A fundamental challenge in developing treatments for autism spectrum disorders is the heterogeneity of the condition. More than one hundred genetic mutations confer high risk for autism, with each individual mutation accounting for only a small fraction of cases. Subsets of risk genes can be grouped into functionally related pathways, most prominently those involving synaptic proteins, translational regulation, and chromatin modifications. To attempt to minimize this genetic complexity, recent therapeutic strategies have focused on the neuropeptides oxytocin and vasopressin, which regulate aspects of social behaviour in mammals. However, it is unclear whether genetic risk factors predispose individuals to autism as a result of modifications to oxytocinergic signalling. Here we report that an autism-associated mutation in the synaptic adhesion molecule Ngln3 results in impaired oxytocin signalling in dopaminergic neurons and in altered behavioural responses to social novelty tests in mice. Notably, loss of Ngln3 is accompanied by a disruption of translation homeostasis in the ventral tegmental area. Treatment of Ngln3-knockout mice with a new, highly specific, brain-penetrant inhibitor of MAP kinase-interacting kinases resets the translation of mRNA and restores oxytocin signalling and social novelty responses. Thus, this work identifies a convergence between the genetic autism risk factor Ngln3, regulation of translation, and oxytocinergic signalling. Focusing on such common core plasticity elements might provide a pragmatic approach to overcoming the heterogeneity of autism. Ultimately, this would enable mechanism-based stratification of patient populations to increase the success of therapeutic interventions.

Social recognition and communication are crucial elements in the establishment and maintenance of social relationships. Oxytocin and vasopressin are two evolutionarily conserved neuropeptides with important functions in the control of social behaviours, in particular pair-bonding and social recognition. In humans, genetic variation of the oxytocin receptor (OXTR) gene is linked to individual differences in social behaviour. Consequently, signalling modulators and biomarkers for the oxytocin or vasopressin system are being explored for conditions with altered social interactions such as autism spectrum disorders (ASDs). In mice, mutation of the genes encoding oxytocin or its receptor results in a loss of social recognition and social reward signalling. Mutation of Cntnap2, a gene linked to ASD in humans, resulted in reduced levels of oxytocin in mice, and the addition of oxytocin improved social behaviour in this model. However, the vast majority of genetic risk factors for autism have no known links to oxytocinergic signalling.

Here we explored oxytocin responses in mice that recapitulate a loss of function in the autism risk gene Ngln3. Ngln3 encodes a synaptic adhesion molecule, and Ngln3 mutant mice exhibit a range of behavioural alterations, including motor stereotypes, alterations in social novelty preference, social reward, and responses to social novelty tests. Despite these alterations in social behaviours, adult Ngln3-knockout (Ngln3KO) mice exhibit normal responses to inanimate objects. In a five-trial social habituation/recognition task, we observed that the social novelty response phenotype is established already in juvenile Ngln3KO mice. Re-expression of Ngln3 selectively in dopaminergic cells restored social novelty responses in juvenile Ngln3KO mice. By contrast, selective inactivation of Ngln3 in VTA DA neurons was sufficient to affect social novelty responses. Social recognition in this assay depends on the function of the oxytocin receptor, as treatment of wild-type mice with the oxytocin receptor antagonist L-368,899 impaired recognition.
The function of VTA DA neurons in social novelty responses and reinforcement is in part dependent on an oxytocin-induced increase in neuronal firing. Specifically, oxytocin released from axons that arise from hypothalamic nuclei increases the firing of VTA DA neurons projecting to the nucleus accumbens (NAC) (J2,13). Thus, we examined whether loss of Nlgn3 might affect the response of this population of neurons to oxytocin. We marked VTA DA neurons projecting to the NAC medial shell and performed electrophysiological recordings from back-labelled neurons in acute slices of the VTA (Fig. 1a, n). Consistent with previous reports, back-labelled neurons showed low hyperpolarization-activated current activities (Ih), and there was no significant difference between genotypes in Ih and other basic biophysical properties (Extended Data Fig. 2). In cell-attached recordings, the frequency of baseline firing of VTA DA neurons from Nlgn3KO mice was slightly reduced compared with wild-type mice (Fig. 1p). Notably, bath application of 1 μM oxytocin significantly increased firing frequency in cells from wild-type mice but had no effect in slices from Nlgn3KO mice (Fig. 1o, q). These findings uncover a requirement for the autism risk factor Nlgn3 for oxytocin responses in the VTA.

A loss of oxytocinergic neurons has been reported in knockout mice for the autism risk factors Cntnap2 and Shank3b (J2,13). In Nlgn3KO mice, we did not detect any alteration in the density of oxytocinergic neurons in the paraventricular nucleus (one of the major oxytocinergic nuclei) or in the density of oxytocinergic fibres in the VTA (Fig. 2a, b, Extended Data Fig. 3a–c). Fluorescent in situ hybridization (FISH) analysis revealed a slight increase in Oxtr mRNA in VTA DA neurons from Nlgn3KO mice compared with wild-type animals (Fig. 2c, d, Extended Data Fig. 3d–f). However, targeted proteomics (parallel reaction monitoring) on micro-dissected VTA tissue did not detect significant alterations in total oxytocin receptor protein, with no change in vasopressin IA receptor mRNA (Avpr1a) or protein (Extended Data Fig. 3g–j, Supplementary Table 1).

Thus, we performed shut-gun proteomics for an unbiased identification of molecular alterations in the VTA of Nlgn3KO mice (Extended Data Fig. 4a–c). Gene Ontology (GO) and network-based functional classification analysis for proteins altered in Nlgn3KO mice identified protein transport, cell adhesion, and mRNA translation as main categories (Extended Data Fig. 4b, c). Dysregulation of membrane trafficking and G-protein-coupled receptor (GPCR) signalling components is consistent with the previously discovered roles for Nlgn3 in synapse organization and GPCR signalling (J2,23,34). However, the alterations in regulators of translation were surprising. Alterations in mRNA translation have been linked to deficits in neuronal plasticity. We previously observed that behaviourally induced plasticity is altered in VTA DA neurons of Nlgn3KO mice (J3). Thus, we compared translation in VTA...
DA neurons of naive and behaviourally exposed wild-type and Nlgn3KO mice. Incorporation of the methionine analogue azidohomoalanine (AHA) in VTA DA neurons (marked by tyrosine hydroxylase) of acute mouse brain slices from naive Nlgn3KO mice was reduced compared with wild-type mice (Fig. 2e–g, Extended Data Fig. 4d). Notably, the incorporation of AHA was increased in VTA DA neurons from Nlgn3KO mice exposed to handling (Fig. 2e–g). This suggests a signalling-dependent disruption of translation homeostasis in the mutant mice.

Disruption of translation homeostasis is thought to broadly modify neuronal proteins resulting in impaired plasticity and neurodevelopmental conditions. Thus, we sought to normalize translation in Nlgn3KO mice and test whether this would restore oxytocin responses in VTA DA neurons. We focused on MAP kinase-interacting kinases (MNKs), which are crucial regulators of signalling-dependent modification of mRNA translation (Fig. 3a). Inhibition of MNK was reported to modify ribosomal protein levels and to ameliorate behavioural and plasticity alterations in Fmr1 knockout (Fig. 3c, Extended Data Fig. 5f, l). Of note, phospho- or protein levels of eIF4E or eIF4G or these compounds, ETC-168, resulted in a dose-dependent reduction of AHA incorporation in naive and behaviourally exposed wild-type and Nlgn3KO mice (Fig. 5g–k). As a proof of concept, we probed the effectiveness of ETC-168 in modifying a behavioural phenotype in mice. Using tandem mass tag (TMT)-based isobaric labelling, we verified the de-regulation of translational machinery in VTA tissue from Nlgn3KO versus wild-type mice treated with vehicle (Extended Data Fig. 7a–d). Gene set enrichment analysis uncovered an increase in core proteins of cytoplasmic but not mitochondrial ribosomes (Fig. 3e, Extended Data Fig. 7e, g). Inhibition of MNK with ETC-168 abolished the increase in core ribosomal proteins in Nlgn3KO VTA (Fig. 3f, Extended Data Fig. 7f, h). In acute slices from Nlgn3KO mice treated with ETC-168, incorporation of AHA was significantly reduced as compared with Nlgn3KO mice treated with vehicle, resulting in similar AHA incorporation levels to slices from vehicle-treated wild-type mice (Fig. 3g–i).

We then tested whether ETC-168 treatment restored oxytocin responses and social novelty responses in Nlgn3KO mice (Fig. 4a). Notably, short-term oral treatment (two applications of 5 mg kg−1 ETC-168 over 26 h) of Nlgn3KO mice recovered the oxytocin-induced increase in firing frequency seen in wild-type mice (Fig. 4b, c). This treatment also fully restored social novelty responses, with no detectable effect on wild-type mice (Fig. 4a, d–f, Extended Data Fig. 8). This pharmacologically recovered social recognition behaviour was dependent on oxytocin receptor function (Extended Data Fig. 9). Notably, ETC-168 treatment was well tolerated and remained effective in a long-term treatment regime (Extended Data Figs. 7a–c, 10). Thus, modification of translation homeostasis in Nlgn3KO mice by MNK inhibition restores oxytocin responses and social novelty responses.

This work uncovers an unexpected convergence between the genetic autism risk factor Nlgn3, translational regulation, oxytocinergic signalling, and social novelty responses. Although loss of Nlgn3 impairs oxytocin responses in VTA DA neurons, the behavioural phenotype does not fully phenocopy genetic loss of oxytocin. Oxytocin knockout mice exhibit impaired habituation in the social recognition task, whereas Nlgn3KO mice habituate normally but exhibit a selective deficit in the response to a novel conspecific. This is probably due to differential roles of Nlgn3 and oxytocin across several neural circuits and over development. Moreover, Nlgn3 loss-of-function also affects signalling through additional GPCRs.

We propose that pharmacological inhibition of MNKs may provide a new therapeutic strategy for neurodevelopmental conditions with altered translation homeostasis. Notably, MNK loss-of-function appears to be overall well tolerated. MNK1/2 double-knockout mice...
Fig. 3 The novel MNK1/2 inhibitor ETC-168 rescues translation in Nlgn3KO mice. a, ETC-168 targets MNK1/2. Note that eIF4E phosphorylation decreases affinity of eIF4E for the mRNA 5′ cap structure. b, Representative western blot (top) and quantification (bottom) of eIF4E phosphorylation in days in vitro (DIV) cortical neurons treated with DMSO or ETC-168 for 3 h. n = 8 replicates, 3 independent experiments. c, Representative western blot (top) and quantification (bottom) of eIF4E phosphorylation in VTA lysate from wild-type mice treated with vehicle or 5 mg kg⁻¹ ETC-168. Numbers on graphs represent mice. d, Pharmacokinetic analysis of ETC-168 concentration in male mice (n = 27) after a single oral dose of 10 mg kg⁻¹. Plasma levels are in red, and brain levels are in blue. The half-life (t₁/₂) and the brain-to-plasma exposure (AUCcpu) are shown. e, Tandem mass tag (TMT) proteomic comparison of VTA from vehicle-treated mice (n = 4 mice per genotype and treatment). Relative frequency of log₂-transformed fold change in all detected proteins, cytosolic, or mitochondrial ribosomal protein abundance is plotted (Nlgn3KO versus wild type). f, Comparison as in e for log₂-transformed fold change in protein abundance in ETC-168-treated Nlgn3KO versus vehicle-treated wild-type mice. g, FUNCAT assay in acute slices from vehicle-treated or ETC-168-treated mice (5 mg kg⁻¹ or vehicle by oral gavage). h, Representative examples of AHA incorporation (green) in TH-positive cells (red). i, Quantitative assessment of AHA incorporation in vehicle (as in Fig. 2g for comparison to untreated mice) versus ETC-168-treated wild-type and Nlgn3KO mice. n = 4 mice per genotype and treatment. Numbers on graphs refer to number of images analysed. Error bars denote s.e.m. P values determined by one-way ANOVA followed by Bonferroni’s post hoc test (b), two-sided unpaired t test (e), Kolmogorov–Smirnov test (f), or Kruskal–Wallis test followed by Dunn’s multiple comparison test (i). See Supplementary Information for additional statistics.

Fig. 4 MNK inhibition restores social novelty responses in Nlgn3KO mice. a, Scheme for drug treatment and analysis. b, Firing frequency at baseline in VTA DA neurons from Nlgn3KO mice untreated (as previously shown in Fig. 1p), treated with vehicle, or treated with 5 mg kg⁻¹ ETC-168. Untreated n = 14 neurons from 8 mice; vehicle n = 8 neurons from 4 mice; ETC-168 n = 14 neurons from 8 mice. The wild-type mouse data from Fig. 1p is presented for comparison (n = 22). c, OXT-induced frequency changes over time in VTA DA neurons from Nlgn3KO mice treated with vehicle or 5 mg kg⁻¹ ETC-168. Wild-type mice from Fig. 1q are presented for comparison. P values shown are for baseline versus OXT at 3 min. Vehicle-treated n = 8 cells from 4 mice; ETC-168-treated n = 11 neurons from 6 mice. d, e, Mean social interaction time in wild-type (d) and Nlgn3KO (e) mice treated with vehicle or 5 mg kg⁻¹ ETC-168. f, Social recognition index for wild-type and Nlgn3KO mice treated with vehicle or 5 mg kg⁻¹ ETC-168. Numbers in brackets indicate mice. All error bars are s.e.m. P values determined by one-way ANOVA followed by Bonferroni’s post hoc test (b), repeated measures two-way ANOVA followed by Bonferroni’s post hoc test (c), repeated measures two-way ANOVA between all genotype and treatment groups followed by Bonferroni’s post hoc test (d, e), or repeated measures two-way ANOVA followed by Bonferroni’s post hoc test for treatment and genotype (f). See Supplementary Information for additional statistics.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2563-7.

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Methods

Mice
Male wild-type, Nlgn3\textsuperscript{360} (ref. \textsuperscript{47}) and Fmr1\textsuperscript{15} (ref. \textsuperscript{48}) mice were used for this study. Note that this strain of Nlgn3\textsuperscript{360} mouse contains a transcriptional stop cassette\textsuperscript{46} preventing genetic compensation events that might be triggered in mutants expressing truncated mRNAs\textsuperscript{47}. For dopamine neuron-specific manipulations, DAT\textsuperscript{-}cre BAC transgenic mice were used\textsuperscript{49}. Mice were kept on a C57BL/6j background. All animals were group housed (weaning at postnatal day (P) 21–P23) under a 12 h light/dark cycle (06:00–18:00) with food and water ad libitum. All physiology and behaviour experiments were performed during the light cycle. Embryos for cortical cultures were obtained from NMRI mice (Janvier). All the procedures performed at University of Lausanne and Biozentrum complied with the Swiss National Institutional Guidelines on Animal Experimentation and were approved by the respective Swiss Cantonal Veterinary Office Committees for Animal Experimentation.

Pharmacokinetics of ETC-168
A group of 27 male C57BL/6 mice were administered with ETC-168 solution formulation in 7.5% NMP, 5% Solutol HS, 10% PG, 30% PEG-400, 47.5% normal saline at a dose of 10 mg kg\textsuperscript{-1}. Blood samples (approximately 60 μl) were collected under light isoflurane anaesthesia from retro orbital plexus at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after drug administration. Plasma samples were separated by centrifugation of whole blood and stored below –70 °C until analysis. Immediately after collection of blood, brain samples were collected from each mouse at PD, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h. Brain samples were homogenized using ice-cold PBS (pH7.4) and homogenates were stored below –70 °C until analysis. Total homogenate volume was three times the tissue weight. All samples were processed for analysis by protein precipitation using acetonitrile and analysed with fit-for-purpose LC–MS/MS method (lower limit of quantification (LLOQ): 2.00 ng ml\textsuperscript{-1} in plasma and 1.00 ng ml\textsuperscript{-1} in brain). Pharmacokinetic parameters were calculated using the non-compartmental analysis tool of Phoenix WinNonlin (v.6.3).

Pharmacological treatment
For in vitro experiments, ETC-168 was dissolved in DMSO. For in vivo treatment, ETC-168 was dissolved in 0.5% methylcellulose (Sigma, M7140) and 0.1% Tween-80 (Sigma, P5188) in MilliQ water to 1.25 mg ml\textsuperscript{-1}, and sonicated for 30 min. Animals were gavaged 24 h and 2 h before behavioural assessment or tissue collection for acute treatment. In the chronic treatment regime, animals were treated every 24 h, 2 h before start of the evaluation. The OCTR antagonist L-368,899 (Tocris, 2641) was dissolved in saline and 10 mg kg\textsuperscript{-1} was applied i.p. injection 2 h before the start of behavioural assessments, or 15 min before the final dose of ETC-168.

Stereotoxic injection
Injections of diluted (1:4) Red Retrobead were done at P19–P23 and performed under isoflurane anaesthesia (Baxter AG). The mice were placed in a stereotoxic frame (Kopf Instrument) and a single craniootomy was made over the NAc medial shell at the following stereotoxic coordinates: ML ±0.53 mm, AP 1.5 mm, DV –4.05 mm from bregma. Injections were made with a 30-G Hamilton needle (Hamilton, 65460-02) for a total volume of 200 nl. Behavioural testing was performed at P28 and injections sites were confirmed post hoc by immunostaining as described previously\textsuperscript{50}. In brief, VTA:DA\textsuperscript{NL3KD} mice were included if a minimum of 20% of TH-positive cells in the VTA were GFP-positive. Mice were excluded from the analysis if their body weight was less than 75% of the mean body weight at the start of behavioural trials or in case post hoc analysis revealed inefficient or off-target viral infection.

Habituation and novelty recognition task
Social recognition is considered to be commonly affected in individuals on the autism spectrum. Autistic individuals perform poorly on face identity recognition tasks, especially when a medium to high time delay (seconds to several minutes) between face presentation is applied\textsuperscript{11,12}. To model social recognition in juvenile male mice, we adopted previous protocols that had been developed for adult rodents\textsuperscript{13,14}. In this test, recognition between juvenile male mice (P26–P32) was tested with 5 min inter-trial intervals that mimic the timescales of recognition tasks from patient studies. An experimental cage similar to the animal’s home cage was used with grid, food, and water removed. The experimental mice were acclimated in the cage for 30 min before the start of the test. At the start of the first trial, a novel same-sex mouse (stimulus mouse: C57BL/6j juvenile male mice, P21–P28) or an object (Lego block) was introduced into the cage for 2 min and mice were left to freely interact. This was repeated for 4 consecutive trials with 5 min in-between trial intervals to allow habituation to the stimulus mouse or object. On the fifth trial, a novel mouse (littermate to the stimulus mouse) or a novel object (dice) was introduced. For the social stimulus, interaction was scored when the experimental mouse initiated the action and when the noise of the animal was oriented towards the social stimulus mouse only. For the object stimulus, interaction was scored when the nose of the mouse was oriented 1 cm or less towards the object. The interaction time was used to calculate the recognition index as: (interaction trial 5) – (interaction trial 4). Social recognition in rodents has been reported to depend on oxytocin signalling\textsuperscript{13,14}. To pharmacologically validate the recognition task, we treated mice with the oxytocin receptor antagonist L-368,899 (10 mg kg\textsuperscript{-1} injected intraperitoneally 2 h before testing). This treatment significantly suppressed recognition of novel conspecifics in this task (Fig. Ik, l).

Place-independent cue discrimination and reversal task
Adult male mice were used for this test. The test box (25 x 35 cm) was divided in a waiting and a reward zone by a gated plexiglass wall. Reward (condensed milk) was associated to one of the two lining patterns of a double tray (white tape versus brown sandpaper). The tray was turned in a pseudorandom fashion between trials in order to present the rewarded pattern on the right and the left 8 times within a daily session of 16 trials. Sliding lids were used to prevent nosepoke in the correct target after the mouse made a wrong choice, and to signal end of a trial and return to the waiting zone after the bait has been consumed. Before the start of the trial, mice were food deprived overnight and the reward was presented in the home cage to habituate the reward. For the duration of the test, mice were food restricted overnight and receive food ad libitum after completion of the discrimination task. After the first night of food deprivation, mice were brought to the testing arena where they find condensed milk droplets (15 μl) in falcon lids similar to those used for the test. First day of habituation takes place in groups of cage mates, second day in individual session. Mice were shaped to shuttle to the waiting compartment of the arena after having consumed the reward. On day 1, mice were trained to find the reward only in one of two adjacent falcon lids that have been lined and mounted on little stages with a different pattern (brown sandpaper vs white tape). Each mouse undergoes 16 daily trials, with a cut-off of 20 min. Mice not completing 16 trials by the third testing day were excluded from further testing. Mice not attaining learning criterion for the first contingency (8 consecutive correct responses over two
days) after 6 days of training did not go to the reversal learning step (or receive any treatment). Mice were trained for 6 consecutive days per week. Feeding was restricted to 1g per mouse overnight. On day 7 (day 1 of reversal learning training), mice received ETC-168 5 mg kg⁻¹ or vehicle by gavage 120 min before starting the tests for the duration of the reversal task. For the contingency reversal learning, mice were trained to nosepoke in the previously non-baited pattern in order to find reward. The training schedule was the same as before: 16 trials per day, until attainment of learning criterion (second day when 8 consecutive correct responses have been performed).

Open field and marble burying
Male mice were placed individually in the centre of a square open field arena (50 × 50 × 30 cm) made of grey plastic for 5 min. Velocity (cm s⁻¹) and time spend in center (s) was analysed using EthoVision10 system (Noldus). The arena was cleaned with 70% ethanol between trials. For the marble burying test, animals were placed in a standard type II cage with 5 cm bedding containing 20 identical black marbles distributed equally for 30 min. A marble was considered buried if at least two-thirds of the marble was covered.

Electrophysiology
Male mice (P28–P34) were deeply anaesthetized with isoflurane (4% in O₂, Vapour, Draeger) before decapitation and brain isolation. Acute horizontal brain slices (250 μm thick) from the midbrain were cut with a vibrating microslicer (Leica VT1200S) in ice-cold oxygenated sucrose-based cutting solution containing: NaCl (87 mM), NaHCO₃ (25 mM), KCl (2.5 mM), NaH₂PO₄ (1.25 mM), MgCl₂ (0.5 mM), MgCl₂ (7mM) and glucose (10 mM) (equilibrated with 95% O₂, 5% CO₂). Slices were immediately transferred to a storage container containing artificial cerebral spinal fluid (ACSF) containing: NaCl (125 mM), NaHCO₃ (25 mM), KCl (2.5 mM), NaH₂PO₄ (1.25 mM), MgCl₂ (2 mM), CaCl₂ (2.5 mM) and glucose (11 mM), pH 7.4, constantly bubbled with 95% O₂, 5% CO₂. Slices were maintained at 35 °C for 60 min and then kept at room temperature before their transfer to the recording chamber. During the recordings, the slices were continuously perfused with ACSF at 35.0 ± 2.0 °C throughout the experiments. Neurons were visualized with a LNScope (Luigs & Neumann) equipped with an oblique illumination condenser, a 60x objective (LUMPlanFL, NA 0.9) and a reflected illuminator (Olympus). Slices were illuminated with a collimated LED infrared light source (Thorlabs) and wLS LED illumination unit (Q-imaging). The recorded neurons in the VTA were identified by their anatomical localization and recorded if they were labelled with red retrobeads (or by morphology for recording from non-retrobead labelled cells). Their dopaminergic identity was sub-

Analysis of mRNA translation
Translation in the VTA was analysed using UNCAT55,56, in which AHA is incorporated into cells and then detected using an alkyne tagged to Alexa 488. Mice were anaesthetized at P28 and 250-μm thick horizontal slices were cut on a vibratome and placed in ACSF for 45 min at 35 °C to recover. The same cutting solution and ACSF as described for electrophysiology was used. Slices were moved to an incubation chamber and incubated for an additional 3 h with 1mM AHA (Jena Bioscience, CLK-AA005). At the end of incubation, slices were transferred to ice-cold 4% paraformaldehyde (PFA) and left overnight at 4 °C. The next day, slices were incubated for 1.5 h in a blocking solution containing 5% BSA (Sigma), 5% normal donkey serum and 0.3% Trition X-100 in 1× PBS at agitation overnight. Slices were then washed 3 × 5 min followed by incubation over night with gentle agitation with 500 μl Click-iT reaction cocktail according to the manual instructions (Click-iT Cell Reaction Buffer Kit, Invitrogen, C10269). Slices were then washed 3 × 10 min with 1× PBS followed by incubation with anti-TH (Millipore, AB1542, 1:1,000) primary antibody at room temperature for 2 h, washed three times in 1× PBS, followed by incubation for 2 h at room temperature with a secondary antibody. The sections were then washed three times in 1× PBS before mounted onto microscope slides with ProLong Gold antifade (Invitrogen, P36930). Images were taken on an Olympus SpinSR spinning disk with a UPLS APO 30× objective (NA 1.5). All images were taken with the same laser power, gain and exposure settings, and analysed using ImageJ. For analysis of AHA incorporation,
10 images were taken of the VTA and the Alexa-488 mean fluorescent intensity of 9–20 regions of interest (ROI) corresponding to TH+ somas were measured. The mean fluorescent intensity of cells per image was used for analysis, and the experimenter was blinded to genotype and treatment. All images within each experiment were processed in parallel using identical settings in ImageJ (NIH) and Adobe Photoshop CS 8.0 (Adobe systems).

For puromycin incorporation, 400-μm thick coronal slices were cut on a vibratome and placed in ACSF (in mM: 125 NaCl, 2.7 kCl, 1 MgCl2, 2 CaCl2, 1.25 NaH2PO4, 20 glucose, 26 NaHCO3, 95% O2, 5% CO2) for 30 min at room temperature followed by 2 h at 32 °C. Puromycin (5 μg ml−1; Sigma) was added for 45 min to label newly synthesized proteins. Sections were snap frozen and subsequently lyzed in 10 mM HEPES, 1% SDS, 1 mM NaF, 1 mM NaVO4 containing protease and phosphatase inhibitor cocktail (Roche Applied Science), sonicated and incubated for 10 min at 95 °C. Puromycin incorporation was measured by western blot (see ‘Western blot and AlphaLISA immunoassay’) using mouse anti-puromycin antibody (EQ0001), kerafast. The results were normalized to signal obtained with anti-calnexin antibodies run at the same time on a different blot.

Western blot and AlphaLISA immunoassay
Cortical neurons and brain tissue were homogenized in lysis buffer containing 137 mM NaCl, 2.7 mM kCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, 5 mM EDTA, 1% Triton X-100 and complete protease and phosphatase inhibitors (Roche Applied Science). Immunoblotting was done with HRP-conjugated secondary antibodies and Pierce ECL Western Blotting Substrate. The following primary antibody was used in this study: p-elf4E (Abcam, ab76256 1:1000), elf4E (Abcam, ab47482 1:1000), p-ERK1/2 (Cell Signaling, 4370S 1:1000), ERK1/2 (Cell Signaling, 4695S 1:1000), p-elf4F (Cell Signaling, 2441S 1:1000), elf4G (Cell Signaling, 2498S 1:1000), p-MNK1 (Cell Signaling, 2111, 1:1000), MNK1 (Cell Signaling, 2195S, 1:1000), GAPDH (Cell Signaling, 5717 1:2000), and calnexin (Stressgen, SPA-865 1:2000). Loading controls were run on the same gel, and for some experiments Mini PROTEAN TGX Stain-Free Gels (Bio-Rad) were used as loading controls. Signals were acquired using an image analyser (Bio-Rad, ChemiDoc MP Imaging System) and images were analysed and prepared using ImageJ.

For additional measurements of elf4E phosphorylation state, the AlphaLISA SureFire Ultra p-elf4E (Ser209) Assay Kits (PerkinElmer) were used according to the manufacturers protocol. AlphaLISA signals were measured using a Tecan SPARK plate reader on recommended settings.

Immunohistochemistry and imaging
Animals were transcardially perfused with fixative (4% paraformaldehyde in 100 mM phosphate buffer, pH 7.4) at P26–P32. Brains were post-fixed overnight at 4 °C, incubated in 30% sucrose in 1× PBS for 48 h, and snap frozen on dry ice. Tissues were sectioned at 35 μm on a cryostat (Microm HM650, Thermo Scientific). Floating sections were washed three times in 1× TBS containing 0.5% Triton X-100 at 4 °C overnight and washed three times in 1× TBS containing 0.5% Triton X-100, followed by incubation for 2 h at room temperature with a secondary antibody. The sections were washed three times in 1× TBS containing 0.5% Triton X-100 before mounted onto microscope slides with ProLong Gold antifade (Invitrogen, p36930). For post hoc confirmation of TH-positive cells after electrophysiology, the sections were fixed in the same fixative as described above, washed three times with 1× PBS before blocking and incubation with anti-TH antibody using same method as above. The following primary antibodies were used for this study: sheep anti-TH (Millipore, AB1542, 1:1000) and mouse-anti neurophysin-1 (Millipore, MABN844, 1:2000). Secondary antibodies used were: donkey anti-sheep IgG-Cy3 (713-165-147), donkey anti-sheep Cy5 (713-175-147), donkey anti-rabbit IgG-Cy3 (711-165-152), goat anti-mouse Cy2 (714-225-150) all from Jackson ImmunoResearch. Streptavidin DyLight 488 (Thermo Scientific 21832, 1 mg ml−1), was used to visualize biocytin. Hoechst dye was co-applied with the secondary antibody at a final concentration of 0.5 μg ml−1. Images were acquired on a custom-made dual spinning disk microscope (Life Imaging Services GmbH) using 10× and 40× objectives. Images were taken bilaterally along the whole VTA and PVN dorso-ventral axis and images from at least four (VTA) or five (PVN) planes were analysed. OXT+ cells in the PVN were counted manually. Neurophysin-1 area coverage and puncta intensity was measured using the particle measurement tool in ImageJ on sum projections. All images within each experiment were processed in parallel with identical settings using ImageJ and Adobe Photoshop CS 8.0 (Adobe systems).

FISH
Mice were deeply anaesthetized using isoflurane inhalation. Brains were quickly removed and snap frozen on dry ice before storage at −80 °C. Brains were cut on a cryostat into 10-μm sections, adhered to Superfrost ultra plus slides (Thermo Scientific) and stored at −80 °C. Sections were fixed for 30 min in 4% PFA before being processed using the RNAseq Fluorescent Multiplex kit (ACD) according to the manufacturers instruction. The following probes were used: Otxr (C3, 412171), Vlavr (C3, 418061), Th (Slc6a3-C2, 315441) and Nlg3m (Cl, 497661). Probes were combined as Oxtr/Th/Nlg3 or Vlar/Th/Nlg3. Amp 4–Alt B were used for all combinations. Sections were imaged on a custom-made dual spinning disk microscope (Life Imaging Services GmbH) using 40× objective, with 12 section z-stacks with 0.2 μm in between z sections. Images were processed in ImageJ by doing sum projection of the z-stacks, followed by analysis of fluorescent intensity and number of puncta. Cell types were identified based on the presence of TH and DAPI. A ROI was drawn around the cell to define the area using DAPI, and only cells with no adjacent DAPI staining was used to avoid false positives from signals from a second cell. Dots in the ROI were manually counted and fluorescent intensity was analysed using ImageJ. Images were assembled with identical settings using ImageJ and Adobe Photoshop CS 8.0 (Adobe systems).

Cell culture
Cortical cultures were prepared from E16.5 mouse embryos. Neocortices were dissociated by addition of papain (130 units, Worthington Biochemical LK003176) for 30 min at 37 °C. Cells were maintained in neurobasal medium (Gibco 21103-049) containing 2% B27 supplement (Gibco 17504-044), 1% Glutamax (Gibco 35050-038), and 1% penicillin/streptomycin (Sigma P4333). At DIV14, the cells were treated with either vehicle (DMSO) or different doses of ETC-168 for 3 h before collecting for western blot.

Proteomic analysis
VTA tissue was microdissected from coronal sections using anatomical landmarks. Dissected tissue was snap frozen in liquid nitrogen. Successful recovery of proteins from dopaminergic neurons was confirmed by quantitative assessment of enrichment of dopaminergic markers (dopamine transporter, tyrosine hydroxylase, dopamine decarboxylase) as compared to other brain regions (Extended Data Fig. 4a).

Sample preparation for LC–MS analysis. Tissue was washed twice with PBS and dissolved in 50 μl lysis buffer (1% sodium deoxycholate, 0.1 M ammonium bicarbonate), reduced with 5 mM TCEP for 15 min at 95 °C and alkylated with 10 mM chloroacetamide for 30 min at 37 °C. Samples were digested with trypsin (Promega) at 37 °C overnight (protein to trypsin ratio: 50:1). To each peptide samples an aliquot of a heavy reference peptide mix containing 10 chemically synthesized proteotypic peptides (Spike-Tides, JPT) was spiked into each sample at a concentration of 5 fmol of heavy reference peptides per 1 μg of total endogenous protein mass. Then, the peptides were cleaned up using iST cartridges.
Targeted PRM-LC–MS analysis of protein isoforms. In a first step, parallel reaction-monitoring (PRM) assays were generated from a mixture containing 100 fmol of each heavy reference peptide and shotgun data-dependent acquisition (DDA) LC–MS/MS analysis on a Thermofisher Orbitrap Fusion Lumos platform (Thermo Fisher Scientific). The setup of the pRPLC–MS system was as described previously (Pubmed-ID: 27345328). Chromatographic separation of peptides was carried out using an EASY nano-LC 1200 system (Thermo Fisher Scientific), equipped with a heated RP-HPLC column (75 μm × 30 cm) packed in-house with 1.9 μm C18 resin (Reprosil-Pur C18–AQ, Dr. Maisch). Peptides were analysed per LC–MS/MS run using a linear gradient ranging from 95% solvent A (0.15% formic acid, 2% acetonitrile) and 5% solvent B (98% acetonitrile, 2% water, 0.15% formic acid) to 45% solvent B over 60 min at a flow rate of 200 nl min⁻¹. Mass spectrometry analysis was performed on Thermo Orbitrap Fusion Lumos mass spectrometer equipped with a nanoelectrospray ion source (both Thermo Fisher Scientific). Each MS1 scan was followed by high-collision-dissociation (HCD) of the 10 most abundant precursor ions with dynamic exclusion (PreOmics) according to the manufacturer's instructions. Samples were dried under vacuum and stored at −80 °C until further use.

Global proteome analysis using tandem mass tags
Sample aliquots (prepared as described above) containing 10 μg of peptides were dried and labelled with tandem mass isobaric tags (TMT-pro 16-plex, Thermo Fisher Scientific) according to the manufacturer’s instructions. To control for ratio distortion during quantification, a peptide calibration mixture consisting of six digested standard proteins mixed in different amounts were added to each sample before TMT labelling as recently described³⁸. After pooling the differentially TMT labelled peptide samples, peptides were again desalted on C18 reversed-phase spin columns according to the manufacturer’s instructions (Macrospin, Harvard Apparatus) and dried under vacuum. TMT-labelled peptides were fractionated by high-pH reversed phase separation using an XBridge Peptide BEH C18 column (3.5 μm, 130 Å, 1 mm × 150 mm, Waters) on an Agilent 1260 Infinity HPLC system. Peptides were loaded on column in buffer A (ammonium formate (20 mM, pH 10) in water) and eluted using a two-step linear gradient starting from 2% to 10% in 5 min and then to 50% (v/v) buffer B (90% acetonitrile/10% ammonium formate (20 mM, pH 10)) over 55 min at a flow rate of 42 μl min⁻¹. Elution of peptides was monitored with a UV detector (215 nm, 254 nm). A total of 36 fractions were collected, pooled into 12 fractions using a post-concatenation strategy as previously described³⁹, dried under vacuum.

Aliquots of 1 μg of peptides were analysed by LC–MS as previously described³⁹. Chromatographic separation of peptides was carried out using an EASY nano-LC 1000 system (Thermo Fisher Scientific),
equipped with a heated RP-HPLC column (75 μm × 37 cm) packed in-house with 1.9 μm C18 resin (Reprosil-AQ Pur, Dr. Maisch). Aliquots of 1 μg total peptides were analysed per LC–MS/MS run using a linear gradient ranging from 95% solvent A (0.15% formic acid, 2% acetonitrile) and 5% solvent B (98% acetonitrile, 2% water, 0.15% formic acid) to 30% solvent B over 90 min at a flow rate of 200 nL min⁻¹. Mass spectrometry analysis was performed on a Q Exactive HF mass spectrometer equipped with a nanoelectrospray ion source (both Thermo Fisher Scientific). Each MS1 scan was followed by high-collision-dissociation (HCD) of the 10 most abundant precursor ions with dynamic exclusion for 20 s. Total cycle time was approximately 1 s. For MS1, 3 × 10⁶ ions were accumulated in the Orbitrap cell over a maximum time of 100 ms and scanned at a resolution of 120,000 FWHM (at 200 m/z). MS2 scans were acquired at a target setting of 1 × 10⁸ ions, accumulation time of 100 ms and a resolution of 30,000 FWHM (at 200 m/z). Singly charged ions and ions with unassigned charge state were excluded from triggering MS2 events. The normalized collision energy was set to 35%, the mass isolation window was set to 1.1 m/z and one microscan was acquired for each spectrum.

The acquired raw-files were searched against a protein database containing sequences of the predicted SwissProt entries of Mus musculus (www.ebi.ac.uk, release date 27 March 2019), the six calibration mix proteins and commonly observed contaminants (in base containing sequences of the predicted SwissProt entries of acquired for each spectrum).

GO, network and gene set enrichment analysis

GO analysis was performed using DAVID classification system (https://david.ncifcrf.gov). Proteins significantly different between vehicle treated wild type and Nlgn3KO (P < 0.05) were compared to all proteins detected in the proteome screen using the GO GOSlim_BP DIRECT annotation dataset with minimum number of hits set to five and maximum P value threshold to 0.05 with Benjamini correction (P < 0.05). Network analysis was obtained using String v11 database. Each node represents a protein altered in Nlgn3KO VTA compared to wild-type VTA in vehicle-treated conditions, and each edge show protein–protein interaction as determined by experiments and databases. The highest confidence (0.900) was used for interaction scores. Disconnected nodes and networks containing more than six proteins were removed. Gene set enrichment analysis for TMT proteomic data was performed using all proteins detected with at least two peptides with PSEA-Quant. The list of GO terms with Q < 0.01 were summarized using REVIGO with small (0.5) allowed similarity, and displayed using Cytoscape.

Statistical analysis

No statistical methods were used to predetermine sample size. The animals were randomly assigned to each group the moment of drug treatment, and a minimum of three independent cohorts were used for behavioural experiments. Statistical analysis was conducted with GraphPad Prism 8. The normality of sample distributions was assessed and when violated non-parametrical tests were used. When normally distributed, the data were analysed with unpaired t-test for comparison between two groups, while for multiple comparisons one-way ANOVA and repeated measures ANOVA were used. For the analysis of variance with two factors (two-way ANOVA, repeated-measures two-way ANOVA and repeated measures two-way ANOVA by both factors), normality of sample distribution was assumed, and followed by Bonferroni post hoc test. Differences in frequency distribution were assessed using the Kolmogorov–Smirnov test. All the statistical tests adopted were two-sided. When comparing two samples distributions similarity of variances was assumed, therefore no corrections were adopted. Outliers were identified using ROUTS test on the most stringent setting (Q = 0.1%). Data are represented as the mean ± s.e.m. and the significance was set at P < 0.05.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Raw mass spectrometry data associated with the manuscript have been deposited in to the ProteomeXchange Consortium via the PRIDE60 partner repository with the dataset identifier PXD018808 and 10.6019/PXD018808. All renewable reagents and detailed protocols will be made available on request. Source data are provided with this paper.

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Article

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Author contributions This work was jointly conceived by H.H. and P.S. and built on initial findings by S.B. and P.S. MNK inhibitors were developed by A.M. and K.N.; behavioural assays were developed by H.H. and F.M.; experimental procedures were performed by H.H., D.S., F.M., L.H.-B., S.B. and E.P.-G.; and data analysis was conducted by H.H., D.S., E.P.-G., F.M., P.S. and E.P.-V. The manuscript was jointly written by H.H. and P.S., with editing provided by E.-P.G., E.P.-V., S.B. and K.N.

Competing interests S.B. P.S. A.M. and K.N. have filed patents on the use of MNK inhibitors for treatment of neurodevelopmental disorders. A.M. and K.N. are current or former employees of the Experimental Drug Development Centre Singapore which has a commercial interest in the development of MNK1/2 inhibitors.

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Correspondence and requests for materials should be addressed to P.S.
Reprints and permissions information is available at http://www.nature.com/reprints.
Extended Data Fig. 1 | Loss of social recognition in Nlgn3KO mice. **a, b**, Mean social interaction time and data for individual mice in the social habituation/recognition test plotted for wild-type (n = 11) (a) and Nlgn3KO (b) mice (n = 12). **c, d**, Mean social interaction time and data for individual mice plotted for DATcre control mice (n = 10) (c) and DATcre::Nlgn3KO mice (n = 11) (d). **e**, Example for validation of targeted gene knockdown (n = 8 mice) from AAV2-DIO-miRNAlgn3-GFP viruses (green) in TH-positive cells (red) in the VTA of DATcre mice. **f**, Quantification of percentage of TH-positive cells in VTA and SNc of DATcre mice that express GFP from the AAV2-DIO-miR-GFP vector (n = 8). **g, h**, Mean social interaction time and data for individual mice plotted for control mice (g, VTA::DA-miR, n = 10) and VTA DA-specific Nlgn3 loss-of-function (h, VTA::DA-NL3, n = 8) in the social habituation/recognition test. **g**, Mean social interaction time and data for individual mice plotted for mice treated with vehicle (n = 12) (i) and OXTR-A (n = 11) (j). All error bars are s.e.m. Repeated-measures one-way ANOVA followed by Bonferroni’s post hoc test for planned multiple comparison (a–c, g–j) or Friedman test followed by Dunn’s post hoc test for planned multiple comparison (d). See Supplementary information for additional statistics.
Extended Data Fig. 2 | Properties of NAc-projecting VTA DA neurons in wild-type and Nlgn3KO mice. a, Representative I_h currents recorded from wild-type (black) and Nlgn3KO (blue) neurons evoked by consecutive hyperpolarizing voltage steps of −10 mV from −50 to −130 mV (bottom). At the end of each voltage step, the voltage command was returned to −130 mV to evoke tail currents (I_h-tail, depicted with an arrowhead). Red lines show fit of a single exponential function used to assess the I_h activation kinetics. b, Averaged I_h amplitudes were plotted against the voltage step. I_h current amplitudes were measured at the steady state (indicated with a filled circle in a) and the leak current values, as defined as the amplitude of the instantaneous currents at the onset of voltage steps (indicated with an asterisk in a), subtracted. c, Voltage-dependency of I_h-tail currents. I_h-tail amplitudes were normalized relative to I_h-tail at −50 mV and −130 mV. Solid lines show fits with a Boltzmann function for least square fit. The P value shows the difference in V50 between datasets. d, Activation kinetics of the I_h as determined by the r values obtained from the exponential fitting, as a function of the voltage commands voltage. Only the values obtained from commands between −130 mV to −90 mV were evaluated. e, Comparison between groups of the resting membrane potential as assessed with current clamp recordings. f, Membrane capacitance (Cm) of wild-type and Nlgn3KO DA neurons. g, Input resistant values (Ri) for wild-type and Nlgn3KO mice. Cm and Ri values were obtained in voltage clamp mode by applying a −5 mV (200 ms) voltage command from a holding potential set at −50 mV. All error bars are s.e.m. The P value represents genotype differences. n = 5 mice per genotype, numbers on graphs represent cells. P values determined by repeated-measures two-way ANOVA (b), Boltzmann sigmoidal test (c), mixed-effects model (d), unpaired two-sided t-test (e, f), or two-sided Mann–Whitney test (g). See Supplementary Information for additional statistics.
Extended Data Fig. 3 | Oxytocinergic innervation to VTA and Avpr1a mRNA levels are not affected in Nlgn3 KO mice. **a**, Representative images from 3 mice per genotype of neurophysin 1 (green), a cleavage product of the oxytocin neuropeptide precursor that is transported in vesicles together with oxytocin, and TH (red) immunofluorescence in the VTA of wild-type and Nlgn3 KO mice. Note that oxytocinergic axons arise from multiple hypothalamic nuclei, including the paraventricular nucleus. **b**, c, Mean VTA area coverage (b) and puncta fluorescence in the VTA (c) from wild-type and Nlgn3 KO mice. n = 3 mice per genotype. Numbers in brackets represent sections. **d**, Quantification of mean Th intensity per TH+ cell. **e**, Quantification of Nlgn3 puncta per 100 μm² TH+ cell. **f**, Quantification of Oxtr puncta per 100 μm² TH+ cell. n = WT: 280 cells from 4 mice; Nlgn3 KO: 265 cells from 3 mice (d–f). **g**, Targeted proteomic (PRM) measurements for oxytocin receptor (OXTR; left) and AVPR1A (right) proteins in VTA. Numbers on bars indicate mice. **h**, Representative images of FISH labelling of Avpr1a (cyan), Th (red) and Nlgn3 (green) in the VTA from wild-type and Nlgn3 KO mice. Experiment was repeated independently twice. **i**, Quantification of mean Avpr1a intensity per TH+ cell. **j**, Quantification of Avpr1a puncta per 100 μm² TH+ cell from wild-type and Nlgn3 KO VTA. n = wild type: 169 cells from 4 animals; Nlgn3 KO: 200 cells from 3 mice (i, j). All error bars are s.e.m. P values determined by unpaired two-sided t test (b, c, g), two-sided Mann–Whitney U test (d–f, i, j). See Supplementary Information for additional statistics.
**Extended Data Fig. 4 | Ribosomal proteins and translation processes are altered in Nlgn3KO mice.**

**a.** TMT proteomics: graphs plotting abundance of dopamine markers and synaptic proteins from VTA, cortex and hippocampus. Dopaminergic markers are strongly enriched in VTA samples. n = 5 mice per brain region.

**b.** Enrichment of GO terms for biological processes for proteins significantly altered (P < 0.05) in Nlgn3KO mice compared to wild-type mice.

**c.** Network-based analysis of proteins altered in Nlgn3KO VTA (P < 0.01). Blue nodes indicate downregulated proteins, red nodes upregulated proteins, light blue lines indicate interactions known from database and purple lines interactions experimentally determined. Disconnected nodes and nodes containing less than six proteins are not shown. See methods for additional information of statistics and analysis parameters.

**d.** Mean puromycin incorporation in acute cortical slices from adult wild-type and Nlgn3KO mice. All error bars are s.e.m. P values determined by two-sided Mann–Whitney U test. See Supplementary information for additional statistics.
Extended Data Fig. 5 | Pharmacological profile of novel MNK1/2 inhibitor ETC-168. a, b, Quantification of p-ERK1/2 (a) and p-eIF4G (b) levels compared to non-phosphorylated protein in cortical neurons at DIV14 treated with ETC-168. n = 8 replicates from 3 independent experiments. c, d, Quantification of eIF4E (c), ERK1/2 (d) and eIF4G (e) levels normalized to calnexin in cortical neurons at DIV14 treated with ETC-168. P values determined by one-way ANOVA. n = 8 replicates from 3 independent experiments. f, Quantification of p-eIF4E compared with eIF4E levels in VTA of wild-type mice and Nlgn3KO mice. n = 7 mice per genotype. i, Normalized p-eIF4E Alphalisa counts from wild-type and Nlgn3KO VTA lysate. n = 7 mice per genotype. j, k, Representative western blot (j) and quantification (k) of p-MNK1 and MNK1 levels in VTA lysate from wild-type mice and Nlgn3KO mice. n = 7 mice per genotype. l, Normalized p-eIF4E Alphalisa counts from VTA from wild-type treated with 5 mg kg⁻¹ ETC-168 for 24 h +2 h. n = 5 mice per genotype. All error bars are s.e.m. P values determined by one-way ANOVA (a–f), Kruskal–Wallis test (b), unpaired two-sided t-test (h, k), or two-sided Mann–Whitney test (i, j).

See Supplementary Information for additional statistics.
Extended Data Fig. 6 | ETC-168 treatment restores cognitive rigidity in Fmr1KO mice. a, Schematics of the place-independent cue discrimination and reversal task. This task was chosen given that phenotypes in cognitive rigidity tasks have been replicated in several studies on this model. b, Mean consecutive correct responses plotted for Fmr1WT/y and Fmr1KO/y mice. c, Treatment schedule of Fmr1WT/y and Fmr1KO/y mice. Mice were treated daily with vehicle during the learning phase and with 5 mg kg⁻¹ ETC-168 during the reversal phase 2 h before the start of the test. d, Mean consecutive correct responses plotted for vehicle treated Fmr1WT/y and vehicle or ETC-168 Fmr1KO/y mice. Numbers in brackets indicate mice. Error bars denote s.e.m. P values determined by repeated-measures two-way ANOVA followed by Bonferroni’s post hoc test. See Supplementary Information for additional statistics.
Extended Data Fig. 7 | Effect of ETC-168 treatment on protein abundance in wild-type and Nlgn3KO mice. a, Experimental outline. b, Representative western blot and quantification of p-eIF4E compared to eIF4E levels in VTA lysate from wild-type mice treated with vehicle or 5 mg kg⁻¹ ETC-168 for 7 consecutive days. n = vehicle: 6; ETC-168: 7. c, Normalized p-eIF4E AlphaLisa counts from wild-type mice VTA treated with vehicle or 5 mg kg⁻¹ ETC-168 for 7 consecutive days. n = vehicle: 6; ETC-168: 7. d, Graphs plotting TMT proteomic-normalized protein expression of dopaminergic markers in VTA from wild-type and Nlgn3KO mice treated with vehicle or ETC-168. Mice were treated for 7 days. n = 4 mice per genotype and treatment. e, f, Graphical representation of molecular function GO terms enriched in Nlgn3KO versus wild-type mice treated with vehicle (e), and Nlgn3KO mice treated with ETC-168 versus wild-type mice treated with vehicle (f). GO terms were summarized using REVIGO and only terms with Q < 0.01 are represented. g, h, TMT proteomic comparison of VTA from Nlgn3KO versus wild-type mice treated with vehicle (g) and Nlgn3KO mice treated with ETC-168 versus wild-type mice treated with vehicle (h). Relative frequency of log₂-transformed fold change in core proteasome abundance (Nlgn3KO/wild type) is plotted. n = 4 mice per genotype and treatment. All error bars are s.e.m. P-values determined by unpaired two-sided t-test (b), two-sided Mann–Whitney U test (e) or Kolmogorov–Smirnov test (g, h). See Methods and Supplementary Information for additional statistics.
Extended Data Fig. 8 | Effect of short-term ETC-168 treatment on social recognition in wild-type and Nlgn$^{3^\circ}$ mice. a–d, Time course of time interacting in the social habituation/recognition test for mice after short-term treatment with ETC-168. a, Wild-type mice treated with vehicle (n = 12). b, Wild-type mice treated with ETC-168 (n = 10). c, Nlgn$^{3^\circ}$ mice treated with vehicle (n = 9). d, Nlgn$^{3^\circ}$ mice treated with ETC-168 (n = 11). Error bars report s.e.m. P values determined by Friedman’s test followed by Dunn’s post hoc test for planned multiple comparison (a, c, d) or repeated-measures one-way ANOVA followed by Bonferroni’s post hoc test for planned multiple comparison (b). See Supplementary Information for additional statistics.
Extended Data Fig. 9 | Effect of ETC-168 treatment is dependent on the oxytocin receptor. a, Experimental outline. b, Mean social interaction time in Nlgn3KO mice treated with 5 mg kg⁻¹ ETC-168 and either vehicle or 10 mg kg⁻¹ OXTR-A. Numbers in brackets indicate mice. c, Social recognition index for Nlgn3KO mice treated with ETC-168 and vehicle, or ETC-168 and OXTR-A. Numbers on graph indicate mice. d, Individual values and mean of time interacting in the social habituation/recognition test after treatment with ETC-168 vehicle (n = 9) (d), or ETC-168 plus OXTR-A (n = 8) (e). Error bars report s.e.m. P values determined by repeated-measures two-way ANOVA followed by Bonferroni’s post hoc test (b), unpaired two-sided t-test (c), repeated-measures one-way ANOVA followed by Bonferroni’s post hoc test for planned multiple comparison (d, e). See Supplementary Information for additional statistics.
Extended Data Fig. 10 | Effect of long-term ETC-168 treatment on behaviour in wild-type and Nlgn3<sup>30o</sup> mice. a, Experimental schematics of chronic ETC-168 treatment and behaviour schedule. Number of animals per treatment conditions for all behaviours in b–p: wild-type vehicle = 9, wild-type ETC-168 = 10, Nlgn3<sup>30o</sup> vehicle = 8, Nlgn3<sup>30o</sup>ETC-168 = 9. b, c, Mean social interaction time in wild-type (b) and Nlgn3<sup>30o</sup> (c) mice treated for 8 days with vehicle or 5 mg kg<sup>–1</sup> ETC-168. d, Social recognition index for wild-type and Nlgn3<sup>30o</sup> mice treated with vehicle or 5 mg kg<sup>–1</sup> ETC-168. Numbers in brackets indicate mice. e, f, Individual values and mean time interacting in the social habituation/recognition test after chronic treatment with ETC-168 for wild-type vehicle (n = 9) (e), wild-type ETC-168 (n = 10) (f). Nlgn3<sup>30o</sup> vehicle (n = 8) (g), and Nlgn3<sup>30o</sup> ETC-168 (n = 9) (h). i, Experimental schematics of object habituation/recognition test in juvenile mice. j–l, Mean object interaction time plotted for wild-type (j) and Nlgn3<sup>30o</sup> (k) mice. P value above graphs report trial. l, Object recognition index. m, Mean velocity (cm s<sup>–1</sup>) in an open field arena during 7 min. n, Time spend in centre of the open field arena. o, Number of marbles buried during a 30 min marble burying test. p, Percentage weight gain in wild-type and Nlgn3<sup>30o</sup> mice treated with ETC-168 or vehicle. P value for treatment is displayed on graphs. Error bars report s.e.m. Repeated measures two-way ANOVA followed by Bonferroni’s post hoc test for genotype and treatment (b, c, j, k); repeated-measures two-way ANOVA followed by Bonferroni’s post hoc test for genotype and treatment (d, l, o), Friedman test followed by Dunn’s post hoc test for planned multiple comparison (e, f, h), one-way ANOVA followed by Bonferroni’s post hoc test for planned multiple comparison (g), or repeated-measures two-way ANOVA (m, n, p). See Supplementary Information for additional statistics.
Reporting Summary

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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- EthoVision10
- Metamorph 7.8.11.0 (Molecular Devises)
- Igor Po V.6.3.7.2 (WaveMetrics)
- CellSens V2.3 (Olympus)
- SparkControl V2.3 (Tecan)

Data analysis

- Phoenix WinNonlin, version 6.3 (Certata)
- ImageJ, 1.49k (NHI)
- Progenesis QI Version 2.0 (Nonlinear Dynanics)
- MASCOT, version 2.4.1 (Matrix Science)
- DAVID 6.8 (NIAID/NIH)
- SpectroMine, 1.0.20235.13.16424 (Biognosys)
- String v11 (Elexir)
- Prism v8 (GraphPad)
- REVIGO (Rudjer Boskovic Institute)
- PSEA-Quant, October 11, 2019 (Yates lab)

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

Proteomic data will be deposited at PRIDE and will be made available upon acceptance of the manuscript. All renewable reagents and detailed protocols will be made available on request.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No statistical methods were used to predetermine the number of animals and cells. Suitable sample sizes were estimated based on previous experience and are similar to those generally employed in the field.

Data exclusions

For VTA-specific Nlgn3 knockdown experiments, animals were excluded by pre-established criteria if less than 20% of cells in the VTA were TH and GFP positive, or if the body weight was less than 75% of the mean body weight at the start of behavior trials. For Retrobead injected experiments, cells were excluded by pre-established criteria if post-hoc immunostaining for TH or retrobead was negative, or if biocytin labeling was unsuccessful (thus preventing post-hoc confirmation). Outliers were identified using ROUT test on the most stringent setting (Q=0.1%). This was used to exclude one DAT-Cre x Nlgn3KO mouse, and two data point for the FUNCAT experiments.

Replication

All animal experiments were done with a minimum of 3 cohorts with animals from several litters. Each cohort showed similar phenotypes. For immunostaining, western blot, FUNCAT and FISH experiments, a minimum of 3 animals per genotype was used to ensure reproducibility. For cell culture experiments, replicates from 3 independent experiments were used with similar results.

Randomization

Animals were randomly assigned to treatment groups at the moment of viral injections, retrobead injections or behavioral tests and treatment.

Blinding

Experimenter was blinded to genotype for all experiments, and most analysis. The exception was FISH analysis where the presence of a Nlgn3 label made blinding impossible. For electrophysiological experiments, the experimenter was blinded for treatment and genotype. Blinding for treatment was not possible for behavioral experiments, as the experimenter had to administer the drug that had a different color to the vehicle treatment.

Reporting for specific materials, systems and methods

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| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a                              | n/a     |
| ☐ Antibodies                     | ☐ ChiP-seq |
| ☒ Eukaryotic cell lines          | ☒ Flow cytometry |
| ☒ Palaeontology                  | ☒ MRI-based neuroimaging |
| ☒ Animals and other organisms    |         |
| ☐ Human research participants    |         |
| ☐ Clinical data                  |         |

Antibodies

Antibodies used

p-eIF4E: Abcam, ab76256, EP2151Y, Lot#GR210598-1
eIF4E: Abcam, ab47482, Lot#GR100774-1
p-ERK1/2: Cell Signaling, 4370S, clone: 137F5, Lot#12
| Antibody/Protein          | Supplier/Code | Lot Number |
|--------------------------|---------------|------------|
| ERK1/2                   | Cell Signaling, 4695S, clone: D13.14.4E, Lot#14 |
| p-ERK1/2                 | Cell Signaling, 2441S, Lot#4 |
| eIF4G                    | Cell Signaling, 2498, Lot#4 |
| p-eIF4G                  | Cell Signaling, 2111, Lot#6 |
| MNK1                     | Cell Signaling, 2195S, Lot#5 |
| GAPDH                    | Cell Signaling, 5174, clone: D16H11, Lot#7 |
| Calnexin                 | Stressgen, SPA-855, Lot#01031711 |
| Puromycin                | Kerafast, EQ0001, clone: 3RH11, Lot#2733311 |
| TH                       | Millipor, A81542, Lot#2982635 |
| Neurophysin-1            | Millipor, MABN844, clone: PS38, Lot#3083532 |
| Donkey anti-sheep IgG-Cy3| Jackson ImmunoResearch 713-165-147 |
| Donkey anti-sheep Cy5    | Jackson ImmunoResearch 713-175-147 |
| Donkey anti-rabbit IgGCy3| Jackson ImmunoResearch 711-165-152 |
| Goat anti-mouse Cy2      | Jackson ImmunoResearch 714-225-150 |

**Validation**

- **p-eIF4E**: WB: validated using positive control 293 cell lysate treated with alkaline phosphatase and HEK293 cell lysate treated with Dexamethasone (abcam.com).
- **eIF4E**: evaluated by western blotting using Breast carcinoma tissue and NIH/3T3 cells extract (abcam.com).
- **p-ERK1/2**: evaluated by western blotting using extracts from COS cells, untreated or treated with either U0126 #9903 (10 μM for 1h) or TPA (cellsignal.com).
- **ERK1/2**: evaluated by western blotting using extracts from HeLa, NIH/3T3 and C6 cells (cellsignal.com).
- **p-eIF4G**: evaluated by western blotting using extracts from 293 cells expressing GST-eIF4GI Ser1192 Ala or GST-eIF4GII Ser1108 Ala mutant protein (cellsignal.com).
- **eIF4G**: evaluated by western blotting using extracts from various cell lines (cellsignal.com).
- **p-MNK1**: evaluated by western blotting using extracts from NIH/3T3 cells serum starved for 24h and then treated or untreated with serum for 30 min (cellsignal.com).
- **MNK1**: evaluated by western blot analysis of extracts from various cell lines (cellsignal.com).
- **GAPDH**: evaluated by western blotting using extracts from various cell lines (cellsignal.com).
- **Calnexin**: evaluated by western blotting using MWM, Vero, 3T3, PC-12, and HeLa cell lines (enzolifesciences.com).
- **Puromycin**: evaluated for western blotting (Kelleher, AR., et al. (2013). Am J Physiol Endocrinol Metab. 304(2):E229-236).
- **TH**: evaluated by Western Blot on mouse brain lysates (merckmillipore.com).
- **Neurophysin-1**: evaluated for IHC (Ben-Barak, Y., et al. (1985). J. Neurosci. 5(1):81-97).

**Animals and other organisms**

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research.

| Category          | Information                                      |
|-------------------|--------------------------------------------------|
| Laboratory animals| Mice: C57BL/6j, male, P5-adult; NMRI, male, female, E16.5 |
| Wild animals      | the study did not involve wild animals           |
| Field-collected samples | the study did not involve field-collected samples |
| Ethics oversight  | Basel Cantonal Veterinary Office Committees for Animal Experimentation; Lausanne Cantonal Veterinary Office Committees for Animal Experimentation |

Note that full information on the approval of the study protocol must also be provided in the manuscript.