Social memory associated with estrogen receptor polymorphisms in women

Sara Karlsson,1 Susanne Henningsson,1 Daniel Hovey,1 Anna Zettergren,1,2 Lina Jonsson,1 Diana S. Cortes,3 Jonas Melke,1 Petri Laukka,3 Håkan Fischer,3 and Lars Westberg1

1Department of Pharmacology, Institute of Neuroscience and Physiology at the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, 2Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology at the Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, and 3Department of Psychology, Stockholm University, Stockholm, Sweden

Correspondence should be addressed to Lars Westberg, University of Gothenburg, Department of Pharmacology, Institute of Neuroscience and Physiology at the Sahlgrenska Academy, Box 431, 40530 Gothenburg, Sweden. E-mail: lars.westberg@pharm.gu.se.

Abstract

The ability to recognize the identity of faces and voices is essential for social relationships. Although the heritability of social memory is high, knowledge about the contributing genes is sparse. Since sex differences and rodent studies support an influence of estrogens and androgens on social memory, polymorphisms in the estrogen and androgen receptor genes (ESR1, ESR2, AR) are candidates for this trait. Recognition of faces and vocal sounds, separately and combined, was investigated in 490 subjects, genotyped for 10 single nucleotide polymorphisms (SNPs) in ESR1, four in ESR2 and one in the AR. Four of the associations survived correction for multiple testing: women carrying rare alleles of the three ESR2 SNPs, rs928554, rs1271572 and rs1256030, in linkage disequilibrium with each other, displayed superior face recognition compared with non-carriers. Furthermore, the uncommon genotype of the ESR1 SNP rs2504063 was associated with better recognition of identity through vocal sounds, also specifically in women. This study demonstrates evidence for associations in women between face recognition and variation in ESR2, and recognition of identity through vocal sounds and variation in ESR1. These results suggest that estrogen receptors may regulate social memory function in humans, in line with what has previously been established in mice.

Key words: face recognition; social memory; estrogen; ESR1; ESR2

Introduction

Social memory refers to the ability to recognize the identity of previously encountered individuals, an ability essential for successful social interactions. While in rodents this skill is based on olfactory and pheromonal signals, in humans it is based mainly on the identification of faces and voices (Belin et al., 2011).

Face recognition, the most investigated facet of social memory in humans, varies considerably in the population (Kennerknecht et al., 2006; Russell et al., 2009). On one end of the spectrum, prosopagnosia is characterized by serious impairments (Hecken and Angelergues, 1962), and on the other hand, exceptional abilities in face recognition have been reported (Russell et al., 2009). The ability to recognize faces has been reported to be highly heritable (Wilmer et al., 2010), indicating that some of the inter-individual variation in face recognition ability can be explained by genetic factors. For congenital prosopagnosia, an autosomal dominant inheritance has even been suggested (Kennerknecht et al., 2006).

On average, women perform better than men in tasks measuring face recognition (Lewin and Herlitz, 2002; Rehnman and...
The chosen SNPs have been shown to have 95% associations between different ethnic groups (Dvornyk et al., 2003; Barzan et al., 2013), subjects of non-Caucasian or unknown ethnicity (n = 91) were excluded from the original sample (n = 582). For 50% of the single nucleotide polymorphisms (SNPs) in our sample, the minor allele in the Caucasian group was the common allele in the mixed group.

Materials and methods

Materials

DNA was extracted from saliva samples using OraGene DNA self-collection kit (DNA Genotek, Inc., Ottawa, ON). Ten SNPs covering ESR1 (rs1999805, rs2504063, rs488133, rs2071454, rs2234693, rs222208, rs3020314, rs2273206, rs2747648, rs1062577) and ESR2 (rs1271572, rs1256030, rs928554, rs4986938, rs6152) were genotyped with KASPar®, a competitive allele-specific polymerase chain reaction SNP genotyping system using FRET quencher cassette oligos (http://www.lgcgenomics.com). The genotyping success rate was >95%. The chosen SNPs have been shown to have consequences for the protein function and/or previously been associated with sex steroid-related disorders or traits (Zettergren et al., 2013).

Methods

Participants

The study included 490 participants for whom both behavioral and genetic data were available, 181 men (age range 18–36 years, mean ± SD: 23.7 ± 3.1) and 309 women (age range 18–34 years, mean ± SD: 23.0 ± 3.2). All participants were Caucasian, right-handed, fluent in Swedish, healthy and had no past or present psychiatric diseases or substance abuse. All participants provided written informed consent in accordance with the declaration of Helsinki. The study was approved by the regional ethical review board of Stockholm. Ethnicity was assessed by asking which country parents and grand-parents were born in. Due to previously reported differences in allele frequencies and associations between different ethnic groups (Dvornyk et al., 2003; Barzan et al., 2013; Fu et al., 2014), subjects of non-Caucasian or unknown ethnicity (n = 91) were excluded from the original sample (n = 582). For 50% of the single nucleotide polymorphisms (SNPs) in our sample, the minor allele in the Caucasian group was the common allele in the mixed group of non-Caucasians.

Genotyping

In a sample from the normal population, genotyped for common polymorphisms in the genes encoding ER-α, ER-β and AR, i.e. ESR1, ESR2 and AR, we measured performance in recognition memory of faces displaying neutral and emotional expressions, as well as recognition of neutral and emotional vocal sounds and recognition in a multimodal condition where participants saw faces and heard the corresponding sounds simultaneously. We hypothesized that genetic variation in these genes would explain variation in social memory.

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Task

The social memory task, measuring the recognition of the identity of faces, vocal sounds and their combination, comprised an incidental encoding session and a recognition session. During the encoding session, participants were presented with 24 photographs of faces, followed by 24 human vocal sounds, followed by 24 multimodal stimuli (photographs of faces presented together with vocal sounds). The order was randomized (within the modality, not between) across subjects and no face or voice identity was presented more than once in each condition. The faces and voices expressed anger, disgust, fear, happiness, sadness or no emotion (four stimuli for each condition). The timing for the presentation was self-paced and participants were asked to indicate with the mouse which emotion was conveyed by the stimulus in a forced-choice task. The response options were the same as the expressed emotions. MediaLab software (Jarvis, 2008) was used for stimulus presentation and recording of responses. The pictures were color photographs from the FACES database (Ebner et al., 2010) and depicted equal numbers of men and women and younger and older faces. The sounds were non-linguistic emotional vocalizations (e.g. crying, laughter, sighs, screams) from the VENEC database (Laukka et al., 2013). At recognition (6–10 min after encoding depending on condition and reaction times) the same stimuli, interspersed with the same number of new distractor stimuli (24 old, 24 new), were presented. The same identity always expressed the same emotion. The participants were asked to indicate (self-paced) if they remembered the stimulus from the encoding session, if they knew the stimulus to be familiar or if they did not recognize the stimulus [the remember/know-paradigm (Gardiner and Richardson-Klavehn, 2000)]. Pooling the correct answers (remember and know answers) and controlling hit frequency for false alarms (Stanislaw and Todorov, 1999) provided four measures of recognition accuracy: d’faces, d’vocal, d’multimodal and the average across presentation modalities, d’all.

Statistical analyses

All analyses were carried out using SPSS (version 22, Chicago, IL). Linear regression models (additive model; add) were used, treating the heterozygote as the intermediate, and t-tests, pooling either the uncommon homozygote (dominant model; dom) or the common homozygote (recessive model; rec) with the heterozygote. Multiple testing was controlled for using Bonferroni correction. The corrected P-value was determined by dividing $s = 0.05$ by 270, i.e. controlling for the 15 SNPs, the three models (additive, dominant and recessive), the three measures (d’face, d’vocal and d’multimodal) and the two sexes (men and women), resulting in a corrected significance threshold of $P_{\text{corrected}} = 0.00019$. For significant associations between polymorphism and social stimulus, post-hoc tests included separating the tests on male and female stimuli. Linkage disequilibrium (LD) between polymorphisms and Hardy–Weinberg equilibrium was assessed by Haploview 4.2. The power of the t-tests was determined in the software R by simulating 10,000 samples with a mean difference of 20% and with minor genotype-group frequencies of 0.2 and 0.4, approximating the recessive and dominant models for the SNPs with a variety of different minor allele frequencies (Table 1).

Results

Allele frequencies are displayed in Table 1. All SNPs were in Hardy–Weinberg equilibrium. T-tests revealed that there was no significant difference in any of the measures of social memory between men and women ($P$-values $>0.4$).

Out of the 10 investigated ESR1 SNPs one showed a significant association with a social recognition measure which survived correction for multiple testing. Specifically, rs2504063 was associated with recognition of identity through vocal sounds in women ($t_{\text{rec}} = 3.95$, Table 1, Figure 1A). Carriers of the uncommon genotype had better social memory than carriers of the common allele. Although there was no trend in men, the sex by polymorphism interaction was not significant in a linear regression model including also rs2504063 and sex as independent variables ($P > 0.1$). The relationship, in women, was observed for both male and female vocal stimuli (male voices: $P = 0.03$; female voices: $P = 0.002$). The low number of repetitions per emotion type did not allow for expression-specific analyses. However, excluding the four neutral vocal expressions showed that also the association between recognition of emotional vocal sounds and the rs2504063 was significant in women ($t_{\text{rec}} = 3.51$). Moreover, there was an
association between vocal sound recognition and ESR1 rs3020314, which did not survive correction for multiple testing \((P\text{-values} > 0.00019)\) in either women or men \((F: P_{\text{dom}} = 0.009, t_{304} = 2.64; M: P_{\text{dom}} = 0.03, t_{179} = -2.2, \text{Table 1})\). The two SNPs rs2504063 and rs3020314 were not in high LD with each other \((D' = 0.48, r^2 = 0.05, \text{Figure 2A})\), indicating that the potential effects may be independent of each other.

Out of the four investigated ESR2 SNPs three, i.e. rs1271572, rs1256030 and rs928554, in high LD with each other \((D' > 0.8, r^2 > 0.6, \text{Figure 2B})\), displayed significant associations surviving correction for multiple testing, with face recognition in women \((rs1271572: P_{\text{dom}} = 0.00003, t_{307} = 4.2; rs1256030: P_{\text{dom}} = 0.0001, t_{305} = 3.9; rs928554: P_{\text{dom}} = 0.00001, t_{304} = 4.46, \text{Figure 1B})\). Post-hoc analyses are presented for rs928554 only since the results for the other two ESR2 polymorphisms were almost identical. The associations were significantly stronger in women than in men \((P = 0.01\) for the rs928554 by sex interaction term). In women, the association strengths were similar for male and female face stimuli \((\text{Male faces: } P_{\text{rs928554}} = 0.007; \text{Female faces: } P_{\text{rs928554}} = 0.01)\). Excluding the four neutral face repetitions showed that the association with the recognition of emotional face expressions also was significant in women \((P_{\text{dom}} = 0.000008, t_{305} = 4.6)\). In men, d’multimodal was nominally associated with the ESR2 SNPs rs1256030 and rs928554, respectively \((rs1256030: P = 0.03, t_{178} = -2.1; \text{rs928554: } P = 0.04, t_{179} = -2.1, \text{Table 1})\).

The AR polymorphism did not display any association that survived correction for multiple testing \((M: \text{rs6152 for } d’\text{all: } P = 0.013, t_{177} = -2.51)\). There were thus no robust associations between any of the SNPs and social memory in men using the current sample. The power of finding an effect in men was above 80% for faces and above 60% for vocal sounds. For women, the power was above 90% for faces and above 70% for vocal sounds.

**Discussion**

This study was motivated by the sex differences in recognition memory reported for faces, the importance of estrogen receptors for social memory in mice and the heredity of social recognition in humans. In a sample of healthy adults, we demonstrated, specifically in women, significant associations between variation in ESR1 and ESR2 and recognition of identity through vocal sounds and faces, respectively. Although many studies suggest that the sex difference in face recognition is strongest for recognition of female faces, the associations between ESR1, ESR2 and recognition reported here were independent of the sex of the stimuli subjects.

ESR1 and ESR2 are situated on chromosomes 6q25 and 14q23, respectively, and are expressed in the human brain \((\text{Osterlund et al., 2000})\). As ligand-dependent transcription factors, ER-\(\alpha\) and \(\beta\) regulate the synthesis of several neurotransmitters and neuropeptides, and trigger multiple signal transduction pathways involved in various types of memory \((\text{Phan et al., 2011; Gabor et al., 2012})\). Due to the crucial role that the neuropeptide oxytocin plays for encoding of social memories \((\text{Ferguson et al., 2002})\), the effects of estrogen on social recognition has been proposed to depend on expression regulation of the neuropeptide oxytocin and its receptor \((\text{Gabor et al., 2012})\). In addition to the genomic effects, the estrogen receptors also affect signal transduction on a rapid time-scale via non-genomic actions \((\text{Gabor et al., 2012})\). In line with our results, studies of ER-\(\alpha\) knock-out mice corroborate differences between males and females in showing that the memory impairments in females are larger and include encoding, short- and long-term memory, whereas male knock-out mice show intact short-term memory \((\text{Tang et al., 2005; Sanchez-Andrade and Kendrick, 2011})\). The impact of ER-\(\beta\) on social recognition in mice is less prominent than that of ER-\(\alpha\), but more important for females.
than for males (Sanchez-Andrade and Kendrick, 2011). This is in line with our finding of an association between ESR2 SNPs and face recognition in women only.

A limitation of our study is that we did not record hormonal contraception or menstrual cycle phase. However, although these factors have been shown to affect face processing (Gingnell et al., 2012; Mareckova et al., 2014), since we have no reason to believe that contraceptive use or cycle phase would be unevenly distributed across genotypes, it is unlikely that the lack of control for these factors would contribute the observed effects. It is more likely that variation in contraceptive use and menstrual cycle phase would add to the noise and thus reduce the power of finding the observed associations. Although the sample, to our knowledge, is one of the largest available including both DNA and experimentally assessed social recognition, it has to be considered as relatively small compared with those often used in studies of the genetics of psychiatric disorders and self-assessed behaviors. The results should be interpreted with caution until independent replications have been reported.

Due to the limited knowledge about differences between the neural mechanisms mediating recognition memory of faces and voices in humans, and how these mechanisms are modulated by estrogen, it is difficult to interpret the modality-specific associations of ESR1 and ESR2, respectively. Interestingly, however, pharmacological and knockout studies in mice suggest that the actions of ER-α and ER-β on social recognition and learning are dependent on the learning paradigm, the steroid concentrations and on the genomic or non-genomic mode of action (Choleris et al., 2006; Clipperton et al., 2008; Phan et al., 2011; Sanchez-Andrade and Kendrick, 2011). Furthermore, although the role of estrogen for voice recognition is only sparsely investigated, it plays an essential role for the memory of songs in zebra finches (Yoder et al., 2012).

Carriers of the uncommon AA-genotype of the ESR1 promoter polymorphism rs2504063 showed higher social memory via voice recognition. To our knowledge, rs2504063 has not been shown to affect the expression or function of the ER-α, but the previous finding of a genome-wide significant association with bone mineral density (Rivadeneira et al., 2009) suggests that this SNP itself, or an adjacent variant, may modulate ER-α function. It is noteworthy that although the investigated ESR1 SNPs are distributed throughout the gene the coverage of the genetic variation of the gene is rather low (Figure 2A), suggesting that ESR1 should be investigated more comprehensively in future studies.

The uncommon alleles of the three ESR2 polymorphisms, i.e. rs1271572, rs1256030 and rs928554, situated in the promoter, intron 1 and the 3’UTR, respectively, were associated with superior face memory. Although the rs1271572 has been clearly shown to affect the expression of ESR2 by affecting the transcription factor binding sites in the promoter region (Chen et al., 2013), and to be associated with various types of hormone-related cancers (Lurie et al., 2011; Ryan et al., 2012; Chen et al., 2013), the consequences of this polymorphism for brain function remain unknown. In line with impaired or abnormal face recognition in patients with autism spectrum disorder (ASD) (Greimel et al., 2014), the alleles of the ESR2 rs1271572 and rs1256030 that were associated with poorer face recognition abilities have previously been associated with ASD (Chakrabarti et al., 2009). As seen in Figure 2B, the investigated SNPs provide good coverage of the variation of ESR2.

Taken together, the studies so far, in rodents as well as in humans, argue that estrogen receptors are important for the effects of sex steroids on social recognition memory. Of note, due to the nature of genetic associations our results offer no proof as to whether the potential influence of estrogens on social recognition should be categorized as organizational, i.e. a permanent influence caused by prenatal effects of sex hormones on brain morphology, or activational, e.g. reversible effects on neurotransmission.

We did not observe an association between the AR polymorphism and social memory that survived correction for multiple testing (Table 1). However, since previous evidence indicate that the AR may modulate social recognition in rodents, and that ARs are important for sexual differentiation of the human brain during early, prenatal development, the AR does deserve further attention in future studies of social memory (Axelson et al., 1999; Pierman et al., 2008; Zuloaga et al., 2008; Gabor et al., 2012).

In conclusion, our results provide evidence for an association between face recognition memory and variation in ESR2, and recognition of identity through vocal sounds and variation in ESR1. Although the results should be interpreted with caution pending replication, they suggest that estrogen receptors may regulate social memory function in humans, similar to what has previously been established in mice.

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Conflict of interest

None declared.

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