

**Within-subject combination of diffusion tensor MRI and CLARITY
reveals alterations in the social brain network resulting from α -
neurexin II deletion**

Supplemental Material

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Supplemental Materials and Methods

Diffusion Tensor MRI

Data Acquisition

Brain MR imaging was performed on a vertical 9.4 Tesla spectrometer (Bruker AVANCE II NMR, Ettlingen, Germany) with an 89 mm wide bore, 3 radio frequency channels with digital broadband frequency synthesis (6-620 MHz) and an imaging coil with diameter of 25 mm for hydrogen (^1H). 3D images for each brain were obtained using a DT-MRI protocol (TE: 35 ms, TR: 700 ms, 10 signal averages). The field of view was set at 128 x 128 x 128, with a cubic resolution of 100 $\mu\text{m}/\text{pixel}$ and a b value of 1200 s/mm^2 . For each brain, diffusion weighted images were obtained in 6 directions, based upon recent published protocols¹⁻⁵. The subject of the number of diffusions gradients has been debated⁶, with studies suggesting limited benefits of using more than 6 directions in biological tissue⁷⁻⁹. The imaging time for each brain was 60 hours.

Image Processing

Parsing of the raw data was semi-automated using DSI Studio, in order to obtain b-values for every normalized gradient vector on the x, y and z orientations. Unwanted background, setting a threshold, smoothing of the data and definition of tissue boundaries was performed prior to the reconstruction of the final 3D image. DTI analysis parameters were calculated as previously described¹⁰.

The *ex vivo* mouse brain 3D diffusion-weighted images were reconstructed from the Bruker binary file using DSI Studio (<http://dsi-studio.labsolver.org>)¹¹. Direction Encoded Colour Map (DEC) images were generated by combining the information from the primary eigenvectors, diffusion images and the FA. Images of the primary vectors and their orientation were reconstructed and superimposed on corresponding FA images to guide the segmentation of discrete anatomical locations according to the brain atlas (Figure 1B-D). Region of interest definition was performed by author EP and corroborated independently by JD, with region area compared between the experimenters (data not shown). The DSI Studio DTI reconstruction characterizes the major diffusion direction of the fibre within the brain^{12, 13}. Extraction of FA (calculated¹⁴) and ADC was performed within selected segmented brain areas for every 3D reconstructed mouse brain.

Regions of Interest (ROIs)

Given the strong social impairments found within *Nrxn2 α* mice, for the current study, we identified the brain regions of interest (ROIs) most closely linked with social behavior, using previously published reports of brain region involvement in social behaviour. Quantification of c-Fos immunoreactivity has highlighted the importance of several amygdala nuclei (including the basolateral) following social exposure¹⁵, but also the anterior cingulate cortex (ACC), prefrontal cortex and the hippocampus¹⁶. Lesions to the primate amygdala alter social interactions^{17, 18}, and amygdala neurons in primates including humans increase firing rates during social scenarios¹⁹⁻²¹. Consistent with these animal studies, amygdala damage in humans²² and amygdala dysfunction in ASD patients²³ impair social responses. Other socially-important brain regions have also been proposed. Notably, several studies have implicated the rodent hippocampus in social behavior, including social memory and sociability²⁴⁻²⁶. For instance, intrahippocampal administration of neurolide-2, which interacts with α -neurexin, specifically impairs sociability, but not anxiety and spatial learning in rats²⁷. These findings are consistent with reports of social deficits in humans with hippocampal damage²⁸ and hippocampal abnormalities in ASD^{29, 30}. Finally, several studies link the frontal cortex, particularly the orbitofrontal cortex, which is strongly anatomically connected with the amygdala³¹, to social processing^{32, 33}, consistent with findings of abnormalities in orbitofrontal cortex in ASD^{31, 34}. Control regions of the primary motor cortex (M1), primary sensory cortex (S1) and the barrel field were chosen for CLARITY (Supp. Figure 5N-O).

CLARITY

Solutions:

Hydrogel solution: 2% PFA 2% acrylamide 0.05% bis-acrylamide and 0.25% VA-044 thermal initiator (2,2'-Azobis[2-(2-imidazolin-2-yl) propane] dihydrochloride) in PBS, pH 7.4.

Clearing buffer: 8% Sodium dodecyl sulfate in 200mM boric acid, pH 8.5.

Multiphoton imaging and analysis

Cleared samples were mounted in custom 3D printed chambers for two-photon imaging. Images were acquired using ZEN Black (Zeiss, Germany). DAPI signal was detected using a 485 nm short pass filter, and neurofilament using a 500-550 nm band pass filter. The power of the excitation laser was varied to maximise the dynamic range for each image, but all other parameters were kept constant. The images were analysed using custom MATLAB (version 9.1, The Mathworks Inc.) scripts. Two-dimensional images were visualised using ImageJ³⁵ and three-dimensional images using Vaa3D³⁶.

Image files were loaded into MATLAB using the BioFormats toolbox³⁷, and the raw image data was obtained, along with the precise voxel dimensions from the metadata. DAPI bleed through into the neurofilament channel was removed by subtraction. Uneven illumination was corrected for, background noise was removed, and the images were smoothed. DAPI images were thresholded using a combination of Otsu's bimodal³⁸ and Rosin's unimodal thresholding algorithms³⁹ and a watershed approach was used to define individual cells. Cell density was calculated as the number of cells per mm^3 . Neurofilament images were segmented firstly by taking the magnitude of the gradient in three dimensions, followed by thresholding. Due to variations in staining intensity of different axons, the thresholding produced segmented axons of various thicknesses that did not necessarily reflect the true structure. To remove this bias, the thresholded images were skeletonised using a homotopic thinning algorithm⁴⁰. The variation in this image was calculated in each dimension, and the standard deviation of this value in each dimension was calculated as the axonal orientation.

Supp. Table 1

Brain Region	DTI Measure	ANOVA Comparison	F Value	P Value
Anterior Hippocampus	FA	Genotype	$F_{(1,10)} = 3.91$	$P = 0.076$
		Hemisphere	$F_{(1,10)} = 3.89$	$P = 0.077$
Mid Hippocampus	FA	Genotype	$F_{(1,10)} = 3.12$	$P = 0.106$
		Hemisphere	$F_{(1,10)} < 1$	$P = 0.515$
Posterior Hippocampus	FA	Genotype	$F_{(1,10)} = 1.99$	$P = 0.192$
		Hemisphere	$F_{(1,10)} = 1.95$	$P = 0.190$
Anterior Hippocampus	ADC	Genotype	$F_{(1,10)} = 3.17$	$P = 0.105$
		Hemisphere	$F_{(1,10)} = 2.20$	$P = 0.169$
Mid Hippocampus	ADC	Genotype	$F_{(1,10)} < 1$	$P = 0.683$
		Hemisphere	$F_{(1,10)} = 1.62$	$P = 0.232$
Posterior Hippocampus	ADC	Genotype	$F_{(1,10)} = 8.88$	$P = 0.014$
		Hemisphere	$F_{(1,10)} < 1$	$P = 0.903$
Anterior Hippocampus	AD	Genotype	$F_{(1,10)} < 1$	$P = 0.976$
		Hemisphere	$F_{(1,10)} = 1.07$	$P = 0.326$
Mid Hippocampus	AD	Genotype	$F_{(1,10)} < 1$	$P = 0.614$
		Hemisphere	$F_{(1,10)} = 1.04$	$P = 0.332$
Posterior Hippocampus	AD	Genotype	$F_{(1,10)} = 3.45$	$P = 0.093$
		Hemisphere	$F_{(1,10)} = 6.19$	$P = 0.032$
Anterior Hippocampus	RD	Genotype	$F_{(1,10)} = 3.03$	$P = 0.113$
		Hemisphere	$F_{(1,10)} < 1$	$P = 0.429$
Mid Hippocampus	RD	Genotype	$F_{(1,10)} < 1$	$P = 0.450$
		Hemisphere	$F_{(1,10)} = 1.33$	$P = 0.275$
Posterior Hippocampus	RD	Genotype	$F_{(1,10)} = 10.83$	$P = 0.008$
		Hemisphere	$F_{(1,10)} = 3.48$	$P = 0.092$

Statistical analysis of the anterior (Bregma -1.94 mm), mid (Bregma -2.46 mm) and posterior (Bregma -3.28 mm) hippocampus for fractional anisotropy (FA), apparent diffusion coefficient (ADC), axial diffusion (AD) and radial diffusion (RD). Analysis was performed using repeated measure two-way ANOVAs for genotype and hemisphere (Sidak correction for multiple comparisons).

Supp. Table 2

Brain Region	DTI Measure	ANOVA Comparison	F Value	P Value
Amygdala-Anterior Hippocampus	AD	Genotype	$F_{(1,10)} < 1$	P = 0.734
		Hemisphere	$F_{(1,10)} = 2.10$	P = 0.178
		Genotype x Hemisphere	$F_{(1,10)} = 12.12$	P = 0.006
Amygdala-Anterior Hippocampus	RD	Genotype	$F_{(1,10)} < 1$	P = 0.519
		Hemisphere	$F_{(1,10)} = 1.32$	P = 0.279
		Genotype x Hemisphere	$F_{(1,10)} < 1$	P = 0.615
Amygdala-Posterior Hippocampus	AD	Genotype	$F_{(1,10)} < 1$	P = 0.455
		Hemisphere	$F_{(1,10)} < 1$	P = 0.987
		Genotype x Hemisphere	$F_{(1,10)} = 4.54$	P = 0.059
Amygdala-Posterior Hippocampus	RD	Genotype	$F_{(1,10)} < 1$	P = 0.608
		Hemisphere	$F_{(1,10)} < 1$	P = 0.412
		Genotype x Hemisphere	$F_{(1,10)} < 1$	P = 0.397
BLA-Anterior Hippocampus	AD	Genotype	$F_{(1,10)} < 1$	P = 0.739
		Hemisphere	$F_{(1,10)} = 6.59$	P = 0.028
		Genotype x Hemisphere	$F_{(1,10)} = 10.53$	P = 0.009
BLA-Anterior Hippocampus	RD	Genotype	$F_{(1,10)} < 1$	P = 0.649
		Hemisphere	$F_{(1,10)} = 2.59$	P = 0.138
		Genotype x Hemisphere	$F_{(1,10)} < 1$	P = 0.812
BLA-Posterior Hippocampus	AD	Genotype	$F_{(1,10)} < 1$	P = 0.763
		Hemisphere	$F_{(1,10)} = 12.79$	P = 0.005
		Genotype x Hemisphere	$F_{(1,10)} = 12.97$	P = 0.005
BLA-Posterior Hippocampus	RD	Genotype	$F_{(1,10)} < 1$	P = 0.727
		Hemisphere	$F_{(1,10)} = 3.11$	P = 0.109
		Genotype x Hemisphere	$F_{(1,10)} < 1$	P = 0.897

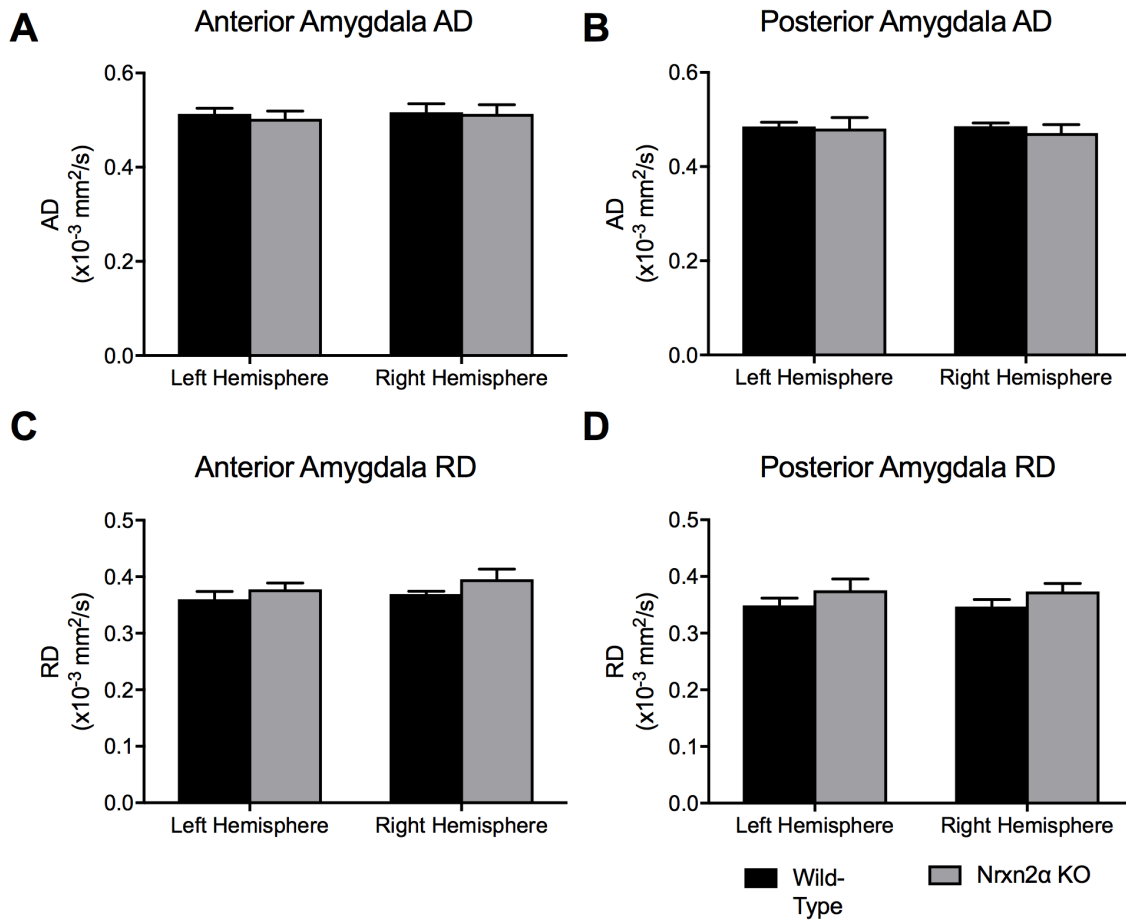
Statistical analysis of the anterior (Bregma -1.94 mm), and posterior (Bregma -3.28 mm) amygdala-hippocampal connections, analysed for axial diffusion (AD) and radial diffusion (RD). Analysis was performed using repeated measure two-way ANOVAs for genotype and hemisphere (Sidak correction for multiple comparisons).

Supp. Table 3

Brain Region	CLARITY Measure	ANOVA Comparison	F Value	P Value
M1	OI	Genotype	$F_{(1,10)} < 1$	P = 0.904
		Hemisphere	$F_{(1,10)} = 1.74$	P = 0.217
M1	Cell Density	Genotype	$F_{(1,10)} = 2.04$	P = 0.184
		Hemisphere	$F_{(1,10)} = 1.41$	P = 0.262
M1	Fibre Density	Genotype	$F_{(1,10)} < 1$	P = 0.791
		Hemisphere	$F_{(1,10)} < 1$	P = 0.860
S1	OI	Genotype	$F_{(1,10)} < 1$	P = 0.919
		Hemisphere	$F_{(1,10)} = 36.86$	P = 0.0001
S1	Cell Density	Genotype	$F_{(1,10)} < 1$	P = 0.401
		Hemisphere	$F_{(1,10)} = 13.73$	P = 0.004
S1	Fibre Density	Genotype	$F_{(1,10)} = 1.73$	P = 0.218
		Hemisphere	$F_{(1,10)} = 8.51$	P = 0.015
BF	OI	Genotype	$F_{(1,10)} < 1$	P = 0.991
		Hemisphere	$F_{(1,10)} = 10.59$	P = 0.009
BF	Cell Density	Genotype	$F_{(1,10)} < 1$	P = 0.408
		Hemisphere	$F_{(1,10)} = 8.70$	P = 0.015
BF	Fibre Density	Genotype	$F_{(1,10)} < 1$	P = 0.460
		Hemisphere	$F_{(1,10)} < 1$	P = 0.356

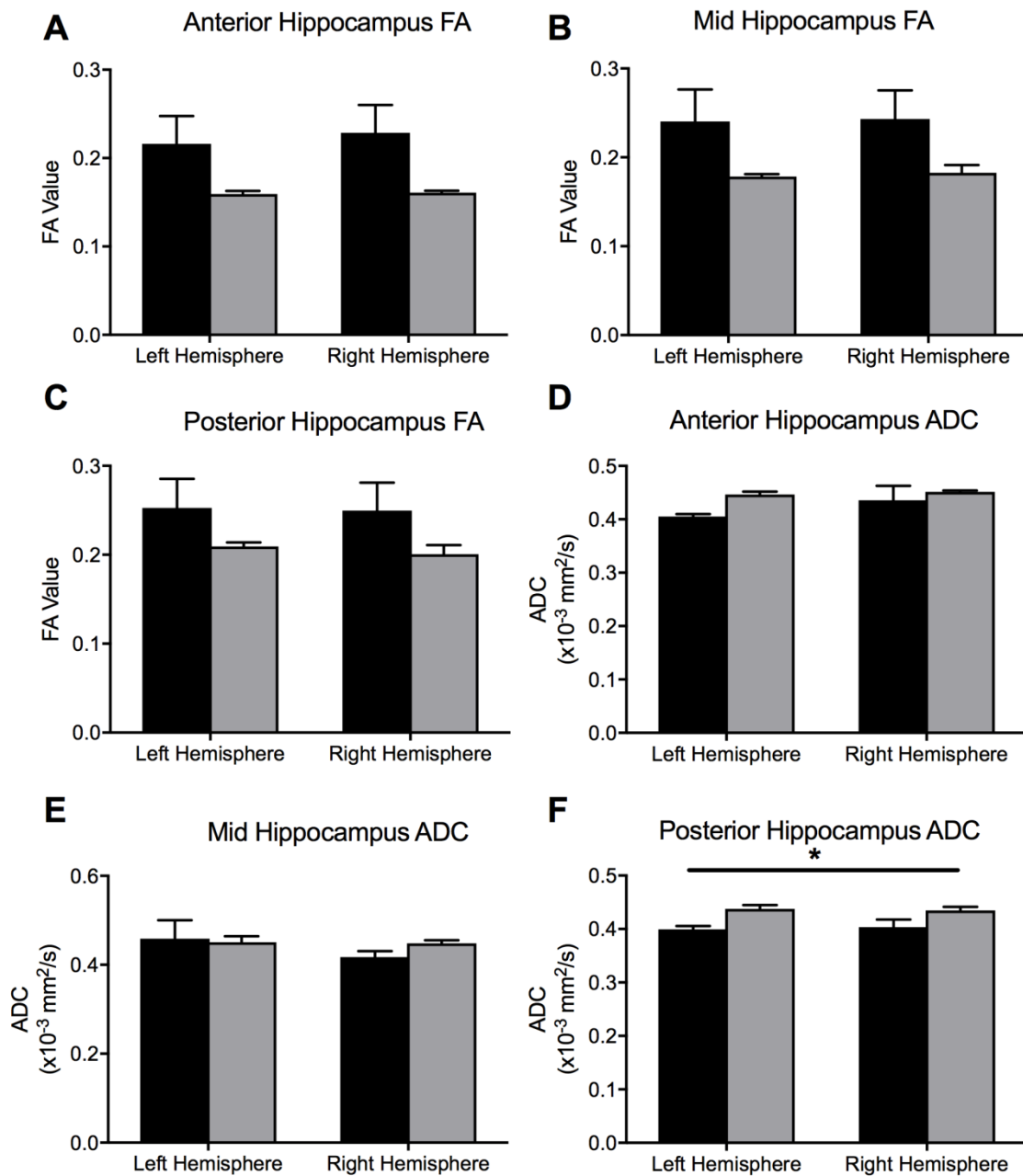
Statistical analysis of the primary motor cortex (M1), primary somatosensory cortex (S1) and the barrel field (BF). CLARITY imaged regions were then analysed for orientation index (OI), cell density and fibre density. Analysis was performed using repeated measure two-way ANOVAs for genotype and hemisphere (Sidak correction for multiple comparisons).

Supp. Figure 1



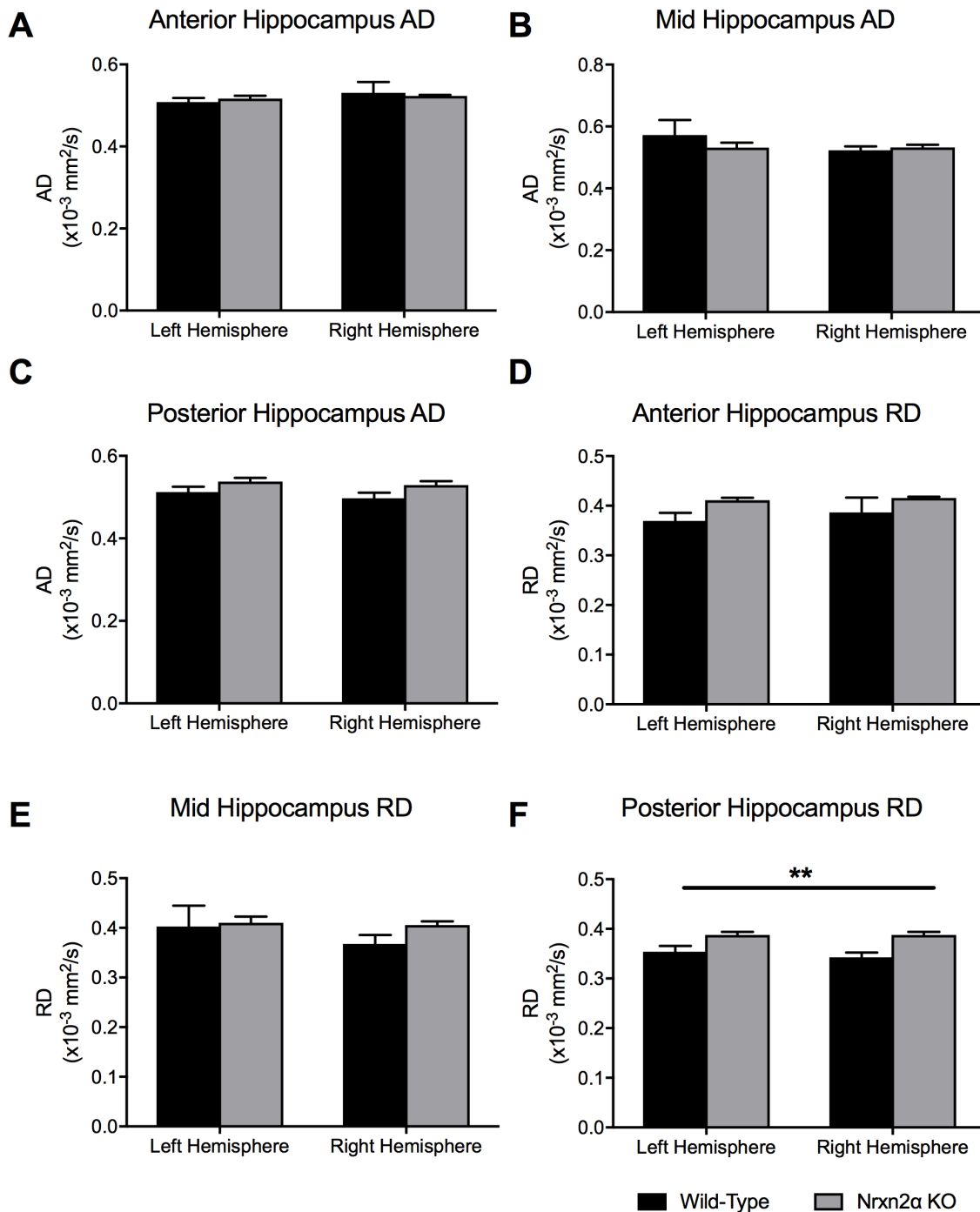
Axial diffusivity (AD) and radial diffusivity (RD) in the amygdala. The entire amygdala was segmented from DTI images at two regions; anterior (**A** and **C**: Bregma -1.94 mm) and posterior (**B** and **D**: Bregma -2.46 mm). No significant differences were observed between the genotypes for anterior or posterior regions. Error bars represent s.e.m. Wild-type n=6, Nrnx2 α KO n=6.

Supp. Figure 2



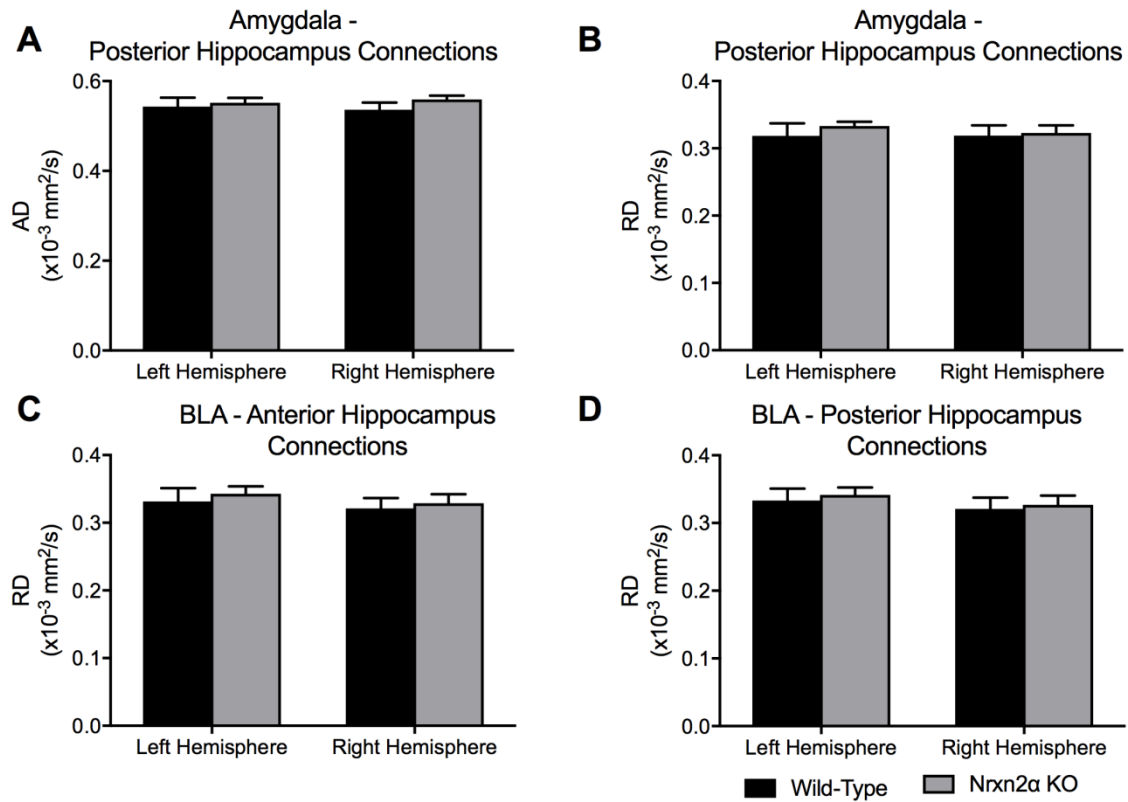
Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) of the anterior to posterior hippocampus. Although *Nrxn2α* KO had trends towards lower FA in the anterior (A), mid (B) and posterior (C) hippocampus, there were no significant differences. Similarly, anterior (D) and mid (E) hippocampal regions did not vary between the genotypes, the posterior hippocampus (F) had significantly increased ADC (f). * = $P < 0.05$. Error bars represent s.e.m. Wild-type n=6, *Nrxn2α* KO n=6.

Supp. Figure 3



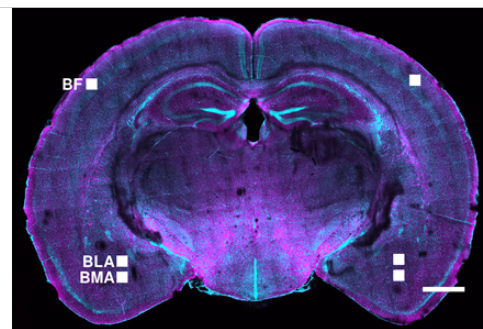
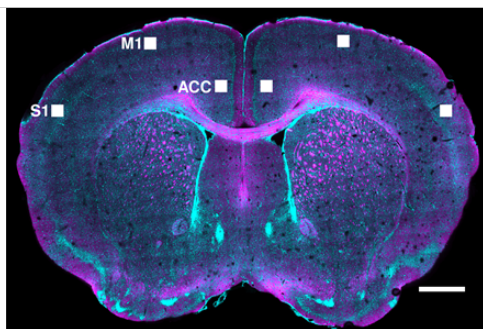
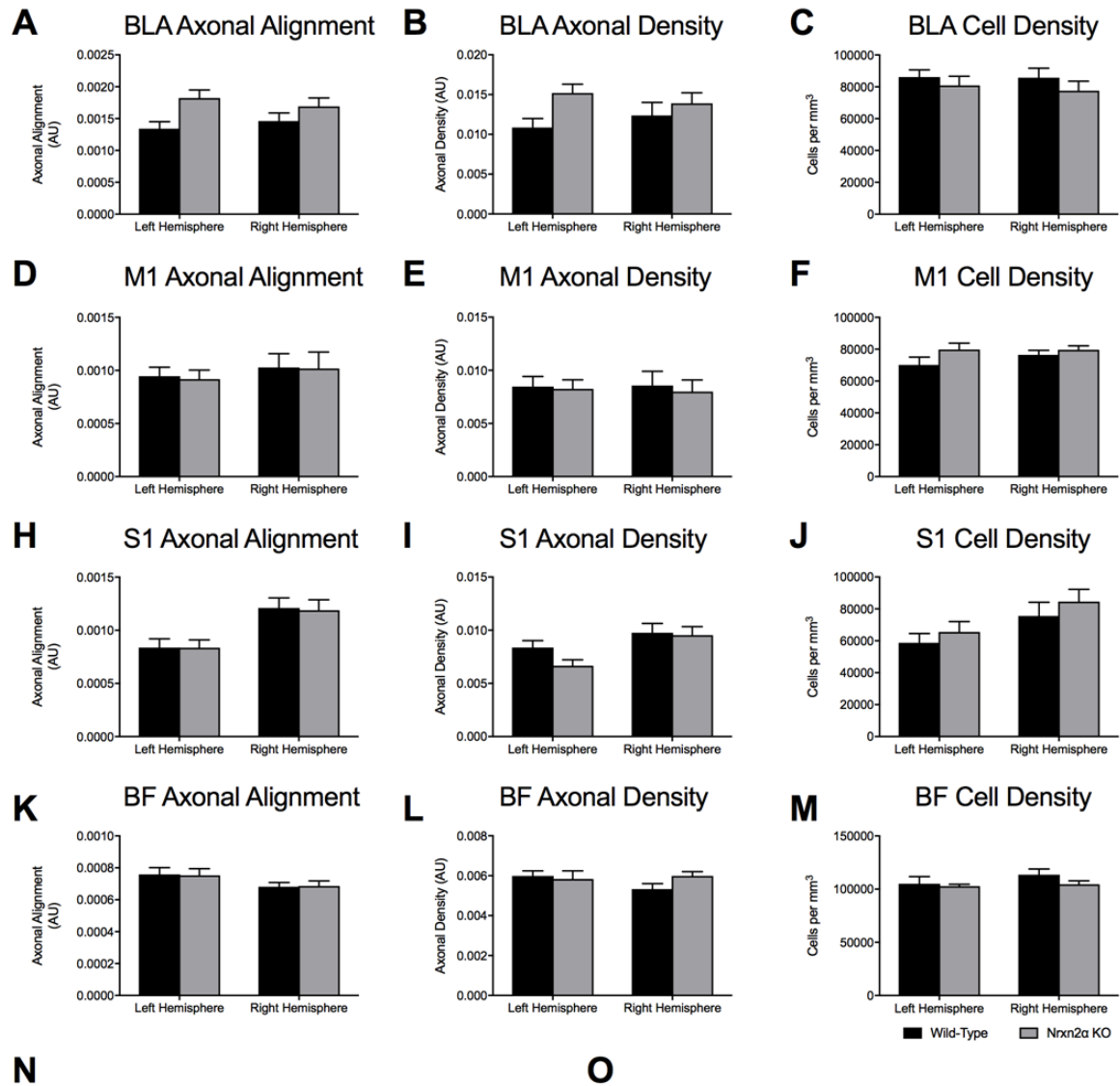
Axial diffusivity (AD) and radial diffusivity (RD) of the anterior to posterior hippocampus. AD did not differ in either the anterior (A), mid (B) and posterior (C) hippocampus. Although the anterior (D) and mid (E) hippocampal regions did not vary between the genotypes, the posterior hippocampus (F) had significantly increased RD (f). ** = $P < 0.01$. Error bars represent s.e.m. Wild-type n=6, Nrnx2α KO n=6.

Supp. Figure 4



Axial diffusivity (AD) and radial diffusivity (RD) of computed tracts of connections from the amygdala to hippocampus. Tracts from the anterior amygdala to the posterior hippocampus (Bregma -2.46 mm) were analysed for AD (**A**) and RD (**B**). No significant differences between the tracts of Nrnx2 α KO mice were observed. No significant differences were found for RD of tracts specifically from the basolateral nuclei of the amygdala (BLA) to the anterior (**C**) or posterior (**D**) hippocampus. Error bars represent s.e.m. Wild-type n=6, Nrnx2 α KO n=6.

Supp. Figure 5



CLARITY-derived quantification of fibres and cell density within the basolateral amygdala (BLA). **(A)** Although there were trends towards increased axonal alignment and fibre density **(B)** in Nrnx2a KO mice, no significant differences were found. **(C)** Cell density in the BLA was similar between the genotypes. Statistical analysis (Supp. Table 4) was performed for the primary motor cortex (M1; **D-F**), primary somatosensory cortex (S1; **H-J**) and the barrel field (BF; **K-M**). No genotypic differences were found for any measure within these cortical regions. **(N-O)**

CLARITY images of the scanned regions of interest. Error bars represent s.e.m.
Wild-type n=6, Nr1h2 KO n=6.

References

1. Wu D, Xu J, McMahon MT, van Zijl PC, Mori S, Northington FJ, *et al.* In vivo high-resolution diffusion tensor imaging of the mouse brain. *NeuroImage* 2013; **83**: 18-26.
2. Vo A, Sako W, Dewey SL, Eidelberg D, Ulug AM. 18FDG-microPET and MR DTI findings in Tor1a^{+/-} heterozygous knock-out mice. *Neurobiology of disease* 2015; **73**: 399-406.
3. Kumar M, Duda JT, Hwang WT, Kenworthy C, Ittyerah R, Pickup S, *et al.* High resolution magnetic resonance imaging for characterization of the neuroligin-3 knock-in mouse model associated with autism spectrum disorder. *PloS one* 2014; **9**(10): e109872.
4. Ruest T, Holmes WM, Barrie JA, Griffiths IR, Anderson TJ, Dewar D, *et al.* High-resolution diffusion tensor imaging of fixed brain in a mouse model of Pelizaeus-Merzbacher disease: comparison with quantitative measures of white matter pathology. *NMR in biomedicine* 2011; **24**(10): 1369-1379.
5. Kim S, Pickup S, Fairless AH, Ittyerah R, Dow HC, Abel T, *et al.* Association between sociability and diffusion tensor imaging in BALB/cJ mice. *NMR in biomedicine* 2012; **25**(1): 104-112.
6. Jones DK. The effect of gradient sampling schemes on measures derived from diffusion tensor MRI: a Monte Carlo study. *Magnetic resonance in medicine* 2004; **51**(4): 807-815.
7. Lebel C, Benner T, Beaulieu C. Six is enough? Comparison of diffusion parameters measured using six or more diffusion-encoding gradient directions with deterministic tractography. *Magnetic resonance in medicine* 2012; **68**(2): 474-483.
8. Ni H, Kavcic V, Zhu T, Ekholm S, Zhong J. Effects of number of diffusion gradient directions on derived diffusion tensor imaging indices in human brain. *AJNR American journal of neuroradiology* 2006; **27**(8): 1776-1781.
9. Hasan KM, Parker DL, Alexander AL. Comparison of gradient encoding schemes for diffusion-tensor MRI. *Journal of magnetic resonance imaging : JMRI* 2001; **13**(5): 769-780.
10. Pervolaraki E, Dachtler J, Anderson RA, Holden AV. Ventricular myocardium development and the role of connexins in the human fetal heart. *Scientific reports* 2017; **7**(1): 12272.
11. Yeh FC, Verstynen TD, Wang Y, Fernandez-Miranda JC, Tseng WY. Deterministic diffusion fiber tracking improved by quantitative anisotropy. *PloS one* 2013; **8**(11): e80713.
12. Basser PJ, Mattiello J, LeBihan D. Estimation of the effective self-diffusion tensor from the NMR spin echo. *Journal of magnetic resonance Series B* 1994; **103**(3): 247-254.

13. Jiang H, van Zijl PC, Kim J, Pearlson GD, Mori S. DtiStudio: resource program for diffusion tensor computation and fiber bundle tracking. *Computer methods and programs in biomedicine* 2006; **81**(2): 106-116.
14. Pervolaraki E, Anderson RA, Benson AP, Hayes-Gill B, Holden AV, Moore BJ, *et al.* Antenatal architecture and activity of the human heart. *Interface focus* 2013; **3**(2): 20120065.
15. Ferguson JN, Aldag JM, Insel TR, Young LJ. Oxytocin in the medial amygdala is essential for social recognition in the mouse. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2001; **21**(20): 8278-8285.
16. Tanimizu T, Kenney JW, Okano E, Kadoma K, Frankland PW, Kida S. Functional Connectivity of Multiple Brain Regions Required for the Consolidation of Social Recognition Memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2017; **37**(15): 4103-4116.
17. Emery NJ, Capitanio JP, Mason WA, Machado CJ, Mendoza SP, Amaral DG. The effects of bilateral lesions of the amygdala on dyadic social interactions in rhesus monkeys (*Macaca mulatta*). *Behavioral neuroscience* 2001; **115**(3): 515-544.
18. Rosvold HE, Mirsky AF, Pribram KH. Influence of amygdectomy on social behavior in monkeys. *Journal of comparative and physiological psychology* 1954; **47**(3): 173-178.
19. Mosher CP, Zimmerman PE, Gothard KM. Neurons in the monkey amygdala detect eye contact during naturalistic social interactions. *Current biology : CB* 2014; **24**(20): 2459-2464.
20. Rutishauser U, Mamelak AN, Adolphs R. The primate amygdala in social perception - insights from electrophysiological recordings and stimulation. *Trends in neurosciences* 2015; **38**(5): 295-306.
21. Rutishauser U, Tudusciuc O, Wang S, Mamelak AN, Ross IB, Adolphs R. Single-neuron correlates of atypical face processing in autism. *Neuron* 2013; **80**(4): 887-899.
22. Adolphs R, Tranel D, Damasio AR. The human amygdala in social judgment. *Nature* 1998; **393**(6684): 470-474.
23. Baron-Cohen S, Ring HA, Bullmore ET, Wheelwright S, Ashwin C, Williams SC. The amygdala theory of autism. *Neuroscience and biobehavioral reviews* 2000; **24**(3): 355-364.
24. Maaswinkel H, Baars AM, Gispen WH, Spruijt BM. Roles of the basolateral amygdala and hippocampus in social recognition in rats. *Physiology & behavior* 1996; **60**(1): 55-63.
25. Kogan JH, Frankland PW, Silva AJ. Long-term memory underlying hippocampus-dependent social recognition in mice. *Hippocampus* 2000; **10**(1): 47-56.

26. Okuyama T, Kitamura T, Roy DS, Itohara S, Tonegawa S. Ventral CA1 neurons store social memory. *Science* 2016; **353**(6307): 1536-1541.
27. van der Kooij MA, Fantin M, Kraev I, Korshunova I, Grosse J, Zanoletti O, *et al.* Impaired hippocampal neuroligin-2 function by chronic stress or synthetic peptide treatment is linked to social deficits and increased aggression. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 2014; **39**(5): 1148-1158.
28. Rubin RD, Watson PD, Duff MC, Cohen NJ. The role of the hippocampus in flexible cognition and social behavior. *Frontiers in human neuroscience* 2014; **8**: 742.
29. Nicolson R, DeVito TJ, Vidal CN, Sui Y, Hayashi KM, Drost DJ, *et al.* Detection and mapping of hippocampal abnormalities in autism. *Psychiatry research* 2006; **148**(1): 11-21.
30. Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, *et al.* The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2004; **24**(28): 6392-6401.
31. Bachevalier J, Loveland KA. The orbitofrontal-amygdala circuit and self-regulation of social-emotional behavior in autism. *Neuroscience and biobehavioral reviews* 2006; **30**(1): 97-117.
32. Beer JS, John OP, Scabini D, Knight RT. Orbitofrontal cortex and social behavior: integrating self-monitoring and emotion-cognition interactions. *Journal of cognitive neuroscience* 2006; **18**(6): 871-879.
33. Watson KK, Platt ML. Social signals in primate orbitofrontal cortex. *Current biology : CB* 2012; **22**(23): 2268-2273.
34. Girgis RR, Minshew NJ, Melhem NM, Nutche JJ, Keshavan MS, Hardan AY. Volumetric alterations of the orbitofrontal cortex in autism. *Progress in neuro-psychopharmacology & biological psychiatry* 2007; **31**(1): 41-45.
35. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, *et al.* Fiji: an open-source platform for biological-image analysis. *Nature methods* 2012; **9**(7): 676-682.
36. Peng H, Ruan Z, Long F, Simpson JH, Myers EW. V3D enables real-time 3D visualization and quantitative analysis of large-scale biological image data sets. *Nature biotechnology* 2010; **28**(4): 348-353.
37. Linkert M, Rueden CT, Allan C, Burel JM, Moore W, Patterson A, *et al.* Metadata matters: access to image data in the real world. *The Journal of cell biology* 2010; **189**(5): 777-782.
38. Otsu N. A Threshold Selection Method from Gray-Level Histograms. *IEEE Transactions on Systems, Man, and Cybernetics* 1979; **9**(1): 62-66.

39. Lee K, Elliott HL, Oak Y, Zee CT, Groisman A, Tytell JD, *et al.* Functional hierarchy of redundant actin assembly factors revealed by fine-grained registration of intrinsic image fluctuations. *Cell systems* 2015; **1**(1): 37-50.
40. Lee T, Kashyap R, Chu C. Building Skeleton Models via 3-D Medial Surface Axis Thinning Algorithms. *CVGIP: Graphical Models and Image Processing* 1994; **56**(6): 462-478.