Impaired perceptual learning in a mouse model of Fragile X syndrome is mediated by parvalbumin neuron dysfunction and is reversible

Anubhuti Goel1, Daniel A. Cantu1, Janna Guilfoyle2, Gunvant R. Chaudhari1, Aditi Newadkar1, Barbara Todisco1, Diego de Alba1, Nazim Kourdougli1, Lauren M. Schmitt2, Ernest Pedapati2, Craig A. Erickson2 and Carlos Portera-Cailliau1,4*

To uncover the circuit-level alterations that underlie atypical sensory processing associated with autism, we adopted a symptom-to-circuit approach in the Fmr1-knockout (Fmr1−/−) mouse model of Fragile X syndrome. Using a go/no-go task and in vivo two-photon calcium imaging, we find that impaired visual discrimination in Fmr1−/− mice correlates with marked deficits in orientation tuning of principal neurons and with a decrease in the activity of parvalbumin interneurons in primary visual cortex. Restoring visually evoked activity in parvalbumin cells in Fmr1−/− mice with a chemogenetic strategy using designer receptors exclusively activated by designer drugs was sufficient to rescue their behavioral performance. Strikingly, human subjects with Fragile X syndrome exhibit impairments in visual discrimination similar to those in Fmr1−/− mice. These results suggest that manipulating inhibition may help sensory processing in Fragile X syndrome.

A typical sensory processing, as observed in Fragile X syndrome (FXS) and across other syndromic and idiopathic forms of autism spectrum disorders (ASD), negatively impacts virtually all activities of daily living. Sensory symptoms are predictive of the subsequent appearance of impaired social behavior and other autistic traits. Thus, a better understanding of the changes in neural circuitry that disrupt perceptual learning could shed light on the mechanistic basis and potential therapeutic avenues for a range of autistic symptoms. FXS is ideally suited to address this issue because it is the leading inherited cause of autism, because it lacks major neuroanatomical defects, and because its well-characterized animal model, the Fmr1−/− mouse, reproduces several aspects of the human disease. Fmr1−/− mice not only manifest the immature synaptic defects seen in humans, but also display multiple symptoms such as anxiety, impaired cognitive flexibility, reduced social interaction, hyperarousal, and sensory over-reactivity that could result from altered sensation. However, at present we lack a clear understanding of how the molecular and synaptic alterations resulting from loss of the fragile X mental retardation protein (FMRP) affect neuronal networks in ways that can explain specific behavioral features in FXS, such as impaired perceptual learning and discrimination. Furthermore, the lack of directly comparable behavioral frameworks in both humans and animal models also limits the translational potential of discoveries in ASD.

Here we examined impairments of perceptual learning and visual discrimination using a similar behavioral task in mice and humans, and then deciphered specific circuit-level disruptions in Fmr1−/− mice that bring about the altered behaviors. This parallel mouse–human perspective, derived from a circuit-level understanding of FXS symptoms, is a distinctive approach to targeting therapeutic interventions. Additionally, although many human psychophysical studies have demonstrated deficits in visual perception in individuals with FXS, whether Fragile X model mice also exhibit similar deficits is not known. We show that Fmr1−/− mice exhibit delayed learning of a visual discrimination task, and two-photon calcium imaging in primary visual cortex (V1) revealed that this impairment correlated with reduced numbers and broader tuning of orientation-selective pyramidal cells. We also found a reduction in the functional output of parvalbumin (PV) interneurons in V1 of Fmr1−/− mice, compared to wild-type (WT) mice. Using an excitatory approach with designer receptors exclusively activated by designer drugs (DREADDs) targeted to PV cells in Fmr1−/− mice, we could restore their visually evoked responses to near-WT levels, and this accelerated their rate of learning. By adapting an analogous visual discrimination experiment for human subjects, we identified a similar deficit in visual discrimination in FXS participants. These findings will help pave the way for new concepts in targeted treatment development for FXS and ASD.

Results

Fmr1−/− mice exhibit delayed learning on a visual discrimination task. To determine whether Fmr1−/− mice manifest perceptual learning deficits associated with abnormal visual sensory discrimination, we trained male and female Fmr1−/− (n = 21) and WT (n = 19) mice (FVB strain) on a go/no-go visual discrimination task. We used separate litters of WT and Fmr1−/− mice rather than littermate controls, because littermates of different genotypes tend to receive unequal attention from the dam (that is, the dam neglects the Fmr1−/− pups), and this often affects the health and behavior of Fmr1−/− pups, which could bias results. To avoid issues with genetic drift, we obtained new WT and Fmr1−/− breeders from Jackson Labs at regular intervals (every 1–1.5 years).

1Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA. 2Department of Psychiatry, Cincinnati Children’s Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA. 3Department of Neurology, Cincinnati Children’s Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA. 4Department of Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA. *e-mail: cpcailiau@mednet.ucla.edu

Corrected: Author correction

https://doi.org/10.1038/s41593-018-0231-0

ARTICLES
https://doi.org/10.1038/s41593-018-0231-0
Following water deprivation, awake head-restrained young adult mice (2–4 months old) were allowed to run on an air-suspended polystyrene ball while they performed the task (Fig. 1a and see Methods). Mice were presented with sinusoidal gratings drifting in two orthogonal directions, 45° (preferred, ‘go’) versus 135° (non-preferred, ‘no-go’) at 100% contrast. Incorrect behavioral responses resulted in a 6.5-s ‘time-out’ period (Fig. 1b). Task performance, as determined by the discriminability index statistic $d'$ (see Methods), was dependent on V1, because pharmacological silencing of V1 with bilateral infusions of muscimol, a GABA$_\text{A}$ receptor agonist, reversibly disrupted discrimination in WT mice (Fig. 1c).

WT mice learned quickly (3–4 sessions) to lick in response to the preferred orientation for a water reward and withhold licking when presented with the nonpreferred orientation (Fig. 1d). In contrast, Fmr1$^{-/-}$ mice exhibited a significantly delayed learning curve, compared to the WT mice (Fig. 1d and Supplementary Fig. 1). In addition, we analyzed their behavioral responses over the course of training and found a significant increase in Hit and correct rejection responses and a significant decrease in Miss and false alarm (FA) responses, as mice of both genotypes learned the task (Supplementary Fig. 2b). Fmr1$^{-/-}$ mice exhibited a significantly higher percentage of FA responses compared to WT mice at session 4 (Fig. 1e and Supplementary Fig. 2b), which likely contributed to their poor performance during early training sessions. This increase in FA rates in Fmr1$^{-/-}$ mice was associated with hyperactivity or abnormal locomotion, as the average running speed was similar between genotypes at session 1, or even after the animals reached $d' > 2$ (Supplementary Fig. 3). Although there was no change in running speed in mice of either genotype during the course of each trial on session 1, by session 4, we observed significantly more slowing down in WT than in Fmr1$^{-/-}$ mice, toward the end of each trial (Supplementary Fig. 3g–i). However, eventually Fmr1$^{-/-}$ mice also learn to slow down for the preferred stimulus at the learned session (Supplementary Fig. 3i). Thus, learning the task was associated with slowing down in the presence of the preferred stimulus (presumably to consume the water reward).

The delay shown by Fmr1$^{-/-}$ mice in learning the visual discrimination task was evident in female and male mice alike (Supplementary Fig. 4). Notably, both WT and Fmr1$^{-/-}$ mice exhibited significant improvements in task performance throughout training (Fig. 1d). Even though Fmr1$^{-/-}$ mice took, on average, 2.5 sessions longer to achieve expert-level performance ($d' > 2$; Fig. 1d), there was no
A reduced-angle task was implemented after mice learned the 90° task (at 100% contrast, as in Fig. 1). This suggests that compared to WT mice, for the reduced angle task, but not for the WT controls in this visual discrimination task.

**Impaired performance of Fmr1Δ/Δ mice in a reduced-angle task.** Fmr1Δ/Δ mice are known to exhibit a broadening of receptive fields in somatosensory cortex7–9. Similar broader tuning in V1, if it exists, could affect the discrimination of visual stimuli with very similar orientations. Therefore, we next tested whether Fmr1Δ/Δ mice would be particularly challenged by a reduced-angle task in which the difference in angle between the preferred and nonpreferred orientations was gradually reduced to 7.5°, after the animals had learned the basic 90° task (Fig. 2a). Mice that had already maintained an expert-level performance (d′ > 2) on the 90° task were tested for only two sessions at each of the reduced-angle tasks (starting at 15°, then 10°, and finally 7.5°). The d′ values shown in Fig. 2b are averages of those two sessions. Overall, there was a significant decrease in performance across both genotypes when the angle between the orientations was gradually reduced (P = 6 × 10−5). However, changes in d′ at different angles suggest that the threshold for discrimination for WT mice was at or below 7.5°, whereas it was 15° for Fmr1Δ/Δ mice (Fig. 2b).

To further probe the extent to which mice were challenged by this reduced-angle task, we assessed their response times and observed a significant delay in the distribution of licking onset in Fmr1Δ/Δ mice, compared to WT mice, for the reduced angle task, but not for the normal (90°) task (Fig. 2c). This suggests that Fmr1Δ/Δ mice take longer to decide only in the face of ambiguous sensory information.

**Orientation-tuning deficits in V1 correlate with task performance in Fmr1Δ/Δ mice.** Having established a defect in perceptual learning in the Fragile X mouse model that is relevant to the human disease, we next adopted a reverse-engineering approach to identify the circuit- and neuronal-level alterations that might underlie the impaired visual discrimination. In light of various reports of cortical hyperexcitability and network hypersynchrony in Fmr1Δ/Δ mice14,17,18, we first investigated whether the perceptual learning deficit we observed in Fmr1Δ/Δ mice, was caused by abnormal orientation tuning of pyramidal cells in V1. To test this, we performed in vivo two-photon calcium imaging in layer (L) 2/3 neurons in awake mice running on a floating polystyrene ball (Fig. 3a–c and see Methods). A recombinant adeno-associated virus (rAAV) for expression of the calcium reporter GCaMP6s19 was injected in V1 following stereotaxic coordinates, and successful targeting was confirmed using intrinsic signal imaging (Fig. 3b). We recorded both spontaneous and visually evoked activity from L2/3 neurons in mice that were fully acclimated to the experimental apparatus and running vigorously throughout the calcium imaging session (Fig. 3c). For the latter, WT and Fmr1Δ/Δ mice (n = 9 and 10, respectively) were presented with four sequential presentations of sinusoidal gratings drifting in 8 different directions (4 orientations) at random (Fig. 3d and see Methods). Although previous studies have reported hyperexcitatory cortical circuits in Fmr1Δ/Δ mice (reviewed in ref. 9), we did not observe a significant increase in either spontaneous or visually evoked activity in Fmr1Δ/Δ mice (Fig. 3e and Supplementary Fig. 6a,b).

Despite the seemingly normal frequency of visually evoked activity in Fmr1Δ/Δ mice, they had a significantly lower percentage of orientation-selective (OS) cells in L2/3 (Fig. 3f). Notably, when we trained these mice on the visual discrimination task, we found a significant inverse correlation between the percentage of OS cells and the number of days it took animals to reach a d′ > 2 (Fig. 3g). This implies that, with fewer available OS cells in V1, Fmr1Δ/Δ mice had more difficulty discriminating between two different orientations, particularly when the difference was small (Fig. 2a–c). In addition, in vivo calcium imaging revealed that L2/3 neurons in V1 of Fmr1Δ/Δ mice had significantly broader tuning compared to those in WT mice (Fig. 3h). This 6.6° difference in the mean tuning width of pyramidal neurons in V1 between WT and Fmr1Δ/Δ mice, though slight, might be sufficient to explain why Fmr1Δ/Δ mice can discriminate at 15° but not at 10°. Additionally, we found a significant correlation between the tuning width of L2/3 cells and the number of days it took the animals to reach a d′ > 2 (P = 0.04; Supplementary Fig. 7), which suggests that the broader tuning of the pyramidal neurons in V1 contributed to the delayed learning in Fmr1Δ/Δ mice.

**Reduced activity of parvalbumin cells in V1 in Fmr1Δ/Δ mice.** Abnormal V1 network dynamics pertaining to orientation selectivity and tuning width could be the result of dysfunction in PV interneurons, the most prevalent inhibitory neuron in V120. PV cells exhibit very broad orientation tuning by simply responding to all
 orientations, since they receive local input from a wide range of orientation tuned pyramidal cells\textsuperscript{[8–21]}. Furthermore, selective stimulation of PV cells in V1 with channelrhodopsin-2 leads to improved feature selectivity and visual discrimination\textsuperscript{[2]}. For these reasons, we tested the hypothesis that PV cells were hypoactive in Fragile X model mice. We used in vivo calcium imaging to record the activity of PV neurons in V1 of WT and Fmr1\textsuperscript{–/–} mice (\(n=6\) and 7, respectively) that expressed tdTomato in PV neurons (PV-Cre mice × Ai9 mice; see Methods). At the time of the cranial window surgery, we injected a Cre-dependent virus into V1 to selectively express GCaMP6s in PV cells (Fig. 4a,b). Our calcium imaging recordings revealed stark differences in the activity of PV cells between WT and Fmr1\textsuperscript{–/–} mice; whereas traces of PV cell activity in WT mice showed the expected broadly tuned, nonselective responses to visual stimuli, traces of PV cells in Fmr1\textsuperscript{–/–} mice exhibited reduced visually evoked activity (Fig. 4c). Fmr1\textsuperscript{–/–} mice exhibited a significantly lower frequency of calcium peaks (see Methods) triggered by visual stimuli (Fig. 4d,e). One of our criteria for selecting PV cells for analysis in V1 was the proportion of active PV cells (Fig. 4f) or the amplitude or frequency of spontaneous calcium transients in PV cells that were significantly different between WT and Fmr1\textsuperscript{–/–} mice (Supplementary Fig. 8; \(P<0.006\), Pearson’s correlation). We also found a significantly lower fraction of PV cells in Fmr1\textsuperscript{–/–} mice (Fig. 4g), which would also ultimately be expected to affect the functional output of V1.

A DREADD strategy that restores PV cell activity and orientation tuning in V1 accelerates learning of the visual task in Fmr1\textsuperscript{–/–} mice. Based on the finding that PV cells were indeed hypoactive in Fmr1\textsuperscript{–/–} mice, we hypothesized that a successful manipulation of PV cell activity that would restore their output in these animals might also improve their performance on the visual discrimination task. Hence, we used a DREADD-based approach\textsuperscript{[24]} (see Methods) to selectively express the excitatory hM3Dq receptor in PV cells of Fmr1\textsuperscript{–/–} mice (\(n=6\); Fig. 5a). We then used the hM3Dq ligand, clozapine-N-oxide (CNO; 5 mg/kg, intraperitoneally), to excite PV cells and increase their output in these Fmr1\textsuperscript{–/–} mice. Overexpressing hM3Dq in PV cells alone (before administering CNO) did not affect visually evoked activity of PV cells in Fmr1\textsuperscript{–/–} mice (Supplementary Fig. 9a–d). In contrast, 30 min after a single CNO injection, we observed a robust increase in visually evoked PV cell output in these Fmr1\textsuperscript{–/–} mice (Fig. 5b,c). Specifically, we observed a significant increase in both the frequency of visually evoked calcium transients in PV cells of Fmr1\textsuperscript{–/–} mice (Fig. 5c) and in the frequency of individual peaks of activity (Fig. 5d). Spontaneous activity was unaffected by CNO (Supplementary Fig. 10). The fraction of stimulus-responsive PV cells in Fmr1\textsuperscript{–/–} mice was also significantly increased by CNO, restoring it to WT levels (Fig. 5e). Notably, the fact that we could increase the activity of PV cells with DREADDs suggests the notion that PV cells were not silent in Fmr1\textsuperscript{–/–} mice due to poor health. Also, the proportion of PV cells that was active did not change after CNO administration
A subset of the DREADD-expressing
able to reverse the delay in learning the visual discrimination task. This
trained on the standard visual discrimination task (90° angle) and
Fmr1–/–,hM3Dq
the properties of pyramidal neurons in the circuit. Calcium imaging
DREADD manipulation on PV cells would be sufficient to affect
cle back to OS pyramidal cells in V1, we also tested whether the
2) on the visual discrimination task compared to
Fmr1–/– mice showed clearly how
mice revealed that
ac
PVMouse #1
GCaMP6s
Fmr1–/–; *
P = 0.03; two-tailed Mann–Whitney test). Horizontal bars indicate mean and error bars
= 7)
NATuRE NEuRoSCIENCE
www.nature.com/natureneuroscience
ARTICLES

Fig. 4 | PV interneurons in V1 in Fmr1–/– mice show reduced visual evoked activity. a. Cartoon of strategy for selective GCaMP6s expression in PV interneurons. b. Representative field of view for in vivo two-photon calcium imaging in PV neurons expressing GCaMP6s (green) and tdTomato (red). Responses to 8 different directions from single trials are shown in gray, while the averages of 4 trials are in black. d. The overall frequency of visually evoked calcium transients (as measured by the mean fluorescence z-scores per s) is significantly lower in Fmr1–/– mice (n = 7 Fmr1–/–, 6 WT mice; z-score per s: 0.27 ± 0.06 for WT vs. 0.15 ± 0.03 for Fmr1–/–; †P = 0.03; two-tailed Mann–Whitney test). e. The frequency of visually evoked peaks of activity in PV neurons is significantly lower in Fmr1–/– mice (z-score per s: 1.8 ± 0.5 for WT vs. 0.3 ± 0.1 for Fmr1–/–; †P = 0.03; two-tailed Mann–Whitney test). f, WT and Fmr1–/– mice showed similar percentages of active PV cells (†P = 0.70, two-tailed Mann–Whitney test). g, The fraction of visually responsive PV cells is significantly reduced in Fmr1–/– mice (0.65 ± 0.02 for WT vs. 0.43 ± 0.03 for Fmr1–/–; †††P < 10–5, unpaired two-tailed Student’s t test). Note that there is an inverse correlation between the fraction of stimulus-responsive PV cells and behavioral performance (see Fig. 5i). Results showing a decrease in mean z-score per s and fraction of stimulus responsive PV cells in Fmr1–/– was independently replicated in Fig. 5 (see data from Fmr1–/– mice before CNO treatment in Fig. 5c–e).

(not shown), suggesting that the DREADD effect on the fraction
of visually responsive PV neurons was not due to simply making
previously silent cells more active.

Having restored visually evoked PV cell activity in Fmr1–/–/AMS1D
mice to near normal WT levels, we hypothesized that we might be
able to reverse the delay in learning the visual discrimination task.
A subset of the DREADD-expressing Fmr1–/– mice were therefore
trained on the standard visual discrimination task (90° angle) and
injected with CNO ~30 min before each training session. This
chemogetic manipulation resulted in a leftward shift in the learning
curve (that is, faster learning) of CNO-treated Fmr1–/–/AMS1D mice
(Fig. 5f), indicating that we were able to rescue the learning impair-
bment by acutely elevating the PV cell output. CNO led to a signifi-
cant reduction in the number of days required to reach expert level
(d’ > 2) on the visual discrimination task compared to Fmr1–/– mice
(Fig. 5g). Notably, the rate of FA responses in these CNO-treated
Fmr1–/–/AMS1D mice was similar to that of WT mice (not shown).
However, injection of CNO alone in the absence of DREADDs in
Fmr1–/– mice did not rescue behavior (Fig. 5g). To come full cir-
cle back to OS pyramidal cells in V1, we also tested whether the
DREADD manipulation on PV cells would be sufficient to affect
the properties of pyramidal neurons in the circuit. Calcium imaging
with rAAV-GCaMP6s in a group of Fmr1–/–/AMS1D mice revealed that
CNO administration significantly raised the proportion of orienta-
tion selective pyramidal cells and showed a trend toward sharper
tuning (Fig. 5h,i). Notably, the relationship between PV cell output
and behavior was apparent from the negative correlation between
the fraction of stimulus responsive PV cells and the number of days
needed to reach a d’ > 2 (Supplementary Fig. 11). This relationship
showed clearly how Fmr1–/–/AMS1D mice treated with CNO were not
distinguishable from WT mice.

Fragile X patients exhibit defects in visual discrimination similar
to those in Fmr1–/– mice. It was recently argued that the absence
of directly comparable behavior frameworks between human
and animal studies is an impediment to progress in translational
research for autism and its associated genetic disorders. It might
even explain, in part, the failure of clinical trials in FXS. To assess
the translational potential of our findings on impaired visual dis-
(Fig. 6a,b) and see Methods). Healthy control human participants and FXS participants (n = 8 each; see Supplementary Tables 1 and 2) were administered the task. Most individuals affected by FXS could complete the task at 90° but
demonstrated, on average, a significantly lower d’ than healthy
controls (Fig. 6c). Performance declined slightly in some healthy
control participants at reduced angles, but on average, this was not
significant (P = 0.2). In contrast, FXS participants showed a clear
trend toward a lower d’ on the 15° task compared with 90° or 45°
Fig. 5 | A DREADD strategy that restores normal PV cell activity in V1 rescues delayed learning in Fmr1−/− mice. a, Cartoon of strategy for selective rAAV-EF1α-DIO-hM3D(Gq)-mCherry expression in PV interneurons of Fmr1−/− mice. b, Example of GCaMP6s traces for 4 representative PV neurons in V1 from 4 different Fmr1−/−-MADQ mice before and ~30 min after intraperitoneal injection of CNO. c, The activity of PV cells, as measured by both e) mean fluorescence (z-score per s: 0.18 ± 0.02 before CNO vs. 0.32 ± 0.05 after CNO; *P = 0.02; one-tailed, unpaired Student’s t-test) or the d) frequency of peaks in calcium transients (0.31 ± 0.04 Hz before CNO vs. 0.59 ± 0.11 Hz after CNO; *P = 0.03; one-tailed, unpaired Student’s t-test), in Fmr1−/−-MADQ mice increases significantly after CNO administration (n = 6 Fmr1−/− mice, before and after CNO). Horizontal bars indicate mean and error bars indicate s.e.m. e, The fraction of stimulus-responsive PV cells also increases significantly after CNO (0.39 ± 0.06 before CNO vs. 0.72 ± 0.04 after CNO; ***P = 0.001; one-tailed, unpaired Student’s t-test). Note that the fraction of visually responsive PV cells was comparable between Fmr1−/− mice expressing DREADDs (before CNO) and Fmr1−/− mice in Fig. 4g, f, Fmr1−/−-MADQ mice treated with CNO 30 min before each session learned the basic 90° task in ~3 d on average. The rate of learning for Fmr1−/− mice (from Fig. 1d) is shown for comparison. The solid line indicates the mean, and the shaded area shows the standard error. f, Dashed line at d′ = 2 indicates the expert performance threshold. g, Fmr1−/−-MADQ mice treated with CNO learned the basic 90° task significantly faster than Fmr1−/− mice and as fast as WT mice (WT: 3.5 ± 0.2 d, Fmr1−/−: 6.0 ± 0.4 d, Fmr1−/−-MADQ with CNO: 3.7 ± 0.3 d; Fmr1−/− with CNO: 6.4 ± 0.8 d; χ² = 1.26, d = 0.05, Kruskal–Wallis test; WT vs. Fmr1−/−: P = 10 × 10⁻⁶; two-tailed Mann–Whitney test; Fmr1−/− vs. Fmr1−/−-MADQ with CNO: P = 0.004; two-sided Mann–Whitney test). In a control experiment, treating Fmr1−/− mice (without DREADD) with CNO had no effect on the time to learn the task (P = 0.8, two-tailed Mann–Whitney test; n = 19 WT, 21 Fmr1−/−, 6 Fmr1−/−-MADQ + CNO, 10 Fmr1−/− + CNO mice). h, The percentage of OS pyramidal cells in Fmr1−/−-MADQ mice was significantly higher after CNO administration (*P = 0.02, unpaired one-tailed Student’s t test; n = 3 Fmr1−/−-MADQ mice, tested before and after CNO). i, There was a nonsignificant trend toward reduced tuning width of pyramidal neurons in Fmr1−/−-MADQ mice after CNO administration (P = 0.09, unpaired one-tailed Student’s t test; n = 3 Fmr1−/−-MADQ mice before and after CNO). ***P < 0.005.

Discussion

Progress in FXS research is limited by the lack of clearly identified circuit-level alterations that can explain the neuropsychiatric phenotype that characterizes the disorder. Though circuit activity in monogenetic murine models of autism can be readily interrogated and manipulated, there is increasing interest in demonstrating both face validity and predictive validity for these translational tasks (P = 0.06). Additionally, the performance of FXS participants was lower than that of controls for all the angles measured. Thus, FXS participants and Fmr1−/− mice exhibit strikingly similar visual discrimination deficits for ambiguous stimuli of similar orientations. This suggests that a discrimination task like the one we used could eventually be used as a biologically based outcome measure of sensory processing in human clinical trials.
IncorrectRight NR

**P = 0.001 **

Fig. 6 | Fragile X patients exhibit defects in visual discrimination similar to those seen in Fmr1<sup>−/−</sup> mice. a, Photograph of a FXS subject performing the visual discrimination task. b, Timeline of an individual trial for the visual discrimination task in human subjects. “Correct” vs. “incorrect” refer to key presses (see Methods). NR, no response. c, Task performance at different angles between preferred and nonpreferred orientation stimuli for FXS subjects and age-matched control participants. Individuals with FXS were able to perform the 90° visual discrimination task with d' > 2 but exhibited a significantly lower d' than controls in the reduced-angle task (90°: P = 0.001; 45°: P = 0.01; 15°: P = 0.001), two-tailed Mann-Whitney test for FXS vs. controls at different angles; Kruskal-Wallis test for comparisons across all angles, P = 0.06; n = 8 subjects with FXS and 8h control subjects). The horizontal bars indicate mean and error bars indicate s.e.m. **P < 0.01, ***P < 0.005.

The lack of changes in spontaneous neuronal activity in V1 of Fmr1<sup>−/−</sup> mice, together with the fact that we could manipulate the gain of PV cell output to restore circuit function and rescue behavior, highlights the important notion that perhaps the basal circuit connectivity may remain 'intact' in adult Fmr1<sup>−/−</sup> mice. However, depending on the computational demands imposed by sensory environment, Fmr1<sup>−/−</sup> mice (and by inference, humans with FXS) may exhibit subtle functional alterations in cortical circuits that render them unable to efficiently process sensory information in ways that impair their ability to properly utilize this information to learn perceptual tasks. For example, one simple interpretation of our findings is that perhaps manipulating orientation tuning in FXS could improve visually evoked behaviors that are critical for playing sports, driving, or judging emotions. In conclusion, our study offers hope that simple therapeutic strategies targeting relevant but relatively subtle circuit defects may be of value in treating specific behavioral impairments in FXS.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41593-018-0231-0.

Received: 17 December 2017; Accepted: 6 August 2018; Published online: 24 September 2018
References

1. Association, A.P. Diagnostic and Statistical Manual of Mental Disorders (DSM-5), Edn. 5th. (2013).
2. Robertson, C. E. & Baron-Cohen, S. Sensory perception in autism. Nat. Rev. Neurosci. 18, 671–684 (2017).
3. Estes, A. et al. Behavioral, cognitive, and adaptive development in infants with autism spectrum disorder in the first 2 years of life. J. Neurodev. Disord. 7, 24 (2015).
4. Roff, M. et al. Incidence of Fragile X in 5,000 consecutive newborn males. Genet. Test. 7, 339–343 (2003).
5. The Dutch-Belgian Fragile X Consortium. Fmr1 knockout mice: a model to study Fragile X mental retardation. Cell 78, 23–33 (1994).
6. Bloch, Y. & Burkhalter, A. Three distinct families of GABAergic neurons in rat visual cortex. Cereb. Cortex 7, 347–358 (1997).
7. Zanin, D. A. et al. Analysis of dendritic connectivity in excitatory and inhibitory neurons in visual cortex. Nat. Neurosci. 17, 1701–1709 (2014).
8. Juczewski, K. et al. Differential sensitivity to visual stimuli in Fragile X mice. Mol. Psychopathol. 27, 360–371 (2016).
9. LeBlanc, J. J. et al. Visual evoked potentials detect cortical processing deficits in Fragile X mice. J. Neurodev. Disord. 7, 120–128 (2015).
10. Chen, T. W. et al. Ultrasensitive fluorescent proteins for imaging neuronal structure in parvalbumin-expressing inhibitory neurons in visual cortex. Neuron 98, 602–615.e8 (2018).
11. Armbruster, B. N., Li, X., Pausch, M. H., Herlitze, S. & Roth, B. L. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc. Natl Acad. Sci. USA 104, 5163–5168 (2007).
12. LaFata, G. et al. FMRP regulates multipolar to bipolar transition of the Fragile X brain. Ann. Neurol. 78, 775–786 (2015).
13. Niel, C. M. & Stryker, M. P. Modulation of visual responses by behavioral state in mouse visual cortex. Neuron 65, 472–479 (2010).
14. Pakan, J. M. et al. Behavioral-state modulation of inhibition is context-dependent and cell type specific in mouse visual cortex. elife 5, e14985 (2016).
15. Robertson, C. E. & Baron-Cohen, S. Sensory perception in autism. Nat. Rev. Neurosci. 17, 1478–1490 (2015).
16. Lee, S. H. et al. Activation of specific interneurons improves V1 feature selectivity and visual perception. Nature 488, 379–383 (2012).
17. Zhang, Y. et al. Dendritic channelopathies contribute to neocortical and sensory hyperexcitability in Fragile X mice. Neuron 98, 1471–1478 (2018).
18. Zupan, B. & Toth, M. Wild-type male offspring of Fmr1(-/-) mothers exhibit characteristics of the Fragile X phenotype. Neuropsychopharmacology 33, 2667–2673 (2008).
19. Arnett, M. T., Herman, D. H. & McGee, A. W. Deficits in tactile learning in a mouse model of autism. J. Neurodev. Disord. 78, 93–103 (2015).
20. Chen, T. W. et al. Ultrasensitive fluorescent proteins for imaging neuronal structure in parvalbumin-expressing inhibitory neurons in visual cortex. Neuron 98, 602–615.e8 (2018).

Acknowledgements

The authors thank K. Cohan, S. Cohen, and M. Hong for help with early behavioral experiments; P. Golshani and M. Einstein for advice on mouse behavior; the Janelia GENIE project (for providing GCaMP6s); F. Yu for building custom lick ports; and D. Buonomano, A. Contractor, and J. Sweeney for feedback on the manuscript. The authors also thank P. Pellionisz and A. Cheng for help assembling the two-photon microscope. K. Battista created the illustration in Fig. 1b. This work was supported by the following grants: W81XWH-17-1-0231 (USAMRMC, DOD), Developmental Disabilities Translational Research Program #20160969 (John Merck), SFARI Award 295438 (Simons Foundation), and 5R01HD054453 (NICHD/NIH) awarded to C.P.-C.; K23 MH12936 (NIMH/NIH) to E.P.; a grant from the Fragile X Alliance of Ohio to C.A.E.; and U01 DD001185 (NCBDD/NIH), U54 HD082008 (NICHD/NIH), and a grant from the Cincinnati Children’s Hospital Research Foundation to E.P. and C.A.E.

Author contributions

A.G. and C.P.-C. conceived the project and designed the experiments with help from J.G., L.M.S., E.P., and C.A.E. for the human studies. A.G. developed the behavioral experiment for mice and humans. A.G. and D.A.C. wrote the Matlab code for analysis. A.G., G.R.C., A.N., B.T., D.d.A., N.K., and J.G. conducted the experiments and analyzed the data. A.G., L.M.S., E.P., C.A.E., and C.P.-C. interpreted the data and wrote the paper with input from other authors.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41593-018-0231-0.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to C.P.-C.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Methods
Experimental animals. All experiments followed US 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. Exploratory tasks in Figs. 1 and 2 used male and female FVB.129P2 WT mice (JAX line 004828) and Fmr1−/− mice1 (JAX line 004624), and experiments in Fig. 3 used male and female PV-Cre mice (JAX line 008069) that were crossed to the A9 tdTomato reporter line (JAX line 007909), and the resulting PV-Cre A9 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 rates of Fmr1−/− pups due to the possibility of littermates with different genotypes receiving unequal attention from the dams7, we used homozygous litters.

Go/no-go visual discrimination task for head-restrained mice. Awake, head-restrained young adult mice (2–4 months) were allowed to run on an air-suspended polystyrene ball while they were trained on the visual discrimination task. Prior to performing the discrimination task the animals were subjected to handling, habituation, and pretrials (Fig. 1a). After recovery from headbar/ cranial window surgery, mice were handled gently for 5 min every day until they were comfortable with the experimenter and would willingly transfer from one hand to the other to eat sunflower seeds. This was followed by water deprivation (giving mice a rationed supply of water once per day) and habituation to the behavior apparatus. During habituation, mice were head-restrained, acclimated to the enclosed soundproof chamber, and allowed to run freely on the 8-cm polystyrene ball (Fig. 1b). Eventually, mice were introduced to the visual stimuli on the screen and the lick port (either a commercial lick port from Island Motion or custom-built at the UCLA electronics shop) that dispensed water (3–4 μL). This was repeated for 15 min per session for 2–3 d. Starting water deprivation before pretrials motivated the mice to lick8. After habituation and ~15% weight loss, mice started the pretrial phase of the training. During pretrials, sinusoidal gratings (temporal frequency of 2 Hz, spatial frequency of 0.01 cycles/degree, and 100% contrast) were displayed on a computer monitor (21-in (53.34-cm) display) for the 135° orientation (that is, ‘no-go’). Licking was recorded during the entire 3-s reward during the 45° presentation (that is, ‘go’) while withholding licking during the 45° and 135° orientations were randomly presented on the screen, but only one orientation was presented per trial (Fig. 1c). An incorrect response resulted in a time-out period of 6.5 s, during which the internal cannula projected further to ~0.6 mm. During the go/no-go task. Once the mice reached >2, 8 μL muscimol solution (1 mg/mL in water; Sigma–Aldrich) was infused bilaterally in isoflurane anesthetized mice through a 28-gauge internal (C313IS, Plastics One) and 22-gauge guide (C313GS, Plastics One) cannula. After full recovery from anesthesia (<15 min), mice were immediately retested on the visual discrimination task. Two mice were also retested the following day to evaluate task performance after washout of muscimol.

Viral constructs. AAV1.Syn.GCaMP6s.WPRE.SV40 and AAV1.Syn Flex.GCaMP6s.WPRE.SV40 were purchased from the University of Pennsylvania Vector Core and diluted to a working titer of 2 × 1013 or 2 × 1012 (to enable a longer period of optimal expression) with 1% filtered Fast Green FCF dye (Fisher Scientific). GCaMP6s was chosen over GCaMP6f because it detects more active neurons and has an improved signal-to-noise ratio for more reliable detection of single action potentials during spontaneous activity9. For DREADD experiments, pAAV.Syn.DIO.hM3D(Gq) (Qn) was purchased from Addgene and diluted to a working titer of 2 × 1013 with 1% Fast Green FCF dye.

Cranial window surgery. Experiments were started with craniotomies performed at 6–8 weeks on the four different mouse lines mentioned above. Mice were anesthetized with isoflurane (5% induction, 1.5–2% maintenance via a nose cone) and placed in a stereotaxic frame. A 4.5-mm diameter craniotomy was performed over the right primary visual cortex (V1) and covered with a 5-mm glass coverslip, as previously described10,11. Before securing the cranial window with a coverslip, we injected ~50 nL of AAV1.Syn.GCaMP6s.WPRE.SV40 (Fig. 3a, in vivo calcium imaging of L2/3 pyramidal neurons), or a mixture of AAV1.Syn.GCaMP6s.WPRE.SV40 and AAV1.Syn Flex.GCaMP6s.WPRE.SV40 (Fig. 4, in vivo calcium imaging in V1) or a cocktail of AAV1.Syn Flex.GCaMP6s.WPRE.SV40 and AAV1.Syn.hDIO.hM3D(Gq) (Qn) (Fig. 5, to activate PV cells with DREADD). A custom-made U-shaped aluminum bar was attached to the skull with dental cement to head-restrain the animal during behavior and calcium imaging.

Optical intrinsic signal (OIS) imaging. Two weeks after cranial window surgery, OIS imaging was used to map the location of V1. Visual stimulation was provided by a piezo-actuator (Physik Instrumente) that deflected light from a red light-emitting diode in front of the contralateral eye. Responses for 30 stimulation trials were averaged; each consisted of 100 Hz deflections for 1.5 s. The response signal divided by the averaged baseline signal, summed for all trials, was used to generate the cortical map.

In vivo two-photon calcium imaging. Calcium imaging was performed on a custom-built two-photon microscope, with a Chameleon Ultra II Ti:sapphire laser (Coherent), resonant scanning mirrors (Cambridge Technologies), a 25× objective (1.05 NA, Olympus), multikaiki polymultiplexer tubes (R3896, Hamamatsu), and...
and ScanImage software\(^\text{\textregistered}\). Prior to calcium imaging, head-restrained mice were habituated to a soundproof chamber and allowed to run freely on a polystyrene ball (Fig. 1b). Eventually, mice were introduced to the visual stimuli on the screen. This habituation phase lasted 3–4 sessions, and there was no difference between genotypes in the time of habituation. Visual stimuli were generated using custom-written Matlab (MathWorks) routines, using Psychtoolbox, that consisted of full-field square-wave drifting gratings (2 cycles/s, 0.005 spatial frequency, 32 random repeats of 8 orientations) presented for 3 s and separated by a 3-s-long gray screen. Both spontaneous and visually evoked responses of L2/3 pyramidal cells from V1 were recorded at 15 Hz in 2–4 fields of view. Each FOV consisted of a median of 63 pyramidal cells (range: WT=54–81; Fmr1\(^{-/-}\)=57–79) or 8 PV cells (range: WT=3–10; Fmr1\(^{-/-}\)=3–8). In each animal, imaging was performed at 2 or 3 depths (150–250 μm), and data was averaged from movies collected across all FOVs.

### Data analysis for calcium imaging

Calcium imaging data were analyzed using custom-written Matlab routines, which included modifications of our previously described Matlab code\(^\text{\textregistered}\). This code is available from the corresponding author upon request. X–Y drift in the videos was corrected using an iterative, cross-correlation-based, nonrigid alignment algorithm\(^\text{\textregistered}\). A semiautomated algorithm\(^\text{\textregistered}\) was used to select regions of interest (ROIs), each representing a single cell body, and to extract the fluorescence signal (F) for each ROI. A modified z-score, \(Z_f(t)\), vector for each neuron was calculated as:

\[
Z_f(t) = \frac{F(t) - \text{mean}(\text{baseline})}{\text{std}(\text{baseline})}
\]

where the baseline period is the 10-s period with the lowest variation (s.d.) in ΔF/ΔF. All subsequent analyses were performed using the \(Z_f(t)\) vectors.

Neuronal subtraction was performed by removing the local fluorescence signal surrounding each ROI\(^\text{\textregistered}\). Peaks of activity were then detected in the z-scores using the PeakFinder Matlab script. These peaks were used to calculate the mean z-score fluorescence (an estimate of amplitude of the fluorescence signal) and the frequency of events. To remove any bias resulting from peak detection, especially in PV cells, we also calculated the frequency of events based on the magnitude of the fluorescence signal (area under the curve). For this analysis, we calculated the area under the curve for each fluorescence trace and divided that by the number of frames during which a stimulus is presented (that is, 15 frames or 3 s). This was then multiplied by the frame rate to get a z-score of fluorescence per s.

Orientation selective cells were defined by an orientation selectivity index (OSI) calculated as

\[
\text{OSI} = \frac{Z_{pref} - Z_{orth}}{Z_{pref} + Z_{orth}}
\]

where \(Z_{pref}\) is the mean response to the orientation orthogonal to the preferred one (\(Z_{orth}\)). A cell was considered orientation-selective if it had OSI ≥ 0.5.

To quantify visually evoked activity, we averaged the responses of neurons during the 3 s of drifting gratings stimulation and the 3 s of gray screen that followed the visual stimulus. To quantify spontaneous activity, we conducted separate recordings during which the animals were presented a static gray screen. To determine whether an individual cell showed a time-locked or stimulus-selective response to a visual stimulus in Fig. 3 and Supplementary Fig. 9c (which examines the correlation between the stimulus and the fluorescence signal in PV cells), we used a probabilistic bootstrapping method as described previously\(^\text{\textregistered}\). First, we calculated the correlation between the stimulus time-course and the \(Z_v\) vector, followed by correlation calculations between the stimulus time-course and 1,000 scrambles of all calcium activity epochs in \(Z_v\) (epoch = consecutive frames wherein \(Z_2 ≥ 3\)). The 1,000 comparisons generated a distribution of correlations (r values), within which the correlation of the unscrambled data and the stimulus fell at any given percentile. If the calculated percentile for a cell was less than 1%, then we described that cell as being stimulus selective. Correlations in Figs. 3g and 5j and in Supplementary Fig. 7 were calculated using a Pearson’s correlation.

Our tuning width calculation procedures are similar to what has been described before\(^\text{\textregistered}\). First, we generate a tuning curve of the mean z-score values of the responses for each orientation presented. The orientation with the largest mean response is considered the preferred orientation. A Gaussian is then fitted to the tuning curve, and the tuning width is determined to be the width at half the maximum value of the fitted Gaussian. The equation of the Gaussian we use is the one used in Akerboom et al.\(^\text{\textregistered}\).

\[
\sigma = \sqrt{2 \ln 2} \cdot Z_{50}
\]

We included only neurons that elicited at least one calcium transient during the duration of the recording; neurons were excluded if they were deemed inactive on the basis of calcium imaging data (pyramidal neurons excluded: WT=0.1%; Fmr1\(^{-/-}\)=0.1%; PV neurons excluded: WT=0.1%; Fmr1\(^{-/-}\)=0.1%).

### Transcardial perfusion and DiI histology

Mice were deeply anesthetized with isoflurane and underwent transcardial perfusion with 4% paraformaldehyde solution in sodium phosphate buffer (composition: 30 mM NaH2PO4, 120 mM Na2HPO4, pH 7.4). Brains were kept overnight at 4°C first in 4% paraformaldehyde solution (pH 7.4) and then stored in 30% sucrose (in phosphate buffered saline) at 4°C until sectioned.

### Human subjects

Eight male with FXS and eight male healthy controls, matched in chronological age, completed the visual discrimination experiment (Supplementary Table 1). 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 (US4). Approval of this study was granted through the Institutional Review Board at Cincinnati Children’s Hospital Medical Center. All participants > 18 years of age provided written consent, and minors provided assent plus written consent from their legal guardians. Additional consent was obtained to use the de-identified photograph of an FXS participant performing the visual discrimination experiment. All FXS participants with ASD were confirmed by genetic testing. No participants had a history of nonfebrile seizures or treatment with an anticonvulsant medication (Supplementary Table 2). FXS participants completed the Abbreviated Battery of the Stanford-Binet Intelligence Scales-Fifth Edition (SB-5). 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 (for example, schizophrenia) in themselves or in first-degree relatives, or for a family history of ASD in first- or second-degree relatives based on a brief screening interview. All study procedures were approved by the local Institutional Review Board.

Human FXS and control participants completed a visual discrimination task that was analogous to the one used with mice, with relatively minor modifications. Due to the additional cognitive demands of a go/no-go experiment, including inhibitory control, which is known to be impaired in FXS\(^\text{\textregistered}\), we designed a forced-choice two-choice visual discrimination task, so that all FXS participants could learn and perform the task in a single day. The participants were instructed that they could have learned the no-go task with subsequent training sessions, just as the mice required consecutive sessions to learn; however, time constraints and burden on the participant limited our ability to do so. Visual gratings were displayed on a 13-inch (33.02-cm) Hewlett Packard laptop computer with a 15-inch (38.1-cm) liquid crystal display and made available by designated keys on the laptop keyboard. During the task, when the visual gratings appeared to move from right to left side, subjects were instructed to press the corresponding left-side key (‘Z’ or ‘A’), and when the visual gratings appeared to move from left to right, subjects were instructed to press the corresponding right-side key (‘L’ or ‘M’). If participants correctly responded to the direction of the stimulus, they received positive visual feedback (for example, images of popular video game cartoon characters were displayed on the computer screen). If participants incorrectly responded to the direction of the stimulus, they received negative visual feedback (for example, a large red ‘X’ was displayed). Visual gratings appeared on screen for 4 s, during which participants could respond. Once the participant responded or at the end of 4 s, feedback was presented for 1 s. The following trial would begin 3 s later. All participants completed the first-order visual task, followed immediately by the second-order visual task. For each of the tasks, visual gratings appeared in four blocks of 30 trials, each block consisted of one condition: 180°/0°, 45°/90°, 67.5°/45°, or 82.5°/15°. The order of the blocks was presented randomly, but participants always received first-order blocks before second-order. Prior to administration of the task, participants completed two practice blocks. During the first practice block, a smiley face emoji moved from left to right on the screen (or right to left), and participants were instructed to press the corresponding key based on the direction the smiley faced moved. In the second block, visual gratings at 50°/80° angles were presented, and participants pressed the key corresponding to direction of movement. Twelve trials of each practice block were administered. If participants did not reach ≥50% correct trials, the block was repeated one time for a total of 24 trials per block. All participants met practice criterion. Depending on the stimulus presented, the subject’s behavioral response was characterized as ‘Right’ (similar to Hit), ‘NR’ (no response) or ‘Wrong’ (similar to FA).

Since this was a two-choice forced visual discrimination task, a modified d’ (discriminability index) was calculated as follows:

\[
d' = \text{norminv}(\text{fraction of Right}) - \text{norminv}(\text{fraction of Wrong})
\]

### Statistical analyses

Statistical analysis of normality (Lilliefors and Shapiro–Wilk tests) were performed on each dataset and, depending on whether the data significantly deviated from normal (\(P < 0.05\)) or a deviation from normality (\(P > 0.05\)), appropriate nonparametric or parametric tests were performed. The statistical tests performed are mentioned in the text and the legends. For parametric two-group analyses, a Student’s t-test (paired or unpaired) was used. For nonparametric tests, we used the following: Mann–Whitney test (two groups), the Kruskal–Wallis test (three or more groups), and Friedman test (repeated measures). In the figures, significance levels are represented with the following convention: *\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.005\). In all figures, we plot the s.e.m.

Graphs show either individual data points from each animal or group means (averaged over different mice) superimposed on individual data points.
Sample size. We did not use statistical methods to predetermine sample sizes. For all main results shown in Figs. 1–3, we used sample sizes of ≥ 10 mice, and subsequent statistics were performed using the number of mice as the sample size. Working with Fmr1−/− mice can be technically challenging for several reasons: (i) Fmr1−/− mothers have a higher incidence of cannibalization, (ii) Fmr1−/− mice can require extra handling 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. Hence, to maintain the feasibility of experiments and ethical use of numbers of animals for most of our experiments, we used at least 10 mice per group.

In Figs. 4 and 5 we used triple transgenic mice: PV-Cre mice were crossed with Ai9 mice and these were then back crossed to FVB WT and Fmr1−/− mice for eight 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.

The sample size we used is consistent with those in previously published studies9–10. For the inactivation of visual cortex experiments (Fig. 1c), the sample size was in agreement with previous studies42–43. For Fig. 6 we used n = 8 humans for each group. Recruiting age- and gender-matched patients with Fragile X syndrome is challenging, but a sample of 8 per group is comparable to the number of human subjects used in previously published studies44–45.

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, subjects with Fragile X syndrome were randomized with control subjects.

Blinding. G.C., A.L., B.T., and D.A. were blinded to the genotype in 60% of mice used in all the experiments during visual discrimination training.

Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary linked to this article.

Code availability. All code used in this manuscript is available from the corresponding author upon reasonable request.

Data availability
All the analyzed data reported in this study is available from the corresponding author upon reasonable request.

References
33. Guo, Z.-Y. et al. Flow of cortical activity underlying a tactile decision in mice. Neuron 81, 179–194 (2014).
34. Mostany, R. & Portera-Cailliau, C. A craniotomy surgery procedure for chronic brain imaging. J. Vis. Exp. https://doi.org/10.3791/681 (2008).
35. Golshani, P. & Portera-Cailliau, C. In vivo 2-photon calcium imaging in layer 2/3 of mice. J. Vis. Exp. https://doi.org/10.3791/681 (2008).
36. Pologruto, T. A., Sabatini, B. L. & Svoboda, K. ScanImage: flexible software for operating laser scanning microscopes. Biomed. Eng. Online 2, 13 (2003).
37. Mineault, P. J., Tring, E., Trachtenberg, J. T. & Ringach, D. L. Enhanced spatial resolution during locomotion and heightened attention in mouse primary visual cortex. J. Neurosci. 36, 6382–6392 (2016).
38. Cottam, J. C., Smith, S. L. & Häusser, M. Target-specific effects of somatostatin-expressing interneurons on neocortical visual processing. J. Neurosci. 33, 19567–19578 (2013).
39. Akerboom, J. et al. Optimization of a GCaMP calcium indicator for neural activity imaging. J. Neurosci. 32, 13819–13840 (2012).
40. Hooper, S. R. et al. Executive functions in young males with Fragile X syndrome in comparison to mental age-matched controls: baseline findings from a longitudinal study. Neuropsychology 22, 36–47 (2008).
41. Hamm, J. P., Peterka, D. S., Gogos, J. A. & Yuste, R. Altered cortical ensembles in mouse models of schizophrenia. Neuron 94, 153–167.e8 (2017).
42. Pinto, L. & Dan, Y. Cell-type-specific activity in prefrontal cortex during goal-directed behavior. Neuron 87, 437–450 (2015).
43. Harvey, C. D., Coen, P. & Tank, D. W. Choice-specific sequences in parietal cortex during a virtual-navigation decision task. Nature 484, 62–68 (2012).
44. Kogan, C. S. et al. Integrative cortical dysfunction and pervasive motion perception deficit in Fragile X syndrome. Neurology 63, 1634–1639 (2004).
45. Plaisted, K., O’Riordan, M. & Baron-Cohen, S. Enhanced visual search for a conjunctive target in autism: a research note. J. Child Psychol. Psychiatry 39, 777–783 (1998).
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

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)

Software and code

Policy information about availability of 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

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

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 Figures 1-3 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 A09 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, 2 and 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 processing. Additionally, to improve the survival of Fmr1-/- pups due to the possibility of littermates with different genotypes receiving unequal attention from the dam 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. |