Haploinsufficiency of the 22q11.2 microdeletion gene Mrpl40 disrupts short-term synaptic plasticity and working memory through dysregulation of mitochondrial calcium

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INTRODUCTION

Schizophrenia (SCZ) is a catastrophic disease that affects approximately 1% of the world’s population and is characterized by multiple symptoms that include cognitive abnormalities such as deficits in working memory, executive function and learning.¹ Mechanisms of cognitive symptoms of SCZ are poorly understood, partly because only weak associations have been identified between any single gene and the disease, and valid animal models have been lacking.² Mouse models of 22q11.2 deletion syndrome (22q11DS) are among the few animal models that replicate abnormalities associated with SCZ. The 22q11DS is the most common multigene syndrome in humans and is considered a genetic risk factor for SCZ. The 22q11DS is caused by the hemizygous deletion of a 1.5- to 3-megabase region on chromosome 22 causing 22q11.2 deletion syndrome (22q11DS), which constitutes one of the strongest genetic risks for schizophrenia. Mouse models of 22q11DS have abnormal short-term synaptic plasticity that contributes to working-memory deficiencies similar to those in schizophrenia. We screened mutant mice carrying hemizygous deletions of 22q11DS genes and identified haploinsufficiency of Mrpl40 (mitochondrial large ribosomal subunit protein 40) as a contributor to abnormal short-term potentiation (STP), a major form of short-term synaptic plasticity. Two-photon imaging of the genetically encoded fluorescent calcium indicator GCaMP6, expressed in presynaptic cytosol or mitochondria, showed that Mrpl40 haploinsufficiency deregulates STP via impaired calcium extrusion from the mitochondrial matrix through the mitochondrial permeability transition pore. This led to abnormally high cytosolic calcium transients in presynaptic terminals and deficient working memory but did not affect long-term spatial memory. Thus, we propose that mitochondrial calcium deregulation is a novel pathogenic mechanism of cognitive deficiencies in schizophrenia.

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Here we report results of our STP screening of the distal region of the 22q11D5 microdeletion, which encompasses six genes: Cldn5, Cdc45l1, Ufd1l1, 2510002D24Rik, Mrpl40, and Hira. Using mutant mice carrying hemizygous deletions of individual genes, we discovered that haploinsufficiency of Mrpl40 (mitochondrial large ribosomal subunit protein 40, also known as Nlvf1) causes abnormal STP and short-term memory deficits via Serca2-independent deregulation of presynaptic Ca2+. Using two-photon Ca2+ imaging of the genetically encoded Ca2+ indicator GCaMP6,29 expressed either in the presynaptic cytosol or in mitochondria, we showed that Mrpl40 haploinsufficiency hindered the extrusion of Ca2+ from the mitochondrial matrix through an impaired mitochondrial permeability transition pore (mPTP). This leads to abnormally high levels of Ca2+ in the presynaptic cytosol and elevated STP. Our data implicate Mrpl40 as a 22q11D5 gene, the haploinsufficiency of which contributes to cognitive deficits in microdeletion-related SCZ.

MATERIALS AND METHODS

Animals

Mature (16–20 weeks) mice of both sexes were used. Production and genotyping of D1(16)35cGrt, D2(16)35cGrt, Cldn5−/−, and Hira−/− mice were previously described.31–36 To generate Cdc45l1−/− and Ufd1l1−/− mice, we obtained SIGTR embryonic stem (ES) cell clones containing gene-trap disruptions of the pGTO1Lxr vector for Cdc45l1 (cell line A0425) and a Ufd1l1 allele (cell line AW0532) from the Mutant Mouse Regional Resource Center (University of California, Davis, CA, USA). The A0425 ES cell line carries a Genetrap insertion in exon 3 of the Cdc45l1 gene. Offspring were genotyped using the following primers: Cdc45lF: GCTGGGTACCTGAGTGTCATTG, and the betageo primer 2:

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\text{Ufd1lF: GTTGACGCTAACGTCCAGTCAC, Ufd1lR: GAAGCAGC} \\
\text{GTTCGCTATGCTGATGTTG, and the betageo primer 2:}
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(University of California, Davis. CA, USA). The AJ0425 ES cell line carries a disruptions of the pGT01Lxr vector for obtaining SIGTR embryonic stem (ES) cell clones containing gene-trap

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\text{GTTGG, producing a 612-bp WT amplicon and a 304-bp mutant amplicon.}
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whole-cell electrophysiology

Electrophysiological data were acquired using a MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA), filtered at 2 kHz and digitized at 10/20 kHz using a Digidata 1440 digitizer (Molecular Devices) controlled by Clampex acquisition software (Molecular Devices). All offline analyses of electrophysiological data were done in Clampfit. To evoke excitatory postsynaptic current amplitudes (EPSCs) in CA1 neurons, we stimulated Schaffer collateral axons with a concentric bipolar stimulating electrode (1.25 μm outer diameter, 1.25 μm inner diameter) connected to a Master 8 stimulator and an Iso-Flex stimulus isolator (both A.M.P.I., Jerusalem, Israel) with 100-μs pulses. Stimulating and recording electrodes were separated by at least 350–400 μm. For STP experiments, a cut was placed between the CA3 and CA1 regions to avoid recurrent stimulation. Recording electrodes were borosilicate glass capillaries pulled in a P-1000 puller (Sutter Instruments, Novato, CA, USA) and had resistances approximately 3.5–5 MΩ. CA1 neurons were whole-cell voltage-clamped using electrodes filled with 130 mM K gluconate, 10 mM EGTA, 2 mM MgCl2, 5 mM NaCl, 2 mM ATP–Na2, 0.4 mM GTP–Na, 10 mM HEPES and 10–25 μM Alexa 594 (pH 7.35, ~290 mOsm). EPSCs were recorded by holding the cells at −74 mV (accounting for a liquid junction potential of ~14 mV). Access resistance was measured using a ~5 mV step before each recording (range, 25–50 MΩ), and cells that displayed unstable access resistance (variations >20%) were excluded from the analyses. All EPSC recordings for STP experiments were done in the presence of the GABAAR antagonist picrotoxin (100 μM) to prevent inhibitory responses and the NMDA receptor antagonist 2-aminophosphonovalerate (D-AP5, 50 μM) to avoid induction of long-lasting plasticity.

STP components were separated using an adapted procedure.30 A baseline of stable EPSCs was established to evoke 150- to 200-pa EPSCs. Stimulation intensities ranged from 30 to 60 μA and were held constant for each cell throughout the experiment. Stimulation intensity did not differ significantly between slices from WT and mutant litters (P > 0.05). The stimulation protocol consisted of the following: 5 pre-train pulses (0.2 Hz), a high-frequency train (100 pulses, 80-Hz), 24 pulses at 0 Hz (to measure recovery from depression) and 24 pulses at 0.2 Hz (to measure augmentation). The 100 (100 Hz) pulses train was chosen because it induced both strong depression and augmentation of EPSP amplitude on the basis of STP induced by trains of different frequencies (data not shown). EPSCs during and after the train were normalized to the average of the five pre-train EPSCs (shown as a single baseline point), and the amplitudes are reported as normalized EPSC peak amplitudes. For each cell, we averaged the data from 3 to 4 trials by using this stimulation protocol. To measure the EPSC amplitudes during the train, we resorted to a binning procedure, instead of the template waveform-based subtraction protocol, as previously described.30 The measured EPSC train of 1250 ms (80-Hz train of 100 pulses) was divided into 200-ms bins, and the peak amplitude within each bin was graphed as the normalized EPSC amplitude during the train. This procedure was sufficient to separate EPSCs in the train.

Augmentation, the slowest component of STP, has negligible contamination from depression and facilitation and is reported as measured without corrections. Statistical comparisons of EPSCs were made using the measured peak augmentation at 5 s after the 80-Hz train, instead of the extrapolated augmentation at 0 s. Recovery from depression (200–4800 ms) after the 80-Hz train was contaminated by augmentation and some residual facilitation. Because the EPSCs measured during the recovery phase had an interstimulus interval of 200 ms, the contamination by facilitation was minimal and corrected only for overlying augmentation. To that end, the measured augmentation decay curve (5–120 s after the 80-Hz train) was extrapolated from 200 to 4800 ms after the train by using a standard exponential fit in Clampfit. The normalized EPSCs during the recovery phase were then corrected for the overlying extrapolated augmentation for each time point. Facilitation was measured in separate experiments by using paired pulses at interstimulus intervals of 20–1000 ms.

To measure excitability, we held CA3 neurons in current clamp mode and added D-AP5 (50 μM) and picrotoxin (100 μM) to the external ACSF bath to avoid possible long-term effects. Action potential (AP) parameters were estimated by clamping the cell at ~65 mV using automatic slow-current injection. Input resistance, AP threshold, rheobase (intensity of current reached at threshold) and number of APs (evoked by holding at the minimum current needed to reach threshold) were measured by injecting 1-s steps of 25-pA current (12 steps from ~25 pA to 250 pA). To measure AP widths, we evoked APs by injecting five short current steps (1500–2000 pA, 1-ms duration, 0.2 Hz). AP durations were calculated as the...
time interval between the up-stroke and down-stroke of the AP waveform at ~10 mV. AP duration during the 80-Hz train was normalized to the AP duration at baseline, as previously described.21 Electrophysiology experiments were done without blinding.

Generation of plasmids and viruses

To generate adeno-associated viruses (AAVs) expressing cytoplasmic GCaMP6f (GCaMP6f) and mitochondrial-targeted GCaMP6f (mitoGCaMP6f), we used PCR to amplify the human synapsin promoter (hSyn) from pAAV-6P-SEWB.20 The pAAV-GFP (Addgene plasmid 32395) was cut with SnbI and SacI to replace CMV with hSyn (pAAV-hsyn-GFP). EcoRI and BamHI were used to replace pAAV-hsyn-mCherry to generate pAAV-hsyn-mCherry. To generate pAAV-hsyn-mCherry-2A-GCaMP6f, oligonucleotides containing the coding sequence for the 2A peptide were used for PCR amplification of GCaMP6f from pGP-CMV-GCaMP6f (Addgene plasmid 40755). To generate pAAV-hsyn-mCherry-2A-mitoGCaMP6f, oligonucleotides containing the coding sequence for the 2A peptide and the mitochondrial-targeting sequence (MSLVTPLLLRLGSRPRLPVRAPRHS) were used for PCR amplification of GCaMP6f from pGP-CMV-GCaMP6f (Addgene plasmid 40755). To generate AAV-Slc25a4 oe, we used PCR to amplify the open reading frame of Slc25a4. Drd4 open reading frame from AAAMKInn-Drd2 OE13 was replaced with the Slc25a4 open reading frame using HindIII. DNA sequencing was used to verify the absence of PCR-induced mutations. Lentivirus vector sRNA plasmids (control sRNA, 5′-TAGCTCCAAGCTGTCCGAGAAAGA-3′; Slc25a4 shRNA1, 5′-GCAAGGATCTTTCCACCGCAAATCCTAA-3′; Slc25a4 shRNA2, 5′-GGTTGAGACTGTGCGTCTGGAAGGATC-3′; Slc25a4 shRNA3, 5′-GCACATATCATGAGCTCAGATGATTGATTGCC-3′) were generated by Applied Biological Materials (Richmond, BC, Canada). Viruses (1.8 × 10^9 to 1 × 10^10 particles ml⁻¹) were produced by either the St. Jude or University of Tennessee Health Sciences Center Viral Vector Cores.

Two-photon imaging of presynaptic calcium and in vivo injections

Two-photon laser-scanning microscopy was performed using a Ultima imaging system (Prairie Technologies, Middleton, WI, USA), a Ti:sapphire Chameleon Ultra femtosecond-pulsed laser (Coherent, Santa Clara, CA, USA) and × 60 (0.9 NA) water-immersion IR objectives (Olympus, Center Valley, PA, USA). Calcium (Ca²⁺) transients in presynaptic terminals were recorded using GCaMP6f expressed in the CA3 hippocampal neurons.

To express GCaMP6f, mice were anesthetized using isoflurane (2% for induction and 1.5% for maintenance) in 100% oxygen, and their heads were restrained on a stereotaxic apparatus. An approx. 1-cm midline incision was made centered about 0.25 cm behind bregma. Vessels were injected into three locations within the CA3 region, in one or both hemispheres. The stereotaxic coordinates for the three injections were as follows, in relation to the bregma: (1) −1.5 mm anteroposterior, 1.8 mm lateral, and 1.7 mm deep; (2) 2.2 mm anteroposterior, 2.3 mm lateral and 1.8 mm deep; (3) 2.5 mm anteroposterior, 2.8 mm lateral and 2.2 mm deep. Craniotomies were made at these locations, and 200 nl of AAVs were slowly (20 nl min⁻¹) injected via a 33G cannula. After each injection, the cannula was left in place for 2–3 min before being retracted. Following injections, the skin was sutured, and the mice were allowed to recover before returning to the holding cages. Imaging experiments were performed 4–7 weeks after AAV injections. During each experiment, care was taken to limit the differences in post-injection durations to a maximum of 2–3 days across experimental groups to avoid substantial differences in the levels of AAV expression.

To visualize GCaMP6f or mitoGCaMP6f, we used brain slices prepared from AAV-injected mice. Schaffer collaterals were stimulated via field stimulation using bipolar stimulating electrodes, as in STP experiments. In addition to D-AP5 and picrotoxin, the AMPA receptor antagonist NBQX (3 μM) was added to the bath ACSF to prevent excitation of postsynaptic neurons. GCaMP6f was visualized at 940 nm, and mCherry was visualized at 1040 nm by two Ti:Si lasers. Presynaptic boutons were identified in a 34 μm × 34 μm region of interest by activity-dependent increase in GCaMP6f fluorescence during time-series scans. Line-scans through identified boutons were then used for experiments. Boutons from 4 to 8 regions of interest in the stratum radiatum of the CA1 area were imaged for each mouse. In some experiments, we used whole-cell recordings from CA3 neurons and filled the cells with Alexa Fluor 594 (30 μM) and Fluor S F (300 μM) at 820 nm to visualize presynaptic Ca²⁺. Axons emanating from the cell bodies were identified based on their morphology and lack of spines. Presynaptic terminals were identified as boutons in secondary and tertiary axonal branches. Those axons could not be tracked beyond 200 μm from the CA3 cell bodies due to the limitation of the approach. APs were evoked by holding the cells at −70 mV (current clamp mode) and injecting depolarizing current (3.5–4.0 nA, 500 μs). We recorded Ca²⁺ transients in line-scan mode from 3 to 7 boutons per cell. For GCaMP6f or mitoGCaMP6f experiments, the baseline fluorescence (F₀) was used to calculate the change in signal (ΔF/F₀). For Fluo 5F experiments, fluorescence changes (ΔG/R) were quantified as an increase in Fluo 5F fluorescence (ΔG) normalized to the respective Alexa 594 fluorescence (R). Imaging experiments were done without blinding.

Two-photon glutamate uncaging

For two-photon glutamate uncaging (TGU), MNI glutamate (2.5 μM) was added to the recording ACSF. The timing and intensity of glutamate uncaging were controlled by TriggerSync (Prairie Technologies). In typical experiments, 0.2-ms pulses from a second Ti:sapphire Chameleon Ultra laser (720 nm) were delivered to the vicinity of a targeted dendritic spine, and TGU-evoked EPSCs (uEPSCs) were recorded. The duration and intensity of illumination of the uncaging laser were then adjusted to induce responses that mimicked spontaneous miniature EPSCs, which were recorded in CA1 neurons and averaged 10–15 pA. After the uncaging parameters (i.e., site, laser duration and laser intensity) were adjusted for a single spine, the parameters remained constant for the STP experiments on the particular spine. An 80-Hz train of 100 TGU pulses was delivered to a single dendritic spine to measure TGU-induced STP.

Electron microscopy

Mice were anesthetized with ethyl carbamate (1.5 g kg⁻¹, 25% solution, intraperitoneal) and perfused transcardially with phosphate-buffered saline for 1–2 min and then a fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.2 M sodium cacodylate). Brains were isolated, stored at 4 °C overnight in the same fixative, and sagittal sections (100-μm-thick) were prepared on a vibratome (Leica Biosystems, Buffalo Grove, IL, USA). Smaller regions (~500 μm × 500 μm) containing the stratum radiatum of the CA1 hippocampal region were processed for three-dimensional (3D) scanning electron microscopy. The samples were stained with a modified heavy-metal-staining method, processed through a graded series of alcohol and propylene oxide, and then embedded in Epon hard resin.22 Sections (0.5-μm-thick) were cut to determine the correct area and then coated with iridium in a Denton Desk II sputter coater (Denton Vacuum, Moorestown, NJ, USA). The 3D EM images were collected on a Helios Nanolab 660 Dualbeam system (FEI, Hillsboro, OR, USA). From the 3D stacks of electron micrographs (10x x 10x x 100 nm voxel size, 250–260 sections of 10-nm thickness and approximately 30 x 20 μm area), synapses were identified based on the presence of postsynaptic densities and presynaptic vesicles. The mitochondria in presynaptic terminals were identified and counted manually.

For transmission electron microscopy, 100-μm-thick vibratome sections containing the CA1 stratum radiatum region were prepared as described above and fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). Sections were post fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer with 0.3% potassium ferrocyanide for 1.5 h. After rinsing in the same buffer, the sections were dehydrated through a series of graded ethanol and propylene oxide solutions, infiltrated and embedded in epoxy resin, and polymerized at 70 °C overnight. Thin sections (0.5 μm) were stained with toluidine blue for examination by light microscopy. Ultrathin sections (80 nm) were cut and imaged using a Tecnai F 20 FEG Electron Microscope (FEI) with a XRX41 Camera (Advanced Microscopy Techniques, Woburn, MA, USA).

Mitochondrial DNA quantification

RNA was isolated from the hippocampus of 4-month-old mice by using the mirVana RNA isolation kit (Ambion, Life Technologies, Foster City, CA, USA). The SuperScript III reverse transcriptase kit (Invitrogen, Life Technologies) was used to synthesize cDNA from 1 μg RNA. The following primers were used in the qPCR experiments: COI (5′-GCCCCAGATATAGCATTCCC-3′ and 5′-GTTCATCTGTTCCTGCTCC-3′); ND2 (5′-CCCTATCCCTGTCTAGATCACC-3′ and 5′-ATAGATCTAGATGGTAGTC-3′); 18 S (5′-TAGAGGCCAACTGTTGGCCCT-3′ and 5′-CGGTGAGCGCGCTAGT-3′). The ratio of mitochondrial (COI and ND2) transcripts to the nuclear 18S transcript was used to quantify relative mitochondrial DNA.

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Mtrp40 is required for mitochondria calcium, STP and memory

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Mitochondrial respiration

Mice were decapitated, and their brains were dissected immediately in cold (4 °C) dissecting ACSF. Hippocampi were removed and washed in a mitochondrial isolation buffer containing 250 mM sucrose, 1 mg/ml bovine serum albumin, 5 mM EDTA and 10 mM Tris-HCl at pH 7.4. A crude mitochondrial fraction was then isolated by differential centrifugation. Briefly, the hippocampal tissue was finely minced on a pre-chilled glass dish, washed several times, resuspended in 1 ml mitochondrial isolation buffer and homogenized in a 2-ml Dounce homogenizer (glass/glass, 5–7 runs in an ice bath). The homogenized tissue was centrifuged at 700 g for 5 min; the supernatant was then transferred to a new 1.5-ml tube and centrifuged at 7000 g for 10 min. The resulting pellet was washed in 1 ml mitochondrial isolation buffer and resuspended in 30 μl mitochondrial isolation buffer. The protein concentration in the pellet was typically 25–35 mg protein ml⁻¹. The mitochondrial integrity was tested by measuring the glutamate/malate-dependent respiratory control ratio (i.e., State III/State IV of respiration). The resulting mitochondrial samples were used immediately to measure respiration (mitochondrial oxygen consumption) and Ca²⁺-retention capacity.

Mitochondrial oxygen consumption was measured using a Clark-type electrode (Hansatech Instruments, Norfolk, UK) in a thermostatically controlled chamber equipped with a magnetic stirring device and a gas-tight stopper fitted with a narrow port for additions via a Hamilton microsyringe. Isolated mitochondria were placed in the respiration chamber at 37 °C in 0.4 ml respiration buffer (250 mM sucrose, 1 g l⁻¹ bovine serum albumin, 10 mM KH₂PO₄, 2.7 mM KCl, 3 mM MgCl₂, 40 mM HEPES, 0.5 mM EGTA, pH 7.1) to yield a final concentration of 0.5 mg ml⁻¹ mitochondrial protein. Mitochondrial respiration was stimulated by the addition of 2 mM ADP (state IV). The measurement protocol involved sequential addition of 5 mM glutamate, 2.5 mM malate (activating the CI–CIV span), 1 μM rotenone, 10 μM succinate (activating the CII–CIII span), 1 μM antymycin A, 10 μM ascorbate, 0.4 mM TMPD (activating the CIV span) and 5 mM KCN. The rates of oxygen consumption were calculated online, as first derivatives of the oxygen-content changes by manufacturer-provided software.

Quantitative real-time PCR

The cDNA was generated from 1 μg hippocampal RNA by using Super Script III (Life Technologies, Waltham, MA, USA). Primers for qPCR were as follows:

- Hira: TCGCCGATCCATCAATTC and CATATCTCCACCAGGCTTAC, Ctdns: GCCACAGACCTGGAGAG and GCGACACAGTCTACTATAC, Mrpl40: CTGGATCTAGGAGATGAGTCAAGG and ATGGCGCGAGTTGCATTG, Ybflf: TCAAGCTATGTTGACCTGC and TTTATTAGCTGACCGAGAA, 2150009224Rik: GTGTCAGGTGAGGATTA and AGAAAGCAAGTAGATGAGGAC, Cdc451: GATTTCCGCAAGGAGTTCTACG and TACTGGACGTGGTCACACTGA, Co1: GCCCGATGAGATCCTCCC and GTGATCCCTGGTCTCCTCC, Nd2: GGCAATCCATTGATGACT and ATGATAGTGGATGAGGCA, 18s: TAGAGGCAAGGTGCGCT and CGTGACAGGCGCTG.

We performed qPCR using SYBR green in an Applied Biosystems 7900HT Fast Real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) and the standard protocol. A serial dilution of cDNA was used to generate a standard curve for each primer set, and this curve was used to calculate gene concentrations for each sample. All samples were run in triplicate.

Mouse behavior

Mature animals (16–20 weeks) were used for all behavior experiments.

Morr's water maze. One hour prior to testing, animals were brought into the testing room and allowed to habituate. Testing was performed during the animal’s inactive phase under dim-light conditions. Mice were allowed to navigate in the maze, and swim patterns were recorded with a video camera tracking system (HVS Image, Buckingham, UK) mounted above the pool. Animals learned to find a hidden, clear platform by using the standard spatial version of the Morris water maze task for four successive days. Each day, animals were given four 1-min trials from each starting position with an intertrial latency of 60 s. The order of the starting locations was counterbalanced each day by using a Latin-square design. A spatial learning (probe) trial was administered 1 h after the completion of spatial training. A spatial memory (probe) trial was administered 48 h after completion of the spatial learning. During both probe trials, the platform was removed, and the mice received a single 1-min trial in which the animals tried to find the escape platform. These trials originated from the starting location that was the furthest from the platform’s location throughout training. Mice also completed a nonspatial learning task at least 7 days after completion of the spatial protocol. In that task, mice were trained to find a black visible platform for two successive days. During day 1, the escape platform was located in the same position used during spatial training. The next day, the escape platform was moved to a new quadrant. Each day, the mouse was given four 1-min trials in the same manner that occurred during spatial training. To avoid hypothermia, immediately after each round of training and testing trials, animals were dried with paper towels and placed in warmed holding cages.

Delayed non-matched-to-position task. To motivate mice to complete the delayed non-matched-to-position task, they were subjected to water restriction for 2 days prior to testing. Specifically, mice were allowed 2 h of free access to water per day. Mice were weighed daily to ensure that weight decrease during deprivation did not surpass the recommended 20% loss. One hour prior to testing, animals were brought into the testing room and allowed to habituate. Testing was performed during the animal’s inactive phase under well-lit lighting conditions. The testing apparatus consisted of a Y-maze (Clever Sys, Reston, VA, USA) with a start arm (20 cm × 16 cm × 7 cm; /x×x×x) leading to two goal arms (20 cm × 16 cm × 7 cm). Mice were allowed to habituate to the maze and were given a positive reinforcer (i.e., Chocolate YooHoo) before behavioral testing. To achieve this, mice were allowed to investigate the Y-maze baited with YooHoo for 15 min. Maze habituation was performed for two consecutive days. After maze habituation was complete and the animal had consumed the food rewards, we conducted a test of spatial working memory. First, the mouse was constrained in the start arm with a guillotine door. Next, a sample arm was determined at random and the choice arm was closed off with a guillotine door. There was a limit of two consecutive same-side sample arms in a 10-trial test. Both arms were then baited with 20 μl YooHoo. The mouse was released from the start chamber and allowed to run to the sample arm and consume the reward. The mouse was then returned to the start arm and the guillotine door to the nonsample arm was removed. All efforts were made to keep the intrtrial interval at 5 s. The mouse was again released from the start chamber and allowed to run to either the previously entered sample arm or new choice arm to consume the reward. A return to the sample arm was counted as an incorrect response. Incorrect responses resulted in no reward and return to the start arm. A total of 10 trials were given.

The Y-maze spatial recognition. The maze was shaped like a ‘Y’, with three equally spaced arms (20 cm × 7 cm × 16 cm) radiating from a triangular center section. The Y-maze was constructed from blue opaque plastic to aid in video detection. The maze was located in a lit room with abundant extra-maze cues. The procedure consisted of an acquisition and a recognition session. During the acquisition session, the mouse was placed facing the distal end of an arm (start arm; determined semirandomly) and allowed to freely explore the maze for 15 min. During acquisition, the mouse was allowed to freely explore two of the three arms (determined semirandomly). After completing the acquisition session, the mouse was returned to its home cage for 1 h. Next, the mouse was given a 2-min recognition session, where all three arms were available for the mouse to freely explore. Time spent in each arm and the triangular center section and the total distance traveled during each trial were automatically recorded using TopScan software (Clever Sys). Mice prefer novelty; therefore, if a mouse recognized and remembered which arms were familiar during the recognition session, the mouse would spend more time in the novel arm (expressed as a percent of total arm time) than would be expected by chance.

Acoustic startle and prepulse inhibition. Each day before testing, the mice were allowed a 1-h habituation in the testing room after being transported from the animal housing room. Before experiments were initiated, the mice were allowed to acclimate to the Plexiglas restraint chamber (6 cm × 6 cm × 4.8 cm) for 20 min. Acoustic startle and prepulse inhibition (PPI) tests were performed in ventilated, sound-attenuated chambers (Med Associates, St. Albans City, VT, USA). For acoustic-startle experiments, the mice had a 5-min acclimation period to a 65-dB background white noise, which played throughout the session. Three stimulus tones (8 kHz, 120 dB, 40 ms) were then delivered at 15-s intertrial intervals. For PPI experiments (conducted on different days than acoustic-startle experiments), mice had a 5-min acclimation period to a 65-dB background white noise.
white noise, which played throughout the session. Three acoustic startles (broadband white noise click, 120 dB, 40 ms) were then delivered separately by a 15-s intertrial interval. The testing session consisted of 39 trials of 5 trial types: pulse alone, in which the startle pulse was presented; the combination of a 40-ms prepulse of 74, 82 or 90 dB preceding the startle pulse by 100 ms; and no stimuli. Trials were separated by 15 s and presented in a pseudo-random order. PPI was calculated as follows: 100×(pulse-alone response – prepulse + pulse response)/pulse-alone response. All mouse behavior experiments were performed in a blind manner in respect to mouse genotypes.

Western blotting
AAV5-hSyn-mCherry-2A-GCaMP6f or AAVS–hSyn-mCherry-2A-GCaMP6f viruses were injected in vivo into the mouse hippocampus as described above. Four weeks after injections, mice were killed and dorsal hippocampi were isolated at 4 °C for fractionation into nucleus/cell debris, cytoplasm and crude mitochondrial fractions. Freshly extracted hippocampi from each mouse were homogenized in isolation buffer (250 mM sucrose, 75 mM mannitol, 1 mM EDTA and 5 mM HEPES, at pH 7.4) by using a glass-teflon tissue homogenizer on ice. Homogenized hippocampi were centrifuged twice at 1400 g for 3 min at 4 °C to separate nuclei and cell debris. The supernatant was then centrifuged at 17 200 g for 10 min at 4 °C to separate cytosol and crude mitochondria. Ice-cold RIPA buffer (Santa Cruz Biotechnology, Dallas TX, USA) with protease inhibitors (Roche, Basel, Switzerland) was added to the mitochondria but not the cytoplasmic fraction, and both were briefly sonicated. Samples were centrifuged at 17 200 g for 20 min at 4 °C, and the pellet was kept at −80 °C for protein quantification. Protein quantification was performed using a Pierce BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). For loading-sample preparation, we used NuPAGE LDS sample buffer (Life Technologies, Thermo Fisher Scientific).

The western blot experiments were performed similar to a previously described method. Briefly, NuPAGE 10% Bis-Tris gels and MES-SDS running buffer (Life Technologies) were used to load mitochondria and cytoplasmic fractions (10 μg total protein/well) and run the gels. The primary antibody used to detect GCaMP6 was rabbit-anti-GFP (1:1000; Abcam, 6556, Cambridge, MA, USA; also used rabbit-anti-Prohibitin 1:1000, Thermo Scientific, PAS-12274) and mouse-anti-β-actin (1:10 000; Sigma-Aldrich, 5316, St. Louis, MO, USA) primary antibodies. Secondary antibodies were Odyssey goat-anti-rabbit IRDye-680LT (1:40 000; LI-COR Biosciences, Lincoln, NE, USA, 926-68021) or Odyssey donkey-anti-mouse IRDye-800CW (1:15 000, LI-COR Biosciences, 926-32212). Membranes were imaged using an Odyssey Infrared Imager (LI-COR Biosciences). Images were analyzed using Odyssey V3.0 software (LI-COR Biosciences).

Subcellular localization of mitoGCaMP6 and GCaMP6
Neuro-2a (N2a) mouse neuroblastoma cells (ATCC, CCL-131, Manassas, VA, USA) were plated in four-well chamber slides (Lab-Tek 177399, Thermo-Fisher Scientific) at 1.25 × 105 cells/well and maintained in culture using Eagle’s medium essential media (ATCC) plus 10% fetal bovine serum (heat inactivated; Life Technologies) and 1 × PenStrep (Life Technologies) in an incubator at 37 °C and 95% O2/5% CO2. Following a 24-h incubation, cells were transfected with hSyn-mCherry-2A-mitoGCaMP6f or hSyn-mCherry-2A-GCaMP6f plasmids (0.5 μg DNA/well) using Fugene HD (1:1,000, Roche, Basel, Switzerland) with protease inhibitors (Roche, Basel, Switzerland) was added to the mitochondria but not the cytoplasmic fraction. Plasma membranes were fixed in 4% paraformaldehyde for 10 min at room temperature. Following fixation, cells were washed three times for 5 min with phosphate-buffered saline and blocked with 0.1% normal goat serum and 0.1% TritonX-100 in phosphate-buffered saline for 1 h at room temperature. We used the following antibodies: chicken-anti-GFP to detect GCaMP6 or mitoGCaMP6 (1:1000; Abcam, 13970) and goat-anti-chicken-Alexa-488 (1:1000; Life Technologies, A11039). Cell imaging was performed using an LSM-780 confocal microscope (Zeiss, Oberkochen, Germany).
Abnormal short-term synaptic plasticity in Df(16)5<sup>−/−</sup> mice is caused by Mrpl40 haploinsufficiency. (a) Diagram depicting genes in the 22.q11.2 genomic region of the human chromosome 22 and the syntenic region of mouse chromosome 16. Red horizontal bar represents genomic regions hemizygously deleted in Df(16)5<sup>−/−</sup> mice, and gray horizontal bar represents genomic regions hemizygously deleted in Df(16)1<sup>+/−</sup> mice. Note that 2510002D24Rik, Mrpl40 and Hira genes are mapped outside the Df(16)1 microdeletion. (b) Input–output relations in Df(16)5<sup>−/−</sup> and wild-type (WT) littersmates. (c) Short-term potentiation (STP, comprising facilitation, depression and augmentation) induced by the high-frequency (80 Hz) train. The first time point represents an average of five baseline excitatory postsynaptic currents (EPSCs) delivered at low frequency. The top inset shows the protocol for measuring STP, recovery from depression and augmentation in the same experiment. Average facilitation tested by paired-pulse ratio in separate experiments (without thapsigargin: |t|<sub>10</sub> = 4.404, P < 0.001; with thapsigargin: |t|<sub>10</sub> = 1.954, P = 0.0396) (Supplementary Figure S2a). Further, Serca2 protein levels were normal (U = 107, P = 0.836) in Df(16)5<sup>−/−</sup> mice (Supplementary Figure S2b), suggesting that haploinsufficiency of genes within the Df(16)5 genomic region resulted in abnormal STP through Serca2-independent mechanisms. To ensure that the STP increase in Df(16)5<sup>−/−</sup> mice originated from the presynaptic CA3 neurons, we next assessed the role of augmentation, which is the longest-lasting component of STP and operates on a time scale of tens of seconds. Using a previously reported approach, we isolated augmentation by applying a single stimulus to Schaffer collaterals every 5 s for 2 min, starting 5 s after the 80-Hz train (Figure 1f). Augmentation was significantly increased (|t|<sub>16</sub> = 4.758, P = 0.0002) in Df(16)5<sup>−/−</sup> mice compared with WT controls (Figure 1f). This increased augmentation in Df(16)5<sup>−/−</sup> mice was maximal at the onset and decayed to normal values after 20 s. Elevated augmentation in Df(16)5<sup>−/−</sup> mutants was not sensitive to the Serca inhibitor thapsigargin (4 μM) (without thapsigargin: |t|<sub>10</sub> = 4.404, P < 0.001; with thapsigargin: |t|<sub>10</sub> = 1.954, P = 0.0396) (Supplementary Figure S2a). Further, Serca2 protein levels were normal (U = 107, P = 0.836) in Df(16)5<sup>−/−</sup> mice (Supplementary Figure S2b), suggesting that haploinsufficiency of genes within the Df(16)5 genomic region resulted in abnormal STP through Serca2-independent mechanisms. To ensure that the STP increase in Df(16)5<sup>−/−</sup> mice originated from the presynaptic CA3 neurons, we

(see Supplementary Methods). This analysis revealed no significant difference (F<sub>1,23</sub> = 1.947; P = 0.182) in recovery from short-term depression between Df(16)5<sup>−/−</sup> and WT littersmates (Figure 1e).

Next, we assessed the role of augmentation, which is the longest-lasting component of STP and operates on a time scale of tens of seconds. Using a previously reported approach, we isolated augmentation by applying a single stimulus to Schaffer collaterals every 5 s for 2 min, starting 5 s after the 80-Hz train (Figure 1f). Augmentation was significantly increased (|t|<sub>16</sub> = 4.758, P = 0.0002) in Df(16)5<sup>−/−</sup> mice compared with WT controls (Figure 1f). This increased augmentation in Df(16)5<sup>−/−</sup> mice was maximal at the onset and decayed to normal values after 20 s. Elevated augmentation in Df(16)5<sup>−/−</sup> mutants was not sensitive to the Serca inhibitor thapsigargin (4 μM) (without thapsigargin: |t|<sub>10</sub> = 4.404, P < 0.001; with thapsigargin: |t|<sub>10</sub> = 1.954, P = 0.0396) (Supplementary Figure S2a). Further, Serca2 protein levels were normal (U = 107, P = 0.836) in Df(16)5<sup>−/−</sup> mice (Supplementary Figure S2b), suggesting that haploinsufficiency of genes within the Df(16)5 genomic region resulted in abnormal STP through Serca2-independent mechanisms. To ensure that the STP increase in Df(16)5<sup>−/−</sup> mice originated from the presynaptic CA3 neurons, we
Figure 2. Normal mitochondrial structure but abnormal presynaptic cytosolic and mitochondrial calcium regulation in $Df(16)5^{+/−}$ and $Mrp40^{+/−}$ mice. (a) Three representative transmission electron microscopy images of mitochondrial ultrastructure in the CA1 area of the hippocampus of wild-type (WT) and $Df(16)5^{+/−}$ mice. (b) The representative fluorescent image of mCherry after infection of the CA3 area with adeno-associated viruses (AAVs) encoding either GCaMP6 or mitoGCaMP6. (c) Line scan of mCherry and GCaMP6 fluorescence in a CA3 presynaptic terminal before and after the 80-Hz stimulation of Schaffer collaterals (arrow). (d–g) Mean normalized cytosolic GCaMP6 (d, f) and mitoGCaMP6 (e, g) fluorescence in CA3 presynaptic terminals imaged in the CA1 area of the hippocampus, before and after 80-Hz stimulation in $Df(16)5^{+/−}$ and WT littermates (d, e) and $Mrp40^{+/−}$ and WT littermates (f, g). (h, i) Normalized mean peak amplitudes of GCaMP6 (h) or mitoGCaMP6 (i) in $Df(16)5^{+/−}$ and WT littermates and $Mrp40^{+/−}$ and WT littermates. Numbers of fluorescent puncta are shown inside columns. *$P < 0.05$. 

Mrpl40 is required for mitochondria calcium, STP and memory
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To identify the culprit gene(s) whose haploinsufficiency underlies the abnormal synaptic plasticity in Df(16)5−/− mice, we tested STP parameters in Df(16)5−/− mice and their WT littermates (Supplementary Figure S4). All transcripts were reduced by approximately 50% in the Df(16)5−/− mice (Cldn5<sup>Hir</sup>−/−: P < 0.001; Cdc45l<sup>Hira</sup>−/−: P = 0.004; 2510002D24Rik<sup>Cdc45l</sup>−/− and Mrpl40<sup>−/−</sup>−/− mice; Supplementary Figure S4). Testing STP in all six mouse mutants revealed that Mrpl40<sup>−/−</sup>−/− mice had elevated STP and augmentation compared with their WT littermates (STP: t<sub>29</sub> = 2.368, P = 0.025; augmentation: t<sub>29</sub> = 3.150, P = 0.0037), whereas Cldn5<sup>Hir</sup>−/−, Cdc45l<sup>Hira</sup>−/−, Udfl1<sup>Hira</sup>−/−, 2510002D24Rik<sup>Cdc45l</sup>−/− and Hira<sup>−/−</sup>−/− mutants had normal STP and augmentation (STP and augmentation, respectively: Cldn5<sup>Hir</sup>−/−: t<sub>12</sub> = 0.349, P = 0.733 and t<sub>12</sub> = −1.205, P = 0.250; Cdc45l<sup>Hira</sup>−/−: t<sub>18</sub> = 0.566, P = 0.579 and t<sub>18</sub> = −0.637, P = 0.532; Udfl1<sup>Hira</sup>−/−: U = 43, P = 0.903 and U = 35, P = 0.438; 2510002D24Rik<sup>Cdc45l</sup>−/−: t<sub>35</sub> = 0.789, P = 0.436 and t<sub>35</sub> = 1.773, P = 0.085; Hira<sup>−/−</sup>−/−: t<sub>11</sub> = −0.644, P = 0.532 and t<sub>11</sub> = −0.687, P = 0.507) (Figures 1g and h and Supplementary Figure S5). Mrpl40 haploinsufficiency did not affect mRNA expression of other genes in the Df(16)5 microdeletion (Gapdh: U = 28, P = 0.871; Mrpl40: t<sub>14</sub> = 4.225, P < 0.001; Cldn5: t<sub>14</sub> = −0.0174, P = 0.986; Udfl1: t<sub>14</sub> = −0.368, P = 0.719) (Supplementary Figure S6). The full complement of the Mrpl40 gene appeared to be essential for prenatal development as its homozygous deletion was embryonically lethal. The enhanced STP and augmentation in Mrpl40<sup>−/−</sup>−/− mice were comparable to those in Df(16)5−/− mice, suggesting that hemizygous deletion of Mrpl40 underlies the abnormal synaptic plasticity in Df(16)5−/− mice.

Because Mrpl40 is thought to be one of the proteins of the mitochondrial ultrastructure imaged with transmission electron microscopy in the CA1 area of the hippocampus (Figure 2a), in total mitochondrial DNA (Co1: U = 89, P = 0.147; Nd2: U = 101, P = 0.318) or in oxidative phosphorylation (F<sub>(1,80)</sub> = 0.108, P = 0.745) in isolated mitochondria from the hippocampus between Df(16)5−/− and WT littermates, suggesting normal energy production in mice with a Df(16)5 hemizygous deletion (Supplementary Figure S7). Furthermore, 3D scanning electron microscopy imaging of the hippocampal CA1 area revealed a normal distribution of mitochondria in presynaptic terminals of Df(16)5−/− mice compared with WT littermates (U = 34.5, P = 0.093) (Supplementary Figure S8), suggesting normal trafficking of mitochondria to presynaptic terminals in Df(16)5−/− mice.

Dysregulation of activity-dependent presynaptic and mitochondrial Ca<sup>2+</sup> in Mrpl40<sup>−/−</sup>−/− mice

Because mitochondria also regulate presynaptic Ca<sup>2+</sup> levels, we measured activity-dependent Ca<sup>2+</sup> changes in response to the 80-Hz stimulation of Schaffer collaterals in presynaptic CA3 terminals in the hippocampal stratum radiatum (CA1 area). To this end, we took advantage of the highly sensitive genetically encoded Ca<sup>2+</sup> indicator GcaMP6 (GCaMP6)25. After infecing the CA3 area of the hippocampus in vivo with recombinant AAVs encoding mCherry and GCaMP6, we observed high expression of fluorescent proteins in neuronal cell bodies in the CA3 area but not in the CA1 area (Figure 2b). In the stratum radiatum we observed fluorescent boutons, which responded to 80-Hz electrical stimulation (10 pulses) of Schaffer collaterals with GCaMP6 fluorescence transients with fast rise and decay (rise time<sub>20</sub>−80%; 100.46 ± 4.03 ms; decay time (τ), 222.71 ± 9.40 ms) (Figure 2c). These activity-dependent kinetics of GCaMP6 fluorescence were similar to those observed in CA3 presynaptic terminals in which the inorganic Ca<sup>2+</sup> indicator Fluo SF was used (data not shown), suggesting that GCaMP6 reliably measures cytosolic Ca<sup>2+</sup> in presynaptic terminals. The activity-dependent increase in GCaMP6 fluorescence during the 80-Hz train of stimulations was substantially higher (U = 537, P < 0.001) in Df(16)5−/− mice than in their WT littermates (Figure 2d), which is consistent with the notion that higher presynaptic Ca<sup>2+</sup> levels lead to elevated augmentation and STP.20

To directly measure Ca<sup>2+</sup> in the mitochondria of CA3 axons and terminals, we expressed GCaMP6 with a mitochondrial-localization signal (mitoGCaMP6) in the CA3 area using recombinant AAVs and imaged in the CA1 area (Figure 2b). We verified the specific localization of mitoGCaMP6 to mitochondria using subcellular fractionation followed by western blotting and immunolocalization with mitochondrial markers (Supplementary Figure S9). We also verified that the activity-dependent increase in mitoGCaMP6 fluorescence was sensitive to an inhibitor of the mitochondrial Ca<sup>2+</sup> uniporter Ru360 (10 μM) (U = 15, P < 0.001) (Supplementary Figure S10). An 80-Hz train (10 pulses) applied to the Schaffer collaterals induced an activity-dependent increase in mitoGCaMP6 but with substantially slower kinetics compared with cytosolic GCaMP6 (mitoGCaMP6: rise time<sub>20</sub>−80%; 210.21 ± 27.03 ms; decay time > 1 s; GCaMP6: rise time<sub>20</sub>−80%: 100.46 ± 4.03 ms; decay time (τ), 222.71 ± 9.40 ms; U = 540, P < 0.001) (Figure 2e). Similar to cytosolic Ca<sup>2+</sup>, mitochondrial Ca<sup>2+</sup> was elevated (U = 889, P = 0.020) in Df(16)5−/− mice in response to the 80-Hz synaptic stimulation (Figure 2e). The increases in cytosolic and mitochondrial Ca<sup>2+</sup> were also observed in Mrpl40<sup>−/−</sup>−/− mice to a similar degree as in Df(16)5−/− mice (GCaMP6: U = 482, P < 0.001; mitoGCaMP6: U = 618, P < 0.001) (Figures 2f–i). These data suggest that Mrpl40 is the gene in the Df(16)5 genomic region that is responsible for the STP phenotype by deregulating activity-dependent mitochondrial and cytoplasmic presynaptic Ca<sup>2+</sup> dynamics.
Mrpl40<sup>−/−</sup> mice are deficient in short-term but not long-term spatial memory or long-term synaptic plasticity.

To test if the hemizygous Mrpl40 deletion affects cognitive function, we compared the performance of Mrpl40<sup>−/−</sup> and WT mice in several behavioral tests. Mrpl40<sup>−/−</sup> mice behaved normally in the acoustic startle test (F(1,2) = 0.0711, P = 0.79) and prepulse inhibition (PPI) (F(1,2) = 1.314, P = 0.274) of acoustic-startle tests (Figures 3a and b), a measure of sensorimotor gating that is believed to be associated with positive symptoms of SCZ. However, Mrpl40<sup>−/−</sup> mice showed no memory deficit compared to WT controls (day 1: t(13) = 0.0425, P = 0.837; Figure 3e). Mrpl40<sup>−/−</sup> mice also performed comparably to WT controls (day 1: t(13) = 0.457, P = 0.651; day 2: U = 149.5, P = 0.921) when the escape platform was visible (Figure 3f), demonstrating that they maintained their spatial memory for 48 h and did not show any memory deficits in the Y-maze (t(13) = 0.160, P = 0.873), where we measured the amount of time a mouse spends in a novel arm 1 h after exploring the other two arms of the maze (Figure 3g).

These data suggest that Mrpl40 haploinsufficiency affects short-term (working) memory but not long-term memory. Consistent with this notion, STP was abnormal in Mrpl40<sup>−/−</sup> mice (Figures 1g and h), but long-term potentiation of excitatory synaptic transmission, a major form of long-term synaptic plasticity at CA3–CA1 synapses, did not differ (t(53) = 0.161, P = 0.873) between Mrpl40<sup>−/−</sup> mice and WT littermates (Figure 3h).
Mitochondrial Ca\(^{2+}\)-extrusion deficit underlies STP and Ca\(^{2+}\) phenotypes in Mrpl40\(^{-/-}\) mice

The abnormally high increase in cytoplasmic Ca\(^{2+}\) induced by the 80-Hz synaptic stimulation in Mrpl40\(^{-/-}\) mice coincided with the enhanced mitochondrial Ca\(^{2+}\) increase. A role for slow mitochondrial Ca\(^{2+}\) extrusion in STP has been implicated in the crayfish neuromuscular junction\(^{16}\) and led us to hypothesize that our results in the hippocampus could also be explained by impaired Ca\(^{2+}\) extrusion from mitochondria. Two major mechanisms extrude Ca\(^{2+}\) from the mitochondrial matrix to the cytoplasm: mitochondrial Ca\(^{2+}\) exchangers and the mPTP.\(^{37,38}\) The selective antagonist of the mitochondrial Na\(^{+}\)–Ca\(^{2+}\) exchanger, CGP 37157 (5 \(\mu\)M), had no effect on STP or augmentation in WT mice (STP: \(t_{(18)} = 0.999, P = 0.333\); augmentation: \(t_{(18)} = 1.007, P = 0.330\)), suggesting that this Ca\(^{2+}\) extrusion mechanism is not required for Ca\(^{2+}\) handling by mitochondria during 80-Hz-induced synaptic plasticity (Supplementary Figure S11). However, BKA (2 \(\mu\)M), a non-selective inhibitor of the adenine nucleotide (ADP/ATP) translocases (ANTs),\(^{39}\) which are required for sensitivity of mPTP to calcium,\(^{40}\) significantly increased STP and augmentation (STP: \(U = 14, P = 0.005\); augmentation: \(t_{(19)} = -3.169, P = 0.005\)) in WT mice (Figure 4a and Supplementary Figures S12a and c). This increase mimicked the STP and augmentation enhancement in Df(16)5\(^{+/-}\) and Mrpl40\(^{-/-}\) mice. Interestingly, BKA did not increase STP or augmentation further (STP: \(t_{(19)} = -0.107, P = 0.916\); augmentation: \(t_{(19)} = -0.928, P = 0.365\)) in Df(16)5\(^{+/-}\) mice.

**Figure 4.** The mitochondrial permeability transition pore (mPTP) inhibitor bongkrekic acid (BKA) mimics the short-term potentiation (STP) and calcium transient phenotypes of Df(16)5\(^{+/-}\) and Mrpl40\(^{-/-}\) mice. (a-f) BKA effect on augmentation, peak GCaMP6 (b, e) and peak mitoGCaMP6 fluorescence intensities (c, f) in wild-type (WT) and Df(16)5\(^{+/-}\) littermates (a-c) or WT and Mrpl40\(^{-/-}\) littermates (normalized to WT levels) (d-f). Numbers of neurons or fluorescent puncta are shown inside columns. *\(P < 0.05\).

DISCUSSION

Approximately 30% of patients with 22q11DS meet the diagnostic criteria for SCZ.\(^{4}\) Cognitive symptoms of SCZ include deficits in...
working memory, attention, executive function, and learning and memory; these symptoms have a more prognostic value than do the positive or negative symptoms of the disease, and they contribute more to the patients’ functional disability. The mechanisms underlying the cognitive deficits in SCZ and 22q11DS are still debated. Here we presented evidence that 22q11DS affects working memory and STP through a novel pathogenic mechanism—abnormal Ca²⁺ handling by mitochondria. We also elucidated several features of this pathogenic mechanism: (1) By screening mice carrying hemizygous deletions of individual genes mapped within the distal part of the 22q11 microdeletion, we identified Mrpl40 as a culprit gene that causes abnormal STP. (2) We identified that the augmentation component of STP is specifically affected. (3) Mrpl40 haplinsufficiency

![Diagram](image-url)
led to abnormal STP through mitochondria-mediated deregulation of presynaptic Ca\(^{2+}\) levels. (4) We pinpointed a functional abnormality in mitochondrial Ca\(^{2+}\) extrusion through the mPTP as a pathogenic mechanism caused by Mrpl40 haploinsufficiency. (5) Mrpl40 haploinsufficiency led to deficits in working memory but not in long-term plasticity or long-term memory.

The hippocampus, prefrontal cortex and interactions between these brain regions all contribute to working memory.45–48 Deficits in working memory in patients with SCZ and/or 22q11DS are well documented.49,50 STP, an associative, short-lived synaptic strengthening, is considered a cellular correlate of short-term memory, a term often used synonymously with working memory.49,51 Consistent with this notion, deficits in STP are well established in animal models of SCZ and 22q11DS.48,52–54 Because symptoms of SCZ typically appear during late adolescence or early adulthood, age appears to be an important variable in synaptic plasticity phenotypes associated with 22q11DS mice. In our experiments, we used mice that were older than 4 months. At this age, STP and long-term potentiation at CA3–CA1 synapses are substantially increased in Df(16)1/− mouse models of 22q11DS, whereas at younger ages, both forms of synaptic plasticity are normal.23 These experiments led to the identification of the microRNA-processing gene Dgcr8 as the culprit gene residing in the proximal part of the microdeletion affecting both short- and long-term synaptic plasticity in the hippocampus. Deletion of one allele of Dgcr8 causes depletion of mir-185 and mir-25 and post-transcriptional upregulation of Serc2a in the forebrain of older but not younger mice.23

Because 22q11DS is a multigene syndrome, it is extremely likely that more than one gene is involved in STP abnormalities. Our present work showed that deletion of one allele of Mrpl40 residing in the distal part of the microdeletion, which is outside of the Df(16)1 genomic region, caused Dgcr8-independent deficits in STP and working memory in 22q11DS. First, we identified the STP increase in Df(16)1/− mice, which were hemizygous for genes that mapped distally to Dgcr8. The STP screen revealed that only Mrpl40+/- mice replicated the Df(16)1/− phenotype. Furthermore, the Mrpl40-related STP increase was mechanistically distinct from the STP increase identified in Dgcr8−/- mice. Dgcr8 haploinsufficiency elevates the Serc2a protein,22 but in the Df(16)1/− mice, Mrpl40 levels were normal, and the Serc4 inhibitor thapsigargin did not rescue the STP defect.

Mrpl40 was originally identified as a nuclear gene involved in mitochondrial function.55 Mitochondrial dysfunctions55 have been strongly implicated in SCZ pathophysiology,56 though the exact connection between SCZ pathogenesis and mitochondria has not been established. For instance, the gene encoding DISC1 (disrupted-in-schizophrenia 1) has been localized to mitochondria and is involved in maintaining mitochondrial morphology and regulating mitochondrial transport.57 Several mitochondrial genes, including Mrpl40, are mapped within the 22q11.2 locus and expressed in the brain throughout development, thus implicating mitochondria in the pathogenesis of 22q11DS.58,59 Mrpl40 haploinsufficiency does not affect mitochondrial ultrastructure and does not reduce mitochondrial numbers in presynaptic terminals or total mitochondrial DNA, which is consistent with the view that SCZ is not a neurodegenerative disease. This is also consistent with our observations that Df(16)1/− or Mrpl40−/− mice develop normally and have normal gross brain morphology.

Mitochondria provide energy through oxidative phosphorylation and regulate Ca\(^{2+}\) dynamics during synaptic transmission in neurons. Because of the mitochondrion’s high capacity for Ca\(^{2+}\) uptake, it acts as a rapid buffering system during periods of intense synaptic activity, then slowly releases Ca\(^{2+}\) when activity subsides. This rapid uptake of Ca\(^{2+}\) into mitochondria occurs through channels (i.e., voltage-dependent anion channel VDAC1) in the outer membrane and the mitochondrial calcium uniporter in the inner membrane. Ca\(^{2+}\) is slowly released from the mitochondria through the calcium exchangers and via the mPTP. Despite Ca\(^{2+}\) has been shown to open during high-frequency stimulation in mammalian neurons, and that transient activation of the mPTP may play a role in the normal contribution of mitochondria to STP.60

Oxidative phosphorylation appeared to be normal, whereas Ca\(^{2+}\) dynamics in presynaptic terminals was substantially altered in Df(16)1/− and Mrpl40−/− mice. Our two-photon Ca\(^{2+}\) imaging in presynaptic terminals revealed that synaptic high-frequency stimulation that induces STP evokes enhanced Ca\(^{2+}\) transients in both the presynaptic mitochondrial matrix and presynaptic cytosol of mutant mice. These data argue for a problem with mitochondrial Ca\(^{2+}\) extrusion rather than with mitochondrial Ca\(^{2+}\) uptake. Indeed, if Mrpl40 haploinsufficiency reduced the Ca\(^{2+}\) uptake into mitochondria, we would expect to see increased amplitudes of cytoplasmic Ca\(^{2+}\) transients but decreased Ca\(^{2+}\) transients within mitochondria. Our data are more consistent with a model for a reduced Ca\(^{2+}\) extrusion from mitochondria. Impaired Ca\(^{2+}\) extrusion from mitochondria will result in Ca\(^{2+}\) accumulation in the mitochondrial matrix, and this will contribute to the fast rise in mitochondrial Ca\(^{2+}\) that we observed with mitoGCaMP6. This will then reduce the effective mitochondrial buffering capacity and lead to enhanced cytoplasmic Ca\(^{2+}\) transients that we observed with the cytoplasmic GCaMP6 (Figure 5d). Moreover, slow Ca\(^{2+}\) extrusion from mitochondria (evident from extremely slow decay times of mitoGCaMP6) is an unlikely contributor to fast cytosol Ca\(^{2+}\) transients that occur during high-frequency synaptic activity.

Because inhibition of mPTP but not the mitochondrial Na\(^+-\)Ca\(^{2+}\) exchanger increased presynaptic Ca\(^{2+}\) transients in WT mice, we concluded that the main route of Ca\(^{2+}\) extrusion from mitochondria during STP is mPTP. In WT mice, the pharmacological or molecular inhibition of mPTP with BKA or SC254a shRNA, respectively, mimicked the STP and Ca\(^{2+}\)-transient phenotypes we observed in Df(16)1/− and Mrpl40−/− mice. Moreover, AAV-Slc25a4 OE rescued the STP abnormality in Mrpl40−/− mice, indicating that deletion of one allele of Mrpl40 causes mPTP deficiency and STP increase.

Although our experiments demonstrated that Mrpl40 haploinsufficiency leads to abnormal Ca\(^{2+}\) handling by mitochondria during STP, the exact connection between Mrpl40 and mPTP function remains unclear. Some pharmacological agents that affect mPTP function56 can be beneficial adjuvants to antipsychotics to alleviate SCZ symptoms.56 However, because mPTP is involved not only in Ca\(^{2+}\) extrusion from mitochondria but also in ATP transport, we cannot rule out the possibility that impaired mPTP regulation leads to the abnormal production of ATP in presynaptic terminals, which in turn could result in abnormal presynaptic function. This theory could be addressed using a novel ATP probe that visualizes ATP dynamics in the presynaptic terminals of neurons in culture, but those experiments have not yet been tailored for acute brain slices.62

In summary, we report that haploinsufficiency of Mrpl40, a gene mapped within the 22q11.2 microdeletion associated with SCZ, causes deficient working memory. This behavioral abnormality, which typically manifests in patients with SCZ, is associated with abnormal STP changes in synaptic transmission in the hippocampus and caused by elevated presynaptic Ca\(^{2+}\) and impaired Ca\(^{2+}\) extrusion from mitochondria.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)