1 **Beyond excitation/inhibition imbalance in multidimensional models of neural circuit**

2 **changes in brain disorders**

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13 **Abstract**

14 A leading theory holds that neurodevelopmental brain disorders arise from imbalances in

15 excitatory and inhibitory (E/I) brain circuitry. However, it is unclear whether this one-

16 dimensional model is rich enough to capture the multiple neural circuit alterations

17 underlying brain disorders. Here we combined computational simulations with analysis of *in*

18 *vivo* 2-photon Ca^{2+} imaging data from somatosensory cortex of *Fmr1* knock-out (KO) mice, a

19 model of Fragile-X Syndrome, to test the E/I imbalance theory. We found that: 1) The E/I

20 imbalance model cannot account for joint alterations in the observed neural firing rates and

21 correlations; 2) Neural circuit function is vastly more sensitive to changes in some cellular

22 components over others; 3) The direction of circuit alterations in *Fmr1* KO mice changes

23 across development. These findings suggest that the basic E/I imbalance model should be

24 updated to higher-dimensional models that can better capture the multidimensional

25 computational functions of neural circuits.

Introduction

The nervous system shows complex organization at many spatial scales: from genes and molecules, to cells and synapses, to neural circuits. Ultimately, the electrical and chemical signaling at all of these levels must give rise to the behavioral and cognitive processes seen at the whole-organism level. When trying to understand prevalent brain disorders such as autism and schizophrenia, a natural question to ask is: where is the most productive level of neuroscientific investigation? Traditionally, most major disorders are diagnosed entirely at the behavioral level, whereas pharmaceutical interventions are targeted at correcting alterations at the molecular level. However even for the most successful drugs, we have little understanding of how pharmaceutical actions at the molecular level percolate up the organizational ladder to affect behavior and cognition. This classic bottom-up approach may even be further confounded if phenotypic heterogeneity in disorders such as autism turn out not to reflect a unique cellular pathology, but rather "a perturbation of the network properties that emerge when neurons interact" (Belmonte et al., 2004). These considerations imply that a more promising level of analysis might be at the level of neural circuits, since the explanatory gap between circuits and behavior is smaller than the gap between molecules and behavior. This circuit-level viewpoint argues for a reverse-engineering approach to tackling brain disorders: rather than start at the molecular level and working up, we should instead start by asking how cognitive and behavioral symptoms manifest as alterations at the circuit level, then interpret these changes at the levels of cells, synapses, and molecules as appropriate.

One prominent circuit-level hypothesis for brain disorders has been the idea of an imbalance in excitatory and inhibitory signaling. First proposed as a model for autism (Rubenstein and Merzenich, 2003), the concept has since been applied to many other brain disorders, including Schizophrenia, Rett syndrome, fragile-X syndrome, tuberous sclerosis, and Angelman Syndrome. However, a major drawback of this model is that it only considers overall activity, which is 1-dimensional. It implies that either too much excitation or too much inhibition is unhealthy (Figure 1A). Although several studies have found evidence that the E/I balance is indeed upset in multiple brain disorders (Bateup et al., 2011; Dani et al., 2005; Gibson et al., 2008; Kehrer et al., 2008; Wallace et al., 2012), a model's usefulness should not be judged on

55 whether it is nominally true or false, but on its explanatory and predictive powers as compared

56 with competing alternative models. In this study we argue that that even if the E/I imbalance

57 model proves correct, its unidimensionality might ultimately limit its applicability, for three

58 reasons.

59 First, by placing all disorders on the same single axis, the E/I imbalance model implicitly lumps

60 together some vastly different disorders, such as epilepsy, schizophrenia and autism (Figure

61 1A) because they share an excess of excitation. By extension it implies that the symptoms of

62 diverse disorders could be normalized solely by either enhancing or reducing the level of, say,

63 GABAergic signaling as appropriate. Although clinical trials for such GABAergic-based

64 interventions are ongoing (Braat and Kooy, 2015), no treatment for a neurodevelopmental

65 disorder based on this principle has yet been approved.

66 A second issue with the unidimensionality of the E/I imbalance model is that it lumps together

67 all excitatory and inhibitory neural circuit components. In Figure 1B we show a schematic

68 diagram of a generic neural circuit with excitatory components colored red and inhibitory

69 components colored blue. The E/I imbalance model implies that varying any of the excitatory

70 components, such as the strength of recurrent excitatory synapses or the input resistances of

71 excitatory neurons, would have the same overall effect on circuit function. In contrast, theorists

72 have found that these equivalences often do not hold even in very simple circuit models

73 (Wilson and Cowan, 1972).

74 Third, because the standard E/I imbalance model is given in terms of circuit components, not

75 circuit function, it does not specify which aspect of a neural circuit's activity should be

76 maintained for healthy performance. For example, it leaves unclear which of neuronal firing

77 rates, synchrony, or reliability of responses might be altered if E/I balance is upset.

78 To motivate our study, we began by investigating which circuit activity properties are altered in

79 a model brain disorder. We re-analyzed published *in vivo* 2-photon Ca^{2+} imaging data we

80 previously recorded from somatosensory cortex in *Fmr1* knockout mice (Gonçalves et al.,

81 2013), a well-studied animal model for fragile-X syndrome (The Dutch-Belgian Fragile X

82 Consortium, 1994). We compared the data from wild-type (WT) mice with *Fmr1* KO mice,

83   across three different developmental time points: just before (P9—11) and after (P14—16) the

84   critical period for heightened activity-dependent synaptic plasticity in L2/3 barrel cortex, and a

85   more mature timepoint (P30—40). Example ΔF/F raster plots from each group are shown in

86   Figure 1C, top left. We binned the data into 1 s timebins (originally imaged at 4 Hz), then

87   transformed each neuron's timeseries of ΔF/F values into a probabilistic sequence of binary

88   ON/OFF values by assuming a Poisson firing model (Methods). We then summarized the

89   neural population activity from each animal with three statistics: the mean ON probability

90   across all recorded neurons, the standard deviation (s.d.) in ON probability across neurons,

91   and the mean correlation between all pairs of neurons (Figure 1C, bar charts right and scatter

92   plots lower left). Together these measures capture both the statistics of the bulk population

93   activity and some indication of the heterogeneity across neurons.
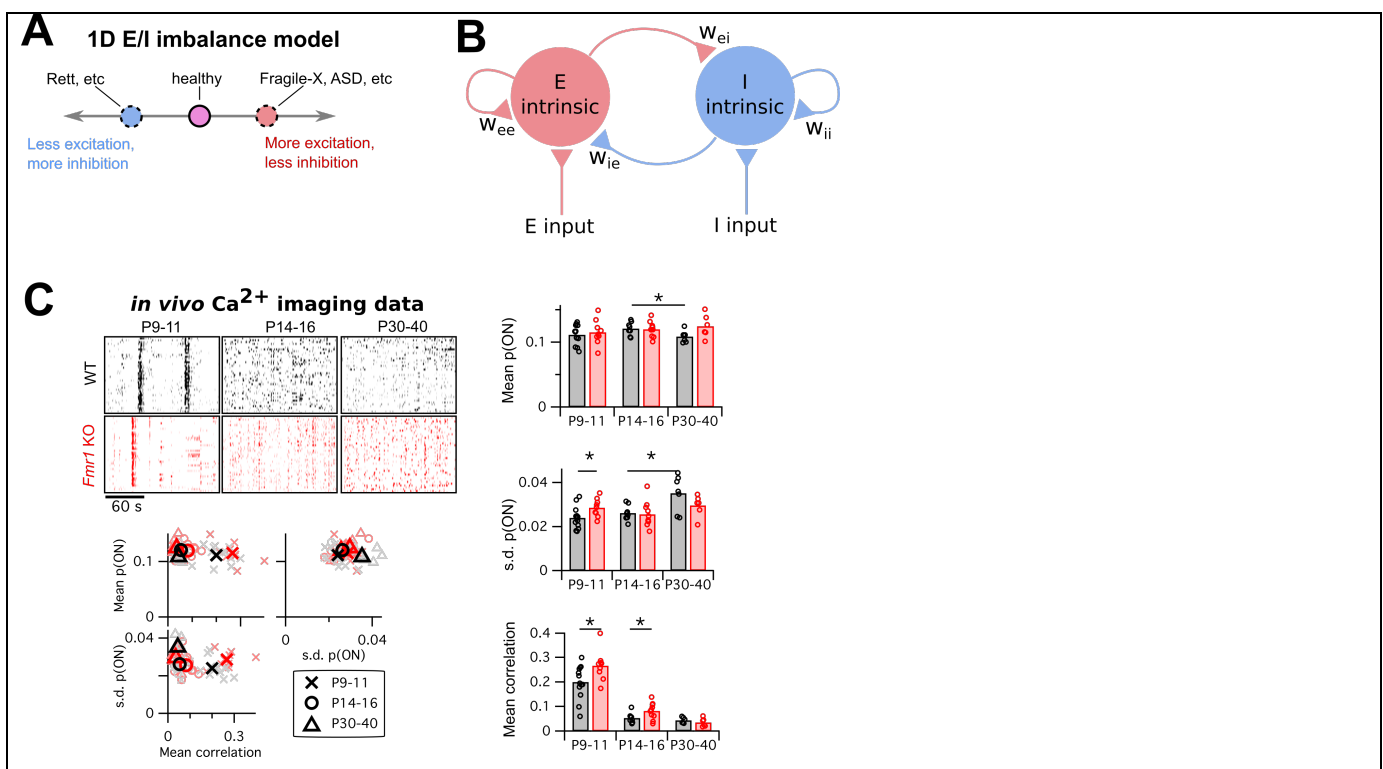
Figure 1. Mismatch between the E/I imbalance model's unidimesionality and the multiple changes in circuit activity in Fragile-X mouse models.

A: Schematic of standard E/I imbalance model as a unidimensional axis.

B: Diagram of a generic neural circuit, showing an excitatory and an inhibitory population of neurons and their interconnections. Although the E/I imbalance model implicitly groups all

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components as either excitatory (red) or inhibitory (blue), in principle any component could separately be altered in brain disorders, and may have a distinct effect on circuit function. **C:** Upper left, example Ca^{2+} imaging dF/F raster plots from a single animal from each of two genotypes, WT and *Fmr1* KO, and three age groups, P9—11, P14—16 and P30—40. In each case 3 minutes of data are shown from 40 neurons. Right and lower left, mean firing probability, standard deviation of firing probabilities, and mean pairwise correlation across all neurons. Same data in scatter plots lower left and bar charts right. * indicates significant difference in group means at p<0.05, by bootstrapping.

94   For mean firing rates, the only change we detected was a decrease in firing probability in WT
95   between P14—16 and P30—40 (p = 0.027), which was coupled with an increased s.d. of firing
96   rates (p = 0.015). We also detected a higher firing rate s.d. in P9—11 KO animals than WT (p =
97   0.031). Finally, as previously reported (Golshani et al., 2009; Gonçalves et al., 2013; Rochefort
98   et al., 2009), we found a substantial decrease in pairwise correlations in both genotypes
99   across development, with slightly higher correlations in KO animals than WT at P9—11 (p =
100   0.029) and P14—16 (p = 0.047).

101   These results show that multiple statistics of cortical circuit activity are altered in *Fmr1* KO
102   mice. However, two questions remain: 1) Which circuit components are responsible for these
103   activity alterations? 2) What is the consequence for circuit computation? In the remainder of
104   this study, we used computational simulations and further data analysis to ask whether the E/I
105   imbalance model could help address these questions.

106   We first built a detailed spiking neural circuit model of mouse L2/3 somatosensory cortex to
107   explore how its various excitatory and inhibitory components affect the circuit's spiking output,
108   and found that the E/I imbalance model was not flexible enough to capture key aspects of this
109   relationship. We then derived an abstract 2-dimensional circuit model that captures more
110   features of the circuit function than the 1-dimensional E/I imbalance model did. Using this new
111   model, we found that certain sets of circuit components have redundant effects on circuit
112   function, and that circuit function is vastly more sensitive to changes in some components over
113   others. To ask how this 2-D model could help interpret brain circuit abnormalities in a particular
114   test case, we fit a version of the model to the Ca^{2+} imaging data from Fragile-X mouse models

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115   presented above. We found that the model predicts opposite changes in *Fmr1* KO circuit

116   properties at different developmental ages. Finally, we applied a new large-scale neural

117   population analysis method (O'Donnell et al., 2017) to the same Ca^{2+} imaging data, and found

118   systematic shifts in the distribution of neural circuit activity patterns in Fragile-X that were not

119   predictable from neural firing rates or correlations alone.

Results

121 Neural circuits consist of many components that typically interact non-linearly to generate
122 complex circuit activity dynamics. Although many properties of cortical circuit components
123 have been found to be altered in animal models of brain disorders, it remains extremely difficult
124 to predict the net effect of varying any one particular parameter on circuit activity. The E/I
125 imbalance model seeks to simplify this problem by projecting all circuit alterations onto a one-
126 dimensional axis (Figure 1A) where the goal is to achieve a 'healthy' balance of excitation and
127 inhibition. Under this model, either too much excitation or too much inhibition leads to improper
128 circuit function.

129 To explicitly test whether the E/I imbalance model can account for the effects of cellular
130 component changes on circuit function, we built a detailed computational model of layer (L) 2/3
131 mouse somatosensory cortex. This circuit has been studied in detail by neurophysiologists,
132 and several of its properties have been found to be altered during development in mouse
133 models of Fragile-X syndrome, including parvalbumin-positive interneuron number (Selby et al.,
134 2007), GABA receptor reversal potential (He et al., 2014), dendritic spine dynamics (Cruz-
135 Martin et al., 2010), and L4 excitatory input (Bureau et al., 2008), reviewed by Contractor et al.
136 (2015). We used numerical computer simulations to perform hypothetical experiments where
137 we perturbed various parameters of the circuit model and observed the resulting changes in
138 circuit-level activity. Although we focused on this particular brain circuit for tractability, our
139 general conclusions and methodology should be readily applicable to other brain circuits (Frye
140 and Maclean, 2016).

141 The L2/3 computational model we built (Figure 2A, see Methods for details) consisted of four
142 interconnected populations of leaky integrate-and-fire neurons: one group of 1700 excitatory
143 (E) pyramidal neurons and three groups of inhibitory neurons: 115 $5HT_{3A}R$-expressing neurons,
144 70 parvalbumin-expressing (PV) neurons, and 45 somatostatin-expressing (SOM) neurons.
145 This L2/3 circuit was driven by a separate population of 1500 L4 excitatory neurons. Cellular
146 numbers, intrinsic properties, synaptic strengths, and connectivity statistics were taken from
147 published *in vitro* data from P17—22 wild-type mice (Avermann et al., 2012; Lefort et al., 2009;

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148 Tomm et al., 2014). We chose this level of detail for the model in order to relate experimentally

149 measureable biophysical properties of neurons to their putative role in the circuit at large.

150 L2/3 neurons of the rodent somatosensory neocortex respond only sparsely to sensory

151 stimulation *in vivo*. For example, twitching a whisker activates, on average, only ~20% of L2/3

152 neurons in its corresponding barrel, each of which typically emits only one action potential

153 (Clancy et al., 2015; Kerr et al., 2007; Sato et al., 2007). Hence any individual neuron carries

154 very little information about the stimulus on its own, implying that information must instead be

155 encoded at the circuit level as the identities of the subset of neurons that respond.
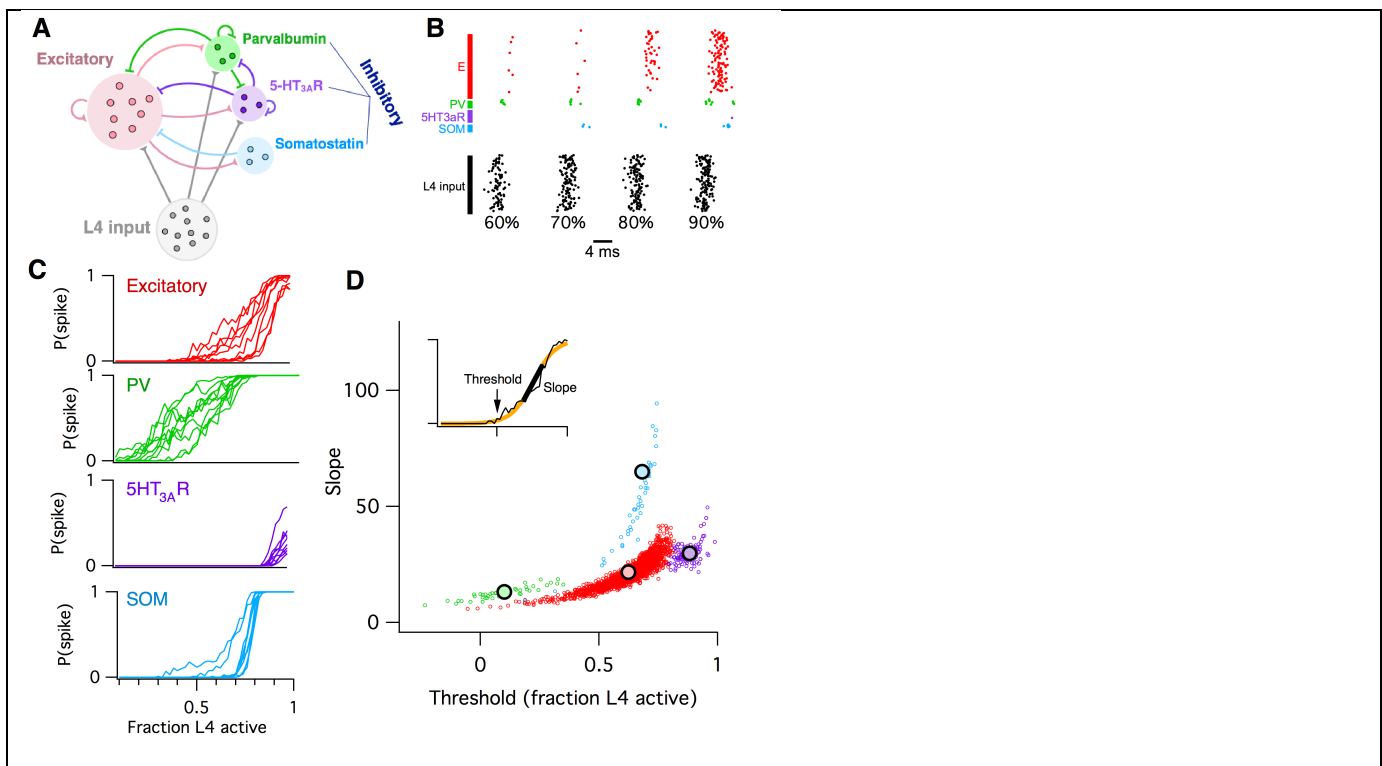
Figure 2. Computational model of L2/3 mouse somatosensory cortex.

A: Schematic diagram of computational circuit model.

B: Example raster plots of spiking responses from a subset of neurons from each cell type (colors as in panel A), for varying fractions of L4 activated (black).

C: Probability of spiking as a function of the fraction of L4 neurons activated. Each curve represents the response probability of a single neuron, averaged over multiple trials and multiple permutations of active L4 cells.

D: Each circle plots the fitted logistic slope and threshold values for a single neuron in the simulation. Circle color indicates cell type: red is excitatory, green is PV inhibitory, purple is 5HT$_{3A}$R inhibitory, blue is SOM inhibitory. Large black circles indicate mean for each cell-type. Inset shows an example fitted logistic response function (orange) to the noisy simulation results from a single excitatory neuron (black).

156   To model whisker stimulation, we simulated a volley of spikes arriving from L4 as input to the
157   population of L2/3 cells. We chose a random subset of L4 neurons as ON, then sent a single
158   spike from each of these L4 cells to their target neurons in L2/3, and recorded the responses
159   of all neurons in L2/3, some of which spiked and some of which did not. We repeated this
160   identical stimulation multiple times, in order to get an average response probability for each
161   L2/3 neuron, given the probabilistic vesicle release at synapses in the model. Then we chose a
162   different random subset of L4 neurons as ON, and repeated the entire procedure. Finally, we
163   varied the fraction of L4 cells active and plotted the probability of response for each individual
164   L2/3 neuron as a function of L4 activity level (Figure 2B—C).

165   We found that the mean response probability of each neuron in the simulation increased from
166   zero to one monotonically with increasing L4 activity level. This sigmoidal-shaped response
167   profile of simulated L2/3 neurons mimics the spiking response of mouse L2/3 pyramidal cells to
168   extracellular L4 stimulation *in vitro* (Elstrott et al., 2014), while the sparse, noisy and distributed
169   network responses were reminiscent of *in vivo* activity following whisker stimulation (Clancy et
170   al., 2015; Kerr et al., 2007). Neurons of all four cell types in the simulation responded to the L4
171   stimulus, including the SOM interneurons which did not receive direct L4 input, but were
172   instead activated by disynaptic connections via L2/3 excitatory neurons. The detailed shape of
173   the response curve varied systematically across cell types, and was heterogeneous for
174   different neurons of a given cell type. To quantify these differences, we used logistic
175   regression to fit the response profile of each neuron with a sigmoid function (Figure 2D, inset),
176   which has just two parameters: the slope (representing the steepness of the response curve)
177   and threshold (representing the minimal fraction of L4 neurons needed to activate the cell).
178   When we plotted the fitted slope and threshold values for each neuron against each other, we
179   found that each cell type falls into a distinct cluster in this 2-dimensional space. For example,

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180 all PV inhibitory neurons had a low slope and low threshold, whereas SOM inhibitory neurons
181 had a steep slope and moderate threshold. We then used these slope-threshold
182 measurements to summarize the circuit-level input-output function of this 'default' model of
183 L2/3 somatosensory cortex. This 2-D logistic model has two benefits over the 1-D E/I
184 imbalance model: first, its extra degree of freedom allows for richer and more flexible fits to
185 data, and second, by describing an input-output mapping for the L2/3 circuit it can capture
186 some aspects of the computation that the circuit performs for the animal. In contrast, the E/I
187 imbalance model is specified purely in terms of circuit components, and so is agnostic to the
188 circuit's computational function.

189 The biophysical circuit model contained 100 parameters (Methods). How sensitive is the
190 circuit's macroscopic input-output function to alterations in its low-level components? To test
191 this, we varied 76 of the model parameters in turn by ±20%, and repeated the entire set of
192 simulations for each case (Methods). For each individual parameter alteration, we fit a new
193 logistic response function for each model neuron. We summarize the results by plotting the
194 logistic slope and threshold parameters and comparing their values to those found with the
195 default model. The outcomes were hugely varied. We show three examples from the set of
196 seventy-six in Figure 3A, chosen to illustrate three qualitatively different effects that neural
197 parameter changes can have on circuit function. First, when we increased the amplitude of
198 postsynaptic potentials (PSPs) of excitatory synapses from L4 to L2/3 excitatory neurons, we
199 found that the logistic threshold parameter of all cell types shifted leftwards to lower values
200 (Figure 3A left), implying that fewer L4 neurons were needed to activate the entire L2/3 circuit.
201 In contrast, when we increased the PSP amplitude of a different excitatory synapse, the
202 recurrent connections between L2/3 excitatory neurons, we found (Figure 3A center) that
203 excitatory and SOM inhibitory neurons had increased slope parameters relative to default, with
204 little change in their threshold parameters. $5HT_{3A}R$ inhibitory neurons had decreased slopes
205 and thresholds, while PV neurons had little change at all. As a third example we increased the
206 probability of inhibitory synaptic connections from L2/3 PV interneurons to L2/3 excitatory
207 neurons (Figure 3A right). In this case we found that excitatory neurons had a lower slope and
208 increased threshold, SOM inhibitory neurons had a lower slope, $5HT_{3A}R$ inhibitory neurons had

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209   both an increased slope and threshold, and PV neurons showed little change, even though

210   their outgoing synapses were the parameter that was altered.
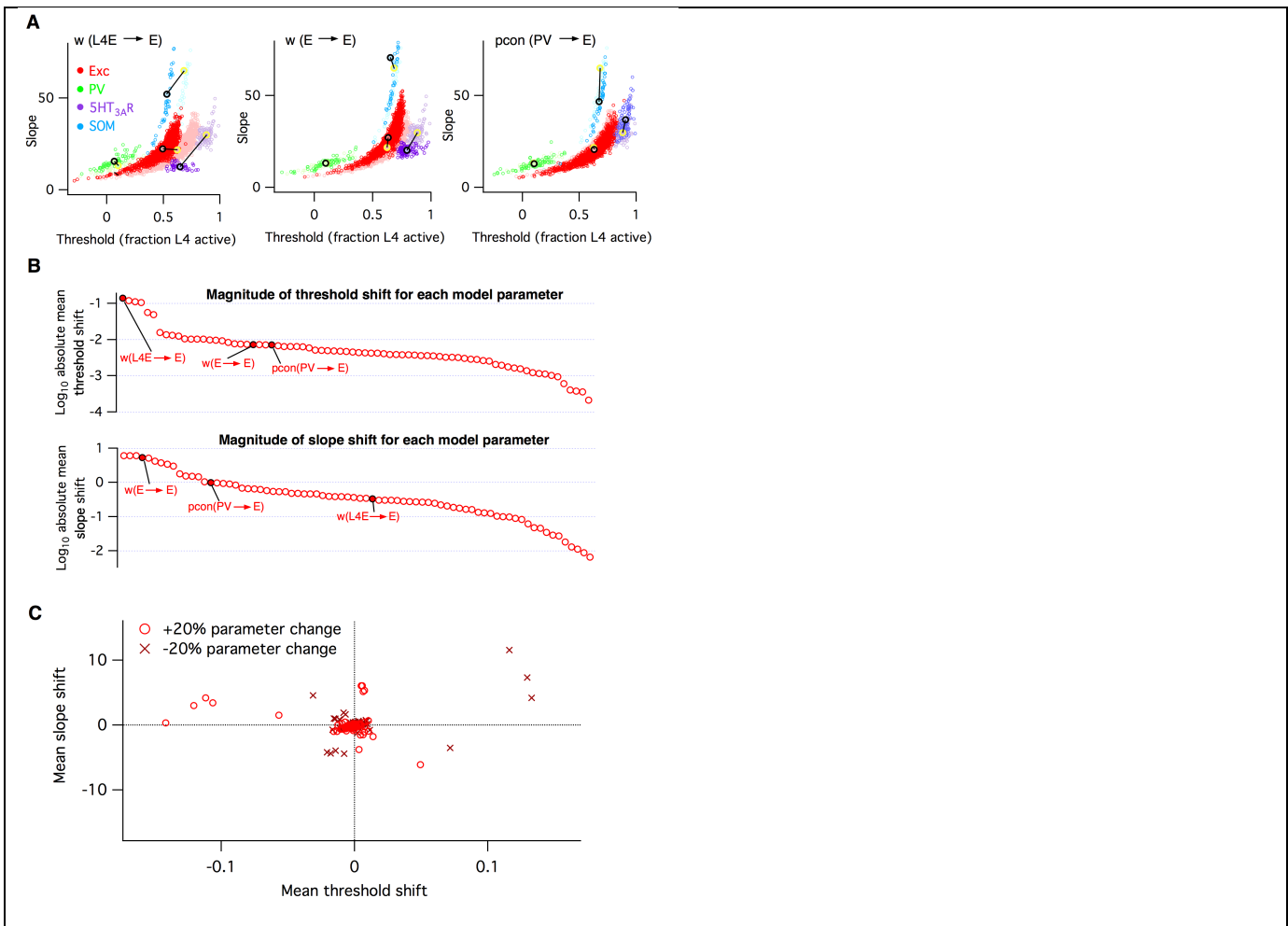
Figure 3. Heterogeneous effects of varying L2/3 parameters on the circuit input-output function.

A: Shifts in the distribution of fitted slope and threshold parameters as a result of increasing the strength of synapses from L4 to L2/3 E neurons (left), increasing the strength of recurrent synapses between L2/3 E neurons (center), or increasing the connection probability between L2/3 PV interneurons and E neurons (right). Transparent circles represent values for default network, heavy circles for altered network. The default and altered group means are large yellow and black open circles, respectively.

B: Absolute values of mean shifts in threshold (upper plot) and slope (lower plot) for Excitatory neurons arising from increasing the value of each parameter by +20%. The three example

parameters from panel A are labeled and indicated as filled red circles. Note that data are presented on a log10 scale.

C: The shift in mean slope-threshold parameter values for E neurons from the default network values, in response to each of the 76 circuit parameter alterations. Light red circles indicate +20% increase in parameter value; dark red crosses indicate a -20% decrease in parameter value.

211   To synthesize the findings from all simulations, for each altered parameter we plotted the shift
212   in mean slope-threshold values for L2/3 excitatory neurons from the mean values found with
213   the default model (Figure 3B). We focused on excitatory neurons because they constitute 90%
214   of the neurons in this layer (Lefort et al., 2009) and are the primary output to downstream
215   circuits (Mao et al., 2011; Petreanu et al., 2007). Overall, we found a very heterogeneous
216   picture. First, the magnitude of the shift in circuit response varied from parameter to parameter
217   (Figure 3B—C). Varying some parameters, such as the first two examples given above, had
218   large effects, whereas varying other parameters such as w_{I5htE} (the strength of synapses from
219   $5HT_{3A}R$ inhibitory neurons to E neurons) or τm_{Isom} (the membrane time constant of SOM
220   inhibitory neurons) had little effect. Second, the direction of shift in 2-D slope-threshold space
221   also depended on parameter (Figure 3C). Increasing some parameters changed either circuit
222   slope or threshold in isolation, while other parameters changed both slope and threshold
223   together. All four quadrants of the slope-threshold plane could be reached by various subsets
224   of the model parameters. Third, basic knowledge of whether a component was 'excitatory' or
225   'inhibitory' was insufficient to predict the direction of slope-threshold change. For example, the
226   two glutamatergic projections considered in Figure 3A had distinct effects on circuit function.

227   In summary, these simulations indicate that the L2/3 somatosensory cortex circuit has
228   extremely varied sensitivities to changes in its cellular components, and that the eventual
229   circuit-level consequences cannot be predicted from knowledge of the class of the perturbed
230   neurotransmitter alone. Since the E/I imbalance model groups all excitatory and inhibitory
231   components as respective equals, it cannot account for these results.

232   ***Firing rates and correlations from the logistic model***

233   In the above analysis, we investigated how low-level circuit components affect a high-level

234   circuit input-output function, as parameterized by the slope and threshold of fitted logistic

235   functions. But how is this logistic input-output function related to more common measures of

236   neural population activity, such as firing rates and pairwise correlations between neurons? To

237   investigate this, we considered the following reduced statistical model of cortical activity. We

238   assumed for simplicity that the magnitude of the total input to the L2/3 circuit can be described

239   by a Gaussian distributed random variable, with zero mean and unit standard deviation (Figure

240   4A lower left). Then we described each L2/3 neuron's input-output as a logistic function as

241   before (Figure 4A upper left), with threshold and slope defined relative to the Gaussian input's

242   mean and standard deviation, respectively. Given this model, we can numerically calculate the

243   probability distribution over a neuron's firing probability, which in general is skewed and non-

244   Gaussian (Figure 4A upper right). From this function we compute (Methods) both the neuron's

245   mean firing probability and the pairwise correlation of two identical neurons following this

246   profile (Figure 4A lower right). Example samples from the model are illustrated in Figure 4B.

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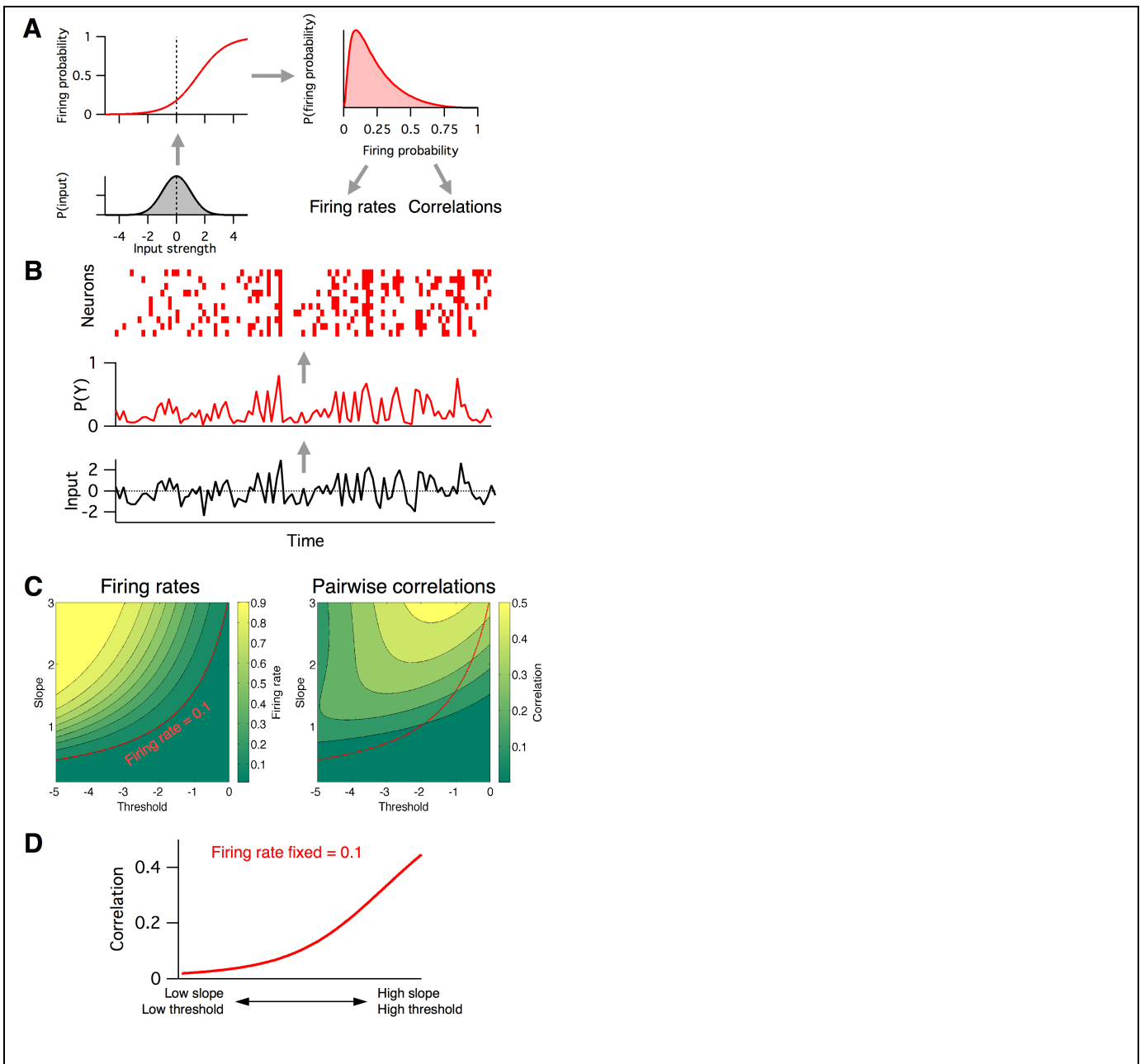
Figure 4. Firing rates and pairwise correlations from the logistic response model.

A: Logistic model components. We assume a normally distributed input drive (gray distribution, bottom left), which is passed through the neuron's probabilistic spike input-output function (red curve, top left), which results in a distribution of spike probabilities (top right) that are determined by the input-output function's slope and threshold parameters. From the output distribution we can directly calculate the mean firing rate and correlation between a pair of such neurons (Methods).

B: Example spikes from the logistic model. The bottom trace (black) shows examples inputs over time drawn randomly from the same normal distribution. This is transformed to spike probability at each time point (red trace). Example spike trains can then be generated from the spike probability trace by drawing Bernoulli samples with the specified probabilities (red ticks, top). If each neuron's spike train is conditionally independent given the same spike probabilities, we can see correlations in their spike trains.

C: Calculated mean firing rate (left) and pairwise correlation (right) color maps as a function of the logistic threshold (x-axis) and slope (y-axis) parameters. Contours indicate lines of fixed firing rate or correlation in the 2D slope-threshold space.

D: Pairwise correlation values along the slope-threshold contour for firing rate = 0.1.

247   Neural firing rates and correlations had qualitatively different dependencies on the underlying

248   logistic model's slope and threshold. Neural firing rate was greatest when threshold was low

249   and slope was high (top left of phase plot, Figure 4C left), whereas correlations were greatest

250   when both threshold and slope were high (top right of phase plot, Figure 4C right). This implies

251   that any change in the circuit's input-output function slope or threshold will in general have

252   distinct effects on firing rate versus correlations, and so could not be captured by a 1-

253   dimensional E/I balance model that sought to for example normalize firing rates alone. To

254   illustrate this fact, we plot the calculated correlation values along a contour where firing

255   probability is fixed at 0.1 (Figure 4D). In the region of parameter space where both the slope

256   and threshold are low (Figure 4C bottom left), correlations are low, ~0.01. However, as we

257   move along the contour for firing rate = 0.1 towards the region of parameter space where slope

258   and threshold are high (Figure 4C top right), the pairwise correlations increase to ~0.4. This

259   shows that a 1-dimensional E/I balance rule that exclusively sought to normalize neural firing

260   rates would leave neural correlations free to achieve arbitrary values.

261   Previous studies have found evidence for an E/I imbalance in ASD (Lee et al., 2017; Nelson

262   and Valakh, 2015). Fragile-X syndrome is the leading inherited cause of ASD, and also carries

263   alterations in excitability (Contractor et al., 2015). We aimed to interpret our *Fmr1* KO Ca^{2+}

264   imaging data (Figure 1C) via the 2D logistic model. Since our earlier analysis found important

265   differences in the heterogeneity in firing rates across neurons in *Fmr1* KOs (Figure 1C), we

266   extended the 2-D logistic model for single neurons to a 5-D neural population version that
267   captured cell-to-cell heterogeneity. The three extra parameters represented the standard
268   deviations and correlation in slope and threshold parameters across the neural population (see
269   Methods for details). We fit the parameters of the logistic model to reproduce the same neural
270   population Ca^{2+} imaging data presented in Figure 1C. Given the three summary statistics from
271   each animal (firing rate mean and s.d., and mean pairwise correlation), we used a gradient
272   descent algorithm to find the five parameters of the population-level version of the logistic
273   model that best matched the activity statistics (see Methods). The output statistics of the fitted
274   models matched well that of the target data (Figure 5 – figure supplement 1). Example neural
275   population activity patterns drawn from the mean model fits for each group are shown in Figure
276   5A, along with the fitted slope-threshold functions (Figure 5A insets), to be compared with the
277   Ca^{2+} imaging rasters in Figure 1C. For each animal, we also plot the full 5-D parameter fits for
278   all animals in Figure 5 – figure supplement 2. For the rest of the analysis, we focus on the
279   mean slope and mean threshold parameters, which showed the most prominent changes.
280   However we also note an increase in the slope s.d. between P14-16 and P30-40 WT animals
281   that was not observed in *Fmr1* KO, mirroring the increased heterogeneity in firing rates in the
282   same animals (Figure 1C). In Figure 5B we plot the mean slope and mean threshold fits on top
283   of the previously calculated (Figure 4C) 2D slope-threshold maps of firing rate and correlation.
284   We found that in young animals, P9—11, most points were scattered at high values of both
285   slope and threshold (Figure 5B left). With age, the parameter fits for both genotypes moved
286   south-west towards the low slope and low threshold region of parameter space (Figure 5B
287   center and right). The mean location of the cloud of points at each developmental age differed
288   between WT and KO. We plot the direction of shift in group mean from WT to KO in Figure 5C.
289   In young animals, P9—11 and P14—16, the KO group had both higher slope and higher
290   threshold than WT, whereas in adult animals, P30—40, the KO group had a lower slope and
291   lower threshold than WT. These results demonstrate an opposite direction of circuit parameter
292   change in young Fragile-X mice compared to adults, which was not be uncovered by
293   measures of neural firing rates and correlations (Figure 1C).
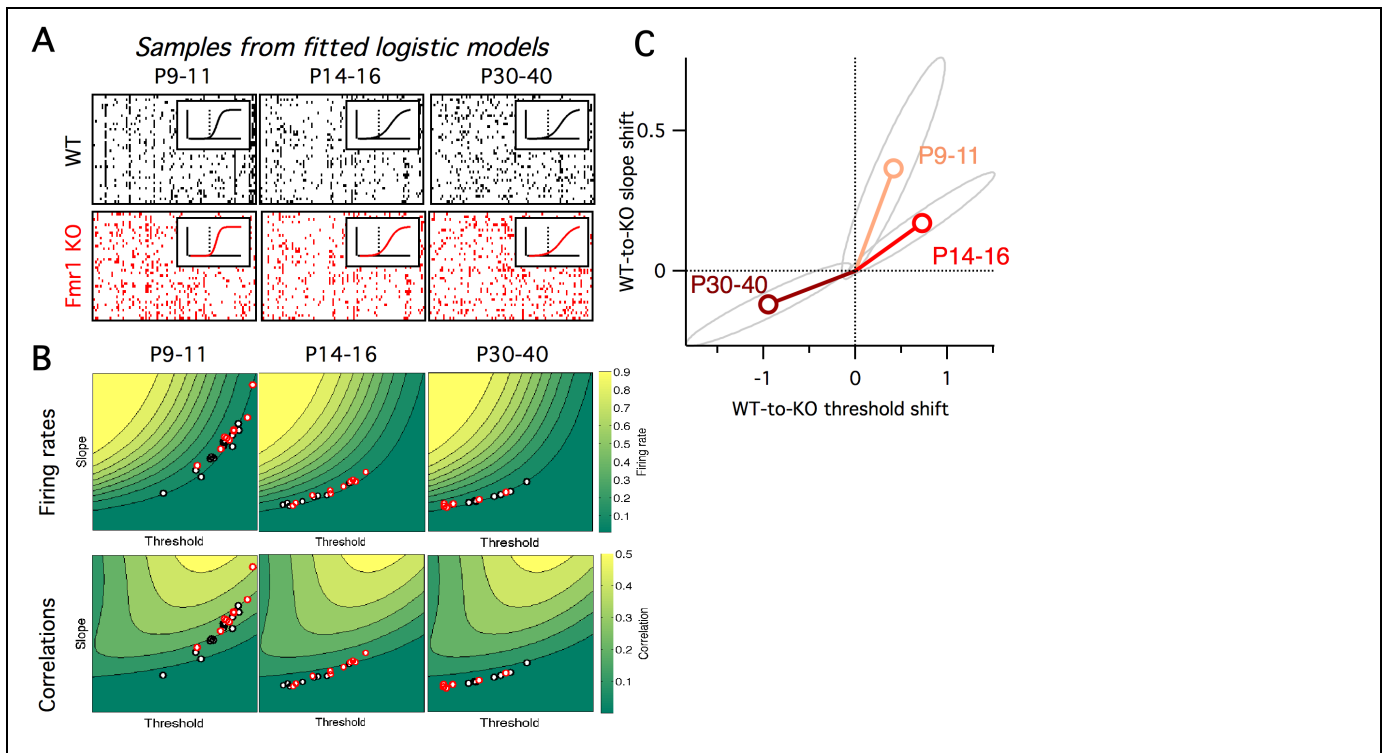
**Figure 5. Fragile-X fits from logistic model.**

A: Example samples from the fitted logistic models, corresponding to the six groups shown in panel A. Inset shows group mean fitted logistic function, dashed vertical line represents zero.

B: Fitted logistic mean slope and mean threshold values for data from each WT (black circles) and *Fmr1* KO (red circles) animal. Values overlaid on same firing rate (top) and correlation (bottom) maps from Figure 4C.

C: Shift in mean logistic slope and threshold values from WT to KO for P9—11 (orange), P14—16 (red) and P30—40 (brown). Grey ellipses represent 95% confidence intervals (Methods).

294 Earlier we asked how sensitive the logistic model slope and threshold parameters were to

295 alterations in the many underlying neural circuit components (Figure 3). In a similar way, we

296 can also ask how sensitive the neural firing rates and correlations are to alterations in the

297 logistic slope and threshold parameters. This is important since inspection of the 2-dimensional

298 maps in Figure 3C shows that these sensitivities will differ depending on starting location within

299 the slope-threshold space. To quantify this effect, we calculated the sensitivity of both the firing

300 rate and correlations to small changes in the slope and threshold (Figure 6A-B, see Methods),

301 quantified as the partial derivatives local to the fitted logistic parameter values for each animal

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302 (black and red circles in Figure 5B). In general, increasing the slope or decreasing the

303 threshold always increased both firing rates and correlations, as can be predicted from Figure

304 5B. However, the magnitude of sensitivities varied across animals. We found only minor

305 differences in sensitivities between genotypes (Figure 5 – figure supplement 2), and as a result

306 we pooled the sensitivity measurements across genotypes to test for statistical differences in

307 sensitivity with developmental age. In young animals, P9—11, changes in the logistic threshold

308 (solid bars in Figure 6) had substantial effects on both firing rates and correlation. This

309 sensitivity decreased with age (p ≤ 0.013 for firing rates, p < 0.01 for correlations from P9—11

310 to P14—16), so that in adult animals, P30-40, changes in threshold had relatively little effect

311 on neural activity statistics. A different picture emerged for the logistic slope parameter (striped

312 bars in Figure 6). There, the firing rate sensitivity increased with from P9—11 to P14—16 (p <

313 1e-6) (Figure 6A), while correlation sensitivity stayed approximately constant (p ≥ 0.18) (Figure

314 6B). These results show that the quantitative relationships between neural activity statistics

315 and the underlying circuit parameters are not fixed across development.
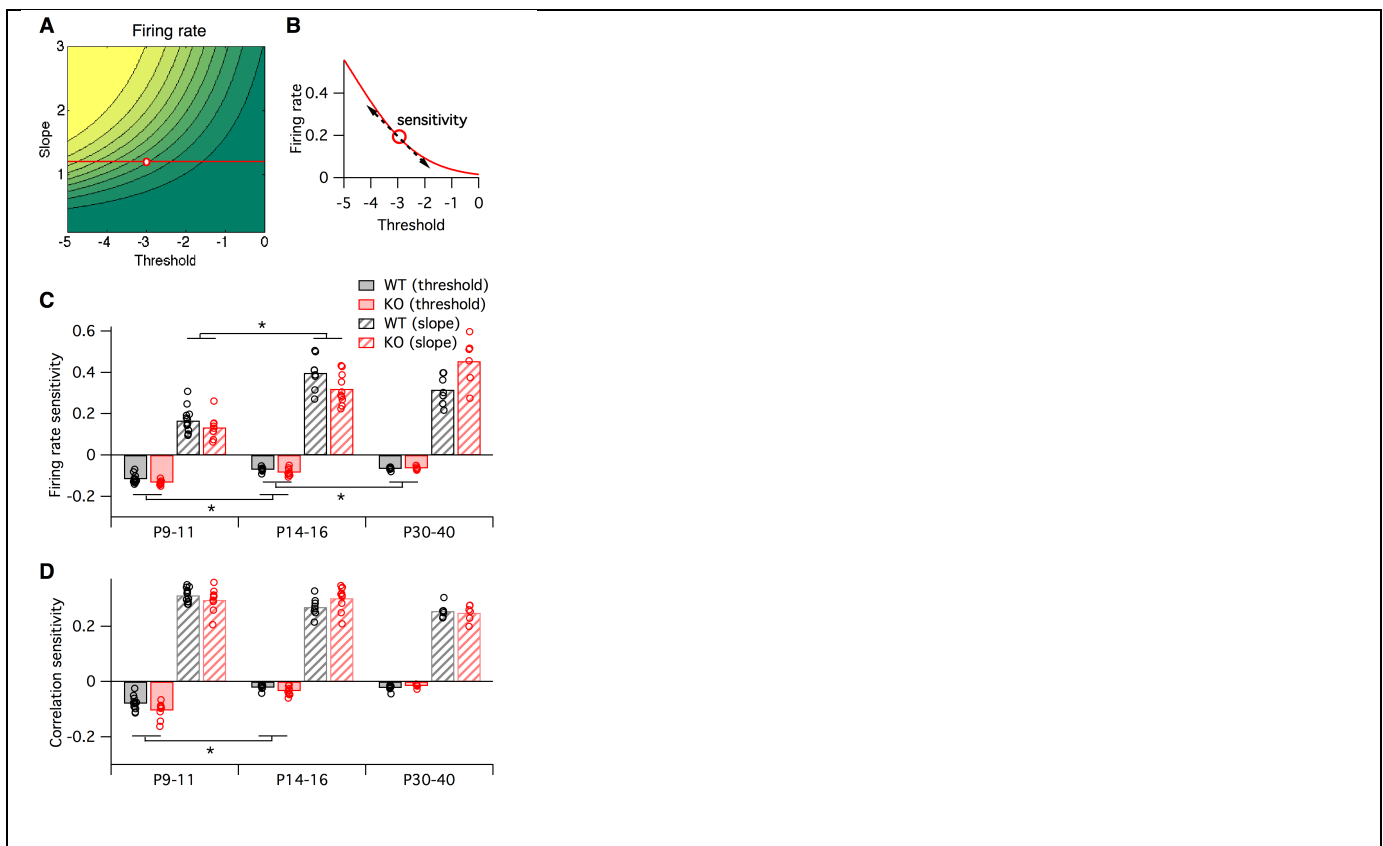
Figure 6. Sensitivity of firing rate and correlations with respect to logistic model parameters, local to the parameter fit for each animal.

A-B: Sensitivity to a parameter is calculated about a given point in parameter space. In this hypothetical example, we plot a slope-threshold parameter fit at the red circle on the firing rate contour map (A). The firing rate varies non-linearly if the threshold is varied away from this point (B). Sensitivity is calculated as the local derivative, or slope of the tangent, about the target point.

C-D: Sensitivity of firing probability (**C**) and pairwise correlations (**D**) to change in threshold (solid bars) and slope (striped bars) parameters of logistic model, about the fitted parameter values for each animal (circles) displayed in Figure 5B. Bars represent group means. Each statistical test compares the mean values between adjacent pairs of age groups, where the data were pooled between genotypes.

316 What are the functional implications of these alterations in firing rates and correlations in
317 Fragile-X mice across development? To address this, we calculated the entropy of the neural
318 population activity for the data from each animal. Entropy is a quantity from information theory,
319 measured in bits, that puts a hard upper bound on the amount of information that can be
320 represented by any coding system (Cover and Thomas, 2006). Intuitively, the entropy
321 measures how uniform the neural population activity pattern distribution is: it is large if the
322 circuit exhibits many different activity patterns over time, and small if only a few activity
323 patterns dominate. Entropy is an appealing measure for the present problem because it is
324 sensitive both to neural firing rates and to correlations at all orders. It is typically highest when
325 firing rates are high and correlations are low. Although entropy is notoriously difficult to
326 calculate for large neural populations because most estimation methods require impractically
327 long data recordings (Quian Quiroga and Panzeri, 2009), we recently developed a new
328 statistical method for this purpose, called the *population tracking model*, that scales well to
329 large numbers of neurons, even for limited data (O'Donnell et al., 2017). This model matches
330 both the synchrony distribution for the number of neurons simultaneously active, and the
331 variations in individual cell-to-cell firing rates. We fit this population tracking model to the same
332 Ca^{2+} imaging data as analyzed above (Figure 7). An intermediate step in estimating the neural
333 entropy involves calculating a low-parameter approximation of the entire probability distribution

334   over all 2^N neural population activity patterns, where N is the number of neurons. The

335   cumulatives of these probability distributions calculated for 50-neuron subsets of the

336   recordings are shown in Figure 7A. In young animals P9—11, a small number of activity

337   patterns accounts for a large fraction of the probability mass (Figure 7A left). For example,

338   based on these curves, 50% of the time we would expect to see the same 1000—10,000

339   patterns out of a possible total $2^{50} \approx 10^{15}$ patterns. In contrast, in older animals P14—16 and

340   P30—40 the cumulative distributions shift rightwards so that more patterns are typically

341   observed (Figure 7A center, right). In these cases, around 1,000,000 patterns are needed to

342   account for 50% probability mass.
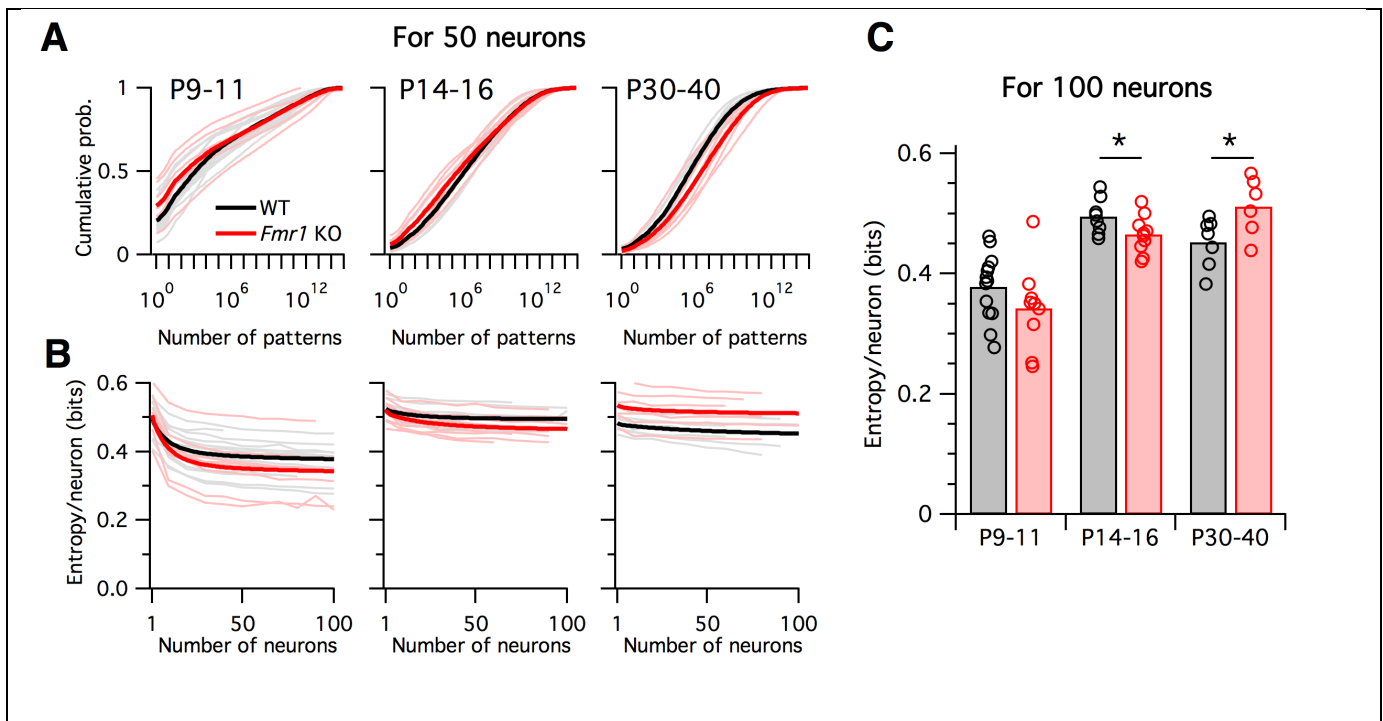
Figure 7. Differing trajectories of WT and KO entropy across development.
A: Cumulative probability mass as a function of the number of patterns. Patterns ordered from most probable to least probable. Thin lines are mean across many randomly-chosen 50-neuron subsets from a given animal, and thick lines represent means across all animals of a given   genotype.
B: Entropy per neuron as a function of the number of neurons analyzed. Thin lines are mean across many randomly chosen subsets for a given animal, thick lines are group mean of

> double exponential fits to the data (see Methods). Age groups (left to right) are as in panel A. **C:** Estimated entropy/neuron for 100 neuron populations. Circles represent individual animals, bars are group means.

343

344 Instead of attempting to quantify these shifts by asking how many patterns are needed to cross
345 an arbitrary threshold of probability mass, we instead calculated the entropy, which takes into
346 account the shape of the entire probability distribution. The entropy depends on the number of
347 neurons analyzed, so we normalized all estimates to calculate the entropy per neuron (Figure
348 7B—C). Since we are treating neurons as binary, the entropy/neuron was bounded between 0
349 and 1 bits. For all age groups, and for both WT and *Fmr1* KO animals, entropy/neuron
350 progressively decreased with the number of neurons analyzed (Figure 7B). Because each
351 imaging session captured a different number of neurons (range 40—198, median 97), we fit
352 the entropy/neuron versus number of neurons data with a double exponential function (see
353 Methods) and use the fit to provide a standardized estimate of the entropy/neuron for 100-
354 neuron populations (Figure 7C). In WT animals, entropy/neuron showed a non-monotonic
355 trajectory across development (O'Donnell et al., 2017). At P9—11 it was low, 0.38 bits (95% c.i.
356 [0.35:0.41]), before increasing at P14—16 ($p < 0.001$) to 0.50 bits (95% c.i. [0.48:0.52]), before
357 decreasing again at P30—40 ($p = 0.028$) to 0.45 bits (95% c.i. [0.42:0.48]). We found a
358 different entropy trajectory in *Fmr1* KO animals. There, although entropy/neuron also began
359 low at 0.34 bits (95% c.i. [0.30:0.39]), not different from WT ($p= 0.19$), when it increased at
360 P14—16 ($p < 0.001$) to 0.465 bits (95% c.i. [0.45:0.48]) it remained lower than for WT ($p = $
361 0.048). Finally, instead of decreasing as in the WT case, entropy continued to increase in
362 P30—40 *Fmr1* KO animals ($p = 0.033$) to 0.51 bits (95% c.i. [0.47:0.55]), higher than WT ($p = $
363 0.034). These entropy values estimated directly from Ca^{2+} imaging data agreed well with
364 entropy estimates for synthetic data sampled from the previously fit logistic models (Figure 7 –
365 figure supplement 1). In summary, unlike WT animals, *Fmr1* KO mice showed a monotonically
366 increasing entropy/neuron from P9—11 to P30—40. Furthermore, the direction of change in
367 entropy between P14—16 and P30—40 was opposite for WT and *Fmr1* KO animals,
368 decreasing in the former and increasing in the latter.

Discussion

The 1-dimensional E/I imbalance model has been widely used for interpreting neural circuit changes observed in animal models of diverse brain disorders (Bateup et al., 2011; Dani et al., 2005; Gibson et al., 2008; Kehrer et al., 2008; Wallace et al., 2012). In the case of Fragile-X syndrome, the hyperexcitability prediction of the E/I imbalance model is consistent with many of the symptoms of the disease (e.g. seizures, hyperarousal, hyperactivity, hypersensitivity to sensory stimuli) and the known pathogenic defects implicated in *Fmr1* KO mice (diminished GABA signaling, exaggerated intrinsic excitability, increased neuronal firing rates; reviewed by Contractor et al., 2015). Here we tested the hypothesis that the E/I imbalance model can account for alterations in other neural activity statistics beyond the mean firing rates; however, our results demonstrated that it was inadequate. The model was too inflexible to account for the joint alterations in both neural firing rates and correlations observed in Fragile-X model mice. This suggests that future studies of brain disorders may need to consider higher-dimensional models of neural circuit dysfunction.

To test how cellular components affect their circuit function, we built computational models of mouse L2/3 somatosensory cortex at two levels of abstraction: a detailed, 100-parameter biophysical model, and a 2-parameter logistic response model. The purpose of the detailed model was to build a representation of the circuit where each parameter has a one-to-one mapping with something that could be experimentally measured in a real animal - indeed many of these parameters have been shown to be altered in *Fmr1* KO mice. The purpose of the logistic model was different: it simple enough to be both derivable from the complex model, and provide a direct link with measurable activity variables in our *in vivo* Ca2+ imaging data, firing rates and correlations. The disadvantages of detailed models are that they contain many parameters, and so are hard to constrain to data – in this case it was only possible because of the large dataset from Petersen et al., for P17-22 WT mice (Avermann et al., 2012; Lefort et al., 2009; Tomm et al., 2014). The disadvantages of the simple 2D model is that its logistic input-output structure implies a very strong and specific assumption about the functional purpose of the circuit – to generate single spikes across a subset of neurons. Although this may be a physiologically relevant computation for this particular brain circuit (Clancy et al., 2015; Kerr et

398   al., 2007; Sato et al., 2007), it is not immediately obvious how to extend this approach to
399   include temporal correlations, for example, or to apply it to other brain circuits where we may
400   have less insight into their natural computations. Nevertheless, our approach demonstrates a
401   new way to tackle such problems.

402   After building the detailed computational model of L2/3 of mouse somatosensory cortex
403   (Figure 2), and asked how sensitive the spiking responses of the overall circuit were to
404   changes in its underlying neural components, many of which are known to be altered in *Fmr1*
405   KO mice (Bureau et al., 2008; Gibson et al., 2008; Gonçalves et al., 2013; Harlow et al., 2010;
406   Hays et al., 2011; Paluszkiewicz et al., 2011; Patel et al., 2013; Testa-Silva et al., 2012). We
407   found that while changing some neural parameters did have a large effect, changing other
408   parameters had little or no effect on circuit function (Figure 3B). This redundancy property has
409   been reported as widely prevalent in computational models of biological systems (Gutenkunst
410   et al., 2007; O'Leary et al., 2015). Its existence has two important implications for studies of
411   brain disorders: first, many of the physiological component changes discovered in animal
412   models may be entirely benign at the circuit level. Second, any treatment designed to correct
413   circuit function is free to push the system by arbitrary amounts along insensitive directions in
414   parameter space without consequence, as long as it makes the correct perturbations along the
415   sensitive directions. The insensitive directions form a null space, which is a subspace of the
416   parameter space.

417   An important caveat to our parameter sensitivity analysis is that it was linear and local to a
418   particular point in the high-dimensional model parameter space, corresponding to WT P17-22
419   mice. Since the circuit dynamics are nonlinear, it is likely that the particular parameter
420   sensitivities would be different in other parts of parameter space, especially near bifurcations
421   where qualitatively different dynamics emerge (Hirsch et al., 2013). However, as long as the
422   redundancy property is widely preserved, as suggested by studies on computational models of
423   other biological systems (Fisher et al., 2013; Gutenkunst et al., 2007; Machta et al., 2013;
424   Panas et al., 2015), then our conclusions for brain disorders remain valid.

425 In addition to the varying magnitudes of circuit components' effect on circuit function, we also

426 found that different components shifted the circuit input-output function in different directions,

427 as defined by our 2D logistic response model (Figures 1 and 2). Even circuit parameters that

428 are nominally of the same type, such as the strength of glutamatergic synapses between

429 excitatory (E) neurons in L4 to E neurons in L2/3 or synaptic strength between E neurons

430 within L2/3, had qualitatively different effects on the circuit response to stimulation (Figure 3).

431 According to the standard E/I imbalance model (Rubenstein and Merzenich, 2003), both of

432 these parameters should have similar effects on circuit function; but according to the logistic

433 response model we studied, their differing effects on slope and threshold parameters must

434 necessarily lead to different magnitudes of change in neural firing rates and correlations

435 (Figure 4C). Indeed, no 1-dimensional model of circuit function could ever capture the

436 heterogeneity in parameter sensitivities that we observed (Figure 3B).

437 Next, we fit the parameters of the logistic response model to match the *in vivo* firing statistics

438 of neural populations from WT and *Fmr1* KO mice of varying age (Figure 5). Previous studies

439 had found that neural correlations decrease during development (Golshani et al., 2009;

440 Rochefort et al., 2009), and that early postnatal *Fmr1* KO mice had higher correlations and

441 firing rates than WT mice (Gonçalves et al., 2013; La Fata et al., 2014). Circuit hypersynchrony

442 may be a general defect in autism disorders, as it is also found in mouse models of Rett

443 syndrome (Lu et al., 2016). However, the relationship between these changes in firing statistics

444 and the underlying neural circuit components were unclear. Our logistic model helps bridge

445 this gap, leading to two findings: first, the direction of circuit parameter change from WT to KO

446 was opposite in young (P9—11 and P14—16) versus mature (P30—40) animals (Figure 5C).

447 Similar opposing switches in sensory cortex properties with age were also recently reported in

448 *Fmr1* KO and WT rats (Berzhanskaya et al., 2016). Second, we found that the sensitivity of

449 neural firing rates and correlations to changes in underlying circuit components depends on

450 developmental age (Figure 6). Taken together, these findings imply that qualitatively different

451 interventions may be needed at different stages of development in Fragile-X, and perhaps

452 other neurodevelopmental disorders, to shift cortical circuit function towards typical wild-type

453 operation.

454 Spontaneous, intrinsic activity is ubiquitously present in mammalian cerebral cortex. It is highly
455 structured at multiple spatiotemporal scales (Mitra et al., 2015; Ringach, 2009) and interacts
456 strongly with the signals evoked by sensory stimulation (Ringach, 2009). Cellular-resolution
457 recordings in animals have shown that the patterns of spontaneous activity in neural
458 populations are representative of the ensemble of activity patterns used by the brain to
459 represent sensory stimuli (Berkes et al., 2011; Luczak et al., 2009; Miller et al., 2014). Here we
460 found that the entropy of spontaneous activity in WT mouse somatosensory cortex follows an
461 inverted-U shaped trajectory across development, and that this trajectory is dramatically
462 altered in the *Fmr1* KO mouse model of Fragile-X (Figure 7). Although we saw no reliable
463 differences across genotypes in early postnatal animals (P9—11), *Fmr1* KO animals showed
464 lower entropy than WT after the second postnatal week (P14—16), while surprisingly switching
465 to show higher entropy than WT in adult (P30—40). Notably, this switch in the direction of
466 entropy change from WT to KO during development mirrors the reversing we saw in logistic
467 model parameter changes in Figure 5C. Together, these findings suggest a perturbed
468 trajectory of cortical development during the critical period in *Fmr1* KO mice (Meredith et al.,
469 2012). However, our results cannot distinguish whether the observed perturbation in L2/3
470 activity statistics reflects a developmental delay, or a permanently altered developmental
471 trajectory. Further studies at later developmental time points are needed.

472 What is the functional significance of these shifts in population entropy? Previous work
473 suggested that the entropy of neural circuit activity may be optimally tuned at intermediate
474 levels as a trade-off between maximizing representational capacity at high entropy, versus
475 maintaining error correction and regularization at low entropy (Schneidman et al., 2006). These
476 properties can also be thought of as trading off between discrimination and generalization,
477 respectively (Qian and Lipkin, 2011). If we assume that WT mice are optimally tuned, our
478 findings predict that young *Fmr1* KO mice should show poorer somatosensory discrimination in
479 behavioral tasks than wild-type animals, while in contrast adult *Fmr1* KO mice should perform
480 more poorly on tasks involving generalization across somatosensory stimuli.

481 If the unidimensional E/I imbalance model is not sufficiently rich to capture the circuit changes
482 observed in neurodevelopmental disorders, what should replace it? How many dimensions or

483   degrees of freedom should a working model for a brain disorder have? Theoretical

484   neuroscientists have long studied E/I balance in generic models of recurrent neural circuits

485   (Brunel, 2000; Tsodyks and Sejnowski, 1995). These models have uncovered important

486   distinctions between 'loose' balanced regimes, where E and I inputs to a neuron are equal only

487   on average, and fine-tuned 'tight' balanced regimes where E and I inputs to a neuron track

488   each other closely on fast timescales (Deneve and Machens, 2016; Hennequin et al., 2017). In

489   principle these generic models could be used to investigate multidimensional E/I imbalances in

490   brain disorders (Vogels and Abbott, 2007). However, it is currently difficult to directly fit these

491   many-parameter network models to data (although see Arakaki et al., 2017; Fisher et al., 2013;

492   Stringer et al., 2016), and they are agnostic to circuit function. Instead we suggest an

493   alternative, complementary approach: start by assuming a computational function for the

494   particular neural circuit under study, then work backwards to design a model that is both

495   sophisticated enough to capture the key information processing features of the circuit, but

496   simple enough to interpret and link to physiological data. In this study we considered a 2-

497   parameter model of L2/3 somatosensory cortex's input-output function, which could account

498   for both neural firing rates and correlations. Other brain circuits may demand models with more

499   degrees of freedom. Crucially, the most informative models need not be those that include the

500   highest level of physiological detail. All models are ultimately wrong in the sense that they

501   make abstractions about their underlying parts, and detailed models carry the additional

502   burden of fitting many parameters, which may be difficult to adequately constrain (O'Leary et

503   al., 2015). Nonetheless, some models are useful (Box, 1979).

504   One potential use of simple parametric circuit models such as the ones we employed here may

505   be as a tool for rationally designing candidate intervention compounds and then screening their

506   effects on neural population activity. For example, the current study could have been extended

507   to fit the logistic model to neural activity data from another cohort of *Fmr1* KO mice that had

508   received a candidate treatment, then ask if the fitted model parameters were closer in value to

509   those from WT animals or *Fmr1* KO controls. Approaches like this could complement the

510   traditional strategy of designing drugs based on reversing molecular deficits and then

511   assessing the drug's impact on animal model behavior. Indeed, our results suggest that given

512   the multi-dimensionality of circuit properties, it may prove difficult or impossible to find a single

513   compound that can correctly reverse deficits at any age. This scenario might require a

514   combination of drugs chosen to push circuit-level properties towards the 'correct' region of

515   parameter space. The framework we have introduced in this study can facilitate this type of

516   high-dimensional intervention analysis for diverse neurodevelopmental disorders.

Materials and Methods

Key Resources Table			
Reagent type (species) or resource	Designation	Source or reference	Identifiers
strain, strain background (mus musculus)	c57bl/6J strain of wild type mice	Jackson Labs	IMSR_JAX:000664
genetic reagent (mus musculus)	Fmr1 knockout mouse on a c57 background	William Greenough (originally from Dutch-Belgian Fragile X Consortium)	RRID:MGI:2665400
chemical compound, drug	OGB1 AM (Oregon Green BAPTA-1 AM)	Molecular Probes (ThermoFisher Scientific)	
Software, algorithm	ImageJ	NIH	RRID:SCR_003070
software, algorithm	BRIAN Simulator	http://briansimulator.org	RRID:SCR_002998
software, algorithm	MATLAB	http://www.mathworks.com/products/matlab	RRID:SCR_001622

Mouse in vivo calcium imaging recording

All Ca^{2+} imaging data were published previously (Gonçalves et al., 2013). Briefly, data were collected from male and female C57Bl/6 wild-type and *Fmr1* KO mice at P9–40. For each group the animal numbers were: P9-11, n=13 WT and n=9 *Fmr1* KO; P14-16, n=8 WT and n=10 Fmr1 KO; P30-40, n=7 WT and n=6 *Fmr1* KO. There were variations in the number of cells recorded from each animal. The range of cell numbers for each group were: P9-11, 49-198 cells in WT and 84-144 cells in *Fmr1* KO; P14-16, 65-119 cells in WT and 40-149 cells in *Fmr1* KO; P30-40, 60-114 cells in WT and 69-105 cells in *Fmr1* KO. Mice were anesthetized with isoflurane, and a cranial window was fitted over primary somatosensory cortex by stereotaxic coordinates. Mice were then transferred to a two-photon microscope and headfixed to the stage while still under isoflurane anesthesia. 2—4 injections of the Ca^{2+} sensitive Oregon-Green BAPTA-1 (OGB) dye and sulforhodamine-101 (to visualize astrocytes) were injected 200 um below the dura. Calcium imaging was performed using a Ti-Sapphire Chameleon Ultra II laser (Coherent) tuned to 800 nm. Imaging in unanesthetized mice began within 30-60 min of stopping the flow of isoflurane after the last OGB injection. Images were acquired using ScanImage software (Pologruto et al., 2004) written in MATLAB (MathWorks;

28

534 RRID:SCR_001622). Whole-field images were collected using a 20× 0.95 NA objective

535 (Olympus) at an acquisition speed of 3.9 Hz (512 × 128 pixels). Several 3-minute movies were

536 concatenated and brief segments of motion artifacts were removed (always <10 s total). Data

537 were corrected for x—y drift. Cell contours were automatically detected and the average ΔF/F

538 signal of each cell body was calculated at each time point. Each ΔF/F trace was low-pass

539 filtered using a Butterworth filter (coefficient of 0.16) and deconvolved with a 2 s single-

540 exponential kernel (Yaksi and Friedrich, 2006). To remove baseline noise, the standard

541 deviation of all points below zero in each deconvolved trace was calculated, multiplied by two,

542 and set as the positive threshold level below which all points in the deconvolved trace were set

543 to zero. Estimated firing rates of the neurons, $r_i(t)$, were then obtained by multiplying the

544 deconvolved trace by a factor previously derived empirically from cell-attached recordings in

545 vivo (Golshani et al., 2009).

Computational methods

547 Data analysis and logistic model calculations were done using MATLAB (Mathworks;

548 RRID:SCR_001622).   All   simulation   code   is   available   online   at

549 https://github.com/cianodonnell/ODonnelletal_2017_imbalances, and the population tracking

550 model   code   (O'Donnell   et   al.,   2017)   is   available   at

551 https://github.com/cianodonnell/PopulationTracking.

Detailed layer 2/3 model simulations

553 Layer 2/3 model simulations (Figures 1 and 2) were implemented with the Python-based

554 simulator Brian 2 (http://briansimulator.org; RRID:SCR_002998) (Goodman and Brette, 2009),

555 and results analyzed with MATLAB (Mathworks; RRID:SCR_001622). The model consisted of

556 four populations of reciprocally connected leaky integrate-and-fire neurons representing a L2/3

557 somatosensory barrel circuit: 1700 excitatory neurons, 70 PV inhibitory neurons, 115 $5HT_{3A}R$

558 inhibitory neurons, and 45 SOM inhibitory neurons, driven by a separate population of 1500

559 excitatory spike sources representing input from L4. Cell numbers were estimated by

560 combining layer-specific excitatory and inhibitory cell count information from (Lefort et al.,

561 2009) with the approximate percentages of the three inhibitory cell groups given by (Petersen

562 and Crochet, 2013). The voltage V of each neuron evolved as

29

563 $\quad \dfrac{dV}{dt} = (R_{in}(g_e(E_{rev,e} - V) + g_i(E_{rev,i} - V)) - (V - V_{rest}))/\tau_m$

564 $\quad$ where R_{in} is the input resistance, $E_{rev,e}$ and $E_{rev,i}$ are the excitatory and inhibitory synaptic

565 $\quad$ reversal potentials respectively, τ_m is the membrane time constant, and g_e and g_i are the

566 $\quad$ summed excitatory and inhibitory synaptic input conductances respectively. Between input

567 $\quad$ events the total excitatory synaptic conductance g_e evolved in time according to the equation

574 $\quad \dfrac{dg_e}{dt} = -g_e/\tau_{syn,e}$

568 $\quad$ where $\tau_{syn,e}$ is the excitatory synaptic time constant. Similar equations governed the inhibitory

569 $\quad$ conductances. When a spike arrived at a synapse, a Bernoulli random number was drawn with

570 $\quad$ release probability set according to the particular synaptic connection type. If this number was

571 $\quad$ equal to one, then the total synaptic conductance for that neuron was instantaneously

572 $\quad$ incremented by the specific amplitude of the chosen conductance for that individual synapse,

573 $\quad$ indexed j: $g_e \rightarrow g_e + \bar{g}_j$.

575 $\quad$ All synaptic connections were formed probabilistically by drawing independent random

576 $\quad$ Bernoulli variables with connection type-specific probabilities. Synaptic PSP amplitudes were

577 $\quad$ drawn independently for each synapse from a log-normal distribution constrained by the

578 $\quad$ experimentally reported mean and median values for each particular connection type. The

579 $\quad$ maximum post-synaptic potential amplitude was set to 8 mV. Synapses in the model were

580 $\quad$ conductance-based, but since synaptic strengths reported in the literature were typically in

581 $\quad$ terms of EPSP/IPSP amplitude, in accordance with how the experiments were performed

582 $\quad$ (Avermann et al., 2012), we set each maximal synaptic conductance as the value needed to

583 $\quad$ generate a PSP of the desired amplitude when the target neuron started at resting potential in

584 $\quad$ the case of EPSPs or -55mV in the case of IPSPs, which we computed analytically. Refractory

585 $\quad$ periods were calculated as the inter-spike-interval corresponding to the maximal

586 $\quad$ experimentally reported firing rate. Release probability and synaptic strength values for

587 $\quad$ unconnected neurons are excluded from Table 1. Excitatory synaptic time constants were set

588 $\quad$ at 2 ms, which is typical for the fast component of AMPA receptor responses, but could not be

589 $\quad$ estimated from the PSP statistics in (Avermann et al., 2012) because of masking by the slower

590 membrane time constant. The mathematical form of our model meant that inhibitory synaptic

591 time constants needed to be equal for all incoming inhibitory synapses to a neuron. We set

592 these to 40 ms for E, $5HT_{3A}R$ and SOM neurons and 16 ms for PV neurons, which were the

593 typical values of the IPSP decay time constants in the (Avermann et al., 2012) dataset. Due to

594 lack of direct data for this circuit, connection probabilities for synapses from L4 E neurons to E,

595 PV and SOM L2/3 neurons was set to a reasonable cortical value of 0.15, while $5HT_{3A}R$

596 neurons did not receive any input from L4 (Gentet et al., 2012). Similarly due to a lack of direct

597 data, we set synaptic release probabilities for connections from L4 to L2/3 neurons to a typical

598 cortical value of 0.25, while mean and median L4 excitatory PSP amplitudes onto L2/3 PV and

599 SOM were set to 0.8 and 0.48 mV, respectively, to match reported data for L4 EPSP

600 amplitudes onto L2/3 E neurons (Lefort et al., 2009). The differential equations were solved

601 using the forward Euler method with an integration timestep of 0.01 ms. Each simulation run

602 was 50 ms long, during which we recorded whether or not each neuron responded. In the rare

603 cases where a neuron spiked more than once, we disregarded the extra spikes. L4 neuron

604 dynamics were not explicitly simulated, but instead modeled only as a set of output spike trains.

605 After selecting the subset of active L4 neurons, spike times were drawn randomly from a

606 Gaussian distribution with standard deviation of 2 ms. We repeated the simulations 10 times

607 for this identical input pattern to average over the noise due to probabilistic vesicle release. We

608 repeated this procedure further 10 times for different random allocations of the 'ON' inputs.

609 Then, a neuron's ON probability was defined as the fraction of these $10 \times 10 = 100$ simulations

610 for which it responded with one or more spikes. Finally, we repeated the entire procedure for

611 varying levels of L4 input sparsity.

612 For the simulations presented in Figure 3 we varied only 76 model parameters, which is 24

613 less than the total number of 100 model parameters listed in Table 1. We excluded the four

614 neuronal refractory periods (because in almost all simulations each neuron spiked a maximum

615 of once, making the refractory period irrelevant), and the six connection probabilities that were

616 fixed at zero. Finally, we grouped together the mean and median PSP amplitudes for each of

617 the fourteen non-zero synaptic connections, so that both parameters were increased or

618 decreased by the same fraction in tandem. Together these choices reduced the number of test

619 parameters from 100 to 76.

620 For all parameters that naturally range from 0 upwards, such as the number of neurons or

621 release probability, we increased or decreased their values during testing in the most intuitive

622 way, by adding +/- 20% of the baseline value. However, this method was less useful for other

623 parameters, such as cell resting voltage, for which we reasoned it made more sense to scale

624 relative to another parameter, such as spike threshold. As a result, we varied 1) resting voltage

625 relative to its difference from spike threshold; 2) spike threshold relative to its difference with

626 resting voltage; 3) excitatory synaptic reversal potentials relative to resting voltage; 4) inhibitory

627 synaptic reversal potentials relative to spike threshold.

Table 1. L2/3 computational circuit model parameters and mean slope & threshold shifts.							
Parameter	Value	Source	+20% effect on slope, thresh	Parameter	Value	Source	+20% effect on slope, thresh
N_E	1700	[1]	slope: 6.4×10^{-3} thresh: 5.16	$pcon_{I5htE}$	0.465	[2]	slope: -3.25×10^{-3} thresh: -0.103
N_{Ipv}	70	[1]	slope: 4.3×10^{-3} thresh: -1.51	$pcon_{I5htIpv}$	0.38	[2]	slope: -7.13×10^{-3} thresh: -0.298
N_{I5ht}	115	[1]	slope: -0.0104 thresh: -0.58	$pcon_{I5htI5ht}$	0.38	[2]	slope: 6.07×10^{-4} thresh: 0.175
N_{Isom}	45	[1]	slope: -0.001 thresh: -0.83	$pcon_{I5htIsom}$	0	No data	Not tested
N_{EL4}	1500	[1]	slope: -0.106 thresh: 3.43	$pcon_{IsomE}$	0.5	[5]	slope: -6.29×10^{-3} thresh: -0.8919
$Vrest_E$	-68 mV	[2]	slope: 0.049 thresh: -6.09	$pcon_{IsomIpv}$	0	No data	Not tested
$Vrest_{Ipv}$	-68 mV	[2]	slope: -0.016 thresh: -0.963	$pcon_{IsomI5ht}$	0	No data	Not tested
$Vrest_{I5ht}$	-62 mV	[2]	slope: -9.48×10^{-4} thresh: 0.205	$pcon_{IsomIsom}$	0	No data	Not tested
$Vrest_{Isom}$	-57 mV	[3]	slope: 3.58×10^{-3} thresh: 0.034	$prel_{EL4E}$	0.25	No data	slope: -0.112 thresh: 4.21
Vth_E	-38 mV	[2]	slope: -3.76×10^{-3} thresh: -0.381	$prel_{EL4Ipv}$	0.25	No data	slope: 0.0103 thresh: 0.682
Vth_{Ipv}	-37.4 mV	[2]	slope: 3.87×10^{-3} thresh: 0.221	$prel_{EL4Isom}$	0.25	No data	slope: -2.06×10^{-3} thresh: -0.537
Vth_{I5ht}	-36 mV	[2]	slope: -1.12×10^{-3} thresh: 0.013	$prel_{EE}$	0.25	No data	slope: 4.99×10^{-3} thresh: 6.074
Vth_{Isom}	-40 mV	[3]	slope: -3.26×10^{-3} thresh: -0.083	$prel_{EIpv}$	0.25	No data	slope: -4.04×10^{-4} thresh: 0.163
Rin_E	160 MΩ	[2]	slope: 1.95×10^{-3} thresh: -0.283	$prel_{EI5ht}$	0.25	No data	slope: -9.32×10^{-3} thresh: -0.532
Rin_{Ipv}	100 MΩ	[2]	slope: -8.38×10^{-3} thresh: -0.283	$prel_{EIsom}$	0.25	No data	slope: -7.32×10^{-3} thresh: -0.3847
Rin_{I5ht}	200 MΩ	[2]	slope: -3.87×10^{-3} thresh: -0.653	$prel_{IpvE}$	0.25	No data	slope: 1.03×10^{-2} thresh: -0.941
Rin_{Isom}	250 MΩ	[4]	slope: 7.55×10^{-3} thresh: 0.465	$prel_{IpvIpv}$	0.25	No data	slope: 1.21×10^{-3} thresh: -0.061

τm_E	28 ms	[2]	slope: -9.59 x10^{-3} thresh: 0.268	$prel_{lpvl5ht}$	0.25	No data	slope: -4.73 x10^{-3} thresh: 0.011
τm_{lpv}	21 ms	[2]	slope: 4.82 x10^{-3} thresh: 0.027	$prel_{l5htE}$	0.25	No data	slope: -1.63 x10^{-3} thresh: -0.379
τm_{l5ht}	10 ms	[2]	slope: -5.09 x10^{-3} thresh: -0.302	$prel_{l5htlpv}$	0.25	No data	slope: -3.41 x10^{-3} thresh: -0.262
τm_{Isom}	30 ms	[4]	slope: -2.89 x10^{-3} thresh: -0.159	$prel_{l5htl5ht}$	0.25	No data	slope: 3.05 x10^{-3} thresh: 0.123
$tref_E$	55.5 ms	[2]	Not tested	$p_{rel,IsomE}$	0.25	No data	slope: -2.13 x10^{-4} thresh: -0.65
$tref_{lpv}$	5.4 ms	[2]	Not tested	$w_{EL4E,mean}$	0.8 mV	[1]	slope: -0.142 thresh: 0.342
$tref_{l5ht}$	21.3 ms	[2]	Not tested	$w_{EL4E,median}$	0.48 mV	[1]	slope: -0.142 thresh: 0.342
$tref_{Isom}$	20 ms	[3]	Not tested	$w_{EL4lpv,mean}$	0.8 mV	=w_{EL4E}	slope: 3.61 x10^{-3} thresh: 6.54 x10^{-3}
$\tau syn_{E,e}$	2 ms	Typical	slope: 1.37 x10^{-2} thresh: -1.79	$w_{EL4lpv,median}$	0.48 mV	=w_{EL4E}	slope: 3.61 x10^{-3} thresh: 6.54 x10^{-3}
$\tau syn_{E,i}$	40 ms	[2]	slope: -7.29 x10^{-3} thresh: 0.48	$w_{EL4Isom,mean}$	0.8 mV	=w_{EL4E}	slope: 5.05 x10^{-3} thresh: -0.329
$\tau syn_{lpv,e}$	2 ms	Typical	slope: -9.79 x10^{-3} thresh: -0.477	$w_{EL4Isom,median}$	0.48 mV	=w_{EL4E}	slope: 5.05 x10^{-3} thresh: -0.329
$\tau syn_{lpv,i}$	16 ms	[2]	slope: 1.56 x10^{-3} thresh: -0.097	$w_{EE,mean}$	0.37 mV	[2]	slope: 7.44 x10^{-3} thresh: 5.34
$\tau syn_{l5ht,e}$	2 ms	Typical	slope: 4.52 x10^{-3} thresh: -0.047	$w_{EE,median}$	0.2 mV	[2]	slope: 7.44 x10^{-3} thresh: 5.34
$\tau syn_{l5ht,i}$	40 ms	[2]	slope: -3.82 x10^{-3} thresh: -0.387	$w_{Elpv,mean}$	0.82 mV	[2]	slope: -3.77 x10^{-4} thresh: -0.297
$\tau syn_{Isom,e}$	2 ms	Typical	slope: -0.0126 thresh: -8.82 x10^{-3}	$w_{Elpv,median}$	0.68 mV	[2]	slope: -3.77 x10^{-4} thresh: -0.297
$\tau syn_{Isom,i}$	40 ms	[2]	slope: -4.88 x10^{-3} thresh: -0.301	$w_{El5ht,mean}$	0.39 mV	[2]	slope: -6.81 x10^{-3} thresh: -0.46
$Erev_e$	0 mV	Typical	slope: -0.056 thresh: 1.53	$w_{El5ht,median}$	0.19 mV	[2]	slope: -6.81 x10^{-3} thresh: -0.46
$Erev_{Ei}$	-68 mV	=$Vrest_E$	slope: 3.2x10^{-3} thresh: -3.77	$w_{EIsom,mean}$	0.5 mV	No data	slope: -3.61 x10^{-3} thresh: -0.359
$Erev_{lpvi}$	-68 mV	=$Vrest_{lpv}$	slope: -0.010 thresh: -0.617	$w_{EIsom,median}$	0.4 mV	No data	slope: -3.61 x10^{-3} thresh: -0.359
$Erev_{l5hti}$	-62 mV	=$Vrest_{l5ht}$	slope: 3.8x10^{-3} thresh: 0.132	$w_{lpvE,mean}$	0.52 mV	[2]	slope: 6.41 x10^{-3} thresh: -1.47
$Erev_{Isomi}$	-57 mV	=$Vrest_{Isom}$	slope: -4.2x10^{-3} thresh: -0.088	$w_{lpvE,median}$	-0.29 mV	[2]	slope: 6.41 x10^{-3} thresh: -1.47
$pcon_{EL4E}$	0.15	No data	slope: -0.121 thresh: 2.99	$w_{lpvlpv,mean}$	-0.56 mV	[2]	slope: -2.52 x10^{-3} thresh: -0.345
$pcon_{EL4lpv}$	0.15	No data	slope: 1.38x10^{-3} thresh: 0.029	$w_{lpvlpv,median}$	-0.44 mV	[2]	slope: -2.52 x10^{-3} thresh: -0.345
$pcon_{EL4l5ht}$	0	No data	Not tested	$w_{lpvl5ht,mean}$	-0.83 mV	[2]	slope: -4.24 x10^{-3} thresh: -0.266
$pcon_{EL4Isom}$	0.15	No data	slope: 7.62 x10^{-3} thresh: -0.245	$w_{lpvl5ht,median}$	-0.6 mV	[2]	slope: -4.24 x10^{-3} thresh: -0.266
$pcon_{EE}$	0.17	[2]	slope: 5.86 x10^{-3} thresh: 6.084	$w_{l5htE,mean}$	-0.49 mV	[2]	slope: -3.61 x10^{-4} thresh: -0.018
$pcon_{Elpv}$	0.575	[2]	slope: -1.17 x10^{-3} thresh: -0.099	$w_{l5htE,median}$	-0.3 mV	[2]	slope: -3.61 x10^{-4} thresh: -0.018
$pcon_{El5ht}$	0.24	[2]	slope: -6.44 x10^{-3} thresh: -0.541	$w_{l5htlpv,mean}$	-0.49 mV	[2]	slope: -1.77 x10^{-3} thresh: -0.187
$pcon_{EIsom}$	0.5	[5]	slope: -4.37 x10^{-3} thresh: -0.27	$w_{l5htlpv,median}$	-0.15 mV	[2]	slope: -1.77 x10^{-3} thresh: -0.187

$pcon_{IpvE}$	0.6	[2]	slope: 7.16×10^{-3} thresh: -1.049	$w_{I5htI5ht,mean}$	-0.37 mV	[2]	slope: -4.12×10^{-3} thresh: -0.416
$pcon_{IpvIpv}$	0.55	[2]	slope: -2.61×10^{-3} thresh: -0.0456	$w_{I5htI5ht,median}$	-0.23 mV	[2]	slope: -4.12×10^{-3} thresh: -0.416
$pcon_{IpvI5ht}$	0.24	[2]	slope: -2.81×10^{-3} thresh: -0.458	$w_{IsomE,mean}$	-0.5 mV	No data	slope: -0.013 thresh: -0.984
$pcon_{IpvIsom}$	0	No data	Not tested	$w_{IsomE,median}$	-0.4 mV	No data	slope: -0.013 thresh: -0.984

628 Source [1] is (Lefort et al., 2009), [2] is (Avermann et al., 2012), [3] is (Fanselow et al., 2008),

629 [4] is (Kinnischtzke et al., 2012), [5] is (Fino and Yuste, 2011). *N* is number of neurons, *Vrest* is

630 resting potential, *Vth* is spike voltage threshold, *Rin* is input resistance, *tref* is refractory period,

631 *τm* is the membrane time constant, *τsyn* is the synaptic time constant with the first subscript

632 indicating the postsynaptic neuron type and the second subscript the neurotransmitter type of

633 the presynaptic neuron (*e* or *i*), *Erev* is the synaptic reversal potential, *pcon* is the synaptic

634 connection probability, *prel* is the synaptic release probability, *w* is the mean or median post-

635 synaptic potential amplitude as indicated. For all neuronal parameters, the subscript indicates

636 the neuron type: *E* is L2/3 excitatory neurons, *Ipv* is PV neurons, *I5ht* is $5HT_{3A}R$ neurons, *Isom*

637 is SOM neurons, and *EL4* is L4 excitatory neurons. For synaptic parameters, the first and

638 second subscripts indicate the pre- and post-synaptic neuron types, respectively.

639 An important caveat is that although this model may be considered detailed by some

640 measures, it also simplifies many aspects of L2/3 circuit. For example, we assumed that all

641 $5HT_{3A}R$ cells were homogeneous, even though they likely separate into different subclasses

642 with type-specific connectivity (Gentet, 2012; Petersen and Crochet, 2013). Layer 2 and layer

643 3 may also consist of distinct cell populations (Petersen and Crochet, 2013). Not all likely

644 connections were included in the model (Dalezios et al., 2002; Pfeffer et al., 2013), and

645 connectivity was assumed to be random, even though it is likely non-random (Tomm et al.,

646 2014). Although these choices will likely not affect the conclusions of the current study, they

647 may be important to consider for future work that seeks to understand the biological function of

648 the L2/3 somatosensory microcircuit.

649 **_Logistic model_**

650 From the L2/3 circuit model simulations, we numerically estimated the probability *q* that each

651 neuron in the model fires a spike as a function of the fraction of L4 inputs that were active, *f*.

652 We then used the generalized linear model regression tool 'glmfit' in MATLAB to find the best

34

653   fit of the two logistic model parameters for each neuron:

654    $q(f) = \frac{1}{1+\exp\left(-\beta(f-f_{1/2})\right)}$,

655   where the parameter β represents the slope, and the parameter $f_{1/2}$ represents the fraction of

656   active L4 neurons at which the response probability $q = 0.5$. For clarity of presentation, in the

657   main text we converted this $f_{1/2}$ parameter to what we termed the 'threshold', f_{thresh}, which we

658   defined as the fraction of L4 neurons needed to reach a specified spike probability, q_{thresh}.

659   Throughout the study we fixed $q_{thresh} = 0.01$. The threshold is related to $f_{1/2}$ via the inverse of

660   the logistic function

661    $f_{thresh} = f_{1/2} + \log\left(\frac{q_{thresh}}{1-q_{thresh}}\right)/\beta$.

662   We computed firing rates and pairwise correlations from the logistic model (Figures 3—4) in

663   the following way. First, we assumed that the fraction of active L4 neurons is described by a

664   normally   distributed   random   variable   with   zero   mean   and   unit   variance:

665    $p(f) = \frac{\exp\left(-f^2/2\right)}{\sqrt{2\pi}} = \mathcal{N}(0,1)$.

666   We defined the β and $f_{1/2}$ parameters relative to the mean and standard deviation of the input

667   distribution. Since q is a monotonically increasing function of f, the probability distribution for q

668   is

676    $p(q) = p(f(q))\left|\frac{df}{dq}\right|$

669   where $f(q)$ is the inverse of the logistic function $q(f)$ and

670    $\frac{df}{dq} = \frac{\left(\exp\left(-\beta(f-f_{1/2})\right)+1\right)^2}{\beta\exp(-\beta(f-f_{1/2}))}$. We calculate a neuron's mean firing rate μ as the expectation of q,

671    $\mu = \mathbb{E}[q] = \int_0^1 [q \times p(q)]dq = \int_0^1 \left[q \times p(f(q))\left|\frac{df}{dq}\right|\right]dq$.

672   We calculate the pairwise covariance of two homogeneous neurons driven by a common input

673   f as

674    $\mathrm{cov} = \mathbb{E}[q^2] - (\mathbb{E}[q])^2 = \mathbb{E}[q^2] - \mu^2 = \int_0^1 \left[q^2 \times p(f(q))\left|\frac{df}{dq}\right|\right]dq - \mu^2$,   then   find   the   pairwise

675   correlation by normalizing the covariance by the neurons' shared variance, $\mathrm{var} = \mu(1-\mu)$.

35

677   For fitting the logistic model to the recorded neural firing rates and correlations (Figure 5), we

678   considered a population model where the joint probability distribution across threshold and

679   slope was specified by a 2D Gaussian, which has five parameters: threshold mean and s.d.,

680   slope mean and s.d., and slope-threshold correlation. The three constraint statistics we

681   considered from the neural population data were the mean neural ON probability, the s.d. of

682   neural ON probabilities, and the mean pairwise correlations. We found the best-fit model

683   parameters for each dataset using stochastic gradient descent (code available at

684   https://github.com/cianodonnell/ODonnelletal_2017_imbalances). Briefly, the fitting procedure

685   followed: 1) initialize the parameters at a starting guess points, 2) compute the predicted three

686   output firing statistics via numerical integration over the model's probability distributions, 3)

687   compute the fitting error as the summed squared difference between the model output

688   predictions and the target values, 4) generate a new set of parameter values by adding a small

689   perturbation of a zero-mean Gaussian random number to each parameter, 5) compute the new

690   output statistics, 5) recompute the fitting error, 6) if the new error is smaller than the old error,

691   accept the updated parameter values, otherwise reject them and revert to the old parameters,

692   7) return to step 4 unless the error is lower than the desired tolerance. We checked for fit

693   convergence by sampling a large number of logistic model parameters from the fitted 2D

694   Gaussian, drawing binary samples from these logistic model neurons and computing the ON

695   probability mean and s.d., and mean pairwise correlation from the synthetic binary samples,

696   and comparing the computed statistical values to the original data statistics (Figure 5 –

697   supplemental figure 1). For the sensitivity analysis presented in Figure 6, we numerically

698   computed the partial derivative in mean firing rate and pairwise correlation with respect to the

699   mean slope and mean threshold parameters in the population logistical model, using standard

700   finite difference methods.

701   ***Statistical tests***

702   To avoid parametric assumptions, all statistical tests were done using standard bootstrapping

703   methods with custom-written MATLAB scripts. For example when assessing the observed

704   difference between two group means $\Delta\mu_{obs}$ we performed the following procedure to calculate

705   a p-value. First we pool the data points from the two groups to create a null set S_{null}. We then

706 construct two hypothetical groups of samples S_1 and S_2 from this by randomly drawing n_1 and

707 n_2 samples with replacement from S_{null}, where n_1 and n_2 are the number of data points in the

708 original groups 1 and 2 respectively. We take the mean of both hypothetical sets $\Delta\mu_1$ and $\Delta\mu_2$

709 and calculate their difference $\Delta\mu_{null} = \Delta\mu_1 - \Delta\mu_2$. We then repeat the entire procedure 10^7

710 times to build up a histogram of $\Delta\mu_{null}$. This distribution is always centered at zero. After

711 normalizing, this can be interpreted as the probability distribution $f(\Delta\mu_{null})$ for observing a group

712 mean difference of $\Delta\mu_{null}$ purely by chance if the data were actually sampled from the same

713 null distribution. Then the final p-value for the probability of finding a group difference of at

714 least $\Delta\mu_{obs}$ in either direction is given by $p = \int_{-\infty}^{-\Delta\mu_{obs}} f(\Delta\mu_{null})d\Delta\mu_{null} + \int_{\Delta\mu_{obs}}^{\infty} f(\Delta\mu_{null})d\Delta\mu_{null}$.

715 For Figure 5C we estimated 2-dimensional 95% confidence ellipses for the shift in mean slope-

716 threshold parameters between Fmr1 KO and WT by computing the sample error variances and

717 covariance through bootstrapping. Then the 95% confidence ellipse can be computed using

718 the Chi-squared distribution. We plotted the confidence interval ellipse using the MATLAB

719 function error_ellipse.m, downloaded from

720 https://www.mathworks.com/matlabcentral/fileexchange/4705-error-ellipse.

721 ### Conversion from firing rate to ON/OFF probabilities for Ca^{2+} imaging data

722 For the Ca^{2+} imaging data, we began with estimated firing rate time series $r_i(t)$ for each neuron

723 i recorded as part of a population of N neurons. For later parts of the analysis we needed to

724 convert these firing rates to binary ON/OFF values. This conversion involves a choice. One

725 option would be to simply threshold the data, but this would throw away information about the

726 magnitude of the firing rate. We instead take a probabilistic approach where rather than

727 deciding definitively whether a given neuron was ON or OFF in a given time bin, we calculate

728 the probability that the neuron was ON or OFF by assuming that neurons fire action potentials

729 according to an inhomogeneous Poisson process with rate $r_i(t)$. The mean number of spikes

730 $\lambda_i(t)$ expected in a time bin of width Δt is $\lambda_i(t) = r_i(t)\Delta t$. We choose $\Delta t = 1$ second. Under the

731 Poisson model the actual number of spikes m in a particular time bin is a random variable that

732 follows the Poisson distribution $P(m=k) = \lambda^k \exp(-\lambda) / k!$. We considered a neuron active (ON) if

733 it is firing one or more spikes in a given time bin. Hence the probability that a neuron is ON is

734 $p_{on}(t)=1-P(m=0)=1-\exp(\lambda)$. This approach has two advantages over thresholding: 1) it

37

735 preserves some information about the magnitude of firing rates, and 2) it acts to regularize the

736 probability distribution for the number of neurons active by essentially smoothing nearby

737 values together.

738 ***Entropy estimation for large numbers of neurons***

739 Entropy was estimated by fitting a statistical model we recently developed, called the

740 population tracking model (O'Donnell et al., 2017), to the binarized Ca^{2+} imaging data. Briefly,

741 the population tracking model fits two aspects of the data: the probability distribution for the

742 number of neurons synchronously active in the population, and also the conditional firing

743 probability that each individual neuron is active given the population count. Hence the model

744 captures both some aggregate statistics of the population activity, and some aspects of the

745 heterogeneity across neurons. See (O'Donnell et al., 2017) for complete details and validation

746 of the method. Code for fitting the model to data is available at

747 https://github.com/cianodonnell/PopulationTracking.

748 The entropy/neuron generally decreased with the number of neurons considered as result of

749 the population correlations (Figure 7B), so we needed to control for neural population size

750 when comparing data from different experimental groups. On the one hand, we would like to

751 study as large a number of neurons as possible, because we expect the effects of collective

752 network dynamics to be stronger for large population sizes and this may be the regime where

753 differences between the groups emerge. On the other hand, our recording methods allowed us

754 to sample only typically around ~100 neurons at a time, and as few as 40 neurons in some

755 animals. Hence we proceeded by first estimating the entropy/neuron in each animal by

756 calculating the entropy of random subsets of neurons of varying size from 10 to 100 (if

757 possible) in steps of 10. For each population size we sampled a large number of independent

758 subsets, calculated the entropy of each. Finally, for each dataset we fit a double exponential

759 function to the estimated entropy/neuron as a function of the number of neurons: $H/N =$

760 $A^*\exp(-b^*N) + C^*\exp(-d^*N) + e$, and used this fit to estimate H/N for 100 neurons.

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38

768   **Competing Interests**

769   The authors have no competing interests to declare.

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976 **Figure Supplements**
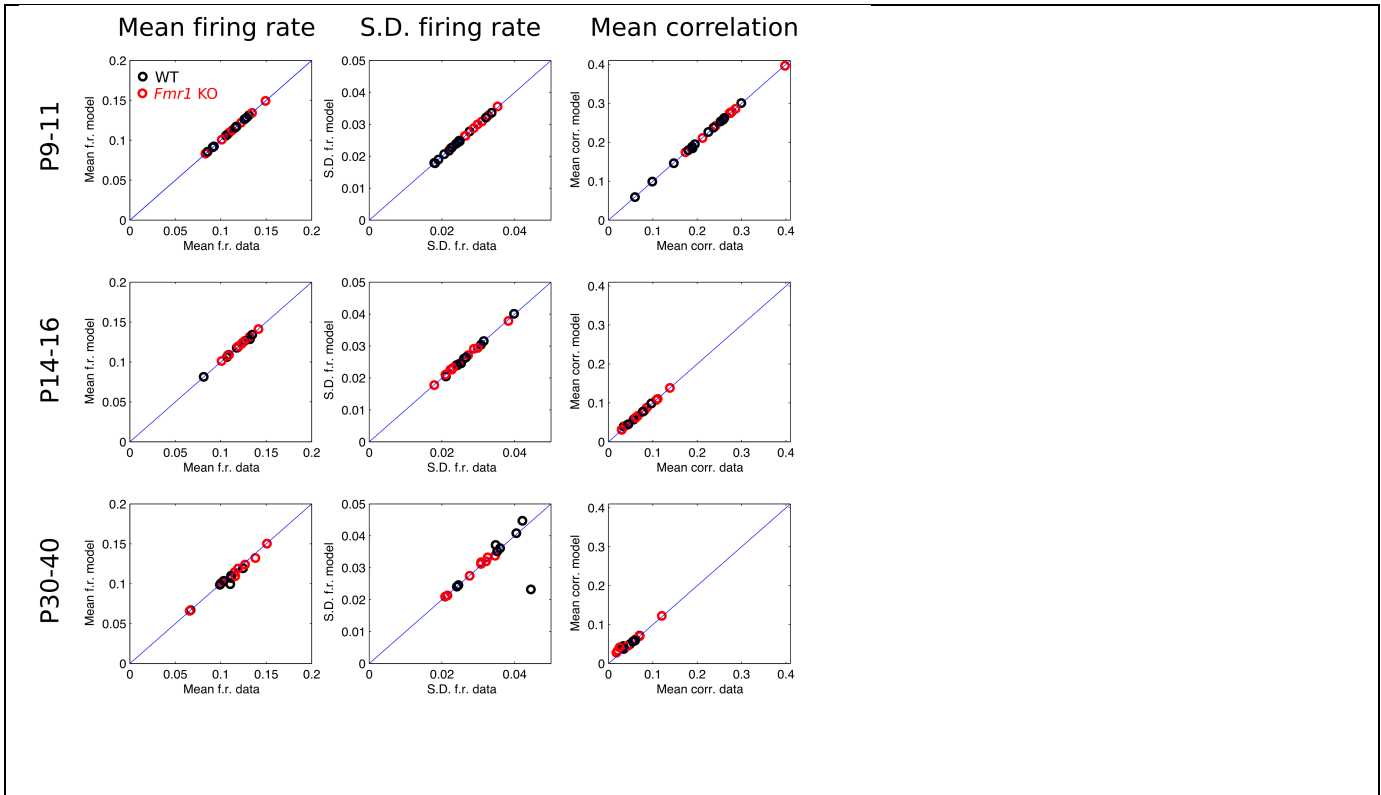
Figure 5 – figure supplement 1. Agreement between population logistic model activity statistics and raw data statistics.

Black circles are WT, red are *Fmr1* KO. Each data point corresponds to a recording from a single animal. Each plot shows model prediction versus raw data target value. Plots in each row correspond to data from a different age group (P9—11, P14—16, and P30—40), and each column corresponds to one of the three target activity statistics (mean firing rate, s.d. in firing rate, and mean pairwise correlation). Blue line is identity.

977
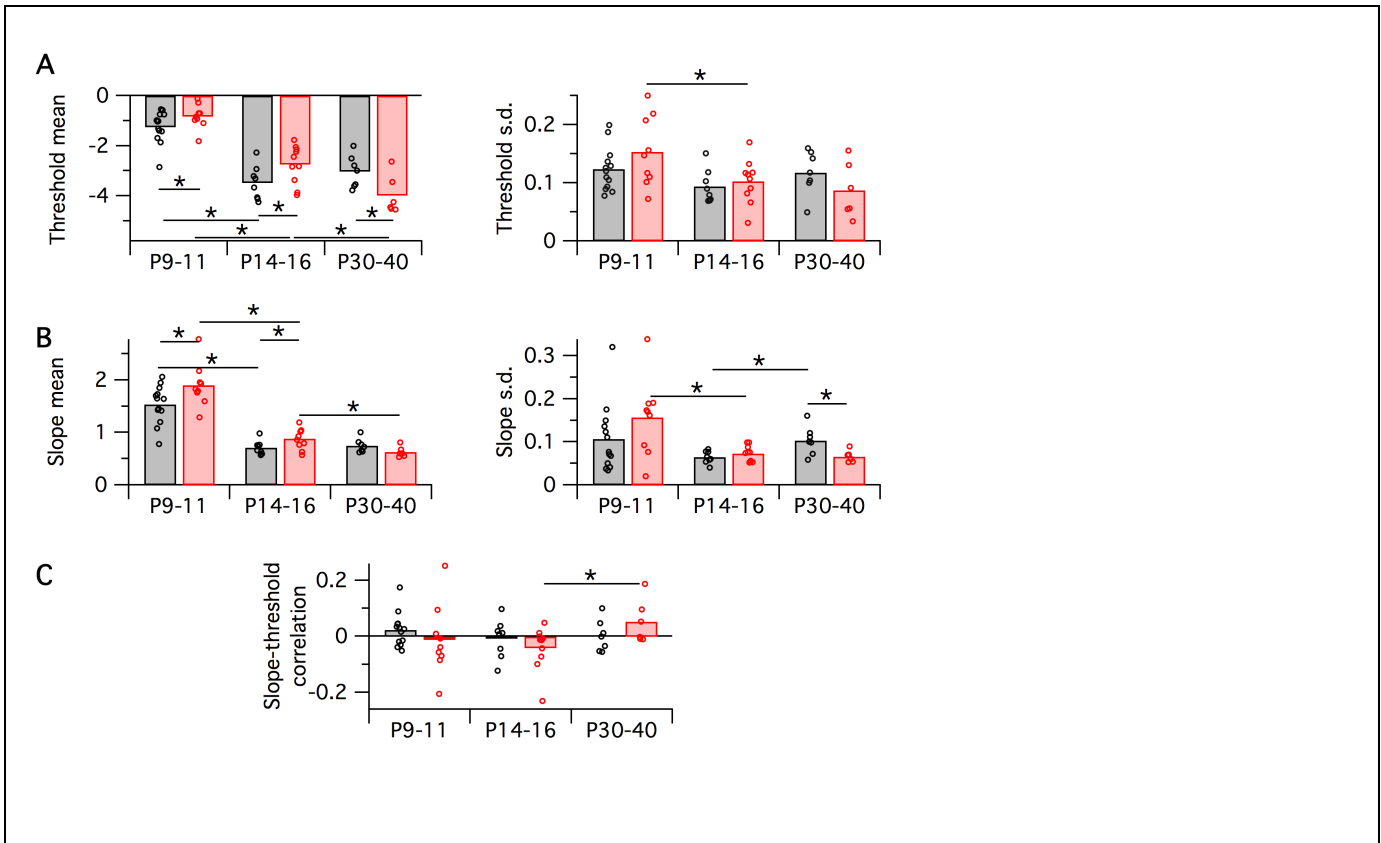
Figure 5 – figure supplement 2. Variation in population logistic model parameter fits with developmental age group and genotype.

Black symbols are WT, red are *Fmr1* KO. Each data point corresponds to a recording from a single animal, bars correspond to group means. In all cases, horizontal bar with asterisk indicates a significant difference in group means (*p*<0.05 via bootstrapping).
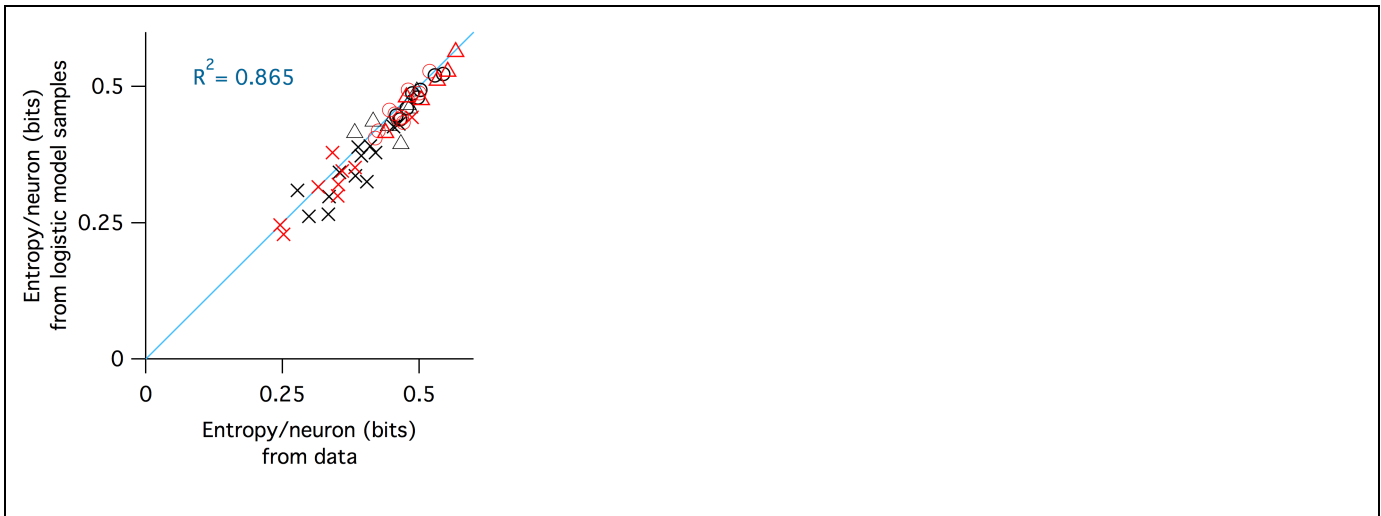
A: Logistic threshold parameter mean (left) and s.d. (right).

B: Logistic slope parameter mean (left) and s.d. (right).

C: Slope-threshold correlation parameter.

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Figure 6 – figure supplement 1. Agreement between entropy estimated from raw data with entropy estimated from samples from fitted logistic models.

Black symbols are WT, red are *Fmr1* KO. Crosses are P9—11, circles P14—16, triangles P30—40. Blue line is identity, the R^2 value of which is reported in the inset.

980