Brunner syndrome associated MAOA mutations result in NMDAR hyperfunction and increased network activity in human dopaminergic neurons

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ABSTRACT

Monoamine neurotransmitter abundance affects motor control, emotion, and cognitive function and is regulated by monoamine oxidases. Among these, Monoamine oxidase A (MAOA) catalyzes the degradation of dopamine, norepinephrine, and serotonin into their inactive metabolites. Loss-of-function mutations in the X-linked MAOA gene have been associated with Brunner syndrome, which is characterized by various forms of impulsivity, maladaptive externalizing behavior, and mild intellectual disability. Impaired MAOA activity in individuals with Brunner syndrome results in bioamine aberration, but it is currently unknown how this affects neuronal function, specifically in dopaminergic (DA) neurons. Here we generated human induced pluripotent stem cell (hiPSC)-derived DA neurons from three individuals with Brunner syndrome carrying different mutations and characterized neuronal properties at the single cell and neuronal network level in vitro. DA neurons of Brunner syndrome patients showed reduced synaptic density but exhibited hyperactive network activity. Intrinsically functional properties and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)-mediated synaptic transmission were not affected in DA neurons of individuals with Brunner syndrome. Instead, we show that the neuronal network hyperactivity is mediated by upregulation of the GRIN2A and GRIN2B subunits of the N-methyl-D-aspartate receptor (NMDAR), resulting in increased NMDAR-mediated currents. By correcting a MAOA missense mutation with CRISPR/Cas9 genome editing we normalized GRIN2A and GRIN2B expression, NMDAR function and neuronal population activity to control levels. Our data suggest that MAOA mutations in Brunner syndrome increase the activity of dopaminergic neurons through upregulation of NMDAR function, which may contribute to the etiology of Brunner syndrome associated phenotypes.

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1. Introduction

Dopamine, serotonin, and noradrenaline all belong to the class of monoamine neurotransmitters. They are prevalent throughout the brain, and their abundance influences brain development, function, and behavior (Levitt et al., 1997; Ruhe et al., 2007). Monoamine neurotransmitter-related activity is tightly regulated, and dysregulation of monoaminergic pathways is associated with several neuropsychiatric disorders including schizophrenia, major depressive disorder, autism spectrum disorder (ASD), and attention deficit/hyperactivity disorder (ADHD). Monoamine oxidases (MAOs) catalyze monoaminergic neurotransmitters (Goda et al., 2016) and thereby regulate the monoamine concentration in the brain. Two isoforms exist that are responsible for this process: MAOA and MAOB. Both show high sequence homogeneity, although they have distinct properties: whilst both show high affinity for catabolization of hydroxylated amines (such as dopamine), MAOB also catabolizes non-hydroxylated amines (Shih et al., 1999). Furthermore, MAOA and MAOB show distinct expression patterns in the human brain, with MAOB being preferentially expressed in glial cells, noradrenergic and serotonergic neurons and MAOA showing additional expression specifically in dopaminergic neurons (Levitt et al., 1982; Vitalis et al., 2002). Disruption of MAO activity can have profound consequences on normal brain function (Bortolato et al., 2008). One disorder in which MAO function is strongly affected is Brunner syndrome: a neurodevelopmental disorder characterized by hemizygous mutations in the X-linked monoamine oxidase-A (MAOA) gene. Brunner syndrome was first described in a large Dutch kindred with non-dysmorphic borderline intellectual disability, prominent impulsivity, and maladaptive externalizing behavior (Brunner et al., 1993a; Brunner et al., 1993b). More recently, three more families have been reported with Brunner syndrome. In all families, individuals carry either nonsense or missense mutations of MAOA (Piton et al., 2014; Palmer et al., 2016), suggesting that impaired MAOA function underlies the reported behavioral phenotypes.

The monoaminergic system has been associated with the regulation of aggressive behavior, both in wildtype animal models (van Erp and Miczek, 2000; Ferrari et al., 2003; Couppis and Kennedy, 2008; Aleskseyenko et al., 2013) and genetic models of neurodevelopmental disorders (Rodriguez et al., 2004; Money and Stanwood, 2013). For example, hemizygous Maa mutant mice show abnormally high levels of aggressive behavior and disturbed monoamine metabolism (Cases et al., 1995; Scott et al., 2008; Bortolato et al., 2011). Furthermore, MAOA-deficient mice display alterations in brain development, with aberrant organization of the primary somatosensory cortex (Cases et al., 1995) and increased dendritic arborization of pyramidal neurons in the orbitofrontal cortex (Bortolato et al., 2011). Postnatal reduction of serotonin levels in MAOA-deficient mice partially corrected some of these developmental abnormalities in the cortex (Cases et al., 1996). On the molecular level, MAOA has been implicated in the regulation of synaptic neurotransmitter receptors, as MAOA knockout mice show increased N-methyl-D-aspartate (NMDA) receptor subunit expression in the prefrontal cortex (Bortolato et al., 2012). Taken together, these data suggest that dysfunction of MAOA in rodents leads to both structural and functional alterations during brain development.

MAOA is expressed in different neuronal as well as glial cell types in the brain (Luque et al., 1995). This complex interplay of multiple monoaminergic pathways in brain function creates a challenge in disentangling the cell-type specific roles of MAOA during neurodevelopment and in the regulation of normal brain activity. Cell-type specific investigation of MAOA expression shows consistent expression of MAOA protein in catecholaminergic (dopaminergic and noradrenergic) neurons (Nagatsu, 2004; Bortolato et al., 2018), whereas expression in serotonergic neurons is variable and decreases during the development of the mouse brain (Vitalis et al., 2002). Abundant expression of MAOA in dopaminergic (DA) neurons (Nagatsu, 2004; La Manno et al., 2016) coincides with the finding that changes in dopaminergic neuronal activity directly affect impulsive and aggressive behavior (de Almeida et al., 2005; Pinto et al., 2014). However, research on the molecular mechanisms affected by MAOA has so far focused on the serotoninergic system (Bortolato et al., 2018), as MAOA dysfunction results in increased serotonin levels in both humans and mice (Brunner et al., 1993a; Cases et al., 1995; Evrard et al., 2002; Bortolato et al., 2011; Piton et al., 2014; Palmer et al., 2016) and dysfunction of the serotonergic system is associated with increased aggression and impulsivity (Seo et al., 2008). As such, how MAOA affects dopaminergic neuron function is not well understood.

Current advances in the generation of human induced pluripotent stem cell (hiPSC) derived neurons enable us to generate cultures of defined cell types, which provides opportunities to disentangle the complexity that underlies interactions of multiple monoaminergic pathways. We generated rodent-astrocyte-supported cultures of hiPSC-derived neurons, which consist predominantly of tyrosine hydroxylase (TH)-expressing DA neurons. We used DA neurons from control individuals, and individuals with Brunner syndrome carrying missense or nonsense mutations, to investigate the cellular and molecular mechanisms underlying MAOA dysfunction. Combining data on morphological analysis, gene expression, single-cell electrophysiology, and neuronal network activity using microelectrode arrays (MEAs), we show that increased network activity in MAOA-deficient DA neuronal networks is associated with increased expression of the N-Methyl-D-Aspartate receptor (NMDAR) subunits GRIN2A and GRIN2B and increased NMDAR function. Rescue of a patient-specific MAOA missense mutation by CRISPR/Cas9 resulted in restoration of GRIN2A and GRIN2B expression, NMDAR function, and neuronal network activity to control levels. This suggests that changes in in vitro neuronal network activity due to mutations in MAOA are established through increased NMDAR activity.

2. Methods and materials

2.1. Background of control and patient material and ethical considerations

Control hiPSC lines were derived from dermal fibroblasts of male healthy volunteers (Mandegar et al., 2016; Soares et al., 2019). The hiPSC lines ME2, ME8 and NE8 were derived from dermal fibroblast biopsies of male individuals diagnosed with Brunner syndrome described previously (Brunner et al., 1993a; Brunner et al., 1993b; Piton et al., 2014; Palmer et al., 2016). Ethical approval for the study was obtained by local ethics committees (Table S1). and written informed consent was given by the parents or legal representatives of the participants. A summary of the demographic information of each individual can be found in Table S1, including references to the extensive description of demographic information, cognitive assessment and further clinical information for all Brunner Syndrome patients.

2.2. Generation of hiPSC lines

All hiPSC reprogramming and characterization of the pluripotency markers was done by the Radboudumc Stem Cell Technology Center (SCTC). Three individual clones were generated for each cell line and were tested for and expression of stem cell markers (ICC, Fig. S1a), activation of stem cell markers (qPCR Fig. S1b) and a negative mycoplasma test. For Control-2, this information can be found in (Mandegar et al., 2016; Frega et al., 2019b). Control-1, ME2, ME8 and NE8 were reprogrammed using retroviral vectors. The hiPSCs were maintained in Essential 8 (EB) or Essential 8 Flex complete medium with 100 μg/ml penicillin/streptomycin or 100 μg/ml Primocin (InvivoGen) on vitronectin coated cell culture plates (Corning) at 37 °C/5% CO2. Cell colonies were passaged by 0.5 M ureapora EDTA treatment for 2 or 3 min in Dulbecco’s phosphate-buffered saline (DBPS) without calcium and magnesium. Unless indicated otherwise, all the reagents were bought from Thermo Fisher Scientific Inc.
2.3. Genotyping of the MAOA promoter VNTR polymorphism

The MAOA 30 bp repeat polymorphism was genotyped with 30 ng Genomic DNA as template, harvested from hiPSCs. The genomic DNA was amplified with 1 × AmpliTaq Gold® 360 Master Mix (Life Technologies) and 0.33 mM of fluorescently labeled forward primer (FAM-5′-AGGCTGACCCTGAGAAG-3′) and reverse primer (5′-GAAGCGACGCTTACATCGGAAG-3′) in a total volume of 7.5 μl using the protocol: 95 °C for 10 min followed by 35 cycles of denaturation for 30 s at 95 °C, 30 s annealing at 60 °C, primer extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Fragment length analysis of the PCR product was performed by an automated capillary sequencer ABI3730 (Applied Biosystems, Nieuwerkerk a/d Ijssel, The Netherlands) using standard conditions (1 μl of the 1:20 diluted PCR product together with 9.7 μl formamide and 0.3 μl GeneScan-600 Liz Size Standard TM (Applied Biosystems). Results were analyzed with GeneMarker version 2.6.7 (SoftGenetics, US).

2.4. MAO activity assay in fibroblasts

MAOA and MAOB activities were determined using a Monoamine Oxidase Assay Kit (MAK136, Sigma-Aldrich). Fibroblasts of Control-1, ME2, ME8 and NE8 were thawed, cultured for one passage and subsequently harvested. Specific MAOA or MAOB activity was assessed by application of the potent MAOB antagonist pargyline (5 μM) or the MAOA inhibitor Clorgyline (5 μM) respectively. The fluorescent response following H2O2 generation by reaction of MAO with p-tyramine was read out after 180 min using a Spark microplate reader (Männedorf, Switzerland) with λex = 535 nm and λem = 585 nm.

2.5. Differentiation of hiPSCs into dopaminergic (DA) neurons

We split hiPSC colonies into single cells by Accutase treatment (5 min/37 °C) two days before the start of differentiation. 1–2×10^5/cm² cells were replated on a vitronectin-coated plate in E8 complete medium with 2 μM thiazovivin (Sigma) or 1 × RevitaCell Supplement. For differentiation, the E8 complete medium was replaced with Knockout DMEM from Days in vitro (DIV) 0 to DIV 5, supplemented with 15% Knockout Serum Replacement, 1 × GlutaMAX, 100 U/ml Penicillin-Streptomycin and 1 × MEM Non-Essential Amino Acids Solution. 0.1 mM β-mercaptoethanol was added freshly to the Knockout DMEM before each medium change. From DIV 6 to DIV 9, the Knockout DMEM medium was gradually replaced by 25%, 50%, 75%, and 100% N2 medium (DMEM/F12 with 1 × N-2 supplement). DA neurons were cultured in N2 medium until DIV 11. From DIV 12 on, the DA neurons were cultured in neurobasal medium supplemented with 1 × B27 and 1 × GlutaMAX and half medium was refreshed every two days. During the differentiation and lineage specification, different combinations of small molecules and/or growth factors were added at different time points: 10 μM LDN-193189 (Stemgent Inc.) from DIV 1 to DIV 11; 10 μM SB431542 (Stemgent Inc.) from DIV 1 to DIV 5; 2 μM Purmorphamine (Stemgent Inc.) and 100 ng/ml recombinant human fibroblast growth factor 8a (FGF-8a, R&D system) from DIV 2 to DIV 11; 3 μM CHIR99021 (Stemgent Inc.) from DIV 3 to DIV 12; 100 ng/ml recombinant human sonic hedgehog (C242I, SHH, R&D system) from DIV 12 to the end of the differentiation. 20 ng/ml recombinant brain-derived neurotrophic factor (BDNF, Peprotech), 20 ng/ml recombinant glial-derived neurotrophic factor (GDNF, Peprotech), 0.5 mM adenosine 3′,5′-cyclic monophosphate (cAMP, Enzo Life Science), 2 ng/ml transforming growth factor beta 3 (TGF-β3, Millipore), 200 μM ascorbic acid (Alpha, Sigma) and 10 nM β-secretase inhibitor IX (DAPT, Millipore, Calbiochem) were added into the medium from DIV 11 to the end of differentiation. The cells were passaged only when they were 100% confluent using accutase treatment. At DIV 20, DA neurons were split into single cells and 1–2×10^5/cm² cells were replated on a poly-L-ornithine (Sigma, 50 μg/ml) and murine Laminin (Sigma, 10 μg/ml) double-coated plate.

From DIV 22 on, 10 μM DAPT was used to promote DA neuron maturation. 2.0×10^4 Rat astrocytes (prepared as previously described(Frega et al., 2017)) were cocultured with DA neuron progenitors from DIV 24 to promote maturation. Heterogeneity in the contribution of astrocytes to DA neuron maturation was controlled by using astrocytes from the same batch and passage number across genotypes for each individual round of DA neuron differentiation.

2.6. Gene expression analysis

RNA was isolated from hiPSCs and differentiated DA neurons with the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. 0.5–1 μg RNA was retro-transcribed into cDNA by the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc) according to the manufacturer’s instructions. The gene expression profile of hiPSCs and differentiated DA neurons was measured by quantitative real-time PCR (qRT-PCR) using the Applied Biosystems 7500 Fast RT-PCR System. Used qRT-PCR primers are listed in Table S1. We decided to use a single reference gene (Beta-2-Microglobulin (B2M)) for our gene expression analyses, as control and patient cell lines are compared at the same in vitro time point. Thus only a difference in the genotype is expected in our neurons, and B2M is regarded as one of the most reliable reference genes for neuronal gene expression(Mathur et al., 2015). The Ct value of each target gene was normalized against the Ct value of the reference gene (ΔCt = Ct(target)-Ct(B2M)). The relative expression was calculated as 2^ΔΔCt and represented as fold change of gene expression when compared to corresponding control conditions [2^ΔΔCt = 2^ΔCt(target)-ΔCt(control)].

2.7. Neuronal reconstruction and quantitative morphometrical analysis

Widefield fluorescent images of MAP2-labeled hiPSC-derived dopaminergic neurons were taken at 20× magnification using a Zeiss Axio Imager Z1 with apotome (Carl Zeiss AG, Germany). The images were stitched using Fiji software and the somatodendritic domains of individual neurons were reconstructed using Neurulucida 360 (Version 2017.01.4, Microbrightfield Bioscience, Williston, USA). Only neurons with at least two primary dendrites and at least one dendritic branch point were selected for reconstruction and further analyses. Care was taken to avoid selection of neurons with strong overlap of multiple neurites or neurons present in neuronal clusters for analysis, as these negatively affect the accuracy of tracing in the neurulucida software. DA Neurons from three individual rounds of differentiation were used for each cell line, and in total 20 neurons per cell line were used for characterization. Sholl analysis(Sholl, 1953) was used to determine the dendritic length of the neurons within a series of concentric circles at 25 μm intervals from the soma. All the morphological data were acquired and analyzed blindly to the genotype of the neurons. For the somatodendritic properties and Sholl analysis, the significance was determined by using Wilks’ Lambda multivariate analysis of variance (MANOVA) followed by the Bonferroni post-hoc (IBM SPSS Statistics, version 24.0, IBM, Armonk, USA).

2.8. Immunocytochemistry

Cells plated on coverslips were fixed with 4% paraformaldehyde/4% sucrose (v/v) (Sigma) in PBS for 15 min at room temperature (RT). Non-specific binding was avoided by incubation in 5% normal goat serum (Thermo Fisher Scientific)/0.4% Triton X-100(Sigma)/1% Glycine (Sigma) in PBS (blocking solution) at RT for one hour. The primary and secondary antibodies were diluted in the blocking solution and applied overnight at 4 °C or 1 h at RT respectively. Cell nuclei were stained with Hoechst 33342 (Molecular probes), and the coverslips were mounted with DAKO fluoromount medium (Agilent). Used antibodies can be found in Table S3. DA neurons were imaged at 63× magnification using a Zeiss Axio Imager Z1 with apotome. Synapse density was assessed
through manual counting using Fiji software (Schindelin et al., 2012).

2.9. Network activity characterization using microelectrode arrays

Neuronal network activity was measured using 6-Well or 24-well microelectrode array devices (Multichannel Systems, MCS GmbH, Reutlingen, Germany). DA progenitor cells (DIV 20) were plated on MEAs and further cultured as previously described. Each well was comprised of either 9 (6-Well MEA), or 12 recording electrodes (24-well MEA) and a grounding electrode. Activity in 6-well MEAs was recorded for 20 min using the MEA60 System (MCS GmbH, Reutlingen, Germany) as described before (Frega et al., 2017) with a high pass filter (Butterworth, 100 Hz cutoff frequency). Recordings on the 24-well MEA system were conducted as described before. During all recordings for the 6-well and 24-well MEAs, the temperature was maintained at 37°C with continuous flow of humidified carbogen (95% O₂, 5% CO₂). Data was sampled at 10 kHz through either MC-Rack software (6-well MEA) or Multiwell-Screen software (24-well MEA) (MCS GmbH, Reutlingen, Germany).

Data analysis from 24-well MEAs was performed off-line by using Multiwell Analyzer (MCS GmbH, Reutlingen, Germany, i.e. software from the 24-well MEA system that allows the extraction of the filtered output data per electrode) and in-house algorithms to average all the data per well as previously described (Bologna et al., 2010; Frega et al., 2017). The extracted parameters encompassed the mean firing rate (MFR, spikes/s) and mean burst rate (MBR, bursts/min). The MFR was computed by averaging the firing rate of each channel, which is averaged for all the active electrodes of the MEA. Spikes were grouped into a burst if at least 4 consecutive spikes were detected with a smaller than 30 milliseconds inter-spike-interval. All bursts were merged that were less than 65 milliseconds apart. Bursts with a duration of lower than 50 milliseconds were removed from analysis.

For 6-well MEA data analysis, spikes and bursts were detected by using the Precise Timing Spike Detection algorithm (PTSD) (Maccione et al., 2009) and the Burst Detection algorithm (Pasquale et al., 2010) embedded in the SpyCode software (Bologna et al., 2010). The mean firing rate (spikes/s) of the network was computed by averaging the firing rate of each channel, which is averaged for all the active electrodes (MFR > 0.1 Hz) of the MEA. The burst was computed as at least 5 spikes in a burst with a maximum inter-spike-interval of 80 milliseconds. The network burst was defined as synchronized bursts that occurs in >50% of the active channels. The network burst rate (burst/min) was calculated as the amount of network bursts per minute. Statistical analysis was conducted in PRISM (Graphpad PRISM 7.0, Graphpad Software, San Diego, CA). For drug application on 6-well MEAs, 100 nM Gleysol (MAO inhibitor, Sigma) was added to a single well and activity was recorded at 24 h and 6 days post drug application.

2.10. Single-cell electrophysiology

All single-cell electrophysiological recordings were conducted on DIV 73 DA neurons. Coverslips plated with neurons were transferred to a recording chamber continuously perfused with oxygenated (95% O₂ / 5% CO₂) and heated (32°C) artificial cerebral spinal fluid (ACSF) containing (in mM): 124 NaCl, 3 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 10 Glucose. Patch pipettes with filament (3.5–7.5 MΩ) were made from borosilicate glass capillaries (Science Products GmbH, Holheim, Germany). For all recordings of intrinsic properties and spontaneous activity, a potassium-based intracellular solution containing (in mM) 130 K-Gluconate, 5 KCl, 10 HEPES, 2.5 MgCl₂, 4 Na₂-ATP, 0.4 Na-GTP, 10 Na-Phosphocreatin, 0.6 EGTA, pH 7.2 and 290 mOsmol) was used. The resting membrane potential (Vrmp) was measured immediately after generation of a whole cell configuration. All other measurements were conducted at a holding potential of ~60 mV. Passive membrane properties were determined via voltage step of ~10 mV. Active intrinsic properties were measured with a stepwise current injection protocol.

For recording of current changes upon exogenous NMDA application coverslip were placed in MgCl₂-free ACSF, a cesium-based intracellular solution containing (in mM) 135 CsMeSO₄, 10 CsCl, 10 HEPES, 2.5 MgCl₂, 4 Na₂ATP, 0.4 NaGTP, 10 Na-Phosphocreatin; 0.6 EGTA, 10 QX-314 was used. Application pipettes (2–4 MΩ) were made from borosilicate glass with filament. NMDA (10 mM) was dissolved in ACSF and locally applied using a pressure ejection system (PDES-2DZ, NPI, Tamm, Germany). The ejection pressure was set to 5 psi/0.4 bar and injection duration was set to 100 ms. CNQX (5 μM, Tocris, Bristol, United Kingdom) was used to block all AMPAR-mediated currents. Cells were visualized with an Olympus BX51WI upright microscope (Olympus Life Science, PA, USA), equipped with a DAGE-MTI IR-1000E (DAGE-MTI, IN, USA) camera. Activity was recorded using a Digidata 1440A digitizer and a Multiclamp 700B amplifier (Molecular Devices). Sampling rate was set at 20 kHz (voltage measurements) or 10 kHz (current measurements) and a lowpass 1 kHz filter was used during recording. Recordings were not corrected for liquid junction potential (~10 mV). Series resistance was monitored on-line and cells were discarded if series resistance increased above 1:10 of membrane resistance. Intrinsic electrophysiological properties were analyzed using Clamptit 10.7 (Molecular Devices, CA, USA), and sPSCs were analyzed using MiniAnalysis 6.0.2 (Synaptosoft Inc., GA, USA).

2.11. CRISPR/Cas9-mediated gene correction

The guide RNA was designed as shown in Fig. S7a and was cloned into the plasmid PX459-V2 (Addgene plasmid #62988 (Ilan et al., 2013)). For correcting the MAOA mutation of ME8, 5*10⁴ cells were electroporated with 2 μg PX459-guideRNA and 1 μl ssODN (10 μM, Fig. S7a) using P3 Primary Cell 4D-nucleofector Kit and 4D Nucleofector Unit X (Lonza, Basel, Switzerland). Single-cell derived colonies were selected by puromycin (0.5 μg/ml) for two or three days to eliminate the non-transfected ones. The selected colonies were verified by sanger sequencing of the targeted region of genomic DNA.

2.12. Statistical analysis

Statistical analysis of the data was performed with GraphPad PRISM (Graphpad Software, Inc., USA). Data is always shown as mean ± SEM. In case data of multiple lines was pooled to increase statistical power, prior to pooling, Student’s t-test was performed to confirm that the lines were not significantly different in any of the parameters investigated (P > 0.05). At least 2 biological replicates (individual clones) and 3 technical replicates (batches of differentiation) were used for all experiments conducted. All data was tested for normality (D’Agostino & Pearson normality test) and the presence of outliers before statistical comparison between genotypes was conducted. A detailed overview of all averaged data can be found in supplementary data tables S4–S9. Mann-Whitney U test, unpaired Student’s t-test or one-way ANOVA with Dunnet’s correction for multiple-comparisons was used for statistical analysis. P < 0.05 was considered significant.

3. Results

3.1. Generation of DA neurons derived from individuals with and without Brunner syndrome

We generated hiPSCs from two healthy subjects and three Brunner syndrome patients from independent families (Fig. 1a, for extended information see Table S1). ME2 and ME8 had a missense mutation in exon 2 (c.133C>T, p.R45W(Palmer et al., 2016)) and exon 8 (c.797_798delinsTT, p.C266F(Piton et al., 2014)), respectively. These mutations are both located in the flavin adenine dinucleotide (FAD)-binding domain of MAOA(Palmer et al., 2016) (Fig. 1b). Both mutations lead to an inactive variant of MAOA, and our previous studies show that
Fig. 1. Differentiation of DA neurons derived from human induced pluripotent stem cells (hiPSCs). (a) Scheme of control and patient hiPSC lines used in the study. The monoamine oxidase A (MAOA) mutations were confirmed by sanger sequencing. (b) Location of the different mutations within the MAOA gene and protein domain. FAD (blue boxes), flavin adenine dinucleotide binding domains; SBD (yellow boxes), substrate-binding domain; CTM (green box), C-terminal membrane region. (c) Schematic overview of the protocol used to generate DA neurons from hiPSCs. (d) Representative images of DIV 55 DA neurons labeled by TH (red) and MAP2 (green) (Scale bar = 20 μm). (e) The percentage of TH-positive neurons (among MAP2-positive cells) at DIV 55. Sample size (across 3 independent differentiations) Control-1 N = 15, Control-2 N = 16, ME2 N = 15, ME8 N = 15, NE8 N = 15. (f) MAOA mRNA expression in control DIV 73 DA neurons. Sample size (at least 3 biological and 3 technical replicates per line): Control-1 N = 13, Control 2 N = 7. (g) Comparison of MAOA mRNA expression between control and patient lines, control data was pooled as Control-1 and Control-2 did not show differences in MAOA mRNA expression. Data is represented as fold change of gene expression compared to control conditions. (Control vs NE8 P = 0.0076). Sample size (at least 3 biological and 3 technical replicates per line): ME2 N = 12, ME8 N = 12, NE8 N = 12. All data represent means ± SEM. One-Way ANOVA with Dunnett’s correction for multiple testing was used to compare between patient lines and control lines. Additional information regarding average values and statistics on Fig. 1 can be found in Table S4. **P < 0.01. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
individuals with these missense mutations have reduced MAOA function (Piton et al., 2014; Palmer et al., 2016). NE8 results in a nonsense mutation in MAOA leading to a premature stop codon (c.886C>T, p.Q296*) (Brunner et al., 1993a)). All patients were known to display either reduced MAOA activity or changes in MAOA protein level, and showed elevated serotonin and disturbed monoamine metabolite levels in serum and urine (Brunner et al., 1993b; Piton et al., 2014; Godar et al., 2016; Palmer et al., 2016). Recently, reduced MAOA activity in fibroblasts of Brunner syndrome patients, including our patient ME8, has been shown using HPLC (Peters et al., 2021). We additionally assessed MAOA and MAOB enzymatic activity in fibroblasts of the control-1 line and all our patient lines. Our data show that MAOA activity is reduced in patient fibroblasts compared to control levels, whilst MAOB activity is similar (Fig. S1A). All selected clones expressed the pluripotency markers OCT4, TRA-1-81, NANOG, and SSEA4 (Fig. S1C,D), and the presence of the MAOA mutation in the patient lines was confirmed by Sanger sequencing of the fibroblast-derived hiPSC lines (Fig. 1a).

We differentiated hiPSCs into a population of predominantly DA neurons as previously described, by application of a combination of growth factors and regulatory compounds at different developmental timepoints (Sundberg et al., 2013) (Fig. 1c). DA neuron progenitors were co-cultured with rat astrocytes to facilitate neuronal development and network maturation. Neuronal identity was confirmed by microtubule-associated protein 2 (MAP2) expression and DA neuron identity by expression of the dopaminergic marker tyrosine hydroxylase (TH) after 55 days in vitro (DIV 55, Fig. 1d). Though we observed variability in percentage of TH+/MAP2 double positive neurons present on each coverslip, all hiPSC lines were able to differentiate into TH+/MAP2 double-positive neurons at similar efficiency: approximately 90% of MAP2 positive neurons expressed TH at DIV55 (Fig. 1e).

MAOA mRNA levels were similar between control lines (Fig. 1f), and the ME2 and ME8 lines (Fig. 1g). As expected, MAOA mRNA levels were reduced in the NE8 line compared to control lines (Fig. 1g). This is likely caused by nonsense-mediated mRNA decay, which has been reported in human fibroblasts with the same mutation (Chen et al., 2004). Of note, ME2 and NE8 carry an allele of the variable number tandem repeat (VNTR) polymorphism in the MAOA promoter region associated with high gene expression (MAOA-H) (Sabol et al., 1998; Vaddodaria et al., 2019), whereas Control-1, Control-2 and ME8 carry an allele associated with low expression (MAOA-L) (Fig. S2). These alleles have previously been suggested to affect MAOA expression differentially using luciferase assays in immortalized cell lines (Petrelli et al., 2020). However, in hiPSC-derived DA neurons, MAOA expression does not seem to be affected by this polymorphism, as the ME2 and ME8 line show the same expression as the Control-1 and Control-2 lines, despite their different VNTR polymorphisms.

3.2. MAOA mutations affect synapse density in DA neurons

It has been shown that dendritic arborization of pyramidal neurons in the orbitofrontal cortex is increased in Maoa hemizygous knockout mice (Bortolato et al., 2011). However, it is unclear whether this is a direct effect of impaired MAOA expression and/or function. We therefore immunostained for MAP2 to identify the soma and dendrites of DA neurons (Fig. S3) and used quantitative morphometric analysis to assess whether MAOA mutations directly affect neuronal somatodendritic morphology (Fig. 2a). We compared DA neurons of a single control line (Control-1) to Brunner syndrome DA neurons at 73 days of differentiation (DIV73). This revealed cell-line specific alterations of DA neuron morphology. ME8 DA neurons showed a significant increase in soma size (Fig. 2b) and dendrite complexity including dendritic nodes, length and Sholl analysis (Fig. 2c–d) compared to Control-1. Furthermore, ME8 and ME2 DA neurons showed a significant increase in the total dendritic span (Convex Hull analysis, Fig. 2g), whereas NE8 DA neurons showed no differences from Control-1 in dendritic complexity. This suggests that mutations in the FAD domain of MAOA might be associated with changes in DA neuron morphology, whereas reduced MAOA expression seems to have no effect.

In addition to alterations in dendritic complexity (Rivero et al., 2015; Chailangkarn et al., 2016), neurodevelopmental disorders have been associated with synaptic deficits in rodents and humans (Zoghbi and Bear, 2012). Changes in synapsin density are a well-described approximation of synapse number in hiPSC-derived human models of neurodevelopmental disorders (Yi et al., 2016; Frega et al., 2019a; Frega et al., 2019b; Meijer et al., 2019; Klein Gunnewiek et al., 2020). We therefore estimated synapse density using the number of presynaptic synapsin1/2 puncta per section dendrite. We found that synapse density was significantly decreased in DA neurons from all three Brunner syndrome-derived lines compared to DA neurons from Control-1 at DIV 73 (Fig. 2h, i). This suggests that, whilst the effect of MAOA dysfunction on DA neuron morphology might be mutation- and/or patient specific, reduced synapse density is a general feature of DA neurons in Brunner syndrome.

3.3. Brunner syndrome-derived DA neurons show increased neuronal network activity

Differences in network activity and organization have been observed in the brain of individuals with neurodevelopmental disorders (Ubbas and Singer, 2006), and changes in synapse density have been shown to underlie these neuronal network changes (Chailangkarn et al., 2016; Marchetto et al., 2017). To investigate the neuronal network phenotypes by means of recording extracellular spontaneous activity at the population level, we generated control and Brunner syndrome DA neuron cultures grown on 6-well MEAs (Fig. 3a). We recorded the network activity at DIV 73, the same in vitro timepoint at which the reduced synapse density was observed, and compared control DA neuron networks with patient networks. At this timepoint, neuronal cultures generated spontaneous activity (Fig. 3b–d), and control lines showed similar, albeit sparse, activity levels. Since network activity was comparable between Control-1 and Control-2 (Fig. 3a–c), we pooled these lines for subsequent analysis and statistical quantification. We furthermore detected no difference in neuronal activity between the ME2 and ME8 lines, and our data on synapse density suggest that dysfunction of the FAD domain similarly affects MAOA function in these lines (Fig. 3a–c). Therefore, we also pooled data from the missense mutation lines ME2 and ME8 for statistical analysis. For each experiment, all lines were measured simultaneously within the same 6-well MEA, and patient line data was normalized to the control values within the same experiment. Control networks mainly showed sporadic random spiking activity (Fig. 3b, e), and synchronous activity at either the single electrode level (burst activity, Fig. 3f) or throughout the entire culture (network burst activity, Fig. 3g) was largely absent. In comparison, Brunner syndrome DA neuronal networks showed significantly higher random spiking activity at DIV 73 (Fig. 3e). Moreover, in Brunner syndrome networks, activity occurred organized into readily observable synchronous events composed of many spikes occurring close in time and across the culture (Fig. 3b–c, g). Lastly, we investigated if inhibition of MAOA activity using the MAOA inhibitor Clorgyline results in increased activity in DIV73 control DA neurons. Though the response to MAOA inhibition was heterogeneous, a trend toward increased activity could be observed, and activity remained increased up to 6 days post Clorgyline application (Fig. 3d–e). Taken together, these data suggest that Brunner syndrome DA neurons are more strongly integrated into a spontaneously active network than control neurons at DIV 73.

3.4. MAOA mutations do not affect intrinsic properties and AMPAR-mediated synaptic transmission

We hypothesized that the increased network activity in Brunner syndrome DA neurons might be caused by changes in intrinsic properties of our DA neurons. Therefore, we investigated passive and active
Fig. 2. Morphological organization and synapse density of DA neurons (a) Representative images of reconstructed DA neurons at 73 days of differentiation (DIV 73). (b-g) Parameters derived from the somatodendritic compartment. Sample size: $N = 20$ for all lines across 3 independent differentiations, information per line can be found in Table S5. (b) Quantifications of synapse density (N positive synapsin puncta/10 $\mu$m, Sample size: Control-1 $N = 25$, ME2 $N = 23$, ME8 $N = 25$, NE8 $N = 26$ across 3 independent differentiations. (i) Representative images of control and patient DA neurons at DIV 73 immunostained for microtubule associated protein 2 (MAP2) (red) and synapsin1/2 (green), scale bar = 20 $\mu$m. Inset shows a single stretch of dendrite (red) with synapses (green), scale bar = 10 $\mu$m. All data is represented as mean ± SEM. One-Way ANOVA with Dunnett’s correction for multiple testing was used to compare between patient lines and control lines in all parameters except Sholl analysis, where MANOVA with Bonferroni correction was used with distance and genotype as factors. *$P < 0.05$; ***$P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
intrinsic membrane properties of DA neurons, which are a measure of neuronal maturity and neuronal health (Chambers et al., 2012). DA neurons from all lines were able to generate action potentials upon application of positive current to the cell soma (Fig. 4a). Since no differences were observed between Control-1 and Control-2, and ME2 and ME8, these lines were again pooled for subsequent analysis (Fig. S6). At DIV 73, membrane capacitance, membrane resistance and membrane resting membrane potential were comparable across all cell lines. Interestingly, the increased soma size for ME8 did not result in an increase in capacitance, suggesting that the reactive surface is not increased in this patient cell line (Fig. S6a). Taken together, these data suggest that all assessed DA neurons show comparable ion channel expression and levels of maturity across different genetic backgrounds. Active properties (related to the action potentials) were also comparable between control and patient neurons (Fig. 4e–g, Fig. S6e–g), which indicates that the cell-autonomous excitability and the intrinsic properties of DA neurons are not affected by mutation of MAOA.

The reduced synapse density seen in all Brunner syndrome DA neuron lines and the increased network activity on the MEA suggest that the reactive surface is not increased in this patient cell line (Fig. S6a). Taken together, these data suggest that all assessed DA neurons show comparable ion channel expression and levels of maturity across different genetic backgrounds. Active properties (related to the action potentials) were also comparable between control and patient neurons (Fig. 4e–g, Fig. S6e–g), which indicates that the cell-autonomous excitability and the intrinsic properties of DA neurons are not affected by mutation of MAOA. The reduced synapse density seen in all Brunner syndrome DA neuron lines and the increased network activity on the MEA suggest that the reactive surface is not increased in this patient cell line (Fig. S6a). Taken together, these data suggest that all assessed DA neurons show comparable ion channel expression and levels of maturity across different genetic backgrounds. Active properties (related to the action potentials) were also comparable between control and patient neurons (Fig. 4e–g, Fig. S6e–g), which indicates that the cell-autonomous excitability and the intrinsic properties of DA neurons are not affected by mutation of MAOA.

Fig. 3. Increased neuronal network activity in Brunner syndrome DA neurons. (a) Example picture of a 6-well MEA. Each chamber is fitted with 9 recording electrodes and separated by a silicon nonconductive wall. (b-d) 60 s example raster plots of spontaneous electrophysiological activity on MEAs with DA neuron cultures at DIV 73 from either healthy controls (b), or individuals with a monoamine oxidase A (MAOA) missense mutation (c) or nonsense mutation (d). Detected spikes are indicated as black bars. Network wide bursting activity is highlighted by colored boxes. (e) Quantification of mean firing rate. (f) Quantification of mean burst rate (g) Quantification of network burst rate. Sample size across 3 independent differentiations: control N = 14, missense N = 10, nonsense N = 5. Details regarding data in Fig. 3 can be found in Table S6. All data represent means ± SEM. One-Way ANOVA with Dunnett’s correction for multiple testing was used to compare between patient lines and control lines. ***p < 0.001.

3.5. MAOA mutations result in NMDAR hyperfunction

Next to AMPAR mediated currents, NMDAR-mediated currents are an important component of balanced network activity both in vitro and in vivo, and changes in NMDAR function have been shown to affect network function in hiPSC-derived neuronal cultures (Frega et al., 2019b; Frega et al., 2020). We hypothesized that aberrant NMDAR function could be responsible for the hyperactive network phenotypes in the Brunner syndrome DA neurons. To test this, we measured the transcripts of the most common NMDAR subunits by RT-qPCR for all hiPSC derived DA neuron lines. We found no significant changes in GRIN1 mRNA expression, which codes for the mandatory subunit present in functional NMDARs (Fig. 4k, S6k). However, we found a two-fold upregulation of GRIN2A and GRIN2B mRNA across all patient lines, which encode NMDAR subunit 2A and subunit 2B, respectively (Fig. 4l–m, Fig. S6l–m). Aberrant expression of GRIN2A and GRIN2B has been shown to directly affect NMDA mediated current responses (Endele et al., 2010; Myers et al., 2019). In order to assess whether the increased NMDAR subunit expression leads to increased NMDAR-mediated currents, we stimulated Control-1, patient ME2 and patient ME8 DA neurons through local (somatic) exogenous application of NMDA in Mg²⁺ free conditions at a membrane potential of -60 mV. Administration of 10 mM NMDA for 100 ms led to saturation of the NMDAR-mediated inward
current (Fig. 4n, Fig. S6n) in all DA neurons. We found that the total current transfer mediated by the NMDA application was significantly increased in missense MAOA mutation DA neurons compared to controls (Fig. 4o-p, Fig. S6o). Taken together, this suggests that increased NMDAR expression or function might underlie the increased network activity observed in Brunner syndrome DA neuronal networks at DIV 73.

3.6. Correction of MAOA mutation restores NMDAR expression and DA neuronal network activity

Our data show that GRIN2A and GRIN2B expression are increased in all patient lines, and functional responses to NMDA are enhanced in the ME2 and ME8 line. This suggests increased NMDAR function is a direct consequence of MAOA mutation, which has also been shown in a mouse model of MAOA dysfunction (Bortolato et al., 2012). In order to further strengthen this possible causality, we generated an isogenic hiPSC line (ME8-CRISPR) in which we corrected the p.C266F mutation present in...
the ME8 line through CRISPR/Cas9 mediated homologous recombination (Fig. 5a, Fig. S7a,b). We found that NMDAR subunit transcript levels in ME8-CRISPR DA neurons were similar to control values at DIV 73 (Fig. 5b-d, Fig. S7c-e). This suggests that rescue of a disruptive mutation in MAOA results in functional rescue that normalizes GRIN2A and GRIN2B expression to control levels. Correction of the ME8 missense mutation also resulted in the normalization of the NMDA-induced NMDAR-mediated current transfer (Fig. 5e, f, Fig. S7f). Finally, we found that restoration of MAOA function normalized activity on the MEA to control values (Fig. 5g, Fig. S5g,h). For each experiment, all lines were measured simultaneously within the same 24-well MEA, and the ME8 and CRISPR-ME8 data was normalized to the control values within the same experiment. Whereas ME8 DA neurons showed increased network activity, this increase was absent in ME8-CRISPR DA neuronal networks as the mean firing rate (Fig. 5h) and mean burst rate (Fig. 5i) were similar to control levels. Therefore, we conclude that rescue of the p.C266F MAOA mutation results in normalization of GRIN2A and GRIN2B expression, which is reflected by a restoration of neuronal network activity to control values. This strengthens the suggestion that aberrant expression or function of NMDARs significantly contributes to the neuronal network phenotypes observed in DA neurons derived from individuals with Brunner syndrome.

4. Discussion

Monoamine aberration through mutations that reduce MAOA function results in Brunner syndrome. Although the syndrome has been described almost three decades ago, insight into the molecular mechanisms of the disorder is still lacking. Here, we developed a hiPSC-derived DA neuron model to assess the molecular and cellular phenotypes underlyng brain dysfunction in Brunner syndrome. Until now, reports on four families have been published in which individuals have Brunner syndrome(Brunner et al., 1993a; Brunner et al., 1993b; Chen et al., 2004; Piton et al., 2014). In the general population, the VNTR polymorphism in the promoter of MAOA can induce different levels of transcriptional activity(Sablo et al., 1998). Low activity alleles of MAOA (MAOA-L) have been associated with increased anti-social behavior in individuals subjected to childhood maltreatment(Caspi et al., 2002; Byrd and Manuck, 2014). Interestingly, we recently showed increased structural and functional connectivity of brain regions associated with emotion regulation in individuals carrying MAOA-L alleles compared to those carrying high activity MAOA (MAOA-H) alleles using magnetic resonance imaging(Harneit et al., 2019). The individuals with Brunner syndrome included here carry both MAOA-L (ME8) and MAOA-H (ME2 and NE8) alleles. Our control individuals both express the MAOA-L genotype. This conservative approach means that differences between these controls and the Brunner syndrome patient lines could be an underestimation of the effect of MAOA mutation on neuronal function compared to the general population. Intriguingly, the ME8 line, which is the only Brunner syndrome line in our study that expresses the MAOA-L genotype, did show an increase in dendritic complexity compared to both controls and the ME2 and NEB patient lines. However, this did not result in differences at the functional level between ME8 and the other Brunner syndrome lines. Furthermore, ME2, ME8 and NEB all showed similar synapse densities and GRIN2A and GRIN2B expression. This suggests the functional phenotypes we show cannot be explained by differences in MAOA-L or MAOA-H VNTR genotypes, but a role for the VNTR in the regulation of DA neuron morphology could still be possible. As such, the increased dendritic complexity might be the consequence of a lack of MAOA activity, possibly aggravated by the presence of the MAOA-L VNTR. Further exploration of whether MAOA-L and MAOA-H alleles affect DA neuron function in healthy subjects can help us understand molecular mechanisms regulated by MAOA in the general population.

Similar to how MAOA mutations affect NMDAR function in human DA neurons, increased expression of the GRIN2A and GRIN2B NMDAR subunits has been observed in prefrontal cortex of M rotated hizygous knockout mice. The prefrontal cortex is a highly heterogenous region, and until now it was unclear whether the changes in NMDAR expression and NMDA mediated currents that were observed in M rotated hizygous knockout mouse were established through cell-autonomous mechanisms. We observed increased network activity and increased NMDAR activity in mouse serotonergic neurons(Vitalis et al., 2002) is a species-specific effect or occurs as well in human serotonergic neurons.

The individuals with Brunner syndrome all carry rare mutations, which lead to either complete loss or reduced activity of the MAOA enzyme(Brunner et al., 1993a; Brunner et al., 1993b; Chen et al., 2004; Piton et al., 2014). In the general population, the VNTR polymorphism in the promoter of MAOA can induce different levels of transcriptional activity(Sablo et al., 1998). Low activity alleles of MAOA (MAOA-L) have been associated with increased anti-social behavior in individuals subjected to childhood maltreatment(Caspi et al., 2002; Byrd and Manuck, 2014). Interestingly, we recently showed increased structural and functional connectivity of brain regions associated with emotion regulation in individuals carrying MAOA-L alleles compared to those carrying high activity MAOA (MAOA-H) alleles using magnetic resonance imaging(Harneit et al., 2019). The individuals with Brunner syndrome included here carry both MAOA-L (ME8) and MAOA-H (ME2 and NE8) alleles. Our control individuals both express the MAOA-L genotype. This conservative approach means that differences between these controls and the Brunner syndrome patient lines could be an underestimation of the effect of MAOA mutation on neuronal function compared to the general population. Intriguingly, the ME8 line, which is the only Brunner syndrome line in our study that expresses the MAOA-L genotype, did show an increase in dendritic complexity compared to both controls and the ME2 and NEB patient lines. However, this did not result in differences at the functional level between ME8 and the other Brunner syndrome lines. Furthermore, ME2, ME8 and NEB all showed similar synapse densities and GRIN2A and GRIN2B expression. This suggests the functional phenotypes we show cannot be explained by differences in MAOA-L or MAOA-H VNTR genotypes, but a role for the VNTR in the regulation of DA neuron morphology could still be possible. As such, the increased dendritic complexity might be the consequence of a lack of MAOA activity, possibly aggravated by the presence of the MAOA-L VNTR. Further exploration of whether MAOA-L and MAOA-H alleles affect DA neuron function in healthy subjects can help us understand molecular mechanisms regulated by MAOA in the general population.

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function in DA neurons from individuals with Brunner syndrome, which are both brought to control values when we rescue the MAOA mutation in our ME8 missense line. This suggests that reversal of the p.C266F mutation leads to restoration of MAOA function, and that reduced MAOA function directly contributes to increased NMDAR mediated activity and network hyperactivity. Benefits of the modulation of NMDAR activity have previously been observed in Maaq hemizygous knockout mice, where NMDAR antagonism improved locomotor function (Bortolato et al., 2012). Lastly, there is evidence that the effects of MAO inhibitors on neuronal activity are highly time-course dependent (Chenu et al., 2009; Garcia-Miralles et al., 2016; Fitzgerald and Watson, 2019). Our preliminary data suggests acute application of the potent MAO inhibitor Clorgyline can increase network activity in DIV73 DA neuronal networks (Fig. 5Sd). Taken together, this suggests that extensive assessment of NMDAR protein expression and function in Brunner syndrome is warranted, as the overlap in mechanisms affected in the prefrontal cortex of these mice and hiPSC-derived DA neurons of individuals with Brunner syndrome shows that DA neuron cultures are a viable in vitro system to investigate possible therapeutic strategies.

This study presents several conceptual strengths and limitations. We present a conservative approach to investigate possible molecular mechanisms that underlie changes in neuronal network activity following mutations in MAOA. We chose to use patient lines with different types of mutations instead of CRISPR-Cas9 mediated generation of isogenic lines, and found that all patient lines result in similar changes at the molecular and functional level. Though we were limited to the generation of only a single CRISPR-Cas9 mediated rescue line, the normalization of both molecular and functional phenotypes to control values suggests reinstatement of MAOA function is sufficient to normalize DA neuronal network development to control values. Furthermore, the use of patient lines as well as possible heterogeneity of culture compositions could be sources of variability. However, we show here that this approach can be used if the functional consequences of a gene mutation are strong enough to supersede interindividual or culture composition variation. Nonetheless, such sources of variation might be the reason of high morphological variability between Control-1 and all patient (Fig. 2). This limited follow-up investigation or morphological phenotypes, and led us to decide to not include Control-2 for further morphological characterization and comparison. Generation of an isogenic knockout line could be an interesting opportunity to reveal if morphological changes in DA neuron complexity, as previously described in MAOA−/− mice (Bortolato et al., 2011; Bortolato et al., 2013), are a more subtle phenotype compared to the changes at the functional level. A final possible limitation could have been our choice to include individuals with both MAOA-H or MAOA-L expression variants in our study, as these variants have been suggested to affect neuronal activity in vivo (Harneit et al., 2019). However, our data shows that we are able to detect a network phenotype generated by DA neurons with MAOA mutations that is not affected by MAOA-H or MAOA-L variant expression. Moreover, this approach strengthens the association between MAOA mutations and NMDAR-mediated changes in DA neuron network activity. Investigation of the interindividual variation of DA neuron morphology and function across controls could be an interesting research direction similar to (Mossink et al., 2021), especially considering that MAOA-H and MAOA-L expression variants are suggested to impact neuronal morphology and connectivity in the human brain (Harneit et al., 2019).

In conclusion, our data suggest that mutations in MAOA that cause Brunner syndrome affect DA neuron function in vitro, and that NMDAR hyperfunction is a key contributor to network dysfunction in Brunner syndrome DA neuron cultures. These alterations on the network level might be able to explain part of Brunner syndrome associated impulsivity and maladaptive externalizing behavior. Manipulation of NMDAR function could be a viable opportunity toward the development of possible therapeutic strategies.

5. Key points and relevance

- Brunner syndrome is caused by mutations that result in loss of MAOA function.
- hiPSC derived DA neurons show mutation and patient specific changes in complexity, different from rodent models of MAOA dysfunction.
- hiPSC derived DA neuronal networks with MAOA mutations show NMDAR hyperactivity and increased network activity.
- Correction of a missense MAOA mutation restores both the molecular and functional NMDAR hyperactivity and network activity to control values.
- Manipulation of NMDAR activity could be an important next step in the development of possible therapeutic strategies for Brunner syndrome.

Author contributions


Declaration of Competing Interest

BF has received educational speaking fees from Medice. The other authors declare to have no competing interests.

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Appendix A. Supplementary data

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