

FMRP Expression in the Visual System

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Background

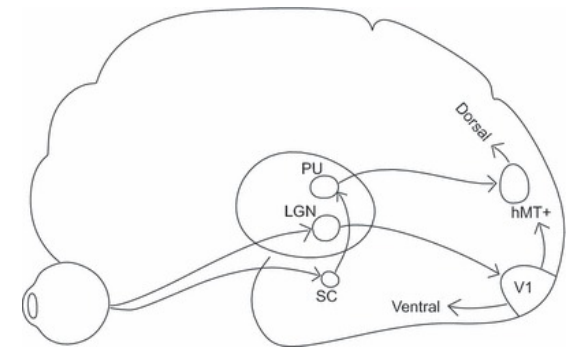
Fragile X Syndrome – a genetic condition caused by a mutation in the FMR1 gene

- Causes a range of symptoms, including developmental delays, learning disability, and sensory processing disorders (such as visual)
- 200,000 US cases per year

FMRP – protein that helps develop synapses; absent in those with Fragile X

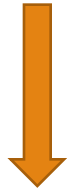
The Visual System:

- Retina – converts light into neural signals
- Visual cortex (layer 5) – receives and processes visual information from retina
- Superior colliculus – receives and processes information from retina and visual cortex

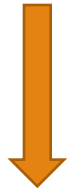


Major Questions

What is the developmental role of FMRP in functional circuit formation?



How does loss of FMRP affect visual circuit organization and function?

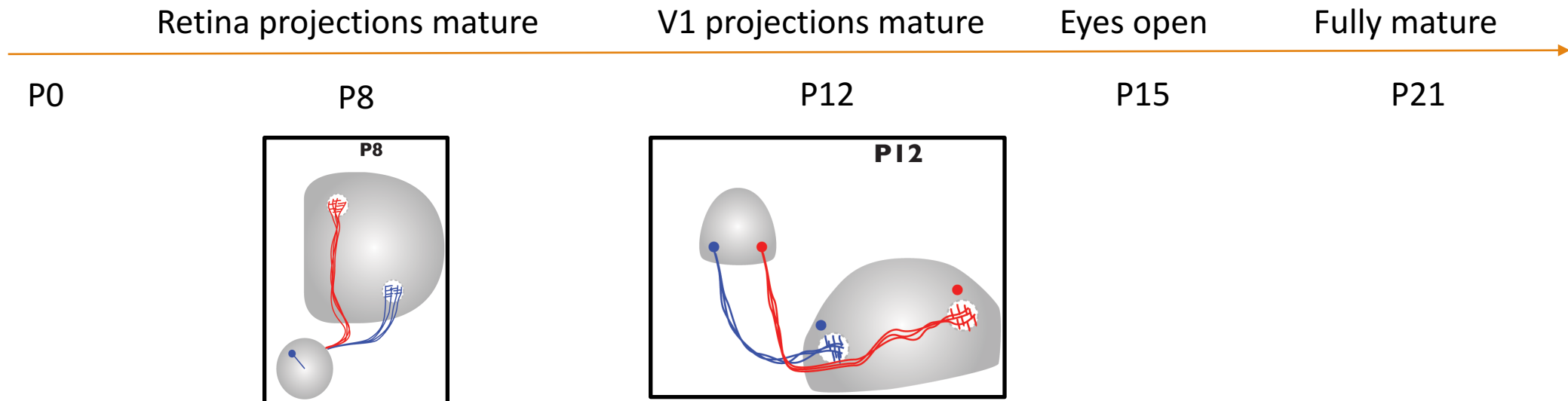


How does this result in visual processing deficits in FXS?

My Role

Where and when during development is FMRP expressed?

Quantify FMRP expression in the visual cortex (V1), superior colliculus (SC), and retina at varying stages of development, P2-P40



Method

Step 1: Sectioning

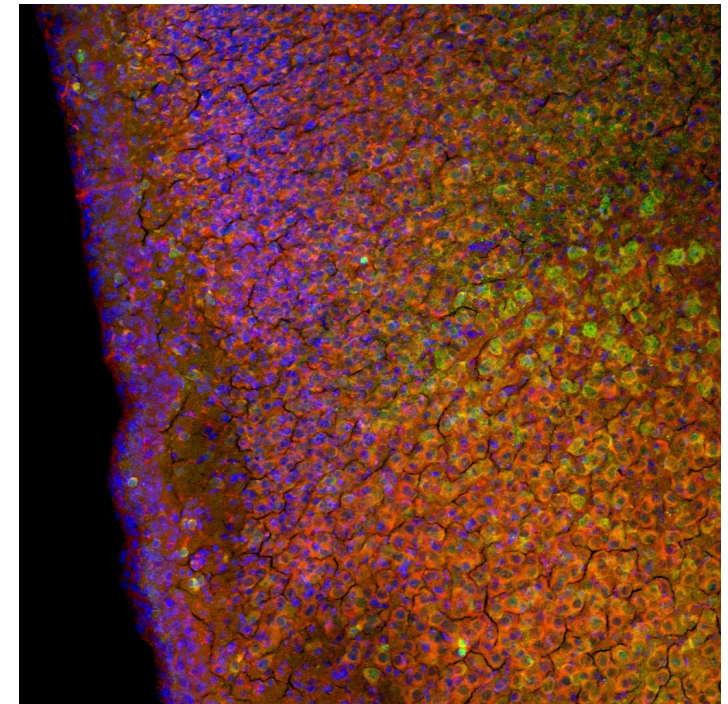
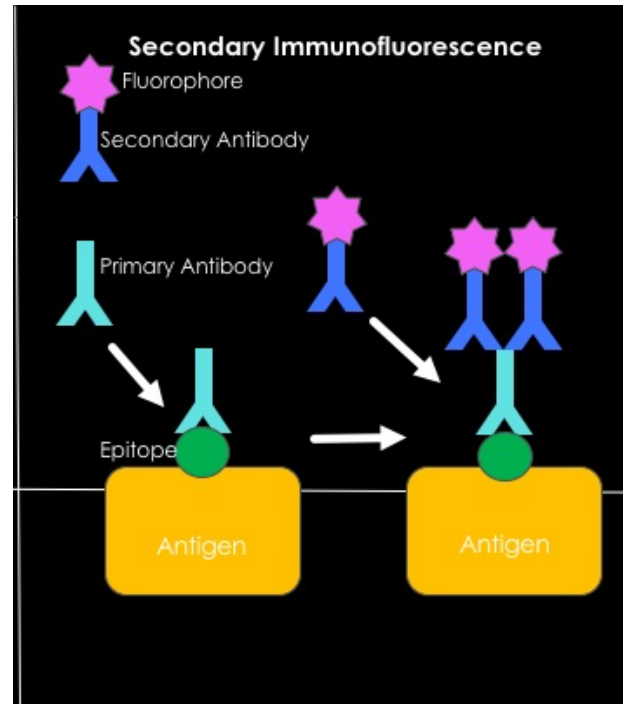
- Cut 50 μm on cryostat (brain; free floating)
- Cut 10 μm on cryostat (eyes; slides)

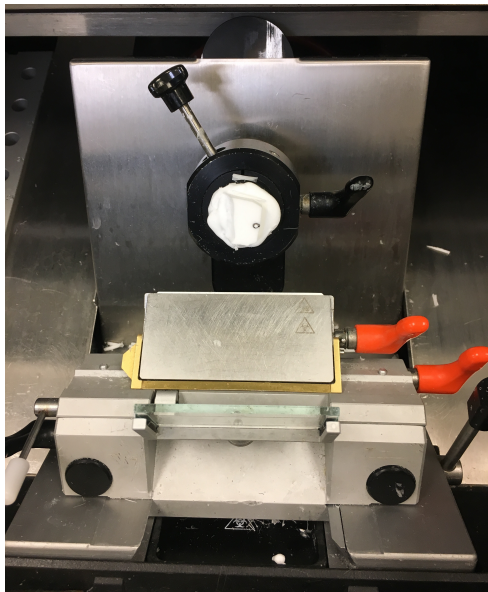
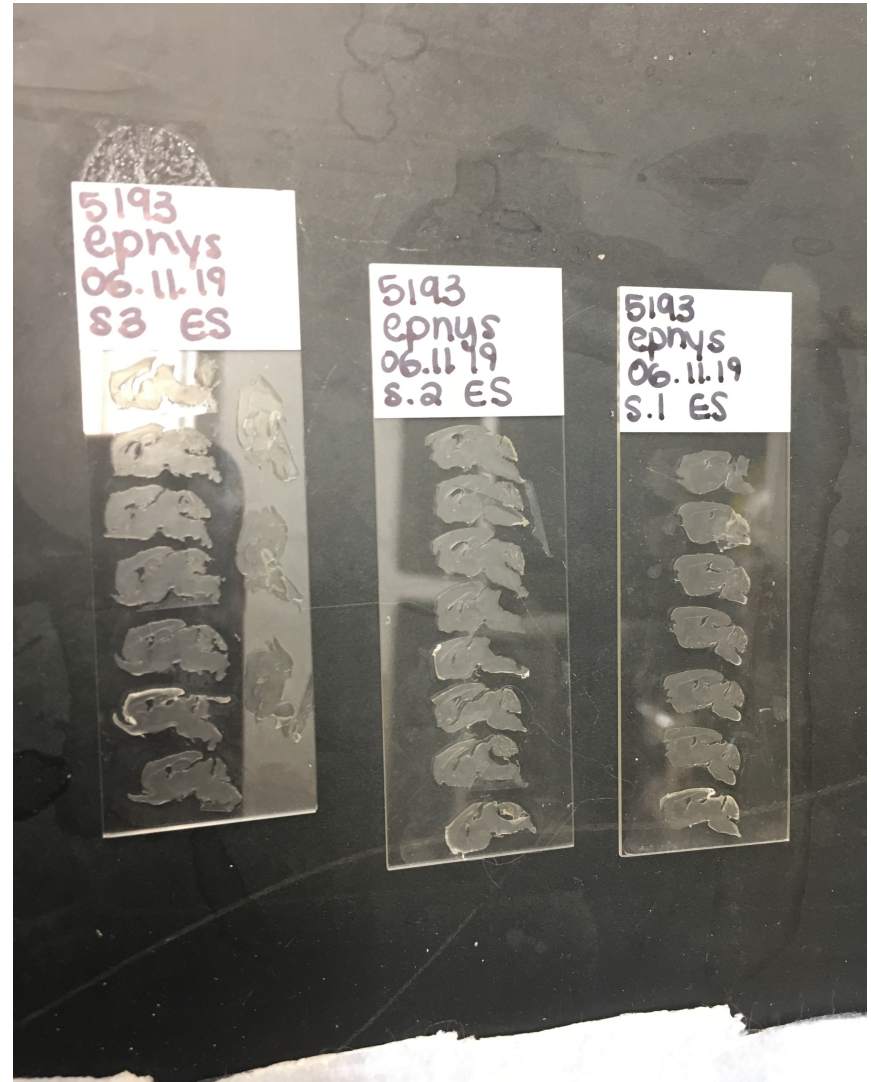
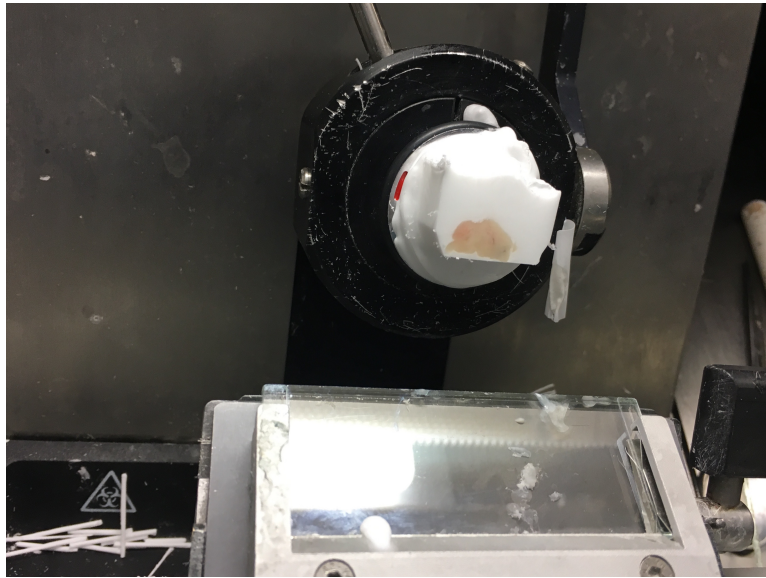
Step 2: Staining

- a) Antigen retrieval
- b) Blocking
- c) Primary & secondary antibody: 2F5 (FMRP), NeuN, DAPI

Step 3: Imaging

Step 4: Analysis





FMSP Data													
Home Insert Page Layout Formulas Data Review View													
Calibri (Body) 12 A A													
B I U Bold Italic Underline Merge & Center Conditional Formatting													
L79 X fx 37													
	A	B	C	D	E	F	G	H	I	J	K	L	M
31	P8	1	SC6	6.1	35	3.4	35	1.3	54	2.4	40	43.25	
32	P8	1	SC7	8.2	44	3.2	41	8.1	40	4.5	35	40	
33	P8	1	SC8	2.7	42	5.8	40	4.6	35	3.2	50	41.75	
34	P8	1	V1	6.3	40	6.5	43	4.7	56	2.4	45	46	
35	P8	1	V2	7.5	29	1.5	38	4.4	43	4.3	37	39.25	
36	P8	1	V3	1.4	34	4.8	40	7.7	46	6.1	41	40.25	
37	P8	1	V4	1.4	38	7.2	43	5.6	48	8.4	45	43.5	
38	P8	1	V5	3.2	47	6.1	56	1.4	44	2.5	57	51	
39	P8	1	V6	7.3	44	6.8	41	7.7	36	3.8	49	43.5	
40	P8	1	V7	3.5	40	1.8	34	8.6	35	3.4	46	38.75	
41	P8	1	V8	4.6	56	2.7	53	3.1	43	4.8	48	50	
42	P8	2	SC1.1	8.7	41	1.8	36	1.5	33	8.3	54	41	
43	P8	2	SC1.2	6.6	35	2.8	42	8.1	41	5.4	35	38.25	
44	P8	2	SC1.3	8.3	27	7.2	33	1.6	40	6.6	51	27.75	
45	P8	2	SC1.4	5.1	35	2.5	52	1.1	33	8.1	35	38.75	
46	P8	2	SC2	8.3	65	7.8	57	7.1	39	7.3	46	51.75	
47	P10	1	SC1.1	7.4	39	4.8	33	5.7	30	3.6	50	40.75	
48	P10	1	SC1.2	4.7	32	5.6	40	1.3	49	1.6	38	39.75	
49	P10	1	SC1.3	2.7	48	5.7	33	3.8	36	4.1	51	42	
50	P12	1	SC1.1	1.5	39	4.2	42	5.1	43	4.7	43	41.75	
51	P12	1	SC1.2	1.4	39	4.7	37	7.2	31	4.5	42	37.25	
52	P12	1	SC1.3	7.1	38	3.5	41	7.7	44	6.5	46	42.25	
53	P12	1	SC1.4	8.4	33	4.2	39	4.6	34	4.6	34	39.75	
54	P12	1	SC1	2.5	38	8.4	33	3.5	42	1.1	35	37	
55	P12	1	V1	3.5	44	2.7	42	1.7	41	7.3	50	44.25	
56	P12	1	V2	8.1	33	6.7	38	5.8	38	5.6	39	38.5	
57	P12	1	V3	3.3	34	8.5	37	4.7	38	3.2	44	38.25	
58	P15	1	SC1	3.3	45	5.1	50	5.7	46	4.4	35	44	
59	P15	1	V1	1.6	29	8.6	36	3.8	47	5.5	30	34.25	
60	P16	1	SC	1.6	32	5.5	37	6.1	37	4.6	31	34.25	
61	P16	1	SC1.1	4.5	45	7.3	36	2.3	39	5.7	39	39.5	
62	P16	1	SC1.2	4.6	37	3.5	33	8.7	34	4.3	32	34	
63	P16	1	SC1.3	3.9	35	7.1	39	6.3	38	8.6	39	37.75	
64	P16	1	SC2.2	6.4	35	6.6	40	4.2	38	4.7	40	38.25	
65	P16	1	SC2.3	8.6	32	6.4	37	7.1	39	4.5	43	37.75	
66	P16	1	SC2.4	4.4	38	6.3	53	1.2	46	6.3	46	45.75	
67	P16	1	SC2.5	6.4	45	4.6	47	1.5	40	6.6	52	46	
68	P16	1	SC2.6	8.8	32	1.3	34	2.7	37	2.1	41	36	
69	P16	1	SC2.7	5.7	43	6.5	36	5.6	47	2.8	48	43.5	
70	P16	1	SC3.1	1.8	36	2.6	44	2.3	27	6.6	38	36.25	
71	P16	1	SC3.2.1	3.8	39	1.1	49	6.1	37	5.2	40	41.25	

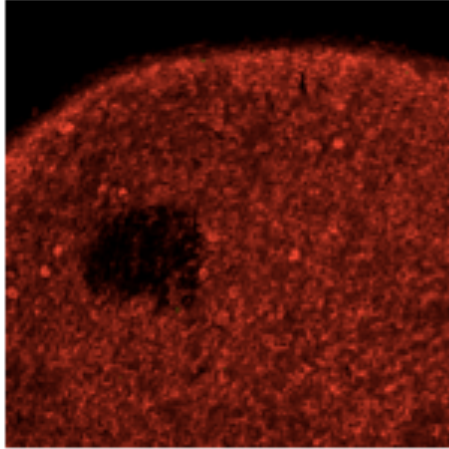
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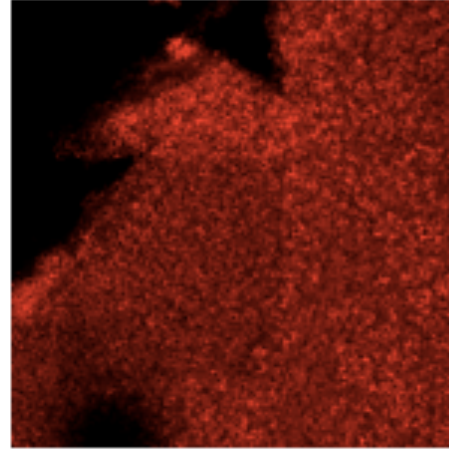
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FMRP Expression in SC

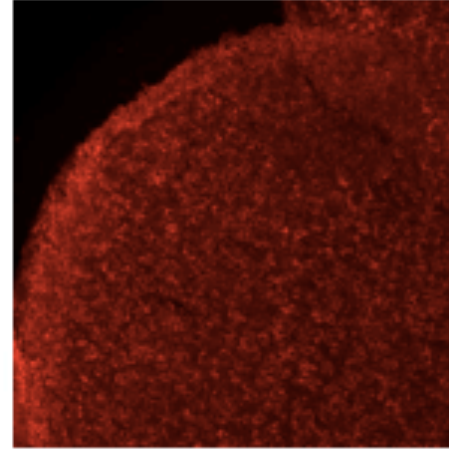
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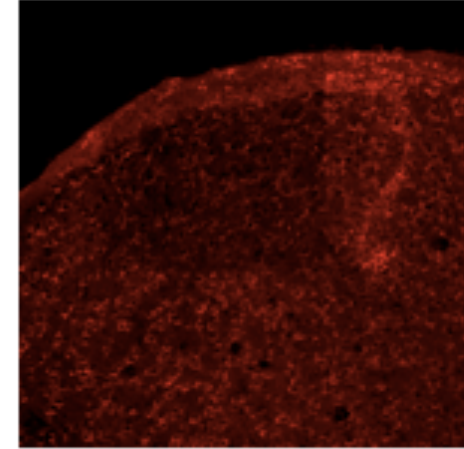
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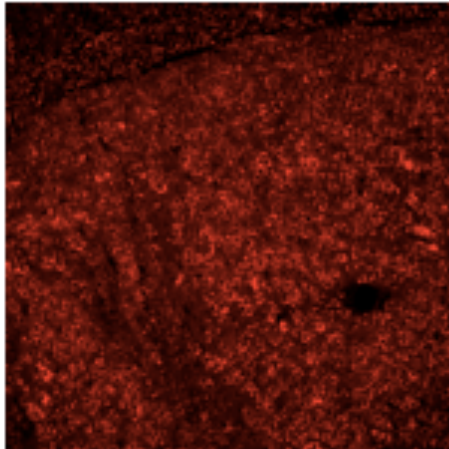
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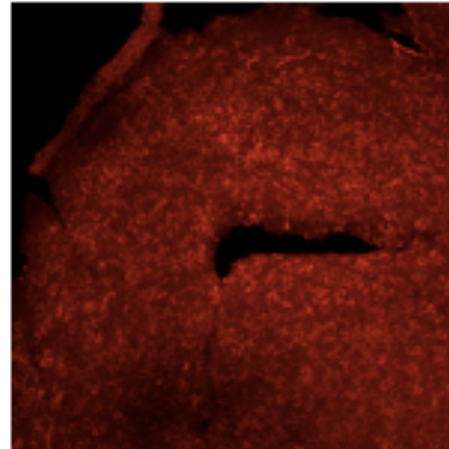
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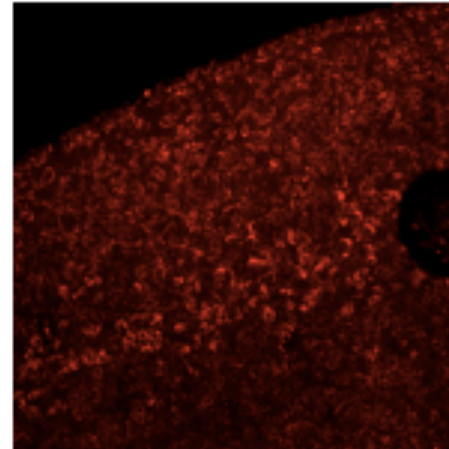
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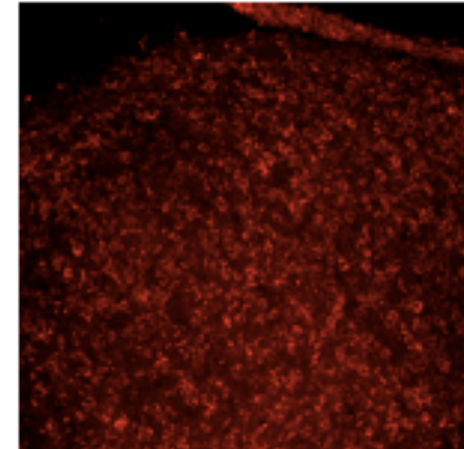
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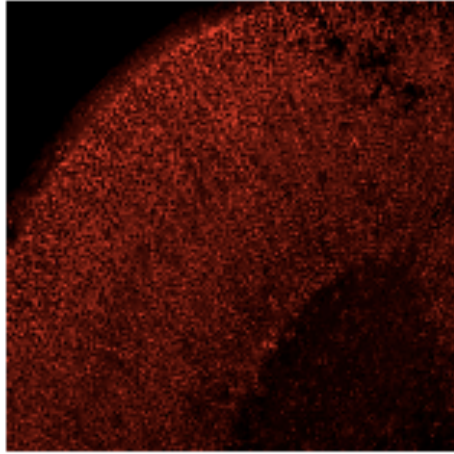


P40

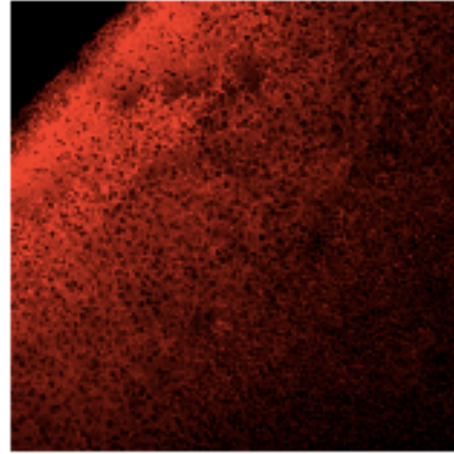


FMRP Expression in V1

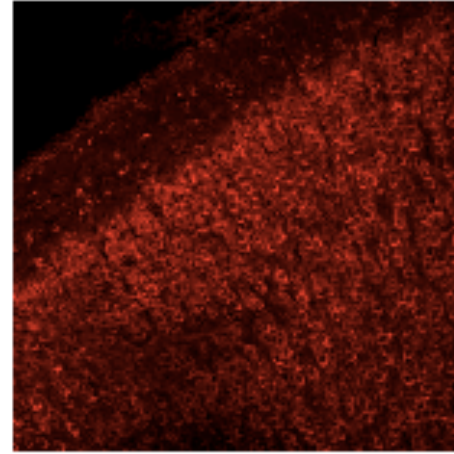
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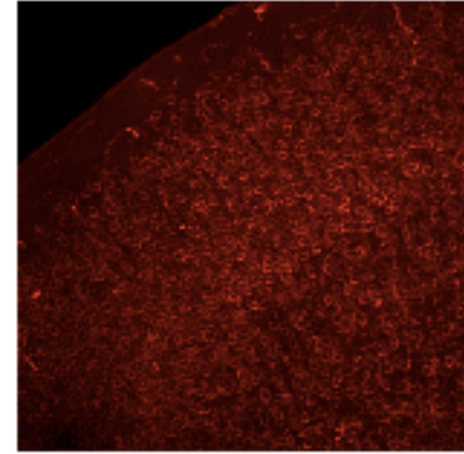
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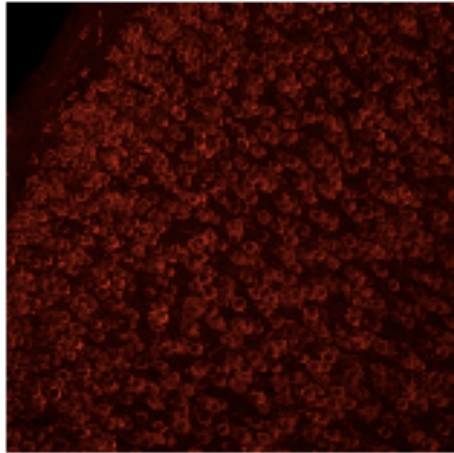
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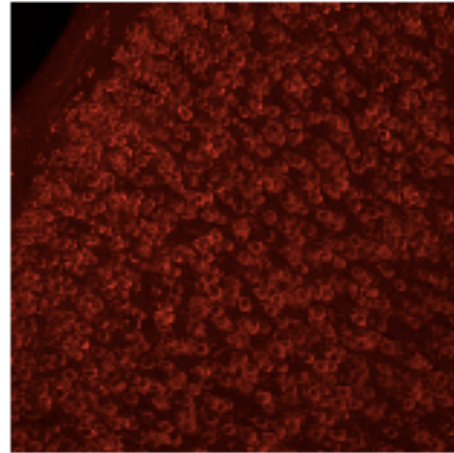
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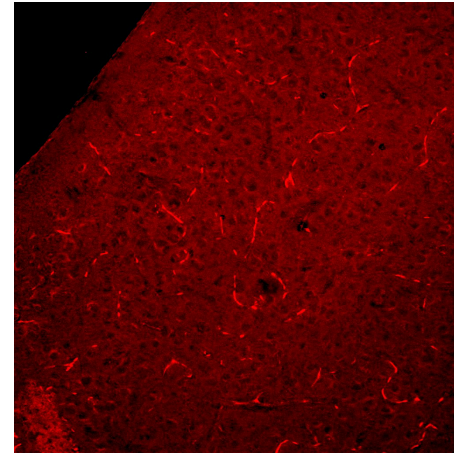
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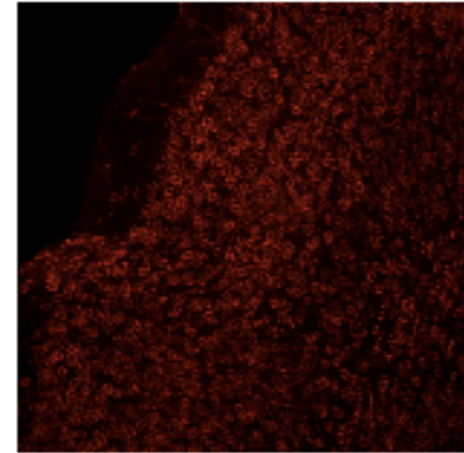
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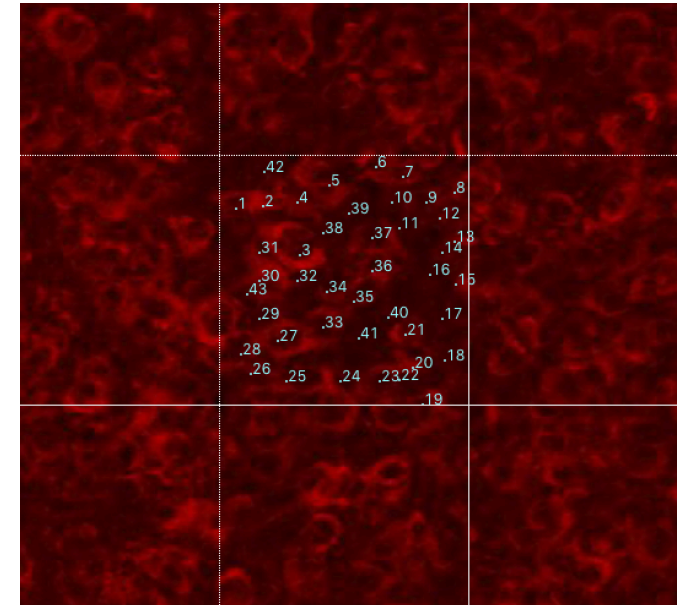
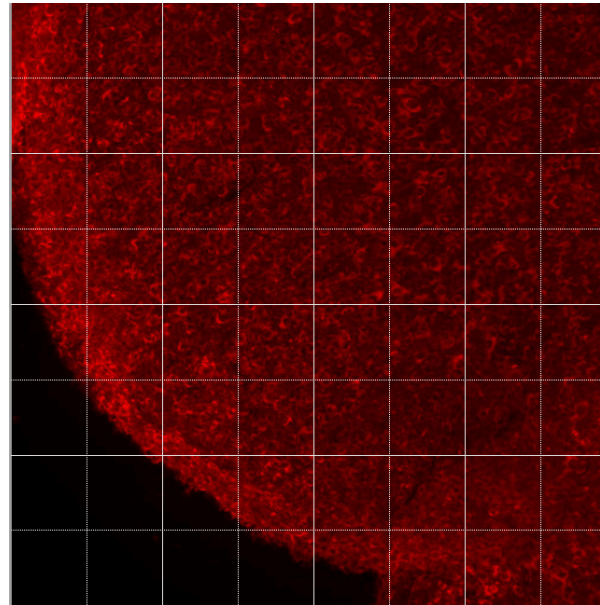
P40



Quantification

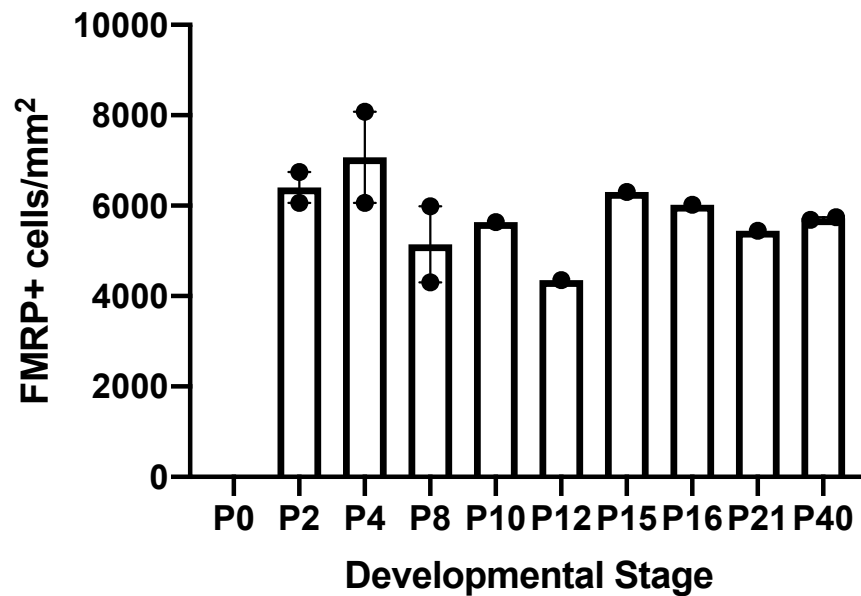
Planned method:

1. Overlay grid onto image in Photoshop
2. Use RNG to randomly select 4 grid squares
3. Count neurons that express FMRP in each square and average the results
4. Convert units to μm^2

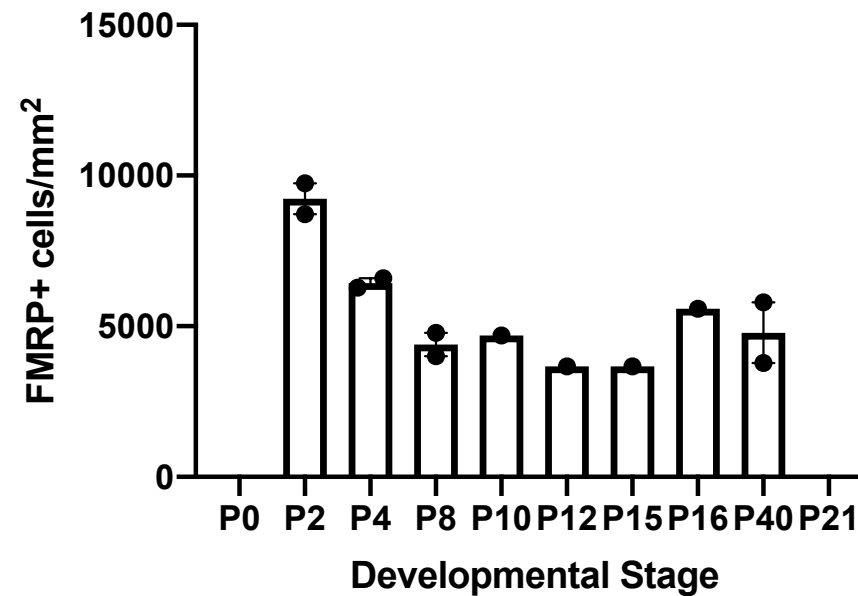


Preliminary Results

Superior Colliculus (Count/Area)



Visual Cortex (Count/Area)



Conclusions

Where and when during development is FMRP expressed?

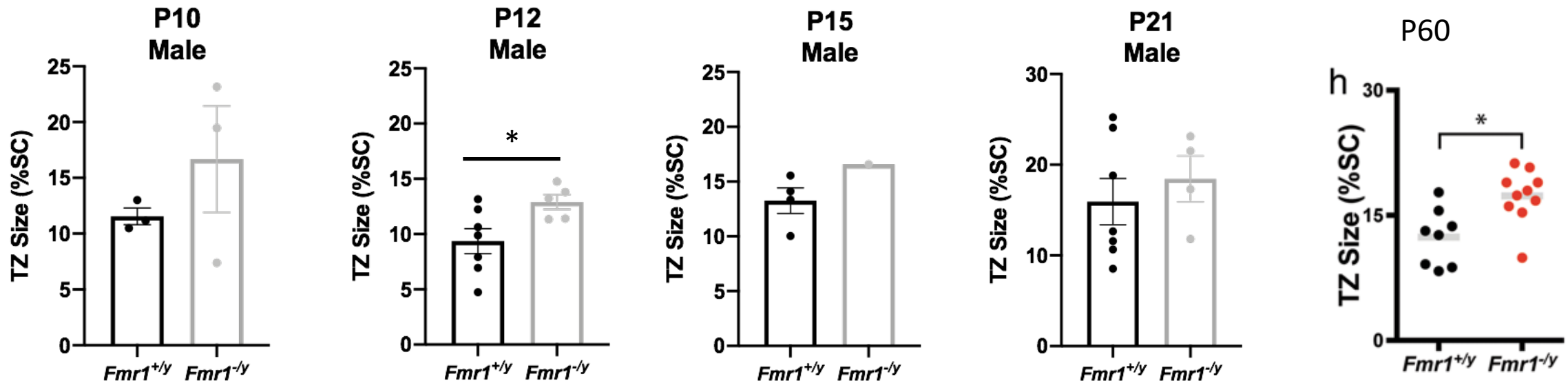
1. Spatial (Is it expressed in V1, SC, and retina?) – Yes, it is expressed in all structures of the visual system that we looked at.
 - However, the V1 could have higher expression than the SC → quantification
2. Temporal (Is it expressed at all stages of development?) – Yes, we see expression consistently from P2 to P40
 - Qualitatively, we see no difference in abundance of FMRP among different stages of development → quantification to confirm

FMRP is likely required for early stages of proper visual map formation and function.

Current hypothesis: The adult phenotype (disrupted function) is likely present in earlier stages of development, because FMRP is always present in our structures of interest.

Future Steps

1. Collect more data on FMRP expression
2. Is the visual circuitry disrupted during development in *Fmr1*^{-/-} mice?
 - Dil injected into V1; termination zone quantified in SC
 - Methods: Genotyping & Termination Zone Quantification (ImageJ)



Acknowledgements



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