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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	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
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Visual discrimination behavior data was acquired using custom written Matlab code using legacy version of Matlab 2014a (code available upon request). Visual stimulus was presented using Psychtoolbox 3.0 that can be downloaded from www.psychtoolbox.org. Calcium imaging data was acquired using commercially available software from Vidriotech called Scanimage 5.0.
Data analysis	Behavior and calcium imaging data was analyzed using custom written Matlab code (code available upon request).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data shown in this paper is available upon request.

Field-specific reporting

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

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

Sample size	We did not use statistical methods to predetermine sample sizes. For all the main results shown in Figures1 -3 we used sample size =>10 mice and subsequent statistics were performed using number of mice as sample size. Working with Fmr1-/- can be technically challenging for several reasons: 1) Fmr1-/- mothers have a higher incidence of cannibalization, 2) Fmr1-/- mice can require extra handling in order to habituate them to any behavioral task and extra care is required during water deprivation because a fraction of them can show adverse effects such as excessive weight loss, which could lead to seizures (see below, Data Exclusions). Hence to maintain feasibility of experiments and ethical use of numbers of animals for most of our experiments we used at least 10 mice per group. In Fig 4 and 5 we used triple transgenic mice where PV-Cre mice were crossed with Ai9 mice and these were then back crossed to FVB WT and Fmr1 -/- mice for 8 generations. Generation of a triple transgenic line was time consuming and resource intensive, and we faced the same technical challenges associated with using Fmr1 -/- mice; hence, we used n => 6 mice. Again statistics were performed using the number of mice as the sample size. For Fig. 6 we used n=8 humans for each group. Recruiting age and gender matched Fragile X patients is challenging, however a sample of 8 is comparable to the number of human subjects used in previously published studies.
Data exclusions	As dictated in our animal protocol mice were prematurely terminated if when they exhibited ruffled fur or became lethargic, which sometimes occurs as a result of water deprivation, or if weight loss exceeded 25% of body weight. As a result, 5 Fmr1 -/- and 1 WT mouse were excluded from the data because they lost > 25% body weight. A loss of >25% body weight can have adverse affects on the health of the mice, makes them listless, they stop grooming and in extreme cases can potentially lead to seizures. This information is included in the Methods.
Replication	We describe our procedures in detail, so that other labs can establish these techniques and reproduce our results. We are also encouraged by the fact that we replicated the deficit in visual discrimination of Fmr1-/- mice within our paper: 1. In Fig 4, a separate cohort of Fmr1 -/- mice and WT was trained on the visual discrimination task following calcium imaging in parvalbumin (PV) cells and Fmr1-/- mice showed a delay in learning that replicates our data in Figs. 1, 2and 3. 2. Separate groups of Fmr1 -/- mice were used to examine calcium imaging in PV cells in Fig. 4 and Fig. 5, with identical results. Note that the mean data from Fmr1 -/- mice for Z score fluorescence/s, frequency of events and fraction of stimulus responsive PV cells in Fig 4 and 5 is similar.
Randomization	We ensured that during a behavior training cycle both WT and Fmr1 -/- were included to exclude any biases introduced by experimenters or the training rig. Similarly on a particular testing day Fragile-X subjects were randomized with control subjects. Since Fmr1-/- mice were obtained from homozygous litters for reasons mentioned in the methods we were not able to randomly pick WT and Fmr1-/- mice from the same litter.
Blinding	G.C., A. N., B.T., D.A. were blinded to the genotype in a 60% of mice used in all the experiments during visual discrimination training. With the calcium imaging data A. G. used semi- automated approaches (MATLAB code) to analyze all the calcium imaging data and the analysis was performed in batches of WT and KO mice simultaneously, rather than all the WT first and then all the KO (to minimize bias).

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	All experiments followed the U.S. National Institutes of Health guidelines for animal research, under an animal use protocol (ARC #2007-035) approved by the Chancellor's Animal Research Committee and Office for Animal Research Oversight at the University of California, Los Angeles. Experiments in Figs. 1 and 2 used male and female FVB.129P2 WT mice (JAX line 004828) and Fmr1-/- mice 5 (JAX line 004624) and experiments in Fig. 3 used male and female PV-Cre mice (JAX line 008069) that were crossed to the Ai9 (Td-Tom) reporter line (JAX line 007909) and the resulting PV-Cre x Ai9 mice were back crossed to FVB WT and Fmr1-/- mice for 8 generations. All mice were housed in a vivarium with a 12/12 h light/dark cycle and experiments were performed during the light cycle. The FVB background was chosen because of its robust breeding, because FVB Fmr1-/- dams are less prone to cannibalizing their pups, and because FVB Fmr1-/- mice have well-documented deficits in sensory processing6. Additionally, to improve the survival of Fmr1-/- pups due to the possibility of littermates with different genotypes receiving unequal attention from the dam32 we used homozygous litters. This information is included in the Methods
Wild animals	No wild animals were used in this study
Field-collected samples	Field collected samples were not involved in this study

Human research participants

Policy information about studies involving human research participants

Population characteristics	Eight males with FXS and eight male healthy controls, matched on chronological age, completed the visual discrimination experiment (Table S1).
Recruitment	Testing was conducted at a regional academic pediatric medical center where the participants with FXS were originally recruited as part of our Center for Collaborative Research in Fragile X (U54). Control participants were recruited through hospital-wide and community advertisements and were excluded for a history of developmental or learning disorders or significant psychiatric disorder (e.g., schizophrenia) in themselves or first degree-relatives, or for a family history of ASD in first- or second-degree relatives based on a brief screening interview.