Neuroscience 192 (2011) 345–350

FRAGILE X PROTEIN EXPRESSION IS LINKED TO VISUAL FUNCTIONS IN HEALTHY MALE VOLUNTEERS

S. KÉRI[a,b]* AND G. BENEDEK[a]

[a]*Department of Physiology, University of Szeged, Szeged, Hungary*

[b]*National Psychiatry Center, Budapest, Hungary*

Abstract—Fragile X syndrome (FXS) is characterized by the impairment of the magnocellular/dorsal visual system. In this study, we explored how fragile X protein (FMRP) expression may affect visual functions in healthy participants. The percentage of FMRP-positive lymphocytes was measured using a rapid antibody test in blood smears of 100 male volunteers. CGG triplet expansion was also determined. Results revealed that participants with fewer FMRP-positive lymphocytes exhibited lower performances on tests biasing information processing toward the magnocellular pathway and dorsal visual stream (contrast sensitivity at low spatial/high temporal frequency and motion coherence). It was not observed in the case of tests biasing information processing toward the parvocellular pathway and ventral stream (contrast sensitivity at high spatial/low temporal frequency and form coherence). These results suggest that healthy persons with lower peripheral FMRP expression display a visual phenotype similar to that described in patients with FXS. **© 2011 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: fragile X syndrome, vision, magnocellular, dorsal stream, motion.

Fragile X syndrome (FXS) is caused by the expansion of a CGG trinucleotide repeat within exon 1 of the *FMR1* gene (Xq27.3) (Verkerk et al., 1991). The full syndrome is characterized by autistic traits and deficits in executive functions, memory, social cognition, and sensory information processing (Koukoui and Chaudhuri, 2007; Bear et al., 2008). The full triplet expansion (>200 CGG) causes methylation of the *FMR1* promoter, resulting in transcription suppression and the absence of the fragile X protein (FMRP) (Oostra and Willemsen, 2009). In contrast to FXS, premutation carriers have a smaller number of CGG triplets (55–200), and they regularly display a subtle cognitive phenotype (Bennetto et al., 2001; Steyaert et al., 2003; Moore et al., 2004; Cornish et al., 2005, 2009; but see Franke et al., 1999). A recent study identified *FMR1* epigenetic regulatory sites where DNA methylation influences gene expression (Godler et al., 2010).

FMRP, which is an RNA-binding protein that controls the translation of numerous genes (De Rubeis and Bagni, 2010), seems to be essential for neuronal plasticity (Oostra and Willemsen, 2009). Mice lacking the *FMR1* gene have a smaller number of new cells in the dentate gyrus and neurons exhibit reduced dendritic arborization. These abnormalities are associated with the dysregulation of the Wnt signaling pathway (Luo et al., 2010). The expression of FMRP is especially rich in brain structures related to the clinical phenotype of FXS (e.g. prefrontal cortex, anterior cingulate cortex, hippocampus, and amygdala) (Zangenehpour et al., 2009).

Patients with FXS and premutation carriers display characteristic visual perceptual dysfunctions (Kogan et al., 2004a,b; Farzin et al., 2008; Kéri and Benedek, 2009, 2010). Specifically, the FXS visual phenotype is dominated by the impairment of the geniculo-striatal magnocellular (M) visual pathway (Kogan et al., 2004a,b). M pathways are sensitive to low luminance contrast, low spatial frequency (coarse resolution of objects), and rapid temporal changes. In contrast, parvocellular (P) pathways can be stimulated with static patterns with medium and high spatial frequency (fine details of objects) and colors (Van Essen and Gallant, 1994; Callaway, 2005). M and P pathways interact in the primary visual cortex (Sawatari and Callaway, 1996; Vidyasagar et al., 2002), but M pathways give a strong input to cortical areas responsible for motion perception, detection of spatial location, and visuo-motor coordination (dorsal occipito-parietal stream). P pathways give afferents to ventral occipito-temporal regions related to color perception and object recognition (Van Essen and Gallant, 1994). Rapid signals of M pathways may modulate information processing in the ventral stream via the facilitation of top–down control (Chen et al., 2007; Kveraga et al., 2007).

Kogan et al. (2004a) and Zangenehpour et al. (2009) found that the M layers of the lateral geniculate nucleus exhibit high FMRP expression, suggesting that these neurons are especially vulnerable in FXS. Consistent with these data, patients with FXS and premutation carriers show deficits on tests of M pathways (contrast sensitivity at low spatial and high temporal frequency gratings) but not on tests of P pathways (high spatial frequency and color processing) (Kogan et al., 2004a; Kéri and Benedek, 2009). These low-level visual deficits are accompanied by abnormal motion perception and visuo-spatial problems (Kogan et al., 2004a,b; Flanagan et al., 2007; Walter et al., 2009).

FMRP levels display a considerable level of individual variability. It was evident even from early studies (Willemsen et al., 1995, 1997), utilizing a rapid antibody test to determine FMRP in peripheral lymphocytes, that approximately 60% of healthy volunteers display 90–100% FMRP-positive lymphocytes, whereas in the other 40% of

*Correspondence to: S. Kéri, Department of Physiology, University of Szeged, Dóm Square 10, Szeged, Hungary. Tel: +36-20-448-3530; fax: +36-62-545-842.
E-mail address: szkeri@phys.szote.u-szeged.hu (S. Kéri).
Abbreviations: ANOVA, analysis of variance; DAB, 3,3′-diaminobenzidine tetrahydrochloride; FMRP, Fragile X protein; FXS, Fragile X syndrome; M, magnocellular; P, parvocellular.

0306-4522/11 $ - see front matter © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2011.06.074

participants the level of expression is lower. This peripheral marker is a reliable measure of FXS in males; in patients with the full syndrome, only a few lymphocytes are positive for FMRP (Willemsen et al., 1995, 1997; de Vries et al., 1998; Ravindran et al., 2005; Romero-Espinoza et al., 2010). However, the potential consequence of individual differences in FMRP in healthy persons is not known. Therefore, the present study was designed to investigate visual functions in individuals with different degrees of peripheral FMRP expression. Based on previous studies that found impaired M pathway function in patients with FXS (Kogan et al., 2004a) and in premutation carriers (Kéri and Benedek, 2009), we hypothesized that M pathway functions are associated with FMRP levels in healthy volunteers.

EXPERIMENTAL PROCEDURES

Participants

One hundred male volunteers were recruited from the community via personal networks and advertisement (mean age: 38.6 years, range: 18–54; mean education: 11.8 years, range: 8–20). None of the participants reported neurological or mental disorders and they had normal or corrected-to-normal visual acuity. The study was approved by the local ethics board and informed consent was obtained from each participant.

FMRP expression in peripheral lymphocytes and DNA analysis

Three blood smears were prepared within 1 h after blood collection. We conducted a 3-step indirect immuno-peroxidase staining using the Zymed Histostain-Plus Kit (Zymed, San Francisco, CA, USA). After fixation in 3% paraformaldehyde (in Sörrensen-Buffer-Solution) and permeabilization with 100% methanol, slides were incubated with monoclonal T1a-antibody (1:1500, 100 μl per slide) and then with biotinylated secondary antibody (Zymed-Histostain Plus Kit "Reagent B," 8596-43) and peroxidase-conjugated streptavidin (Zymed-Histostain Plus Kit "Reagent C," 8596-43) (100 μl per slide). The slides were then incubated with 3,3′-diaminobenzidine tetrahydrochloride (DAB) substrate (DAKO Liquid DAB substrate—chromogen system, K3466-11; 20 μl DAB per 1 ml buffer, 100 μl per slide) and counterstained with Nuclear Fast Red (3 g Nuclear Fast Red plus 150 g $Al_2(SO_4)_3.18H_2O$ in 3000 ml warm aqua dest) (for the detailed protocol, see http://www2.eur.nl/fgg/ch1/fragx/). Smears were examined with a Zeiss Axioskop 20. One hundred lymphocytes were analyzed for each participant and scored for FMRP expression by two independent raters. CGG triplet expansion was assessed using a Southern blot analysis (Steyaert et al., 2003).

Visual contrast sensitivity

Stimuli were vertical sinusoidal luminance-contrast gratings presented on a gamma-corrected ViewSonic PF815 monitor, which was controlled by an IBM-compatible PC. The gratings used to test M and P pathways had different spatiotemporal properties (M pathway: spatial frequency: 0.3 cycle/degree, temporal frequency: 10 Hz; P pathway: spatial frequency: 10 cycles/degree, temporal frequency: 1 Hz) (Fig. 1). The mean luminance of the circular stimulus area was 31 cd/m^2. The diameter of the stimulus area was 8 degrees of visual angle. At the beginning of the test, the luminance was counterphase-modulated by 12% Michelson contrast. This level was increased or decreased according to a Yes/No one-up/two-down staircase procedure. Responses were entered by pressing one of two keys on the computer keyboard. The staircase was terminated when the slope and SD of the last 12 trials was less than the step size. Detection thresholds were the mean value of the last 12 reversals (for methodological details, see Kogan et al., 2004a; Kéri and Benedek, 2009).

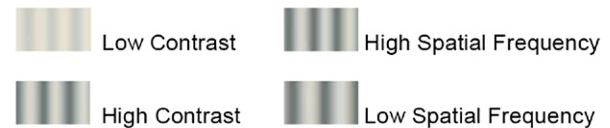

Fig. 1. Illustration of gratings with low/high contrast and low/high spatial frequency.

Motion coherence

The stimulus display was the same as described in the visual contrast sensitivity measurements. Stimuli consisted of two random dot kinematograms (high luminance white dots on a black background, density 4 dots/degree). One of the kinematograms was localized on one side of the screen and was divided into three horizontal strips. The direction of the coherent motion in the middle target strip was the opposite to that of the two outer strips. The kinematogram on the opposite side of the screen displayed a uniform direction of coherent motion. The task was to indicate the side (left or right) where the target strip exhibited coherent motion. At a trial a variable proportion of the dots oscillated horizontally across each array forming the coherent motion (velocity: 6 degrees/s; lifetime of dots: 70 ms), whereas the remaining dots moved in random directions. The direction of coherent motion reversed every 240 ms. Coherence level was adjusted using a two-up/one-down staircase procedure starting with 100% coherent motion in the target strip. Trials with 100% coherent motion were randomly presented during the experiment to control for spurious responding. Coherence thresholds were the mean value of the last 10 reversals (Kogan et al., 2004a).

Form coherence

We adopted the method of Atkinson et al. (1997). Stimuli consisted of a static array of randomly orientated short white line segments on a black background (density: 1.3 segments/degree). The stimulus contained a target area on one side of the screen where segments were oriented tangentially and hence formed circles. The coherence value was defined as the proportion of tangentially oriented segments embedded in randomly oriented noise segments in the target area. The task was to indicate the side (left or right) where the target exhibited a coherent form. The coherence level was modified according to a two-up/one-down staircase procedure beginning with 100% concentricity on the target side. Similarly to the motion coherence task, catch trials with 100% concentricity were randomly exposed to control for spurious responding. Coherence threshold was the mean value of the last 10 reversals.

General cognitive functions

All participants received the revised version of the Wechsler Adult Intelligence Scale (WAIS-R) (Wechsler, 1981).

Data analysis

Participants were divided into three groups according to the percentage of FMRP-positive lymphocytes (90–100%, 80–89%, and 70–79% of lymphocytes). Repeated measures analysis of variance (ANOVA) was used to compare these groups (group was the

Download English Version:

https://daneshyari.com/en/article/6276081

Download Persian Version:

https://daneshyari.com/article/6276081

Daneshyari.com