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Examining the neural correlates of emergent equivalence relations in Fragile X syndrome

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Abstract

The neural mechanisms underlying the formation of stimulus equivalence relations are poorly understood, particularly in individuals with specific learning impairments. As part of a larger study, we used functional magnetic resonance imaging (fMRI) while participants with fragile X syndrome (FXS), and age- and IQ-matched controls with intellectual disability, were required to form new equivalence relations in the scanner. Following intensive training on matching fractions to pie charts (A=B relations) and pie charts to decimals (B=C relations) outside the scanner over a 2-day period, participants were tested on the *trained* (A=B, B=C) relations, as well as emergent *symmetry* (i.e., B=A and C=B) and *transitivity/equivalence* (i.e., A=C and C=A) relations inside the scanner. Eight participants with FXS (6 female, 2 male) and 10 controls, aged 10 to 23 years, were able to obtain at least 66.7% correct on the *trained* relations in the scanner and were included in the fMRI analyses. Across both groups, results showed that the emergence of *symmetry* relations was correlated with increased brain activation in the left inferior parietal lobule, left postcentral gyrus, and left insula, broadly supporting previous investigations of stimulus equivalence research in neurotypical populations. On the test of emergent *transitivity/equivalence* relations, activation was significantly greater in individuals with FXS compared with controls in the right middle temporal gyrus, left superior frontal gyrus and left precuneus. These data indicate that neural execution was significantly different in individuals with FXS than in age- and IQ-matched controls during stimulus equivalence formation. Further research concerning how gene-brain-behavior interactions may influence the emergence of stimulus equivalence in individuals with intellectual disabilities is needed.

Keywords

Fragile X syndrome; Stimulus equivalence; Functional magnetic resonance imaging; Intellectual disabilities; Mathematical processing

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1. Introduction

The ability to associate a stimulus presented in one modality (e.g., a number) to an equivalent stimulus presented in another modality (e.g., a picture of a quantity) is a fundamental component of learning a new skill. For example, when teaching number skills, an instructor may use three sets of corresponding stimuli: numerals (set A), pictures of quantities (set B), and number words (set C). Children may first be taught to associate the numbers to their corresponding picture quantities (A=B training) and then to associate the picture quantities to the number words (B=C training). Interestingly, it has been shown that once A=B and B=C relations are trained, new stimulus relations can *emerge* without explicit training, for example, C=A (the ability to associate word numbers to numerals) and C=B relations (the ability to associate word numbers to picture quantities) (Sidman, 1971; Sidman and Cresson, 1973). These emergent relations have been suggested to occur due to the properties of *symmetry* (if A=B, then B=A) and *transitivity* (if A=B and B=C, then A=C). Thus, if the child can demonstrate proficiency on symmetry (B=A, C=B), and transitivity (A=C) as well as C=A relations, the child can be considered to have demonstrated "stimulus equivalence" (Sidman, 1994). The stimulus equivalence paradigm therefore offers a useful rubric to gauge an individual's capacity to form new concepts. Hence, the ability to achieve stimulus equivalence could be an important correlate or predictor of more advanced cognitive capacity.

Over the past few decades, several theories have been advanced concerning the potential behavioral and/or neuroanatomical mechanisms that may be involved in the emergence of stimulus equivalence relations. To test these theories, neuroimaging studies conducted with neurotypical individuals have investigated the neural correlates of emergent stimulus equivalence relations following training on arbitrary sets of pictures (Dickins et al., 2001), colored ellipsoid shapes (Heckers et al., 2004), sets of symbols (Schlund et al., 2007) and consonant-vowel-consonant triplets (Schlund et al., 2008). In each case, individuals were trained on these associations outside the scanner and were then tested for the emergence of new stimulus relations inside the scanner. Increased activation during tests of symmetry and/or transitivity/equivalence relations has been detected in the dorsolateral prefrontal cortex (DLPFC), posterior parietal regions, the insular cortex and the left caudate nucleus (Dickins et al., 2001), the right anterior hippocampus (Heckers et al., 2004), the right and left inferior frontal gyrus (dorsolateral), the inferior parietal lobule (Schlund et al., 2007), and the parahippocampal gyrus (Schlund et al., 2008). Overall, the results from neuroimaging studies of stimulus equivalence provide valuable, but mixed, information about the neural architecture involved in the emergence of derived stimulus relations in healthy adults.

Fragile X syndrome (FXS) – the most common known form of inherited intellectual disability (Crawford et al., 1999) – may provide a useful model for understanding the pathogenesis of learning impairments commonly shown by children with intellectual disabilities. FXS is caused by mutations to a single gene (*FMR1*), located on the long arm of the X chromosome at Xq27.3 (Verkerk et al., 1991) in which excessive methylation in the promoter region of the gene compromises production of the "Fragile X Mental Retardation Protein" (FMRP), the protein product of the gene. FMRP is thought to actively participate in

the translational machinery that converts messenger RNA into protein (Verkerk et al., 1991; Brown et al., 2001), and low levels of FMRP therefore contribute to aberrant neuronal development and brain function. FXS is also a risk factor for autism spectrum disorder (ASD), accounting for up to 6% of cases of ASD (Freund and Reiss, 1991; Fombonne, 2005). A distinct cognitive profile that includes weaknesses in visual spatial processing, writing skills, spatial memory and mathematical reasoning, but strengths in verbal labeling and comprehension, has been demonstrated in both boys and girls with FXS (Freund and Reiss, 1991; Roberts et al., 2005; Schneider et al., 2009).

Mathematical reasoning impairments in FXS have been reported to begin in early childhood, with toddlers demonstrating significant deficits in processing ordinal numerical sequences when compared to typically developing toddlers (Owen et al., 2013). Problems with counting and number sense have also been reported in females with FXS during late elementary school (Murphy and Mazzocco, 2008a). For example, Murphy and Mazzocco (Murphy and Mazzocco, 2008b) required high-functioning girls with FXS to rank-order sets of 10 fractions, pie charts, and decimals. They found that while girls with FXS were able to rank-order the set of pie charts at grade-level performance, they evidenced impaired performance when attempting to rank-order the fractions, suggesting that girls with FXS demonstrate a relative strength in rote memory of numerical operations, but an impaired ability to understand numerical concepts and applied mathematics. In a functional magnetic resonance imaging (fMRI) study of mathematical reasoning skills, Rivera and colleagues (Rivera et al., 2002) found that when female subjects with FXS, aged 10 to 23 years, were given subtraction and addition tasks to complete in the scanner, activation in the angular gyrus and bilateral prefrontal regions was significantly increased relative to typically developing controls. These authors suggested that individuals with FXS were either employing compensatory strategies or required greater neural resources to complete the task compared with controls.

In a recent study conducted by our group, we examined whether stimulus equivalence relations would emerge in individuals with FXS following training on matching fractions to pie chart and pie charts to decimals (Hammond et al., 2012). Participants comprised 11 individuals with FXS, aged 10 to 23 years, and 11 age- and IQ-matched controls who were taught to match these relations (A=B and B=C training) over a 2-day period. They were then tested for the emergence of symmetry (B=A, C=B) and transitivity/equivalence (A=C, C=A) relations. Results showed that performance improvements on the *symmetry* test were significantly correlated with performance improvements on the *transitivity/equivalence* test in controls, but not in individuals with FXS, suggesting that individuals with FXS demonstrated an impairment in forming equivalence classes. Further investigation of the neural components involved in the emergence of stimulus equivalence could provide important information about how the brain makes logical inferences (generalizability) about stimulus relations. However, to our knowledge, no studies have assessed the underlying neural mechanisms involved in the emergence of equivalence relations in children diagnosed with disorders associated with intellectual impairment such as FXS.

Additional information concerning the neurobiological processes underlying the emergence of stimulus equivalence in FXS may therefore add to our understanding of how gene-brain-

behavior interactions contribute to learning problems in this unique genetic disorder associated with intellectual disabilities. In the present study, we examined the underlying neural mechanisms involved in the emergence of stimulus equivalence relations in individuals with FXS compared to age- and IQ-matched individuals. Given previous research, we predicted that activation would be significantly greater in individuals with FXS than in age- and IQ-matched controls during tests of emergent equivalence relations.

2. Methods

2.1. Participants

Participants with FXS were recruited nationally through postings on parent support group websites, the National Fragile X Foundation, and our lab's database. Control participants were recruited locally within a 50-mile radius from the Stanford University campus through online parent support groups and agencies serving individuals with developmental disabilities. The care providers of potential participants completed a phone screen and demographic questionnaire in order to determine whether their child/ward met initial inclusion criteria. The inclusion criteria were as follows: age between 10 and 23 years old, IQ > 50, ability to travel to Stanford, and the absence of possible MRI contraindications such as orthodontia or other metallic materials in the body. Eligible families were subsequently mailed a brief paper-and-pencil screening test containing fraction, pie chart, and decimal equivalencies using the stimuli shown in Fig. 1 to ensure that participants were unfamiliar with these stimuli before entering the study. Chance responding on this test was 33.3%, and individuals who obtained less than 50% correct on the test were invited to travel to Stanford for the study.

All participants were recruited as part of a larger study evaluating a brief 2-day intensive behavioral intervention for children with FXS (see Hammond et al., 2012; Hall et al., 2014). Participants with FXS had a confirmed genetic diagnosis (i.e., > 200 CGG repeats on the FMR1 gene and evidence of aberrant methylation) and all control participants either had a current clinical diagnosis or qualified for special education services under a diagnosis of developmental delay. None of the control participants had a known genetic basis for developmental delay or a history of seizures and/or premature birth. All participants demonstrated the ability to communicate verbally and were right-handed. Inclusion criteria were satisfied by 20 individuals with FXS and 20 controls. They received an 8-min resting-state scan (see Hall et al., 2013) before the functional scan. For the purpose of the present study, only those participants who were able to obtain at least 66.7% correct on the *trained* relations on the functional scan (8 participants with FXS and 10 controls) were included in the present study. The demographic, cognitive, and behavioral data for individuals included in the present study are shown in Table 1. The groups were matched on age, IQ, and degree of autistic symptomatology.

2.2. Assessment

Following participant assent and parental consent, participants completed an assessment battery which included the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) and the Social Communication Questionnaire (SCQ) (Rutter et al., 2003). Assessment

sessions and training were conducted in one of two rooms located within the Department of Psychiatry and Behavioral Sciences at Stanford University. Session rooms contained a table or desk, chairs, a laptop computer, and a computer mouse. The Stanford University Institutional Review Board (IRB) approved all procedures.

2.3. Training on A=B and B=C stimulus relations

All participants were trained on A=B (fraction-to pie-chart) and B=C (pie-chart to decimal) stimulus relations using the stimuli shown in Fig. 1 over a 2-day period. Briefly, learning trials were presented in 10-min training sessions until participants were able to obtain >80% correct in a session. On each trial, a sample stimulus from the stimulus set shown in Fig. 1 was displayed above three comparison stimuli that were arranged in randomized order in a horizontal row, one of which was the correct matching stimulus, the other two being distracter stimuli. Correct responses were immediately reinforced with specific verbal praise and tokens (e.g., “Great job! You found one-fourth!” and delivery of one token). When participants had accumulated five tokens, he or she was allowed to take a brief break in order to play a computer game. Incorrect responses resulted in verbal and visual feedback (e.g., “The correct answer is one-fourth” while simultaneously removing the two distracter stimuli and moving the correct answer below the sample stimulus), token removal, and if necessary, position prompting until the correct response was displayed. Because children with FXS commonly engage in escape behaviors when required to complete demanding tasks with others (Hall et al., 2006), for the majority of participants, the training was administered on a computer using the *Discrete Trial Trainer* software program (Accelerations Educational Software, 2007). For the remaining participants, training was conducted with a behavior therapist using flash cards that matched the dimensions of the digital stimuli. A=B and B=C learning trials were intermixed during each session. There were no differences in learning rates between those who received computerized training versus those who received in-person training (Hall et al., 2014).

2.4. fMRI task

The in-scanner task was similar to the pre-training task with the exception that only three of the six fraction-pie chart and pie chart-decimal pairs were used, and tokens, feedback and reinforcement were no longer forthcoming following a response on each trial. Approximately 33% of participants were presented with stimulus sets corresponding to one-third, three-quarters, and four-fifths, approximately 33% of participants were presented with stimulus sets corresponding to two-thirds, one-quarter, and one-fifth, and approximately 33% of participants were presented with stimulus sets corresponding to one-fifth, two-thirds and three-quarters. The task used an event-related design, and all six trial types (i.e., A= B, B=A, B=C, C=B, A=C, C=A) were presented in a pseudorandom order with each trial lasting for 7 sec. On each trial, a sample stimulus from one of the three stimulus sets was displayed above three comparison stimuli that were arranged in randomized order in a horizontal row at the bottom of the screen, one of which was the correct matching stimulus, the other two being distracter stimuli. If the subject chose one of the matching stimuli within 7 s, the stimuli were removed from the screen and a blank screen was presented for the remaining seconds. If the subject failed to make a response within 7 s, the stimuli were removed from the screen and the trial was completed. Each trial type was presented 9 times

(i.e., 3 presentations for each of the 3 stimulus pairs), resulting in a total of 54 trials. A pseudorandom interstimulus interval (ISI) was jittered across trials and lasted between 1 and 11 s (total task time = 8 min, 40 s). A fixation cross was displayed in the center of a black screen during the ISI. Participants were given feedback about their performance (i.e., overall percentage correct score) immediately after the task was completed. The task was administered using E-Prime 2.0 (Schneider et al., 2002) and was projected onto a mirror attached to the fMRI head coil. Participants responded on each trial using a non-magnetic, three-button response-recording box and had been pre-trained to press the left button with their index finger to choose the comparison stimulus on the bottom left of the screen, the middle button with their middle finger to choose the comparison stimulus on the bottom middle of the screen, and the right button with their third finger to choose the comparison stimulus on the bottom right of the screen. Given that the order of the comparison stimuli were randomized on each trial, the participant would be required to use each finger 33.3% of the time to obtain 100% correct. We checked for potential response biases by determining whether any subject pressed a particular button more than 50% of the time or whether a particular button had not been pressed. The E-Prime software automatically recorded response time and the accuracy of responses. Trials were arranged into sets *of trained (A=B* and B=C), *symmetry* (B=A and C=B) and *transitivity/equivalence* (A=C and C=A) relations for analysis.

2.5. fMRI data acquisition

Before scanning, all participants underwent a mock scanning session to familiarize them with the scanning environment and to help minimize head movements. Participants were shown a movie of their choice and if the participant moved his/her head > 1 mm, the movie immediately shut off for 3 s. All participants were able to meet the motion criterion (i.e., no movements over 1 mm) during at least one 10-min mock scanning session.

All participants were scanned at the Lucas Center for Magnetic Resonance Spectroscopy and Imaging (Stanford University, Palo Alto, CA) on a 3.0T General Electric Healthcare whole body MR system (GE Healthcare Systems, Milwaukee, WI) using a standardized head coil. High-resolution anatomical brain images using a fast spoiled gradient recalled acquisition in the steady state (FSPGR) echo pulse sequence were acquired for each subject (TR = 8.5 s, TE = 3.4 s, flip angle = 15°, matrix 256 256 pixels, FOV = 220 mm 165 mm) and used for localization and co-registration of functional data. A T2-weighted gradient echo spiral-in/out pulse sequence (Glover and Law, 2001) was used to obtain functional images (TR = 2 s, TE = 30 ms, flip angle = 80°, matrix 64 64 pixels, FOV = 220 mm 220 mm). A total of 259 whole brain volumes were collected (4 mm thick, 1 mm skip). Total functional scan duration was 526 s. A higher-order shimming protocol preceded functional scans in order to correct B0 heterogeneity and avoid blurring and signal loss (Kim et al., 2002). Heart rate and respiration rate were recorded with a scanner safe pulse-oximeter and a respiration belt.

2.6. fMRI data analysis

Data were pre-processed and analyzed using FEAT (FMRI Expert Analysis Tool) Version 4.98, part of FSL. The following preprocessing steps were applied: motion correction using

MCFLIRT (Jenkinson et al., 2002), non-brain removal using BET (Smith, 2002), spatial smoothing using a Gaussian kernel of FWHM 5 mm, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, and highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 100.0 s). Additionally, sharp motion peaks were detected using fsl_motion_outliers script (supplied with FSL) and were regressed out in addition to the six motion parameters (from MCFLIRT). Registration to each participants' own high-resolution structural scan and standard space images was carried out using FLIRT (Jenkinson and Smith, 2001). Time-series statistical analysis was performed using FILM with local autocorrelation correction. There were no significant differences between participants with FXS and controls in terms of absolute head displacement (FXS group: $M = 0.28$ mm; Control group: $M = 0.26$ mm), relative head displacement (FXS group: $M = 0.072$ mm; Control group: $M = 0.065$ mm), heart rate (FXS group: $M = 78.0$ beats/min; Control group: $M = 80.50$ beats/min) or respiration rate (FXS group: $M = 18.43$ breaths/min, Control group: $M = 22.77$ breaths/min) during the in-scanner task.

2.6.1. Individual subject analyses—Task-related brain activation was identified using a general linear model (GLM). Individual subjects' analyses were first performed by modeling task-related conditions. Specifically, only those trials that resulted in a correct response (from trial onset to the time of the participant's correct response) were included. For each of the tests (i.e., *trained*, *symmetry*, and *transitivity/equivalence*), brain activity was convolved using a double gamma hemodynamic response function. A temporal derivative was used to account for voxel-wise latency differences in the hemodynamic response and temporal filtering was applied. Voxel wise *t* statistics maps for each comparison were generated for each participant.

2.6.2. Group analyses—Analyses were performed by entering the individual-subject contrast maps into a random effects analysis that was carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) stage 1 with automated outlier detection. Z Gaussianized T/F statistic images were thresholded using clusters determined by $Z > 1.96$ and a (corrected) cluster significance threshold of $p = 0.05$. To examine brain activation associated with the emergence of *symmetry* relations, we contrasted activation obtained on the *symmetry* trials with activation obtained on the *trained* trials (i.e., *symmetry* > *trained*). To examine brain activation associated with the emergence of *transitivity/equivalence* relations, we contrasted activation obtained on the *transitivity/equivalence* trials with activation obtained on the *symmetry* trials (i.e., *transitivity/equivalence* >*symmetry*). These analyses were conducted irrespective of group. To examine potential group differences in brain activation on each test, we contrasted activation obtained on each of the *trained*, *symmetry* and *transitivity/equivalence* tests between the groups (FXS > Controls, Controls > FXS). Age and IQ were demeaned and entered into the GLM as group level covariates in all analyses. Brain regions were converted from MNI space to Talairach *x*, *y*, and *z* coordinates and subsequently confirmed on the Talairach atlas. MRIcron (http://www.mricro.com/) was used to visualize neuroimaging results on the standard anatomical brain.

3. Results

3.1. In-scanner behavioral performance

Table 2 shows the mean performance accuracy (% correct) and response times (in seconds) obtained for the *trained*, *symmetry* and *transitivity/equivalence* tests for each group.

For accuracy scores, the results of a 2 (group) 3 (stimulus type) mixed-model ANOVA showed a significant main effect of stimulus type ($F(2,32) = 20.92$, $p < 0.001$), indicating that scores obtained on the *trained* relations were significantly higher than scores obtained on the *symmetry* and *transitivity/equivalence* relations in both groups (*p's* < 0.001). Similarly, for response times, there was a significant main effect of stimulus type ($F(2,32) = 22.51$, $p < 0.001$), indicating that response times obtained on the *trained* relations were significantly shorter than response times obtained on the *symmetry* and *transitivity/equivalence* relations in both groups (*p*'s < 0.001). Given that the *symmetry* and *transitivity/equivalence* trials were novel (i.e., had not been presented to the participant's before), a reduction in performance accuracy and an increase in response time on these trials was to be expected. There were no main effects of group and no interaction effect in either analysis indicating that in-scanner performance was therefore comparable between the two groups.

To examine whether age, IQ, or degree of autistic symptomatology were associated with in-scanner performance, for each group, we computed correlations between the percentage accuracy scores obtained on the *trained, symmetry, and transitivity/equivalence* tests and age, IQ, and SCQ score. The results of the correlational analyses showed that autistic symptomatology was significantly negatively associated with performance accuracy on the trained ($r = -0.73$, $p = 0.017$) and symmetry relations ($r = -0.74$, $p = 0.014$) for the control group only. Age and IQ were not associated with in-scanner performance for either group. These data indicated that individuals with higher levels of autistic symptoms obtained lower accuracy scores in the control group only.

3.2. Brain activation

3.2.1. Emergent symmetry and transitivity/equivalence contrasts—Fig. 2 and Table 3 show the results of the contrast in which activation on the emergent *symmetry* relations was compared to activation on the *trained* relations (*symmetry >trained*). For the *symmetry >trained* contrast, activation was significantly increased within the left insula, the left pre/postcentral gyrus and the left inferior parietal lobule. There were no differences in activation for the *transitivity/equivalence* > *symmetry* contrast.

3.2.2. Between-group contrasts—Fig. 3 and Table 4 show the results of the contrasts in which activation in the two groups were compared on the *trained*, *symmetry* and *transitivity/equivalence* tests. The figure shows that for the *transitivity/equivalence* test only, activation was significantly greater for participants with FXS compared to controls within the middle temporal gyrus, the superior frontal gyrus, the precuneus, and the paracentral lobule. These regions have previously been shown to be involved in math processing (Dehaene et al., 2003; Wintermute et al., 2012). There were no differences in brain activation on the *trained* and *symmetry* tests between participants with FXS and controls.

4. Discussion

The primary goal of the study was to examine the neural correlates underlying the emergence of stimulus equivalence relations in individuals with specific learning impairments. To achieve this goal, we trained individuals with FXS, the most common known form of inherited intellectual disability, and age- and IQ-matched controls to match fractions to pie-charts (A=B relations) and pie-charts to decimals (B=C relations) outside the scanner. We then examined differences in brain activation patterns during tests of the *trained* relations (A=B, B=C), and emergence of *symmetry* (B=A, C=B), and *transitivity/equivalence* (A=C, C=A) relations inside the scanner. We used mathematical proportions (fractions, pie charts, and decimals) to avoid the potential confound associated with using stimuli that could have obvious semantic connections, and because previous research has shown that children with FXS experience difficulties learning new mathematical concepts (Murphy and Mazzocco, 2008a, 2008b).

Results showed that the emergence of symmetry relations was correlated with increased brain activation in the left inferior parietal lobule, left postcentral gyrus, and left insula across participants. These results are broadly similar to those of Dickins and colleagues (Dickins et al., 2001) and Schlund and colleagues (Schlund et al., 2007) who also reported increased activation in similar regions during tests of emergent *symmetry* and *transitivity/equivalence* relations. It seems likely that any differences in findings would likely be due to differences in study design, task design, the stimuli used, as well as the study samples. Our finding that the insula was activated during the formation of symmetry relations is interesting because the insula is an important part of the salience network – one of several large-scale resting-state networks – that has been suggested to play a role in initiating dynamic switches between the executive control network and the default mode network (Menon and Uddin, 2010).

When brain activation was compared between the groups, no significant differences in activation were obtained on the tests of the *trained* and *symmetry* relations. However, activation was significantly greater for participants with FXS than for controls on the test of the *transitivity/equivalence* relations in the middle temporal gyrus, superior frontal gyrus, precentral gyrus, precuneus and paracentral lobule. These regions have previously been shown to be involved in math processing. Given that participants with FXS have been shown to exhibit impairments in stimulus equivalence formation, it seems likely that participants with FXS may have compensated for deficits in deriving transitivity/equivalence relations by recruiting resources from math processing regions (Hammond et al., 2012). Reliance upon math processing regions during transitivity/equivalence formation suggests that individuals with FXS may fail to make logical inferences about mathematical stimulus relations. The results from this study therefore provide a potential neurobiological explanation for deficits observed in stimulus generalization in participants with FXS.

There are several limitations of the study however that should be mentioned. First, only those individuals who demonstrated at least intermediate mastery of the *trained* relations (i.e., obtaining 66.7% correct or greater on this relation in the scanner) were included in the present study. Previous studies investigating the neural correlates of stimulus equivalence

have included neurotypical individuals who did not require significant amounts of training to demonstrate mastery of the relations prior to entering the scanner. In those studies, the mastery criterion was also somewhat higher (e.g., 90% correct responding; Schlund et al., 2008). It seems likely that degree of intellectual disability was a limiting factor in the ability of our participants to form equivalence relations in the scanner. In both groups, performance accuracy decreased, and response times increased when participants were presented with the novel stimulus relations. In previous studies involving individuals with intellectual disabilities, participants have been trained on A=B and B=C relations over variable periods of time (e.g., several times per week, over the course of several weeks, or even over months; for a discussion, see (Hall et al., 2006). It therefore seems likely that some participants in the present study may have benefited from longer training times.

A second limitation concerns the fact that motivational variables used during the training (such as reinforcement, feedback, and tokens) were not available to participants during the tests conducted in the scanner (i.e., the tests were conducted under extinction conditions). It is possible that the shift from a highly reinforcing learning environment outside the scanner to a highly restrictive testing environment inside the scanner may have influenced the performance levels of the participants in the scanner. This can be seen by the decrease in performance accuracy on the *trained* relations from the criterion of >80% correct outside the scanner to ∼ 67% correct inside the scanner for individuals with FXS. Future studies could examine whether providing contingent feedback (i.e., providing information concerning whether the response by the participant was correct or incorrect on each trial) in the scanner could increase percentage correct responding on tests of emergent relations.

Finally, we compared individuals with FXS to a group of age- and IQ-matched individuals with intellectual disability rather than neurotypical individuals. This was done to ensure that any differences between individuals with FXS and controls were not simply due to other explanatory variables such as IQ. Performance accuracy and degree of autistic symptomatology was also similar between the two groups, thus those factors could also be ruled out. However, we are unable to ascertain whether similar brain regions would have been recruited in neurotypical individuals during these tests.

Despite these limitations, to our knowledge, this is the first study to evaluate differences in the neural correlates of stimulus equivalence relations between individuals with FXS and age- and IQ-matched controls. In addition, this is also the first study to examine the neural correlates of mathematical proportional processing in those with intellectual disabilities. Taken together, the results suggest that there may have been significant differences in the neural execution between participants with FXS and controls.

It should be pointed out that, as used in the present study, the fMRI data only reveal spatiotemporal differences between the groups. The fMRI data alone cannot tell us what strategies our participants were employing. However, as a starting point, it can be assumed that there is a core network or ‘semantic network’ of brain regions employed during equivalence formation. In our between group comparison, results showing that the FXS group evidenced more activation in specific brain regions does not mean that the control group did not (and vice versa). It only means that the FXS group response was greater (or

lesser). Indeed, it may be the case that both groups actually recruited the exact same network (and by extension used the same 'cognitive strategy') but that neural execution was just different for the FXS group. In addition to providing more information about the brain mechanisms involved in the emergence of stimulus equivalence relations, the results from this study provide information concerning how gene-brain-behavior interactions may influence stimulus equivalence formation in those with intellectual disabilities. Further study of stimulus equivalence relations in FXS may also provide further insight into the neurobehavioral bases of math deficits in FXS.

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Highlights

We used fMRI to investigate the neural correlates of emergent equivalence relations in individuals with intellectual disabilities.

Participants with FXS, and matched controls, were required to match fractions, pie-charts and decimal equivalences in the scanner.

The emergence *of symmetry* relations was correlated with increased brain activation in the left inferior parietal lobule, left postcentral gyrus, and left insula.

Brain activation was significantly greater in individuals with FXS compared with controls during transitivity/equivalence formation.

Gene-brain-behavior interactions may influence the emergence of stimulus equivalence in individuals with FXS.

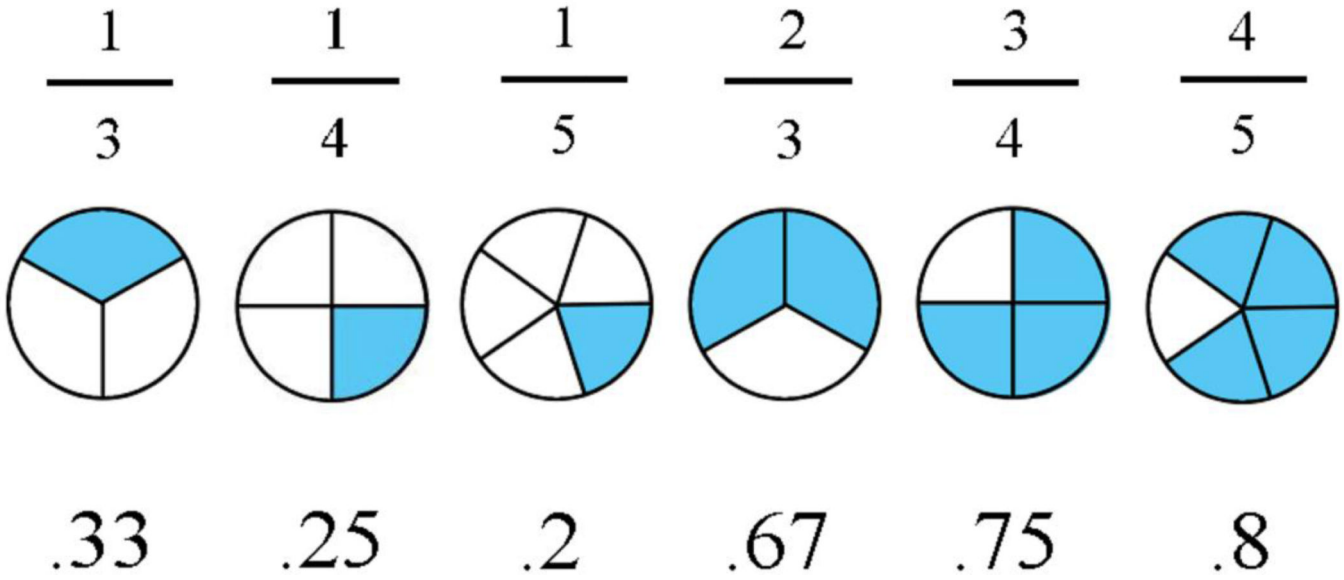

Fig. 1. Stimuli employed in pre-training and testing in the scanner

The figure shows sets of equivalent fractions (top row), pie charts (middle row), and decimals (bottom row) representing one-third, one-quarter, one-fifth, two-thirds, three-quarters and four-fifths (from left to right).

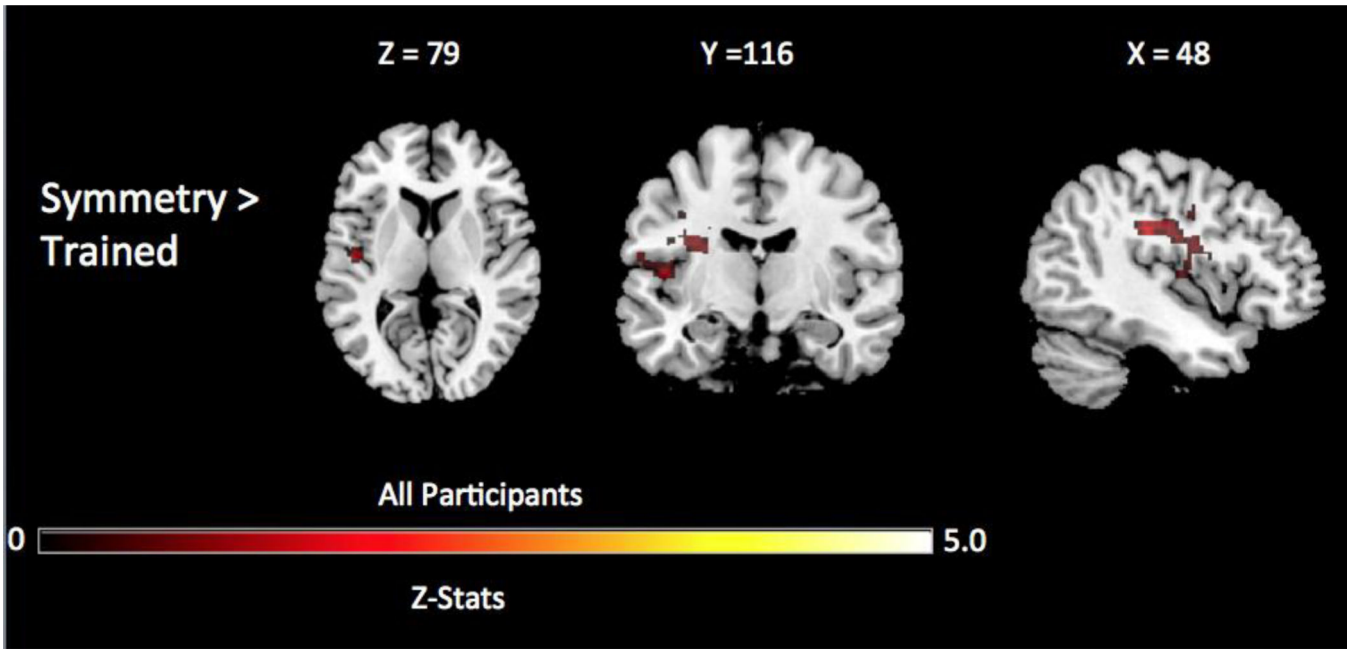

Fig. 2. Activation map obtained from the *symmetry* > *trained* contrast

The figure shows regions where activation was significantly greater on symmetry trials compared to trained trials across all participants (*N*=18).

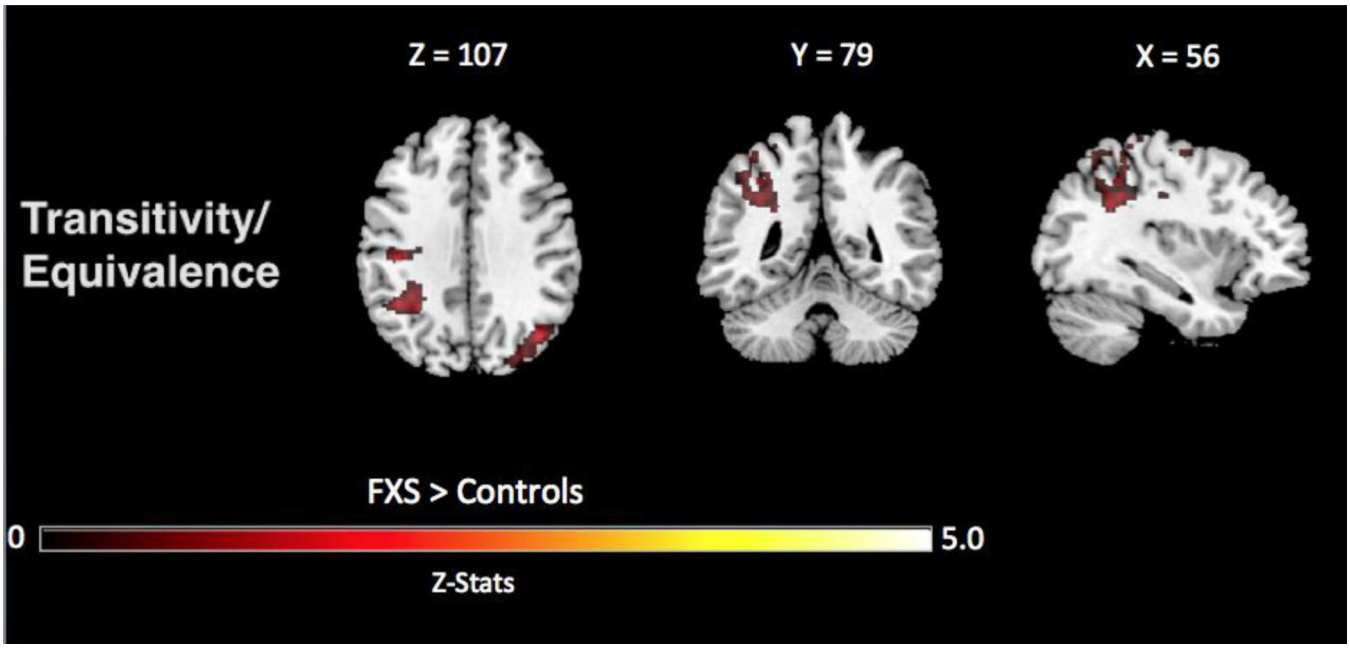

Fig. 3. Activation map obtained from the FXS > Controls contrast on the Transitivity/ equivalence test

The figure shows regions where activation was significantly greater in participants with FXS (*N*=8) compared with controls (*N*=10) on the transitivity/equivalence test.

Table 1

Demographic characteristics

Characteristic	FXS (*N*=8)	Controls (*N* = 10)	χ^2/t	*p*
Sex (f:m)	6:2	2:8	3.4	0.06
Age (years)	18.88 (4.11)	17.04 (3.49)	1.03	0.32
IQ	73.75 (8.41)	68.00 (13.67)	1.04	0.31
SCQ total score	7.63 (6.05)	10.20 (7.38)	−0.80	0.44

Note. SCQ = Social Communication Questionnaire.

The table shows the background characteristics for participants with fragile X syndrome (FXS) and controls. Means, SD's and tests to evaluate differences between the groups are shown for each characteristic.

Table 2

In-scanner behavioral performance for each group.

Measure	Test	FXS (*N* =8)	Controls (*N* = 10)
Accuracy (%)*	Trained	90.28 (9,27)	85.56 (13.41)
	Symmetry	84.03 (10.04)	81.11 (17.21)
	Transitivity/ equivalence	58.33 (26.40)	59.44 (33.74)
Response time (s)	Trained	3.35 (.71)	3.58 (1.02)
	Symmetry	3.71 (.87)	3.79 (1.18)
	Transitivity/ equivalence	4.90 (1.23)	4.64 (1.58)

*Note that 33.3% denotes chance responding for this task.

The table shows the background characteristics for participants with fragile X syndrome (FXS) and controls. Means, SD's and tests to evaluate differences between the groups are shown for each characteristic.

Table 3

Brain regions obtained from the *symmetry* > *trained* contrast.

Region	Cluster size	Peak *Z*	Side	BA	Coordinates		
					x	*y*	*z*
Inferior parietal lobule	683	2.99	L	40	−45	−31	26
Postcentral gyrus		2.89	L	2	−41	−29	28
Insula		2.74	L	13	−43	−17	25

BA = Brodmann Area; L = left. In regions with more than one cluster of activation, coordinates are listed for the cluster with highest activation. Number of voxels and peak activation are listed only for main clusters; activation is not listed for local maxima regions within clusters. The table shows regions where activation was significantly greater on symmetry trials compared to trained trials across all participants (*N*=18).

Table 4

Brain regions obtained from the FXS > Controls contrast on the transitivity/equivalence test.

Region	Cluster size	Peak *Z*	Side	BA	Coordinates		
					x	*y*	*z*
Middle temporal gyrus	3834	3.60	R	39	37	−62	27
Superior frontal gyrus		3.17	L	6	−18	−7	66
Precentral gyrus		3.12	L	4	−18	−24	63
Precuneus gyrus	-	3.12	L	7	−18	−58	56
Paracentral lobule		3.08	L	6	−3	−30	65

BA = Brodmann Area. R = right; L = left. In regions with more than one cluster of activation, coordinates are listed for the cluster with highest activation. Number of voxels and peak activation are listed only for main clusters; activation is not listed for local maxima regions within clusters. The table shows regions where activation was significantly greater in participants with FXS (*N*=8) compared with controls (*N*=10) on the transitivity/equivalence test.