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ORIGINAL ARTICLE

Sex Differences in Oestrogen-Induced p44/42 MAPK Phosphorylation in the Mouse Brain *In Vivo*

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In addition to the classical direct genomic mechanisms of action, oestrogen also exerts poorly understood, nonclassical effects on the signalling system in neurones. In the present study, we investigated whether sex differences exist in gonadectomy- and oestrogen-induced effects on p44/42 mitogen-activated protein kinase (MAPK) phosphorylation in specific brain regions of mice. We demonstrate that MAPK immunoreactivity was not altered by gonadectomy or oestrogen treatment in either sex. However, we show that the level of phosphorylated MAPK (pMAPK) within the anteroventral periventricular nucleus (AVPV) was consistently higher in males than females irrespective of gonadal steroid hormone status. In addition, gonadectomy was found to decrease pMAPK immunoreactivity within the piriform cortex of males. Oestrogen increased pMAPK immunoreactivity in the medial preoptic area and AVPV of females, but failed to have the same effect in male mice. Overall, these results demonstrate a marked sex difference in oestrogen-induced alteration of MAPK phosphorylation in the brain *in vivo*.

Key words: oestrogen, gonadectomy, sex differences, MAPK phosphorylation.

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Oestrogen effectively alters the neuronal function in the central nervous system. Besides the well-established oestrogen receptor (ER)-mediated direct genomic effect, oestrogen also exerts nonclassical actions via multiple different signalling pathways (1, 2). The nonclassical actions of oestrogen can represent an 'indirect genomic' effect, because the downstream signalling cascades can result in changes in gene transcription in a pathway-specific manner. Previously, several groups, including our own, have demonstrated that oestrogen can induce a rapid activation of signalling pathways regulating transcription factors such as cAMP responsive element binding protein (CREB) in the female anteroventral periventricular nucleus (AVPV) and medial preoptic area (mPOA) *in vivo* (3–5, 6). A potential signal transduction pathway that might mediate oestrogen hormone action and play pivotal role in CREB activation is the mitogen-activated protein kinase pathway. The mitogen activated protein kinase pathway is an evolutionarily conserved signalling cascade involved in physiological responses, including cell proliferation, survival and differentiation, and it also takes part in synaptic plasticity in neurones (7–9). Increasing evidence demonstrates that this pathway plays a crucial role in cognitive functions, learning and memory formation (10, 11). The key molecule of this pathway is the p44/42 mitogen-activated protein kinase (MAPK, also called ERK1/2). On activation, MAPK is phosphorylated (pMAPK), and then phosphorylates CREB via mitogen-and stress activated protein kinase-1 or ribosomal SG kinases (RSK1–3) (12–14). Oestrogen can activate MAPK via phosphorylation (6, 15, 16).

Many effects of oestrogen on brain function exhibit sex differences. Oestrogen regulates reproduction via its effects on the hypothalamo-pituitary axis and sexual behaviour (17). In adulthood, oestrogen stimulates massive gonadotrophin-releasing hormone (GnRH) secretion, inducing ovulation in females but has no similar effect in males (18). Oestrogen also acts differently on several processes, such as cell proliferation and cell death, cognitive and memory functions, brain plasticity, neuronal morphology (19–21), in males and females. During development, oestrogen-dependent mechanisms are responsible for a larger sexually dimorphic nucleus of the mPOA in male rodents providing a structural basis to sex differences in reproductive behaviour in adulthood (22). However, details of the mechanisms through which oestrogen exerts its sexually dimorphic effects on the brain are not clear. One possibility is

that sex differences exist at the molecular level: some brain areas exhibit different distributions of oestrogen receptors (23). Tissue-specific expression patterns of coactivator and corepressor genes (24, 25) that enhance or inhibit transcription through oestrogen receptors also show sexual dimorphism. Sex differences at molecular level are supported by recent data on mice where oestrogen induced CREB phosphorylation in the female but not male mPOA (26).

In the present study, we examined whether sex differences in oestrogen action upon CREB phosphorylation are dependent upon sex differences in the up-stream mitogen-activated protein kinase pathway. Therefore, using the same *in vivo* model as that used for our CREB studies, we examined the effects of endogenous differences and gonadectomy and oestrogen administration for MAPK phosphorylation in different brain areas of male and female mice *in vivo* using quantitative immunohistochemistry. Five brain regions were selected for analysis: mPOA, AVPV, oval subnucleus of the bed nucleus of the stria terminalis (BNST), piriform (Pir) and cingulate cortex (Cg). These areas were selected on the basis of their known ER or MAPK expression and the ability of oestrogen to phosphorylate CREB (5, 6, 27, 28).

Materials and methods

Animals

Adult female and male wild-type mice (C57BL6/J; Charles River, Budapest, Hungary) were bred and housed in our animal house. All mice were maintained under a 12 : 12 h light/dark cycle and supplied with food and water *ad lib*. The breeding and the experiments were carried out on the basis of Local Animal Care Committee at the Eötvös Loránd University in accordance with the EU conform Hungarian Act of Animal Care and Experimentation (1998, XXVIII, section 243/1998).

Treatments and tissue preparation

Experiment 1

C57BL6/J female and male mice were gonadectomised (GDX group: female, n = 5; male, n = 9) or sham-operated (SHAM group: female, n = 5; male, n = 9) at 62–79 days of age under avertin anaesthesia. The oestrous cycle of sham-operated female mice was assessed by daily vaginal smear and mice in the dioestrous stage were selected for perfusion. Two weeks later, all animals were transcardially perfused and 0.2-ml blood samples were taken for luteinising hormone (LH) radioimmunoassay (RIA). Brains were quickly removed and postfixed for 2 h, and placed into 30% sucrose Tris-buffered saline 0.1 M (TBS) (Sigma, St Louis, MO, USA) solution overnight at 4 °C. Four series of 30 μm-thick coronal sections were cut on a freezing microtome.

Experiment 2

C57BL6/J female and male mice were GDX at an average of 65–80 days of age under avertin anaesthesia. On postgonadectomy day 14, 1 μg 17β-oestradiol (E_2; Sigma; in 0.1 ml ethyloleate vehicle, GDX + E_2 group) or vehicle alone (GDX + V group) was administered subcutaneously to female and male mice. One hour later, all animals were deeply anaesthetised by overdose of Avertin and they were transcardially perfused with 20 ml ice-cold 4% paraformaldehyde (Sigma) in phosphate buffer (PB; pH 7.6). In previous studies, we have demonstrated that oestrogen administered in this manner in female mice significantly increases the MAPK phosphorylation in the brain within 1 h and remains elevated up to 4 h following treatment (6). Before perfusion, 0.2 ml blood was collected for LH RIA and brains were processed in the same manner as described above.

Immunocytochemical detection of pMAPK and MAPK

Immunocytochemistry was performed as described previously (6) with slight modification. Briefly, free-floating sections were incubated in mouse MAPK primary antibody (pan ERK, 1 : 2000, BD Transduction Laboratories, San Jose, CA, USA). The other set of slices was incubated in rabbit pMAPK primary antibody (1 : 1000, Cell Signalling, Denvers, MA, USA). This was followed by biotinylated goat antimouse IgGs or biotinylated goat anti-rabbit IgGs (1 : 200, Vector Laboratories, Peterborough, UK) and the Vector Elite avidin-biotin-HRP complex (1 : 500). Peroxidase labelling was visualised with nickel-diaminobenzidine tetrahydrocloride (Ni-DAB) using glucose oxidase. The pMAPK antibody detects endogenous levels of the 42- and 44-kDa isoforms of the mitogen-activated protein kinase when catalyticaly activated by phosphorylation, while the MAPK antiserum also recognises the 56- and 85-kDa members of the mitogen-activated protein kinase family. The specificities of the antibodies have been reported previously in rodent species including the mouse (6, 29, 30). The omission of primary antibodies resulted in a complete absence of immunoreactivity.

Image analysis of MAPK and pMAPK immunoreactivity

For qualitative evaluations, the number of MAPK and pMAPK immunoreactive-cells was counted using a computer-based imaging system (AnaliSYS, Soft Imaging System GmbH, Munster, Germany) with the images captured using a digital camera. An investigator blind to the experimental groupings evaluated all sections. For each region, two sections were selected at the appropriate level from each animal and bilateral cell counts were undertaken by counting all immunoreactive cells. The pMAPK immuno-stained cells were counted at ×10 magnification, on the basis of Franklin and Paxinos mouse brain atlas (31): mPOA, rectangle size, 0205 mm^2, Plate 29; AVPV, 0009 mm^2, Plate 28; BNST, 0122 mm^2, Plate 30; Pir, 0322 mm^2, Plate 38; Cg, 0195 mm^2, Plate 26. Because a large number of cells was stained for MAPK and they completely or partially overlapped one another in most cases (Fig. 1), we encountered difficulties in precise cell counting. Therefore, optical density (OD) was measured for estimation of quantitative differences in MAPK immunoreactivity between experimental groups. Using NIH Image software (NIH Image, Bethesda MD, USA), the OD value was obtained by averaging OD values of each section in the case of each area after background subtraction and grey scale threshold determination.

LH RIA

Plasma LH concentrations were determined with RIA using a mouse LH RIA kit obtained from the National Hormone and Pituitary Programme and are expressed in terms of NIADDK rat LH RP-3. The least detectable LH concentration was 0.16 ng/ml for 50 μl plasma, and the intra-assay coefficient of variation was 5.6%. Two parallels were assayed from each plasma sample in a single RIA.

Statistical analysis

Data are expressed as mean ± SEM. To examine the differences between experimental groups, a two-way ANOVA was performed ($P < 0.05$) with Tukey's post-hoc test (Statistica 7.0, StatSoft Inc., Tulsa, OK, USA).

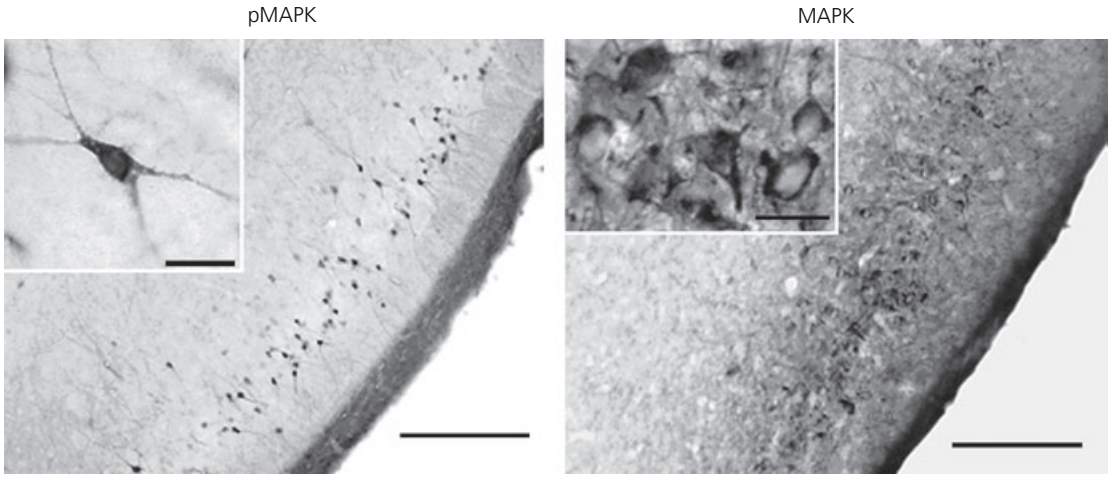

Fig. 1. Photomicrographs depicting phosphorylated p44/42 mitogen-activated protein kinase (pMAPK) and p44/42 mitogen-activated protein kinase (MAPK) immunoreactivity in piriform cortex. Scale bar = 100 μm (50 μm in insert).

Results

Experiment 1: effect of gonadectomy on MAPK phosphorylation in female and male mice

To examine the sex differences in the effect of endogenous oestrogen state on MAPK phosphorylation, we evaluated the expression of pMAPK and MAPK in the brain of intact and GDX female/male mice.

MAPK immunoreactivity was restricted to the cytoplasm of neurones while pMAPK expression was detected in both the nucleus and the cytoplasm (Fig. 1).

Quantitative analysis revealed that gonadectomy did not alter the intensity of MAPK immunoreactivity in any of the brain regions analysed and there were no sex differences (Table 1).

In female mice, gonadectomy significantly elevated ($P < 0.05$) the number of pMAPK positive cells exclusively in AVPV (Fig. 2A). By contrast, gonadectomy significantly reduced pMAPK positive cell numbers only in Pir (Fig. 2A,B) in male mice. Our data also demonstrate that the level of pMAPK immunoreactive cells in AVPV is significantly higher in GDX and intact male mice compared to the GDX and intact female mouse ($P < 0.05$) (Fig. 2A).

The reliability of experimental groups has been verified by the fact that LH levels were significantly higher in GDX mice than in SHAM animals (SHAM female: 2.11 ± 0.14 ng/ml, GDX female: 7.24 ± 0.1 ng/ml, SHAM male: 2.99 ± 0.1 ng/ml, GDX male: 8.08 ± 0.24 ng/ml; $P < 0.05$).

Experiment 2: effect of oestrogen on MAPK phosphorylation in GDX female and male mice

To examine whether oestrogen has a sexually dimorphic action on MAPK phosphorylation, we evaluated the effect of oestrogen injection on pMAPK immunopositive cell numbers in the brain of GDX female and male mice.

The OD of MAPK-immunoreactive cells was not altered by oestrogen treatment and there were no differences in female and male mice in any of the areas examined (Table 1).

Administration of oestrogen to GDX female mice significantly ($P < 0.05$) increased the number of pMAPK immunopositive cells in mPOA (Fig. 3A,B) and in AVPV (Fig. 3A), while there was no change in Pir, in BNST, and in Cg (Fig. 3A). In GDX male mice, oestrogen had no significant effect on the number of pMAPK immunoreactive cells in any of the regions analysed (Fig. 3A). Interestingly, in the vehicle-treated groups, the level of pMAPK expression of AVPV in male mice was significantly higher than in female mice ($P < 0.05$) (Fig. 3A).

Table 1. Summary of Effect of Oestrogen and Gonadectomy on p44/42 Mitogen-Activated Protein Kinase Expression in Different Brain Areas (n = 6 per group).

	♀	♀	♂	♂	♀	♀	♂	♂
Areas	GDX + E_2	GDX + V	GDX + E_2	GDX + V	SHAM	GDX	SHAM	GDX
mPOA	666.7 ± 136.9	765.6 ± 103.9	539.5 ± 52.1	670.1 ± 36.8	434.8 ± 27.1	461.2 ± 29.6	642.7 ± 67.2	642.7 ± 47.1
AVPV	910.5 ± 85.5	902.5 ± 43.5	1023 ± 172.4	1059 ± 138.1	464.1 ± 52.4	524.2 ± 54.4	681.7 ± 67.8	752.6 ± 118.9
BNST	877.7 ± 69.3	832.8 ± 44.8	772.9 ± 23.6	892.9 ± 75.5	524.2 ± 26.5	477.5 ± 19.6	531.1 ± 17.2	591.6 ± 53.6
Cg	790.4 ± 44.2	812.3 ± 27.1	753.4 ± 31.4	765 ± 83.5	636.3 ± 55.9	546.4 ± 11.5	598.3 ± 31.5	418.6 ± 21
Pir	569.1 ± 11.8	504.5 ± 13.9	883.5 ± 31.4	763.4 ± 54.8	640.8 ± 33.8	611.2 ± 34.2	683.6 ± 62.8	727.5 ± 62.8

Data are expressed in optical densities (pixel/area) ± SEM. GDX, gonadectomised; E_2, 17β-oestradiol; V, vehicle; SHAM, sham-operated.

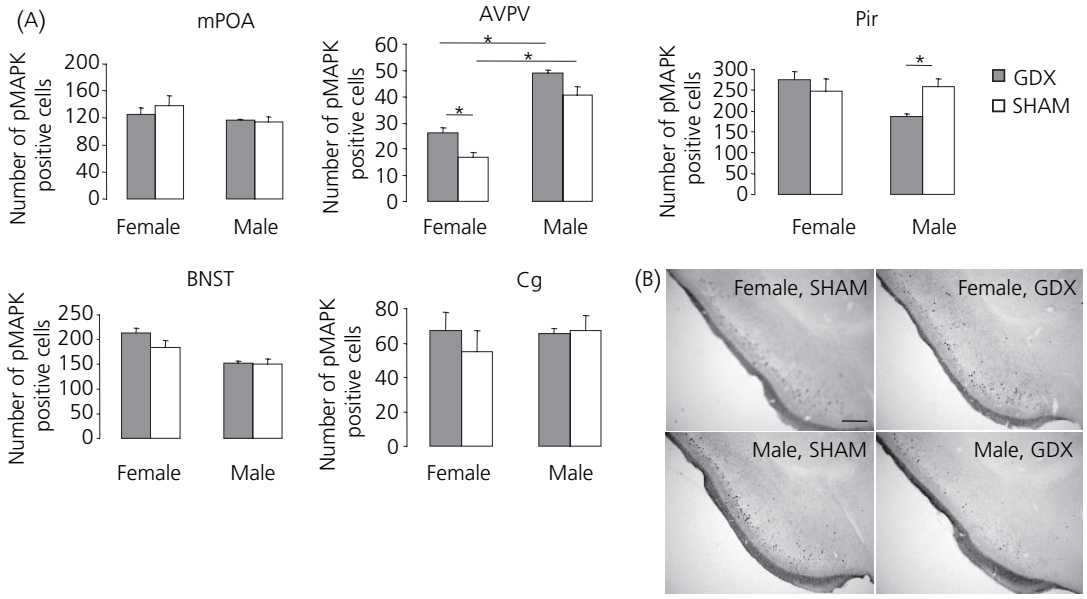

Fig. 2. Effect of gonadectomy on p44/42 mitogen-activated protein kinase (MAPK) phosphorylation in the brain of female and male mice. (A) Histograms show mean ± SEM number of phosphorylated MAPK (pMAPK) immunoreactive cells detected per unit area in the medial preoptic area (mPOA), in the anteroventral periventricular nucleus (AVPV), in the piriform cortex (Pir), in the bed nucleus stria terminalis-oval subnucleus (BNST) and in the cingulate cortex (Cg) of gonadectomised (GDX) and sham-operated (SHAM) female and male mice. *$P < 0.05$ ($n = 5$–9 per group). (B) Photomicrographs demonstrate the changes in pMAPK immunoreactivity in Pir of female and male mice after gonadectomy or sham surgery. Scale bar = 200 μm.

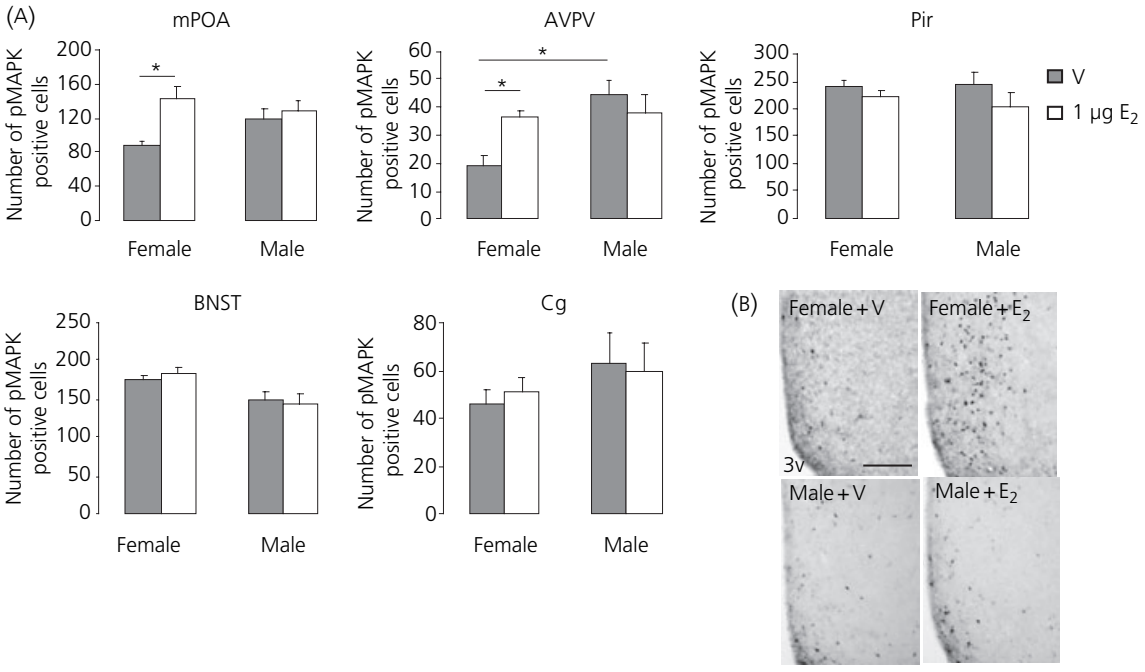

Fig. 3. Effect of oestrogen on p44/42 mitogen-activated protein kinase (MAPK) phosphorylation in the brain of gonadectomised female and male mice. (A): Histograms show mean ± SEM number of phosphorylated MAPK (pMAPK) immunoreactive cells detected per unit area in the medial preoptic area (mPOA), in the anteroventral periventricular nucleus (AVPV), in the piriform cortex (Pir) in the bed nucleus stria terminalis-oval subnucleus (BNST) and in the cingulate cortex (Cg) of gonadectomised female and male mice treated with vehicle (V) or with 17β-oestradiol (E_2; 1 μg, 1 h). *$P < 0.05$ ($n = 6$–6 per group). (B) Photomicrographs demonstrate the E_2-induced changes in pMAPK immunoreactivity in mPOA of gonadectomised female and male mice injected with vehicle (V) or 17β-oestradiol (E_2). Scale bar = 100 μm.

The oestrogen effectively reduced plasma levels of LH in GDX-female as well as GDX-male mice (GDX female: 8.3 ± 1.9 ng/ml, GDX female + E_2: 2.8 ± 0.5 ng/ml, GDX male: 7.3 ± 0.27 ng/ml, GDX male + E_2: 3.01 ± 0.11 ng/ml; $P < 0.05$).

Discussion

The present data provide experimental evidence for clear sex- and region-specific differences in the effects of gonadectomy and oestrogen treatment on MAPK phosphorylation in the mouse brain. Males had higher pMAPK immunoreactivity in the AVPV compared to females, whereas gonadectomy decreased pMAPK positive cell numbers selectively in the Pir of males. GDX increased positive cell numbers only in the APV. Oestrogen treatment of GDX animals was without effect in males but, in females, rather than reversing the effects of GDX, pMAPK cell numbers were increased in the AVPV and also the mPOA. There were no sex differences on effects of oestrogen treatment on MAPK immunoreactivity in specific regions of the brain.

The possible mechanism and physiological consequence of sexually dimorphic oestrogen-induced action on MAPK phosphorylation in mPOA and AVPV

In agreement with previous results (6), our present findings demonstrate a robust effect of oestrogen on MAPK phosphorylation in mPOA and AVPV. However, Bryant *et al.* (32) did not find an increase in MAPK phosphorylation in these areas following oestrogen administration. The divergence between these findings may be explained by the method and timing of detection of pMAPK expression. Bryant *et al.* (32) determined the MAPK phosporylation in microdissected samples by means of Western blot at 20 min following oestradiol administration (32) whereas we performed our analysis on fixed brain tissue using immunohistochemistry at 1 h following oestradiol injection. The possible mechanism underlying the sex differences in oestrogen-induced MAPK phosphorylation in mPOA and AVPV may be that of sex differences in the distribution and density of different ER types in these areas. We have previously demonstrated that oestrogen-induced MAPK phosphorylation is dependent on the coexpression of ERα and ERβ in the female mouse hypothalamus, which suggest a possible importance of ERα/ERβ ratio in the mechanism of oestrogen-induced MAPK phsophorylation (6). More ERβ mRNA and protein are present in the mPOA and AVPV of male mice compared to females (33, 34), whereas the expression of ERα was found to be similar in both sexes (27, 34). The importance of the value of ERα/ERβ ratio over the total amount ERs is also supported by the fact that oestrogen-induced increase in pMAPK expression does not correlate with the total ER density in the brain, as recently demonstrated by Bryant *et al.* (32). Indeed, our present finding confirmed this observation because, in the AVPV and mPOA, where densities of ERα and ERβ are high (27), pMAPK immunoreactivity was sensitive to oestrogen administration. By contrast, in the BNST, no change was observed in pMAPK cell numbers after oestrogen treatment, despite substantial ERα and ERβ expression in this region (27, 35). These data suggest that although the ERα/ERβ ratio might play an important role in MAPK signalling the occurrence of oestrogen-induced MAPK phosphorylation is not an invariable consequence of the coexpression. However, it should be noted that the available oestrogen receptor density data relate to nuclear-located oestrogen receptors and the results were dependent on the primary antibody used. As has been proposed, only membrane or cytosolic ERs can be involved in oestrogen-induced alteration in MAPK phosphorylation (36, 37). Previously, we demonstrated that classical ERs mediate oestrogen actions on MAPK and CREB phosphorylation in the mouse brain (6), and we suggest that the location of ERs inside the cell highly determines their involvement in nonclassical effects of oestrogen. It can be speculated that ratio of membrane/cytosolic ER subtypes is a significant factor underlying sex differences in oestrogen-induced MAPK phosphorylation.

The other possible origin of the sexually dimorphic oestrogen-induced MAPK phosphorylation in the hypothalamus may relate to GABAergic neurones because regulation of GABA release is different in the two sexes (38) and GABAergic cells are involved in the modulation of sexual dimorphic pattern of LH release (39). Oestrogen elicits GABA release in the hypothalamus (40), which effectively reduces MAPK phosphorylation in the brain via its metabolic product, gamma-hydroxybutirate (GHB) (41). GABA-ergic interneurones project to a variety of neuronal phenotypes in the hypothalamus and the expression of the rate limiting enzyme of GABA (GAD65) was found to be lower in female rodent hypothalamus (42). Therefore, the oestrogen-induced inhibition via GHB may be less effective in MAPK phosphorylation in female than in male mice. Consequently, sex differences in GABA-ergic systems may partly contribute to the observed sex difference in oestrogen-induced MAPK phosphorylation in the hypothalamus. In addition to the role of GABA-ergic neurones, the sex dependent diversity in the number of MAPK expressing neuronal phenotypes may play an important role in the AVPV. Indeed, our data clearly demonstrate that more pMAPK expressing neurones can be found in SHAM or GDX males than in females in AVPV. Considering the fact that the AVPV contains less neurones in males than in females (43), our data suggest a saturation in MAPK phosphorylation in the male AVPV, which may contribute to the relative oestrogen insensitivity of MAPK phosphorylation in this brain area of male mice.

The most pressing question related to the sexual dimorphism uncovered in the effects of oestrogen upon MAPK phosphorylation in AVPV and mPOA is that of identifying the physiological relevance of observed differences. Most recent findings demonstrate that the mitogen activated protein kinase pathway participates in the facilitation in reproductive behaviour of female rodents (44). AVPV and mPOA play important roles in the regulation of reproduction and sexual behaviour, respectively (45–47). Small lesions in AVPV block the cyclic release of LH in the female rat (45, 46). This effect being absent in males, whereas the mPOA is primarily involved in the control of male sexual behaviour (47). Furthermore, neuronal circuits in the mPOA are pivotal sites for oestrogen action in the regulation of sexually differentiated behaviour (48). Therefore, our

present findings support the view that the oestrogen-induced MAPK phosphorylation in mPOA and AVPV may play role in sexually dimorphic action of oestrogen in the regulation of reproduction and sexual behaviour.

Consequences of sexually dimorphic action of gonadectomy in Pir

Our data provide the first evidence for a sexually dimorphic effect of gonadectomy on MAPK phosphorylation in Pir. Interestingly, the gonadectomy-induced alterations in pMAPK cell numbers detected in our experiments cannot be correlated with the effect of oestrogen in either sex. For example, in the Pir of male mice, gonadectomy reduced pMAPK cell numbers whereas oestrogen administration had no effect. In the female AVPV, more pMAPK immunopositive cells were revealed following GDX compared to intact controls, yet oestrogen treatment of ovariectomised mice also increased pMAPK cell numbers. Why these findings appear contradictory to expectations is not clear, but it is possible that other gonadal steroids such as progesterone or testosterone may also act as potential regulators of MAPK phosphorylation in the brain (49, 50).

As an olfactory-related brain region, Pir receives input from the olfactory bulb and is involved in cortical information processing of odour sensation. Human data indicate that women outperform men in assessing olfactory information such as odour discrimination (51). MAPK is involved in olfactory sensation within the Pir because MAPK phosphatase gene expression rapidly increases after olfactory stimulation (52) or exposure to female odours and leads to a rapid activation of MAPK in the vomeronasal system in male mice (53). Our results are in accordance with the suggestion that sexually differentiated gonadal steroid-induced MAPK phosphorylation in Pir may be involved in the sexually dimorphic olfactory signal processing as well. However, we emphasise that the functional role of the observed sex differences in MAPK phosphorylation remains unanswered until the specific target genes and gene products of oestrogen-induced MAPK phosphorylation are identified.

The possible role of MAPK in the sexually dimorphic action of oestrogen on CREB phosphorylation and methodological considerations

The present data are in agreement with previous experiment (6) where oestrogen does not have an effect on MAPK phosphorylation in Cg. In view of previous findings, our present results suggest possible role of MAPK in sexually dimorphic oestrogen action on CREB phoshorylation in the brain. Previously, we demonstrated that oestrogen has no effect upon CREB phosphorylation in Cg (26) in both sexes. Furthermore, we showed that oestrogen treatment significantly increased pCREB-immunoreactive cell numbers in the mPOA in female but not in male mice (26). Similarly, in the present study, we demonstrate that oestrogen had no effect on pMAPK expression in Cg in either female or male mice and oestrogen-induced MAPK phosphorylation of mPOA was only found in female mice. Although our data do not allow us to state whether CREB and MAPK phosphorylation occurred in same neuronal phenotypes of Cg or mPOA, our findings support the view that sex differences in oestrogen-induced MAPK phosphorylation as an upstream signalling process may play role in sex differences in oestrogen actions upon CREB phosphorylation.

Similar to Cg, gonadectomy or oestrogen treatment did not alter the pMAPK positive cell numbers in the BNST. As a part of sexually dimorphic descending pathway, this nucleus transmits olfactory information from the accessory olfactory bulb to mPOA and hypothalamic ventromedial nucleus (22). Although our results obtained in the Pir suggest that oestrogen-induced MAPK phoshorylation is involved in the sexual dimorphic olfactory signal processing, our finding in the BNST suggest a tissue specificity in this mode of signalling. However, it is worth noting that identification of neuronal phenotypes expressing pMAPK and evaluation of the time profile of MAPK phoshporylation following oestrogen administration were not performed in this study. Thus, any failure to detect changes in pMAPK expression may be explained by the limitation of the methods used in these experiments.

In summary, the present study reveals that sex differences exist in the ability of oestrogen to phosphorylate MAPK within specific brain regions *in vivo*. Our data suggest that oestrogen-dependent, indirect genomic mechanisms are likely to be involved in modulating sexually differentiated networks that regulate reproductive functions and higher order cognitive information processing.

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