptor related 1 protein (Nurr1) by i.p. injection of amodiaquine (AQ) for 2 weeks. Furthermore, we employed lentiviral system to inhibit the Nurr1 expression and provide evidence for the role of Nurr1 in social behavior.

Results

Transcriptomic analysis showed higher levels of genes related to synaptic function of neurons in striatal tissues from the prenatally VPA-exposed mice. Among those genes, Nurr1 expression was significantly upregulated. The treatment with AQ, which has been known to be a ligand for Nurr1, to saline-exposed mice mimicked social deficits and synaptic abnormality observed in prenatally VPA-exposed mice. Moreover, viral inhibition of Nurr1 markedly improved social deficits in prenatally VPA-exposed mice.

Limitations

This study did not identify the mechanism of how Nurr1 activation regulates striatum-related circuit. Identification of the mechanism will provide explanation for behavioral impairments in prenatally VPA-exposed mice.

Conclusions

Taken together, these results suggest that the increase in Nurr1 expression in the striatum is a mechanism related to the changes in synaptic deficits and behavioral phenotypes of VPA-induced ASD mouse model.

Introduction

ASD is a group of neurodevelopmental disorders characterized by behavioral symptoms, including deficits in social interaction and social communication, repetitive behaviors, and restricted interest. Nationwide population-based study in the USA reported that the ASD prevalence was 2.76% among US children and adolescents in 2016 [1]. Genetic, epigenetic, and environmental factors are reported to be
related to the induction of ASD. The concordance rates for ASD reach up to 88.1% in monozygotic twins and 30.5% in dizygotic twins [2]. Studies have investigated the genetic or epigenetic factors that likely contribute to ASD pathogenesis, using brains from the ASD patients and animal models, and patient-derived neurons. The investigation on ASD postmortem brains using RNA-Seq provided evidence for the enrichment of immune related genes and the downregulation of neuronal and synaptic genes in autism [3,4]. The induced pluripotent stem cell-derived neurons from patients with idiopathic ASD showed dysregulation of genes involved in neuronal differentiation, axon guidance, cell migration, DNA and RNA metabolism, and neural region patterning [5]. Comparative gene expression profile of the hippocampus from 2 genetic mouse models of ASD (BTBR and En2-/- mice) represented a specific enrichment profile in neuronal and glial genes, as well as in genes associated to ASD comorbidities [6]. Overall, these studies suggest implication that there is convergent pathway between ASD patients and mouse models.

The striatum, as the major input structure for basal ganglia, integrates excitatory inputs from various brain regions including the cortex and the thalamus to control diverse functions [7,8]. Although the striatum has long been recognized to control motor movement, recent studies have confirmed that this region is related to goal-directed actions, habitual actions and motivation [9,10]. MRI studies have implicated an activation of the striatum in response to social behaviors, including social rewards [11,12] and a positive correlation with the reciprocal social interaction and communicative skill (social interaction + communication Autism diagnostic observation schedule- generic score) in the bilateral medial caudate head [13]. The striatum consists of dorsomedial striatum (DMS), dorsolateral striatum (DLS), and ventral striatum (VS). DMS receives afferent projections from the associative cortices, thalamus, hippocampus, and amygdala. DLS receives projections from sensorimotor cortices and thalamus, and VS receives projections from cortex, thalamus, and brainstem [14].

Medium spiny neurons, which account for approximately 90-95% of all neurons in the striatum and use γ-aminobutyric acid (GABA) as a neurotransmitter, have 2 subtypes differentiated by their enrichment of dopamine receptor 1 (D1) or D2. Several ASD mouse model studies reported synaptic defects in both D1 or D2 (expressing) medium spiny neurons in the striatum. In the striatum of 16p11+/- mice, numbers of D2 medium spiny neurons were increased [15], while repetitive behaviors of Shank3B-KO mice were rescued by enhancing D2 medium spiny neuron activity [16]. Notably, neuroligin-3 mutations caused a proxy for acquired repetitive behaviors in mice via a synaptic impairment in D1 medium spiny neurons of VS [17]. Mice with a loss-of-function mutation in Chd8, a de novo mutation associated with ASD, displayed altered synaptic physiology in medium spiny neurons and improved acquired motor learning behavior [18]. A transcriptome analysis on the striatal tissues of adult Shank3 overexpressing mice showed the abnormal striatal mTORC1 activity [19].

So far, a large amount of researches have defined genetic contributions to disease risk, but finding a convergent neurobiological mechanisms and therapeutic targets for ASD is still limited due to the high heterogeneity. In this study, we used striatal tissues obtained from prenatally VPA-exposed mice to investigate the transcriptomic dysregulation and its role in the pathophysiology of ASD. The genes related to synaptic function and ASD phenotypes were upregulated, while the genes related to biosynthesis and
bone formation were downregulated. Among differentially expressed genes, Nurr1 was significantly upregulated in the transcriptional and protein level in the striatum from prenatally VPA-exposed mice.

Nurr1 has been known to be an orphan receptor and plays an essential role in dopaminergic neuron development, maintenance, differentiation and survival [20]. Nurr1 is important for cellular homeostasis, proliferation and differentiation of neurons, and cognitive function [21,22]. Moreover, Nurr1 regulates multiple necessary proteins and activates proto-oncogene tyrosine-protein kinase receptor Ret (Ret) to help the dopaminergic neuron growth and survival [23,24]. In addition, Nurr1 was found to diminish the expression of pro-inflammatory factors through nuclear factor–κB-p65 (NF-κB-p65) [25]. In relation to this, Nurr1 dysregulation was reported in some neurodevelopmental diseases. In particular, genome sequencing data and exome sequencing data comparisons from ASD families, showed a frameshift variant in Nurr1 [22]. Based on other studies, several de novo deletions covering Nurr1 were reported in patients with ASD and intellectual disability [26,27].

In order to delineate the functional role of Nurr1 in pathophysiology of ASD, we used pharmacological activation of Nurr1 by AQ in saline-exposed mice. Here we show for the first time that Nurr1 is increased in the striatum from prenatally VPA-exposed mice, and Nurr1 activation by its ligand, AQ, in saline-exposed mice reduced spine density in the striatum and impaired social interaction as shown in VPA-exposed mice. Conversely, abrogating the expression of Nurr1 in striatal neurons of VPA-exposed mice ameliorated the sociability deficits. Therefore, the Nurr1 expression level might be used as a therapeutic target for ASD.

Methods

Animals  Eight-9-week-old male and female BALB/c mice were purchased from Koatech (Pyeong-Taek, Korea) and mated. On TP 12.5, pregnant BALB/c mice were subcutaneously injected with a single dose of VPA (600mg/kg in saline) or vehicle saline (SAL). Mice were maintained under a 12 h light/dark schedule in specific pathogen-free facility at Seoul National University College of Medicine. All animal experiments were approved by the Animal Care Committee of Seoul National University, Seoul, Republic of Korea (Approval number: SNU-190426-10-1). Striatal tissues of PatDp+/− mice were generous gift from Dr. Jong-Cheol Rah (Korea Brain Research Institute).

RNA-Seq  Ten-week-old male mice were anesthetized using Zoletil mix with Xylazine. The striatum was then isolated from ~1 mm thick coronal section, which was located 0.86--0.14 mm anterior to the bregma as previously described [28]. Total RNAs were extracted by Qiazol reagent (Qiagen, Hilden, Germany). RNA library preparation, cluster generation, and sequencing were performed by TheragenEtex BiO Institute (Suwon, Korea). RNA quality was assessed by analysis of rRNA band integrity on an Agilent RNA 6000 Nano kit (Agilent Technologies, CA, USA). Ahead of cDNA library construction, the 2 μg of total RNA and magnetic beads with Oligo dT were used to enrich poly A mRNA from it. Then, the purified mRNAs were disrupted into short fragments, and the double-stranded cDNAs were immediately synthesized. The cDNAs were subjected to end-repair, poly A addition, and connected with sequencing
adapters using the TruSeq RNA Library prep Kit (Illumina, CA, USA). The suitable fragments automatically purified by BluePippin 2% agarose gel cassette (Sage Science, MA, USA) were selected as templates for PCR amplification. The final library sizes and qualities were evaluated electrophoretically with an Agilent High Sensitivity DNA kit (Agilent Technologies, CA, USA) and the fragment was found to be between 350–450 bp. Subsequently, the library was sequenced using an Illumina HiSeq2500 sequencer (Illumina, CA, USA). Statistics for each gene in each of the differential expression analysis, including FDR corrected \( p \)-values are found in Table S1.

**Bioinformatic analysis** The gene ontology analyses of differentially expressed genes were performed using DAVID software (version 6.8). Differentially expressed genes were also analyzed for phenotypes using mouse genome informatics mammalian phenotype analysis in Enrichr (http://amp.pharm.mssm.edu/Enrichr/). \( p \)-value 0.05 was used as cutoff.

**AQ treatment** For an *in vitro* study, primary striatal neuron cultures were treated with 100 nM of AQ (Sigma-Aldrich, MO, USA) for 24 h. AQ was diluted in 0.9% saline and prepared before treatment.

For in vivo study, 6-week-old prenatally SAL or VPA-exposed mice were intraperitoneally injected with AQ (20 mg/kg), twice per day at 12 h intervals, for 2 weeks. The AQ dose (20 mg/kg) used in this study was referred from previous report regarding the activating effect of AQ on Nurr1 in rodents [29]. AQ was diluted in 0.9% saline and prepared before administration. Mice underwent behavioral testing 1 week after the final injection.

**Stereotaxic injection of lentivirus** Nurr1 shRNA lentiviruses and its control shRNA lentiviruses were purchased from Sirion Biotech (Martinsried, Germany). Mice received bilateral stereotaxic injections of virus (1.5 \( \mu l \) per side) into the striatum (coordinates: AP +0.3, ML ±1.9, DV -3.25 mm) at rates of 0.15 \( \mu l/min \) at each site (Kopf instruments, CA, USA). The needle was left in place for an additional 5 min and then was withdrawn gently.

**Golgi staining** After mice were transcardially perfused using heparin (100 U/ml) PBS solution, brains were dissected. Golgi staining was performed using the FD Rapid GolgiStain Kit (FD Neurotechnologies, MD, USA) according to manufacturer’s instructions to label neurons. The brains were sectioned in the coronal plane at 100 \( \mu \)m thickness on a cryostat. Images of neurons in the DMS or DLS were acquired using a LSM 510 confocal microscope (Zeiss, Oberkochen, Germany) with a Plan-Neofluar 100x/1.30 N.A. oil immersion objective and the bright-field setting. To assess spine density and phenotype, 8–10 cells of each slice were randomly selected. 2-3 dendrites per neuron were analyzed. Stacks of 512 x 512 pixel 3-D images with an interval of 1 \( \mu \)m were then taken for each cell to include all visible dendritic branches in the Zen software. After 3D neuronal reconstruction, the secondary and tertiary dendrite spines were measured, wherein the distance to the soma varied from 20–80 \( \mu \)m.

**Quantitative reverse transcription polymerase chain reaction (qPCR)** Total RNA was extracted from the whole striatum using the Qiazol reagent (Qiagen, Hilden, Germany). RNA was converted to cDNA using AccuPower RocketScript RT PreMix (Bioneer, Daejeon, Korea). qPCR was performed using a CFX96 (Bio-
Rad, CA, USA). Results are presented as △△Ct-values normalized to the 18S rRNA. Primers were designed using NCBI primer blast software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The specificity of the primer pairs was tested by PCR, and the PCR product were examined by agarose gel electrophoresis.

**Western blot** The striatum of mice was homogenized in ice-cold RIPA buffer (Elpis Biotech, Daejeon, Korea) with freshly added protease inhibitors (Roche, IN, USA), and phosphatase inhibitors (1 mM PMSF, 1 mM Na3VO4, 5 mM NaF). Protein was quantified using a bicinchoninic acid assay kit (Thermo Fisher Scientific, IL, USA). 20-50 μg of proteins were resolved on a 10% SDS-PAGE gel or tris-tricine gel and transferred to nitrocellulose or polyvinylidene fluoride membrane, followed by blocking with 5% skim milk. Antibodies used were Nurr1 (#PA5-13416, Thermo Fisher Scientific, IL, USA), Cbln1 (#ab-64184, Abcam, Cambridge, UK), D2 (#AB5084P, Millipore, CA, USA), D1 (#ab20066, Abcam, Cambridge, UK), Vesicular glutamate transporter 1 (VGLUT1, #48-2400, Invitrogen, CA, USA), Vesicular glutamate transporter 2 (VGLUT2, #135 403, Synaptic systems), Dopamine transporter (DAT, #MAB369, Millipore, CA, USA), Glutamate decarboxylase 67 (GAD67, #ab26116, Abcam, Cambridge, UK). Relative intensity of blots was quantified using ImageJ software.

**Behavioral assays**

- Self-righting test was held on postnatal day 5-9 (P5-9) as described in previous literature [30]. Each mouse was placed in the supine position and gently held with all four limbs extended outwards at which time it was released. Time taken to right was recorded by the latency for all four paws touching the surface. A maximum score of 30 sec was recorded when the mouse failed to right in that period.

- Maternal scent preference test was conducted on P14 as described in previous research [31]. Each pup was moved from home cage to a fresh transparent polycarbonate cage (20 × 30 × 15 cm). The left third of the test cage was filled to a depth of 3 cm with litter from the mother's cage, the center third contained clean litter, and the right third contained litter from the cage of a stranger dam. The placement of the test litters (mother and stranger) was alternated across subjects to control for any side preferences. Three 1 min trials, with inter-trial intervals of 10 sec, were administered for each pup. For the first trial, pups were placed in the center of the fresh litter facing the back wall of the test cage. For the second trial, pups were placed in the center of the fresh litter facing the section containing its mother's cage litter. For the third trial, pups faced the section containing the litter of the stranger dam. Time spent in each section of the cage was recorded and averaged across the 3 trials. The pup was considered to be inside a section when all four paws were touching the litter within the specified region.

- Social interaction was assayed using the 3 chamber test. The apparatus was constructed of a Plexiglas box (60×45×22 cm) partitioned into 3 chambers with retractable doorways. Openings between the compartments allowed the animals access all three chambers. In the first phase, a mouse was placed in the center chamber and was allowed to freely explore with an age-matched male (familiar) for 10 min. In the second phase, the test mouse was gently guided to the center chamber, and the entrances were blocked. An age-matched stranger mouse then was placed in apposite chamber from the familiar mouse,
and then the test mouse was similarly allowed to explore with the familiar and stranger mouse for an additional 10 min. The apparatus was cleaned with 70% ethanol between trials.

- Self grooming was performed at 9-10 weeks of age, and each mouse was placed individually into a clean transparent polycarbonate cage (20 × 30 × 15 cm) with a video camera placed 15 cm away from the cage. The duration of the test was 10 min after 10 min habituation. The time spent grooming was measured.

- Rotarod test was conducted with mice (9-10 weeks) following previously published research [17]. The test consisted of three trials per day over the course of 3 days. Rotarods were accelerated from 4–40 rpm in 300 s. Each trial ended when a mouse fell off, made one complete backward revolution while hanging on, or reached 300 sec.

- Open field test was conducted at 9-10 weeks of age. A square plastic box (100 cm × 100 cm × 40 cm) was used for this general locomotor activity test. The mice were put into the arena and its movements monitored with a video camera for 30 min. Tracking of mouse behavior was done using EthoVision XT (Noldus) tracking system. The open field was thoroughly cleaned with 70% alcohol between test animals.

**Immunofluorescence and image analysis** Mice were anesthetized using Zoletil/Xylazine and perfused using heparin (100 U/ml) phosphate buffered saline (PBS) solution. The brains from 9 to 10-week-old prenatally VPA- or saline-exposed mice were then removed and post-fixed in 4% paraformaldehyde (PFA) at 4 °C for 24 h before they were transferred to 30% sucrose-PBS 0.1 M, pH 7.3 solution at 4 °C. Afterwards, the brains were sectioned into 30 μm-thick coronal sections using a cryostat (Thermo Fisher Scientific, IL, USA), and three slices per mouse were used in all IF analyses (n=3–4 mice/staining).

The brain slices were incubated in 10 mM sodium citrate buffer (pH 6.0) for 10 min at 95°C for antigen retrieval, and blocked in PBS containing 2% BSA or 10% serum and 0.3% Triton X-100 for 1 h at room temperature (RT). Sections were then incubated in blocking buffer containing a primary antibodies diluted in blocking buffer at 4°C for overnight. Next, sections were incubated with the secondary antibodies in PBS for 2-3 h at RT protected from light. Finally, sections were stained with Topro3 (diluted 1:1000; Thermo Fisher Scientific, IL, USA) or DAPI (1:1000, D1306, Thermo Fisher) in PBS. After final rinsing, sections were mounted and cover-slipped using mounting medium (#345789, Merck, Darmstadt, Germany). The images were acquired on an LSM510 confocal microscope (Zeiss, Oberkochen, Germany) using a Plan-Neofluar 40x/0.90 N.A. with a water immersion objective or on a Nikon A1 confocal microscope (Nikon, Melville, NY, USA) with a Plan fluor 20 × lens (0.75 numerical aperture). For quantification, 2–3 striatal regions were randomly selected for confocal imaging, wherein the intensity of each region was analyzed. The primary antibodies used were Nurr1 (#PA5-13416, Thermo Fisher Scientific, IL, USA), NeuN (#MAB377, Millipore, CA, USA), Iba-1 (#NB100-1028, Novusbio, CO, USA). Secondary antibodies used were goat anti-rabbit Alexa 555, goat anti-mouse Alexa 488, goat anti-rabbit Alexa 488, and donkey anti-goat Alexa 555 (Thermo Fisher Scientific, IL, USA).
Primary striatal neuron culture On E16-17, embryonic striatal tissues were dissected, dissociated with 0.25% trypsin, and plated onto plates or coverslips coated with poly-L-lysine. Neurons were grown in Neurobasal medium (Gibco, CA, USA) supplemented with B27 (Gibco, CA, USA), 2 mM GlutaMAX-I supplement (Gibco, CA, USA) and 100 μg/ml penicillin/streptomycin (Gibco, CA, USA) at 37 °C in a humidified environment of 95% O2/5% CO₂.

(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay To determine cell viabilities, cells were treated with the MTT solution (0.5 mg/ml, Duchefa Biochemie, Haarlem, Netherlands) for 3 h at 37 °C. Formazan grains were solubilized with DMSO and the absorbance was measured at 570 nm using a microplate reader (Molecular Devices, CA, USA).

Statistical analysis The data are expressed as means ± SEM values and were analyzed with the SPSS 23 software (IBM, Chicago, IL, USA) using the Kruskal–Wallis test, one-way-ANOVA with LSD post-hoc analysis, two-way-ANOVA with LSD post-hoc analysis, or repeated measures (RM)-ANOVA with Bonferroni post-hoc analysis. The results were considered to be statistically significant if p < 0.05. n means a number of mice analyzed unless stated otherwise.

Results

Abnormalities in the density and morphology of dendritic spines in the striatum of prenatally VPA-exposed 10-week-old mice

Morphological abnormality of the striatum has been one of the most consistent abnormalities reported in ASD [32,33]. Spine number and morphology can act as a marker of synaptic plasticity. To analyze the effects of VPA on dendritic structure in the striatum, Golgi staining was performed with the brain tissues of 10-week-old mice. Notably, VPA mice had a significantly reduced spine density compared to SAL mice in DMS (p = 0.036), not in DLS (Fig. 1a). Fig. 1b shows representative images taken from Golgi-stained neurons in the DMS and DLS. Next we investigated the morphology of the spines. Dendritic spines are classified by their length and the width of the spine head as filopodia, thin, stubby, mushroom and branch type [34]. The filopodia, and thin types were categorized as immature spines, and the stubby, mushroom, and branch types were categorized as mature spines. The mature spine density was significantly decreased (mature, p = 0.033) (Fig. 1c). In contrast, the immature spine density in DMS of VPA mice was not different as compared to that of SAL mice (Fig. 1d).

The principal neuron type in the striatum is the medium spiny neurons expressing dopamine receptors. We further analyzed differential expression of D1 or D2 to examine whether the prenatal VPA exposure is involved in dysregulation of dopamine receptor expression. Expression level of D2, which could indicate the population of D2 medium spiny neurons, was increased in the striatal tissues of prenatally VPA-exposed mice (p = 0.038) (Fig. 1e). D1 expression level was decreased (p = 0.032) (Fig. 1f).
Main inputs to the striatum are glutamatergic signal from cerebral cortex and thalamus and dopaminergic signal from substantia nigra / ventral tegmental area (SN/VTA). Vesicular glutamate transporter 1 (VGLUT1) and VGLUT2 are localized to the excitatory nerve terminals projecting from the cortical and the thalamic area, respectively, in the striatum [35]. Expression of VGLUT1, which represents corticostriatal terminals in the striatum, was found to be decreased in the striatum of VPA mice (\( p = 0.017 \)) (Fig. 1g). Since the terminal marker for the input from cortex to striatum (VGLUT1) was found to be decreased, we performed the dendritic spine counting with the prefrontal cortex sections. Prefrontal cortex has been well known to be implicated in a social recognition. It was observed that VPA mice exhibited lower total and mature spine density (total, \( p = 0.016 \), mature, \( p = 0.00001 \)) in the prefrontal cortex compared to SAL mice (Fig. S1). Expression of VGLUT2, which represents thalamostriatal terminals in the striatum, was found to be decreased in the striatum of VPA mice (\( p = 0.042 \)) (Fig. 1h). Expression of DAT, which is the marker for nigrostriatal dopaminergic nerve terminal, was found to be decreased in the striatum of VPA mice (\( p = 0.021 \)) (Fig. 1i). The dysfunction of glutamatergic and dopaminergic terminals in the striatum may cause reduction in spine density in DMS of VPA mice.

**Striatal transcriptome analysis of prenatally VPA-exposed 10-week-old mice**

To identify transcriptome profiles in the striatum of prenatally VPA or saline-exposed mice, RNA-Seq with the striatal mRNA from 10-week-old VPA mice was performed. Specifically, 348 genes were upregulated, and 258 genes were downregulated in the striatum of the VPA mice compared to that of SAL mice (Fig. 2a). Top 10 differentially expressed genes are listed in Fig. 2b.

Next, we analyzed gene ontology (GO) of genes that had \( p \)-value less than 0.05 to identify molecular and physiological signature. Interestingly, upregulated genes were related to “ion channel activity” (potassium channel and cation channel) and “chemical synaptic transmission” which are important to excitability of neurons, and “axogenesis”. Analyzing GO term by cellular component showed that upregulated genes are expressed mostly neuron specific part like “dendrite membrane”, “axon”, “dendrite”, and “node of Ranvier”. Upregulated genes for enrichment of mouse phenotype terms were also tested. Two ASD related phenotypes (“abnormal anxiety related response” and “excessive scratching”) were enriched. In the additional GO term analysis with downregulated genes, “protein targeting to endoplasmic reticulum or membrane”, “biosynthesis”, “mRNA catabolic process” by biological process, “binding of platelet-derived growth factor, actin, tropomyosin, and calcium ion”, and “neuropeptide hormone activity” by molecular function, and “cytosolic ribosome” by cellular component were related. Downregulated genes showed relevance with bone development-related (“exostosis”, “abnormal ischium morphology”, “abnormal pubis morphology”, and “absent common crus”) mouse phenotype (Fig. 2c, d).

Since upregulated gene sets displayed more relevance with neuronal function and ASD symptoms than downregulated gene sets, we specifically focused on upregulated genes. In order to verify those genes, top 10 upregulated genes were analyzed using qPCR with striatal mRNA of 10-week-old mice. Nurr1 mRNA (\( p = 0.007 \)) and Cbln1 mRNA (\( p = 0.014 \)) expression levels were found to be increased by qPCR.
Hspa1a mRNA level was found to be increased by RNA-Seq, but showed only tendency to increase by qPCR (Fig. 2g).

To demonstrate the changes in protein expression, we performed a western blot analysis on the two genes which were confirmed to be increased with RNA-Seq as well as qPCR. We identified that only the Nurr1 protein level was significantly increased in the striatum of 10-week-old VPA mice ($p = 0.047$) (Fig. 2h-i).

**Effects of AQ, an Nurr1 agonist, on molecular changes in primary striatal neuron cultures**

Next, we tested whether Nurr1 activation induce the molecular changes in the primary striatal neuron cultures, which was shown in the striatum of VPA mice. To find an optimal concentration that doesn't induce cytotoxicity, the cell viabilities were evaluated after treatment of AQ at various concentrations (10 nM-10 μM) for 24 h by performing an MTT assay. Treatment of concentration over 500 nM AQ was significantly cytotoxic to primary striatal neurons (VPA; $p = 0.003$) (Fig. S2a, b). Based on these results, primary striatal neuron cultures from SAL mice or VPA mice were treated with 100 nM of AQ for following experiments. D2 expression level was increased by 100 nM of AQ in primary striatal neuron cultures of SAL mice, not in cultures of VPA mice ($p = 0.043$) (Fig. S2c, d).

**Administration of AQ induces ASD-like behaviors in 10-week-old mice**

In order to characterize the effect of Nurr1 activation on behavior, SAL mice were injected with 20 mg/kg of AQ for 14 days and subjected to a battery of behavior tests. The body weight and the brain weight of the AQ-injected SAL mice was similar to that of the vehicle-injected SAL mice (Fig. 3a-c). To examine the role of AQ in ASD-like behaviors, social behavioral abnormality and repetitive behavior were investigated by three chamber and self-grooming tests, respectively. The AQ-injected SAL mice displayed no difference in time spent in familiar zone and stranger zone (SAL Veh; $p = 0.00005$, SAL AQ; $p = 0.317$) (Fig. 3d). The AQ-injected SAL mice showed no significant alterations in repetitive grooming behavior (Fig. 3e). The rotarod test was performed to evaluate motor coordination and accelerated motor learning. The performances of the AQ-injected SAL mice on trials 1, 2, and 3 were significantly worse than those of the vehicle-injected SAL mice (T1; $p = 0.04$, T2; $p = 0.014$, T3; $p = 0.028$) (Fig. S3a). During open field test, the AQ-injected SAL mice showed comparable motor function with vehicle group (Fig. S3b). Collectively, these results indicate that Nurr1 affects sociability, rather than repetitive behavior.

**The synaptic and molecular changes in the striatum of AQ-injected 10-week-old mice**

To elucidate the underlying functional mechanisms responsible for the decreased social activity in AQ-injected SAL mice, the dendritic spine density in DMS was measured. The density of mature spines was decreased in DMS of AQ-injected SAL mice ($p = 0.007$) (Fig. 4c). The density of total spines and immature spines weren't significantly different (Fig. 4a, d). Fig. 4b displays representative images taken from Golgi-stained neurons in the DMS.
To determine the molecular change related to medium spiny neurons, expression of GAD67, D2, and D1 were examined in the SAL and VPA mice injected with vehicle or AQ. GAD67 expression level was significantly increased by AQ injection in SAL mice ($p = 0.021$) (Fig. 4e). D2 and D1 expression level and the relative expression level of D2 compared to D1 ($p = 0.095$) was not significantly different (Fig. 4f-h).

**Lentiviral Nurr1 knockdown in the striatum rescues autism-like social deficits in prenatally VPA-exposed 10-week-old mice**

To examine the therapeutic potential of Nurr1 knockdown, we stereotaxically injected lentiviruses expressing shRNA targeting Nurr1 into striatum and assessed the resulting behavioral consequences. Given that $84.51 \pm 2.33\%$ were neurons and $8.02 \pm 1.08\%$ were microglia ($p = 1.43 \times 10^{-14}$) among Nurr1 expressing cells (Fig. S4), we designed the vector expressing shRNA under Syn promoter. Astrocytes were found to reside only in or around the striosomes, and they do not colocalize with Nurr1 positive cells in striatum (data not shown). The knockdown of Nurr1 was confirmed in viral-infected striatum tissue from VPA mice ($p = 4.75 \times 10^{-8}$) (Fig. 5a-c). Effect of shRNA was insignificant since the basal intensity was so low in SAL mice. In 3-chamber test, VPA sh-Nurr1 mice exhibited the significantly increased social interaction time, compared to VPA sh-sc mice (Fig. 5d, SAL sh-sc, $p = 0.020$; SAL sh-Nurr1, $p = 0.014$; VPA sh-sc, $p = 0.205$; VPA sh-Nurr1; $p = 0.039$). Taken together, these results indicated that Nurr1 knockdown could rescue the social deficits in VPA mice.

To investigate the effects of Nurr1 knockdown on dendritic structure in DMS, we analyzed the spine density, finding that total ($p = 0.007$) and mature ($p = 0.00003$) spine densities were significantly increased in DMS of VPA sh-Nurr1 mice. In contrast, the immature spine density in DMS of VPA sh-Nurr1 mice was not different as compared to that of VPA sh-sc mice (Fig. 6a-d). Additionally, we performed a western blot analysis, wherein D1 ($p = 0.04$) and DAT ($p = 0.009$) expression levels were found to be significantly increased in the striatum of VPA sh-Nurr1 mice as compared to that of VPA sh-sc mice (Fig. 6e, f). D2 expression level was not significantly different (Fig. 6g). However, the relative expression level of D2 compared to D1 was significantly increased ($p = 0.023$) (Fig. 6h).

**Nurr1 expression is also increased in the striatum of PatDp+/- mice, a genetic animal model of ASD**

To probe the importance of Nurr1 in pathophysiology of ASD, we also assessed the Nurr1 expression in a commonly used ASD genetic mouse model, PatDp+/- mice that carries a 6.3 Mb paternal duplication homologous to the human 15q11-q13 locus. Chromosomal abnormalities in this region are known to cause ASD, Prader-Willi syndrome, and Angelman syndrome in humans [36]. Interestingly, Nurr1 expression level was also increased in the striatal tissues from PatDp+/- mice ($p = 0.008$) (Fig. S5).

**Discussion**

MRI study with high-functioning ASD subjects aged between 6 years and 25 years revealed that the volume of caudate increased with development [37]. The growth rate of striatal structures for individuals
with ASD increased compared to control subjects in a longitudinal MRI research. This effect was specific
to caudate nucleus and correlated with the insistence on same cluster of repetitive behavior at the
preschool age [38]. Striatal functional connectivity is also aberrant in ASD patients. A resting-state
positron emission tomography study detected weaker correlations in glucose consumption between the
frontal cortical regions and the striatum in young adults with ASD [39]. However, a resting-state functional
MRI implicated the functional connectivity between the striatum and the associative and limbic cortex
increased in children with ASD [40]. These suggest the connectivity between the striatum and other brain
regions is differed in ASD patients and is related to autistic behavioral phenotype. Changes in the dorsal
striatum input has been reported to be involved in promoting sociability deficits and repetitive
behaviors [14]. It has also been reported that glutamatergic innervation from the neocortex and the
thalamus is known to modulate dendritic morphology in medium spiny neurons [41]. Moreover, the
cortical and thalamic glutamatergic input also potentiates the output of neurotransmission of the striatal
GABAergic neurons [42]. In fact, human functional MRI experiments have shown that the striatum
becomes active in relation to other’s reward situations and during social learning [11]. Corticostriatal
synapses in strisomal neurons were reduced in the striatum of prenatally VPA-exposed mice [43]. Mice
with autism-linked mutation in the DAT, a presynaptic transporter for DA reuptake from synaptic cleft,
which is located at the membranes of dopaminergic nerve terminals, exhibited repetitive behaviors and
deficits in social interaction [44]. Based on these reports, we checked the synaptic alteration in the
striatum and input signals to the striatum. Dendritic spine density was decreased in prenatally VPA-
exposed striatum and this effect was specific to mature type of spines. Furthermore, glutamatergic and
dopaminergic inputs to the striatum were decreased in prenatally VPA-exposed mice (Fig. 1). Although
several reports used VGLUT1 and VGLUT2 as presynaptic markers for the corticostriatal and
thalamostriatal circuits and DAT as a presynaptic marker for dopaminergic inputs to the striatum, our
data could not be sufficient to interpret the exact significance of alterations in synaptic innervation into
the striatum in the contribution to behavioral phenotypes of VPA mice. Further research seems to be
needed regarding the contribution of the alterations in striatal circuit in VPA-induced AS phenotypes in
near future. In our behavioral studies, VPA mice showed a developmental delay (Fig. S6a, g, h), an
impairment of social interaction (Fig. S6b-c), a tendency of increased grooming (Fig. S6d), a significantly
increased repetitive motor routine learning rate (Fig. S6e), and a significantly decreased motor function
(Fig. S6h). Taken together, our findings indicate that altered synaptic plasticity and altered glutamatergic
and dopaminergic inputs in the striatum could modulate the striatal neurotransmission output. This
could be relevant regarding the behavioral phenotypes in prenatally VPA-exposed mice.

In this study, our RNA-seq analyses identified the upregulation of synaptic and neuronal function related
genes and the downregulation of genes related to binding and process of protein, and bone development.
Upon validating the top 10 differentially upregulated genes using qPCR, only two genes were upregulated.
This was because we made the list of differentially upregulated genes following the order of \( q \)-value, not
fold change. In our results, prenatally VPA-exposed mice show increased Nurr1 mRNA and protein levels
in the striatum (Fig. 2). Nurr1 (\( \text{Nr}4\alpha2 \)), Nur77 (\( \text{Nr}4\alpha1, \text{NGFI-B} \)), and Nor-1 (\( \text{Nr}4\alpha3 \)) are orphan nuclear
receptors and conform Nur subfamily. Nurr1 is expressed exclusively in brain tissue, unlike Nur77 and
Nor-1. Several lines of evidence have indicated that Nurr1 is important in the development and differentiation of dopaminergic neurons, neurogenesis, and learning and memory. Nurr1 is first expressed at E10.5 in the mouse [45], and interacts with Pitx-3 inducing the differentiation of dopaminergic precursor cells to tyrosine hydroxylase positive dopaminergic neurons [46].

Dysregulation of Nurr1 expression was reported in some neurodevelopmental diseases. Recently, it has been reported that Nurr1 is also involved in the pathogenesis of schizophrenia by regulating the expression of Cnr1 which codes for the cannabinoid receptor 1, which is involved in brain functions such as emotional responses, motivated behavior, cognitive processing and motor control [47]. In addition, comparing genome sequencing data and exome sequencing data from ASD families, a frameshift variant in Nurr1 was discovered [22]. Several de novo deletions covering Nurr1 were reported in patients with ASD and intellectual disability [26,27]. Moreover, a meta-analysis of de novo variants in 4773 published ASD trios and 465 SPARK trios revealed de novo damaging variant in Nurr1 gene [48]. Combining the bisulfite-seq data of placenta samples and RNA-Seq data of chorionic villus samples from patient with Down syndrome, Zhang et al. reported that only Nurr1, Egr2, Egr3, Runx1 and Hnf4g were involved in the protein-protein interaction network [49]. Thus, our findings support the hypothesis that Nurr1 dysregulation has a role in neuropsychiatric disorders.

A Nurr1 agonist, AQ, is an anti-malaria drug that stimulates the transcriptional function of Nurr1 through physical interaction with its ligand binding domain [29]. Recently several studies reported that the pharmacological stimulation of Nurr1 using AQ improved cognitive function via enhancement of hippocampal neurogenesis [50,51]. Interestingly, AQ-injected SAL mice displayed dysfunctions in social interaction, whereas the injection did not affect repetitive behavior or motor function significantly (Fig. 3). In humans, activity in the striatal circuits has been correlated with social deficits relevant to autism [13,52]. In our results, AQ-injected SAL mice showed decreased dendritic spine density in DMS and this effect was specific to mature type of spines. In addition, the administration of AQ increased the expression of GAD 67, GABAergic neuron marker (Fig. 4). Furthermore, it has been reported that striatal D2 overexpression leads to a deficit in inhibitory transmission and dopamine sensitivity [53], suggesting that the molecular changes in AQ-injected SAL mice striatum could contribute to prefrontal cortex GABAergic system hypofunction and to reduced social novelty.

To further illustrate the roles of Nurr1 in social behavior, we performed intrastriatal injections of Nurr1-shRNA expressing lentiviruses. As depicted in Fig. 5, VPA mice injected with sh-Nurr1 lentiviruses showed reduced expression of Nurr1 in striatal neurons and rescued social interaction. Next, we confirmed that VPA mice injected with sh-Nurr1 lentiviruses exhibited increased total and mature spine density in DMS. In addition, the expression levels of DAT and D1 were increased in the striatum of VPA mice injected with sh-Nurr1 lentiviruses. We also found that the relative expression level of D2 compared to D1 was significantly decreased. As well known, medium spiny neurons are composed of D1 and D2 expressing GABAergic neurons. D1s (excitatory) are predominantly expressed on GABAergic medium spiny neurons in the dorsal striatum as part of the “direct pathway” to globus pallidus interna (GPI) and substantia nigra pars reticularis (SNpr) whereas D2s (inhibitory) are predominantly expressed on medium spiny neurons
that primarily project to globus pallidus externa (GPe). A recent study with the post-mortem brains of ASD reported significant increases in D2 mRNA within medium spiny neurons in both the caudate and putamen, in correlation with our results with lentivirus injection shown in Fig. 6. These results indicate alterations in the indirect pathway of the basal ganglia, with possible implications for the E/I balance in the direct/indirect feedback pathways through thalamic and motor cortical areas [54]. Our study is the first study that displays Nurr1 involved in the pathogenesis of ASD, indicating that the upregulation of Nurr1 expression in the striatum from VPA mice is the cause of the altered dendritic spine density of mature forms and may contribute to the autistic behavior. These findings imply that the regulation of Nurr1 could be a therapeutic strategy for ASD.

Limitations

In this study, the relevance of the increase in Nurr1 expression with synaptic and behavioral deficits in prenatally VPA-exposed mice was investigated. To understand how Nurr1 regulates behaviors and synaptic plasticity in striatum, future studies on the changes in striatum-related brain circuits are needed.

Conclusions

Transcriptional analysis and its validation showed increased expression of Nurr1 in the striatum of prenatally VPA-exposed mice. Here we describe a novel role of Nurr1 in synaptic and behavioral phenotypes of ASD. Nurr1 expression was also increased in another ASD mouse model, PatDp+/− mice (Fig. S4). Taken together, our findings implicate Nurr1 as a potential therapeutic target of ASD.

Declarations

Ethics approval

Study procedures were approved by the Animal Care Committee of Seoul National University, Seoul, Republic of Korea (Approval number: SNU-190426-10-1).

Consent for publication

Not applicable

Availability of data and materials

Please contact author for data requests.

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**Competing interest**

The authors declare that they have no competing interests.

**Author's contributions**

HS.K. and HJ.K. conceptualized the project. HJ.K., EJ.Y., HB.K., and EH.J. performed experiments and analyzed data. HS.K. and HJ.K. prepared the manuscript with contribution from all authors.

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Not applicable

**References**

1. Xu G, Strathearn L, Liu B, Bao W. Prevalence of Autism Spectrum Disorder Among US Children and Adolescents, 2014-2016. JAMA. 2018;319(1):81-82.

2. Rosenberg RE, Law JK, Yenokyan G, McGready J, Kaufmann WE, Law PA. Characteristics and concordance of autism spectrum disorders among 277 twin pairs. Arch Pediatr Adolesc Med. 2009;163(10):907-14.

3. Werling DM, Parikshak NN, Geschwind DH. Gene expression in human brain implicates sexually dimorphic pathways in autism spectrum disorders. Nat Commun. 2016;7:10717.

4. Gupta S, Ellis SE, Ashar FN, Moes A, Bader JS, Zhan J, et al. Transcriptome analysis reveals dysregulation of innate immune response genes and neuronal activity-dependent genes in autism. Nat Commun. 2014;5:5748.

5. DeRosa BA, El Hokayem J, Artimovich E, Garcia-Serje C, Phillips AW, Van Booven D, et al. Convergent Pathways in Idiopathic Autism Revealed by Time Course Transcriptomic Analysis of Patient-Derived Neurons. Sci Rep. 2018;8(1):8423.

6. Provenzano G, Corradi Z, Monsorno K, Fedrizzi T, Ricceri L, Scattoni ML, et al. Comparative Gene Expression Analysis of Two Mouse Models of Autism: Transcriptome Profiling of the BTBR and En2 (-/-) Hippocampus. Front Neurosci. 2016;10:396.

7. Pan WXX, Mao TY, Dudman JT. Inputs to the dorsal striatum of the mouse reflect the parallel circuit architecture of the forebrain. Front Neuroanat. 2010;4.

8. Radulescu A, Herron J, Kennedy C, Scimemi A. Global and local excitation and inhibition shape the dynamics of the cortico-striatal-thalamo-cortical pathway. Sci Rep-Uk. 2017;7.

9. Ito M, Doya K. Distinct neural representation in the dorsolateral, dorsomedial, and ventral parts of the striatum during fixed- and free-choice tasks. J Neurosci. 2015;35(8):3499-514.
10. Pauli WM, O'Reilly RC, Yarkoni T, Wager TD. Regional specialization within the human striatum for diverse psychological functions. P Natl Acad Sci USA. 2016;113(7):1907-12.

11. Baez-Mendoza R, Schultz W. The role of the striatum in social behavior. Front Neurosci. 2013;7:233.

12. Bhanji JP, Delgado MR. The social brain and reward: social information processing in the human striatum. Wires Cogn Sci. 2014;5(1):61-73.

13. Qiu A, Adler M, Crocetti D, Miller MI, Mostofsky SH. Basal ganglia shapes predict social, communication, and motor dysfunctions in boys with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry. 2010;49(6):539-51, 51 e1-4.

14. Fuccillo MV. Striatal Circuits as a Common Node for Autism Pathophysiology. Front Neurosci. 2016;10:27.

15. Portmann T, Yang M, Mao R, Panagiotakos G, Ellegood J, Dolen G, et al. Behavioral abnormalities and circuit defects in the basal ganglia of a mouse model of 16p11.2 deletion syndrome. Cell Rep. 2014;7(4):1077-92.

16. Wang W, Li C, Chen Q, van der Goes MS, Hawrot J, Yao AY, et al. Striatopallidal dysfunction underlies repetitive behavior in Shank3-deficient model of autism. J Clin Invest. 2017;127(5):1978-90.

17. Rothwell PE, Fuccillo MV, Maxeiner S, Hayton SJ, Gokce O, Lim BK, et al. Autism-associated neuroligin-3 mutations commonly impair striatal circuits to boost repetitive behaviors. Cell. 2014;158(1):198-212.

18. Platt RJ, Zhou Y, Slaymaker IM, Shetty AS, Weisbach NR, Kim JA, et al. Chd8 Mutation Leads to Autistic-like Behaviors and Impaired Striatal Circuits. Cell Rep. 2017;19(2):335-50.

19. Lee Y, Kim SG, Lee B, Zhang Y, Kim Y, Kim S, et al. Striatal Transcriptome and Interactome Analysis of Shank3-overexpressing Mice Reveals the Connectivity between Shank3 and mTORC1 Signaling. Front Mol Neurosci. 2017;10:201.

20. Shao QH, Yan WF, Zhang Z, Ma KL, Peng SY, Cao YL, et al. Nurr1: A vital participant in the TLR4-NF-kappaB signal pathway stimulated by alpha-synuclein in BV-2 cells. Neuropharmacology. 2019;144:388-99.

21. Zhang CL, Han QW, Chen NH, Yuan YH. Research on developing drugs for Parkinson's disease. Brain Res Bull. 2021;168:100-09.

22. Guo H, Duyzend MH, Coe BP, Baker C, Hoekzema K, Gerdts J, et al. Genome sequencing identifies multiple deleterious variants in autism patients with more severe phenotypes. Genet Med. 2019;21(7):1611-20.

23. Decressac M, Volakakis N, Bjorklund A, Perllmann T. NURR1 in Parkinson disease—from pathogenesis to therapeutic potential. Nat Rev Neurol. 2013;9(11):629-36.

24. Smidt MP, Burbach JP. How to make a mesodiencephalic dopaminergic neuron. Nat Rev Neurosci. 2007;8(1):21-32.

25. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, et al. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. Cell.
2009;137(1):47-59.
26. Leppa VM, Kravitz SN, Martin CL, Andrieux J, Le Caignec C, Martin-Coignard D, et al. Rare Inherited and De Novo CNVs Reveal Complex Contributions to ASD Risk in Multiplex Families. Am J Hum Genet. 2016;99(3):540-54.
27. Levy J, Grotto S, Mignot C, Maruani A, Delahaye-Duriez A, Benzacken B, et al. NR4A2 haploinsufficiency is associated with intellectual disability and autism spectrum disorder. Clin Genet. 2018;94(2):264-68.
28. Biever A, Puighermanal E, Nishi A, David A, Panciatici C, Longueville S, et al. PKA-dependent phosphorylation of ribosomal protein S6 does not correlate with translation efficiency in striatonigral and striatopallidal medium-sized spiny neurons. J Neurosci. 2015;35(10):4113-30.
29. Kim CH, Han BS, Moon J, Kim DJ, Shin J, Rajan S, et al. Nuclear receptor Nurr1 agonists enhance its dual functions and improve behavioral deficits in an animal model of Parkinson's disease. P Natl Acad Sci USA. 2015;112(28):8756-61.
30. Kim JI, Jeon SG, Kim KA, Kim YJ, Song EJ, Choi J, et al. The pharmacological stimulation of Nurr1 improves cognitive functions via enhancement of adult hippocampal neurogenesis. Stem Cell Res. 2016;17(3):534-43.
31. Rosenberg RE, Law JK, Yenokyan G, McGready J, Kaufmann WE, Law PA. Characteristics and Concordance of Autism Spectrum Disorders Among 277 Twin Pairs. Arch Pediat Adol Med. 2009;163(10):907-14.
32. Schuetze M, Park MT, Cho IY, MacMaster FP, Chakravarty MM, Bray SL. Morphological Alterations in the Thalamus, Striatum, and Pallidum in Autism Spectrum Disorder. Neuropsychopharmacology. 2016;41(11):2627-37.
33. Zhou Y, Kaiser T, Monteiro P, Zhang X, Van der Goes MS, Wang D, et al. Mice with Shank3 Mutations Associated with ASD and Schizophrenia Display Both Shared and Distinct Defects. Neuron. 2016;89(1):147-62.
34. Hering H, Sheng M. Dendritic spines: structure, dynamics and regulation. Nat Rev Neurosci. 2001;2(12):880-8.
35. Nakata Y, Yasuda T, Fukaya M, Yamamori S, Itakura M, Nihira T, et al. Accumulation of alpha-synuclein triggered by presynaptic dysfunction. J Neurosci. 2012;32(48):17186-96.
36. Choi Y, Kim H, Choi M, Yang EJ, Takumi T, Kim HS. Fetal neural stem cells from a mouse model of 15q11-13 duplication syndrome exhibit altered differentiation into neurons and astrocytes. J Pharmacol Sci. 2019;139(3):249-53.
37. Langen M, Schnack HG, Nederveen H, Bos D, Lahuis BE, de Jonge MV, et al. Changes in the developmental trajectories of striatum in autism. Biol Psychiatry. 2009;66(4):327-33.
38. Langen M, Bos D, Noordermeer SD, Nederveen H, van Engeland H, Durston S. Changes in the development of striatum are involved in repetitive behavior in autism. Biol Psychiatry. 2014;76(5):405-11.
39. Horwitz B, Rumsey JM, Grady CL, Rapoport SI. The cerebral metabolic landscape in autism. Intercorrelations of regional glucose utilization. Arch Neurol. 1988;45(7):749-55.

40. Di Martino A, Kelly C, Grzadzinski R, Zuo XN, Mennes M, Mairena MA, et al. Aberrant striatal functional connectivity in children with autism. Biol Psychiatry. 2011;69(9):847-56.

41. Buren C, Tu G, Parsons MP, Sepers MD, Raymond LA. Influence of cortical synaptic input on striatal neuronal dendritic arborization and sensitivity to excitotoxicity in corticostriatal coculture. J Neurophysiol. 2016;116(2):380-90.

42. Paraskevopoulou F, Herman MA, Rosenmund C. Glutamatergic Innervation onto Striatal Neurons Potentiates GABAergic Synaptic Output. J Neurosci. 2019;39(23):4448-60.

43. Kuo HY, Liu FC. Valproic acid induces aberrant development of striatal compartments and corticostriatal pathways in a mouse model of autism spectrum disorder. FASEB J. 2017;31(10):4458-71.

44. DiCarlo GE, Aguilar JI, Matthies HJG, Harrison FE, Bundschuh KE, West A, et al. Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors. Journal of Clinical Investigation. 2019;129(8):3407-19.

45. Saucedo-Cardenas O, Quintana-Hau JD, Le WD, Smidt MP, Cox JJ, De Mayo F, et al. Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. Proc Natl Acad Sci U S A. 1998;95(7):4013-8.

46. Jankovic J, Chen S, Le WD. The role of Nurr1 in the development of dopaminergic neurons and Parkinson's disease. Prog Neurobiol. 2005;77(1-2):128-38.

47. Torretta S, Rampino A, Basso M, Pergola G, Di Carlo P, Shin JH, et al. NURR1 and ERR1 Modulate the Expression of Genes of a DRD2 Coexpression Network Enriched for Schizophrenia Risk. J Neurosci. 2020;40(4):932-41.

48. Feliciano P, Zhou XY, Astrovaaskaia I, Turner TN, Wang TY, Brueggeman L, et al. Exome sequencing of 457 autism families recruited online provides evidence for autism risk genes. Npj Genom Med. 2019;4.

49. Zhang J, Zhou W, Liu Y, Li N. Integrated analysis of DNA methylation and RNAsequencing data in Down syndrome. Mol Med Rep. 2016;14(5):4309-14.

50. Kim JI, Jeon SG, Kim KA, Kim YJ, Song EJ, Choi J, et al. The pharmacological stimulation of Nurr1 improves cognitive functions via enhancement of adult hippocampal neurogenesis. Stem Cell Res. 2016;17(3):534-43.

51. Moon M, Jung ES, Jeon SG, Cha MY, Jang Y, Kim W, et al. Nurr1 (NR4A2) regulates Alzheimer's disease-related pathogenesis and cognitive function in the 5XFAD mouse model. Aging Cell. 2019;18(1).

52. Lee PS, Yerys BE, Della Rosa A, Foss-Feig J, Barnes KA, James JD, et al. Functional Connectivity of the Inferior Frontal Cortex Changes with Age in Children with Autism Spectrum Disorders: A fcMRI Study of Response Inhibition. Cereb Cortex. 2009;19(8):1787-94.
53. Li YC, Kellendonk C, Simpson EH, Kandel ER, Gao WJ. D2 receptor overexpression in the striatum leads to a deficit in inhibitory transmission and dopamine sensitivity in mouse prefrontal cortex. Proc Natl Acad Sci U S A. 2011;108(29):12107-12.

54. Brandenburg C, Soghomonian JJ, Zhang K, Sulkaj I, Randolph B, Kachadoorian M, et al. Increased Dopamine Type 2 Gene Expression in the Dorsal Striatum in Individuals With Autism Spectrum Disorder Suggests Alterations in Indirect Pathway Signaling and Circuitry. Front Cell Neurosci. 2020;14:577858.

Figures
Abnormalities in dendritic spine density and inputs in the striatum of prenatally VPA-exposed 10-week-old mice

a  Quantification of total dendritic spine density (F(1, 60) = 6.103, p = 0.016, interaction; F(1, 60) = 0.027, p = 0.870, group; F(1, 60) = 1.88, p = 0.175, region, two-way ANOVA, SAL DMS, n = 21; VPA DMS, n = 20; SAL DLS, n = 16; VPA DLS, n = 10).

b  Representative images of Golgi-Cox stained neurons in DMS and DLS from SAL and VPA mice. Scale bars: 10 μm. 

c-d  Quantification of mature type dendritic spines,

e  Quantification of D2 receptor expression (F(1, 60) = 2.06, p = 0.154), SAL DMS, n = 21; VPA DMS, n = 20; SAL DLS, n = 16; VPA DLS, n = 10).

f  Quantification of D1 receptor expression (F(1, 60) = 0.92, p = 0.343), SAL DMS, n = 21; VPA DMS, n = 20; SAL DLS, n = 16; VPA DLS, n = 10).

g  Quantification of VGLUT1 expression (F(1, 60) = 9.43, p = 0.003), SAL DMS, n = 21; VPA DMS, n = 20; SAL DLS, n = 16; VPA DLS, n = 10).

h  Quantification of VGLUT2 expression (F(1, 60) = 1.26, p = 0.266), SAL DMS, n = 21; VPA DMS, n = 20; SAL DLS, n = 16; VPA DLS, n = 10).

i  Quantification of DAT expression (F(1, 60) = 2.14, p = 0.147), SAL DMS, n = 21; VPA DMS, n = 20; SAL DLS, n = 16; VPA DLS, n = 10).

Figure 1
and immature type dendritic spines (SAL, n = 7; VPA, n = 9). n means a number of neurons analyzed. e-i Densitometric analysis of striatal D2 receptor level (SAL, n = 11; VPA, n = 11), D1 receptor level (SAL, n = 20; VPA, n = 20), VGLUT1 level (SAL, n = 8; VPA, n = 8), VGLUT2 level (SAL, n = 20; VPA, n = 24), and DAT level (SAL, n = 11; VPA, n = 11). Data are presented as mean ± SEM. *p < 0.05 compared to SAL mice, unpaired t-test.
Striatal transcriptome analysis of prenatally VPA-exposed 10-week-old mice. a Volcano plot depicting 348 significantly upregulated and 258 significantly downregulated genes (Log2 fold change). (n = 3 / group) b List of top 10 upregulated and downregulated differentially expressed genes (based on the fold change) from the RNA-Seq analysis. c-d GO and mouse genome informatics mammalian phenotype analysis of the upregulated genes and downregulated genes. e-g mRNA expression of Nurr1 (SAL, n = 9; VPA, n = 9), Cbln1 (SAL, n = 11; VPA, n = 11), and Hspa1a (SAL, n = 7; VPA, n = 8) in the prenatally VPA-exposed striatum determined both in RNA-Seq and qPCR. *p < 0.05, **p < 0.01 compared to SAL mice. h-i Protein level of Nurr1 (SAL, n = 12; VPA, n = 12) and Cbln1 (SAL, n = 18; VPA, n = 18) in the striatum of VPA mice. ***p < 0.001 compared to SAL mice, unpaired t-test or Mann-Whitney test.

Figure 3
Administration of AQ induces the deficit in social interaction in 10-week-old mice. a An experimental scheme. b-c Body weight and brain weight (SAL Veh, n = 16; SAL AQ, n = 14), unpaired t-test. d The interaction time with familiar and stranger mouse (F(1, 40) = 4.506, p = 0.040, interaction; F(1, 40) = 0.216, p = 0.645, group; F(1, 40) = 14.469, p = 0.0005, familiar vs. stranger, two-way ANOVA, SAL Veh, n = 11; SAL AQ, n = 11). ***p < 0.001 compared to time in zone with familiar mouse, unpaired t-test. e The duration of stereotypical self-grooming, unpaired t-test.
The synaptic and molecular changes in the striatum of AQ-injected 10-week-old mice. a Quantification of total dendritic spine density (SAL Veh, n = 12; SAL AQ, n = 18). b Representative images of Golgi-Cox stained neurons in DMS from SAL Veh and SAL AQ mice. Scale bar represents 10 μm. c-d Quantification of mature dendritic spine density and immature dendritic spine density (SAL Veh, n = 12; SAL AQ, n = 18). ***p < 0.001 compared to SAL Veh mice, unpaired t-test. n means a number of neurons analyzed. e The expression of GAD67, the marker of GABAergic neurons (SAL Veh, n = 8; SAL AQ, n = 8). f-g The expression of D2 (SAL Veh, n = 10; SAL AQ, n = 8) and D1 (SAL Veh, n = 13; SAL AQ, n = 12), unpaired t-test. h The relative expression of D2 compared to D1 (SAL Veh, n = 11; SAL AQ, n = 8). *p < 0.05 compared to SAL Veh mice, Mann-Whitney test.
Lentiviral Nurr1 knockdown in the striatum rescues autism-like social deficits in prenatally VPA-exposed 10-week-old mice. a A scheme illustrating the experimental paradigms of lentiviral injection and behavior tests. c Quantification of Nurr1+ immunofluorescent intensity (n = 5-7). ***p < 0.001 compared to SAL sh-sc mice, ###p < 0.001 compared to VPA sh-sc mice, one-way ANOVA. d The interaction time with familiar and stranger mouse (F(2, 38) = 1.329, p = 0.277, interaction; F(1, 38) = 0.056, p = 0.946, group; F(1, 40) =
Lentiviral Nurr1 knockdown in the striatum rescues the abnormalities in dendritic spine density and inputs in the striatum of prenatally VPA-exposed 10-week-old mice. a Quantification of total dendritic spine density. b Representative images of Golgi-Cox stained neurons in DMS from VPA sh-sc and VPA sh-Nurr1.
mice. Scale bar represents 10 μm. c-d Quantification of mature dendritic spine density and immature dendritic spine density (VPA sh-sc, n = 15; VPA sh-Nurr1, n = 11). n means a number of neurons analyzed. **p < 0.01, ***p < 0.001 compared to VPA sh-sc mice, unpaired t-test. e-g The expression of DAT (VPA sh-sc, n = 7; VPA sh-Nurr1, n = 8), D1 (VPA sh-sc, n = 6; VPA sh-Nurr1, n = 8), and D2 (VPA sh-sc, n = 7; VPA sh-Nurr1, n = 8). h The relative expression of D2 compared to D1 (VPA sh-sc, n = 5; VPA sh-Nurr1, n = 8). *p < 0.05, **p < 0.01 compared to VPA sh-sc mice, unpaired t-test.

**Supplementary Files**

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