Brain Research Bulletin 87 (2012) 511–520

Contents lists available at SciVerse ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

The restructuring of dopamine receptor subtype gene transcripts in c-fos KO mice

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ARTICLE INFO

Article history:
Received 16 February 2012
Accepted 17 February 2012
Available online 25 February 2012

Keywords:
c-fos
Dopamine receptors
Cerebral cortex
Cerebellum
Hypothalamus
Striatum
Hippocampus

ABSTRACT

Although c-Fos protein is one of the principal molecules in intracellular signaling, c-fos gene disruption is associated with alterations in neuronal functions that do not correspond to its importance in function. The aim of the study was to evaluate the changes of dopaminergic system together with acetylcholinesterase (AChE) in c-fos disruption (KO). KO male mice showed an increase in D_1-like receptor (279% of WT) and D_2-like receptor (345% of WT) binding sites in the cortex. On the gene expression level (assessed by real-time PCR), lower quantities of D_1R-mRNA (0.64) and D_5R-mRNA (0.6) were found in females when compared to males in the frontal cortex, higher D_2R-mRNA in the parietal (1.43) and temporal (2.64) cortex and lower AChE-mRNA (0.67). On the contrary, female striatum contained higher level of D_2R-mRNA (1.62) and AChE-mRNA (1.57) but lower level of D_3R-mRNA (0.73). Hypothalamic D_1R-mRNA, D_2R-mRNA and D_4R-mRNA were higher in females (1.38, 1.63, and 1.68, respectively). Disruption of c-fos increased selectively D_5R-mRNA (1.31) in male parietal cortex, D_2R-mRNA (1.72) in male temporal cortex, and cerebellar D_2R-mRNA in both males (1.43) and females (1.42), respectively. In females, we found rather decrease in DR-mRNA. Multiple correlations in mRNA quantities (in WT mice) were found, which changed considerably upon c-fos KO. Main interactions in WT were inter-regional, CNS of KO underwent an extensive restructuring comprising intraregional interactions in the frontal cortex, hypothalamus, and cerebellum. These changes in DR (between others) could be considered as one of the adaptive mechanisms in c-fos KO mice.

1. Introduction

C-Fos protein is a molecule that can be viewed from many points. From the signaling point of view, it is a third messenger that activates its target genes [25], it can be comprehended as an inducible transcription factor [13] or as a product of immediate early genes [16]. It is important to note that all these aspects cannot be clearly separated. Usually, c-fos is considered as an ubiquitous molecule that can give the picture of the cell activation, especially neurons [31]. In respect to G-protein coupled receptors, c-Fos is activated by multiple pathways. For example, it can be activated both by proteinkinase C (that is caused by diacylglycerol increase from enhanced phospholipase C activity) and by Ca^{2+} increase (that is caused by inositoltrisphosphate increase from enhanced phospholipase C activity) [4]. The expression of c-Fos can be enhanced by an activation of muscarinic receptors [6], α-adrenoceptors, β-adrenoceptors [42], D_1-like dopamine receptors, D_2-like dopamine receptors and many others [32]. Similarly, D_1 agonists are able to change c-fos gene expression [20]. In conclusion, c-Fos can be considered as very important molecule in cell signaling participating in many physiological processes [9,24].

In nineties of 20th century and at the beginning of 21st century, the knock-out of the target gene was considered to be the superior method for studying the functions of the target gene. On the other hand, many knock-out (KO) animals that were first believed not to be viable, as the deleted gene was considered to be essential for survival, were surprisingly able to live till the adulthood (AChE KO mice [29], for example).

Similarly, despite important role of c-Fos in the CNS function (c-fos is target of many signaling systems, activated by huge amount of receptors, transcription factors and c-fos could be considered as essential in neuronal function), c-fos KO animals are fully viable with disruption of CNS functions that is not fully in accordance with the principal role of c-Fos in the cell. These animals have changes in the acquisition and/or consolidation, but do not have long-term memory deficits in aversive taste learning [40]. Moreover, these

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doi:10.1016/j.brainresbull.2012.02.006

authors [40] compared acute fos inhibition (using microinfusion of antisense oligodeoxynucleotide directed against c-fos mRNA) and permanent deletion of fos gen (c-fos KO) and concluded that the genetic deficiency in c-fos −/− mice caused normal acquisition and retention and that some other factors could compensate conditioned taste aversion mechanism when Fos mediated transcription is not available.

In addition to that, KO mice do not reveal dramatic changes in REM sleep, in the core temperature, although they have more weakfullness and less slow wave sleep, and the null mice are awake more over a 24 h period than WT mice [35]. Also, mice lacking c-Fos specifically in the CNS showed normal general and emotional behavior but were specifically impaired in hippocampus-dependent spatial and associative learning tasks [8].

Moreover, c-fos KO mice do not reveal changes in basal neurotensin, neuromedin, preprotachykinin and preproenkephalin levels in the striatum as well [34]. These peptides are tightly connected with dopamine receptor system. Similarly, dopamine system is also connected with learning and memory processes [38] as well as it is connected (together with c-Fos) with biorhythm formation [39]. Dopamine receptors are implicated in many neurological processes, including motivation, pleasure, cognition, memory, learning [30], and fine motor control, as well as modulation of neuroendocrine signaling [11].

Taken together, since there is discrepancy between the importance of c-Fos in the signaling processes and CNS function impairment (i.e. no changes in emotional behavior, in REM sleep, in the core temperature) that does not correspond to the c-Fos signaling significance, we hypothesized that functional adaptation in c-fos KO mice should exist.

In order to evaluate a dopamine receptor signaling status and its role for maintaining non adequately impaired CNS function in c-fos KO mice, we have employed the mice with targeted disruption of c-fos gene [37] and evaluated dopamine receptor binding and dopamine receptor mRNA levels (dopamine receptor subtype gene transcripts) in selected central nervous system regions (i.e. brain areas: frontal, parietal, temporal and occipital cortex, cerebellum, and subcortical structures: hypothalamus, striatum and hippocampus). Moreover, we strived to analyze pre-existing correlations in dopamine receptor gene transcripts (in wild type mice) and to compare these to the c-fos KO counterparts. Hence, we have hypothesized that restructuring of dopamine receptor signaling can help the brain neurons to maintain intracellular signaling balanced. The change in intracellular gene-gene and signaling molecules interactions could be the basis of relatively preserved CNS functions in this genetic mutant.

2. Material and methods

2.1. Animals

The c-fos mutation was produced by gene targeting [21]. This line was then transferred in the Institute for Molecular Pathology, University of Vienna, Austria from which we obtained this line. The line was maintained on mixed genetic background derived from (C57BL/6Jx129/SvJ)F1 mice. Mice were kept at constant temperature (21 °C ± 1) in 12-h light/12-h dark cycle (lights on at 6:00 AM) with food and water ad libitum. Wild-type (WT, +/+) and knock-out (KO, −/−) were obtained directly by crossing heterozygotes (+/−), adult (2-months old) male and female control ($n = 16$) and knock-out ($n = 11$) mice (20–25 g of body weight) were used for the study. WT and KO genotype were confirmed by PCR as described previously [21]. Although the KO mice had lower weight, the viability of both lines was comparable and there was no difference in the brain morphology (as assessed by light microscopy). Mice were sacrificed by decapitation and exsanguination and specific parts of cerebral cortex, cerebellum, hypothalamus, striatum, and hippocampus were dissected, flash frozen in liquid nitrogen and stored at −80 °C for the further analysis. For binding experiments, the whole cerebellum and cortex was taken, for Real-Time PCR the specific brain regions were isolated by punch method isolating as much tissue as possible. All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experiments were agreed by Committee for Animal Care of the Institute of Experimental Endocrinology in Bratislava, Slovakia.

2.2. Binding experiments

Binding experiments were performed similarly as described previously [14]. Briefly, in preliminary experiments, the receptors were bound with increasing concentrations of radioligand in order to ascertain the saturating concentration of radioligand, and the affinity of receptor to radioligand (K_D). The

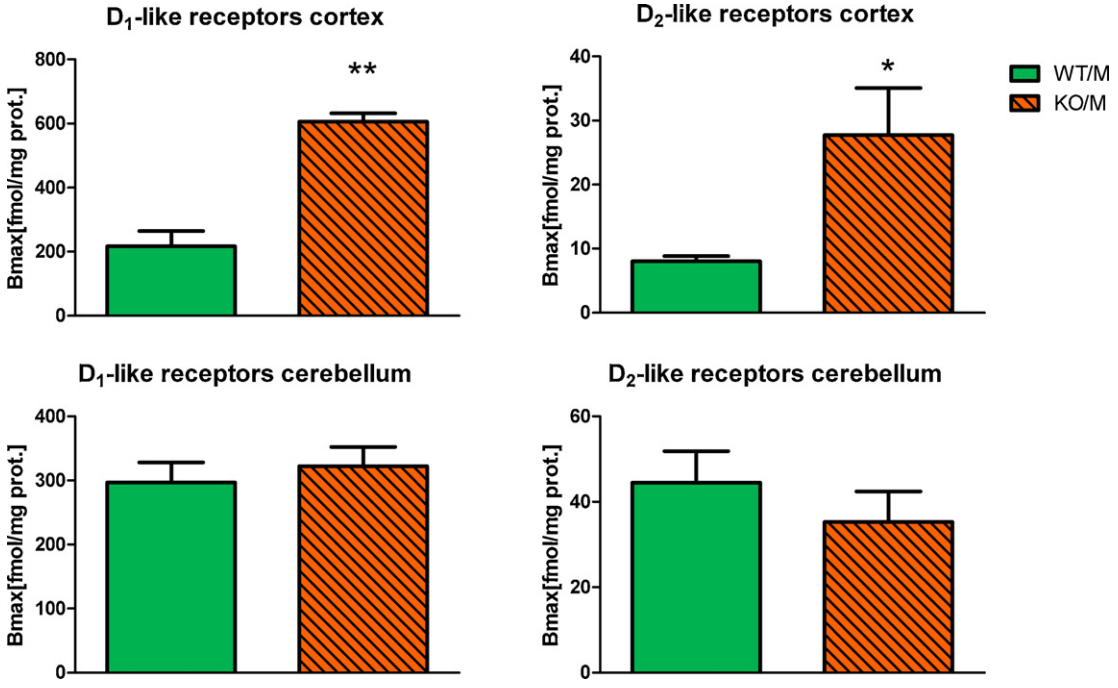

Fig. 1. The changes in receptor binding characteristics in cortex and cerebellum of c-fos KO mice. See inserted legend for explanation. WT/M males of wild type mice, KO/M males of c-fos KO mice, $^{*}p < 0.05$, $^{**}p < 0.01$; differences from wild type.

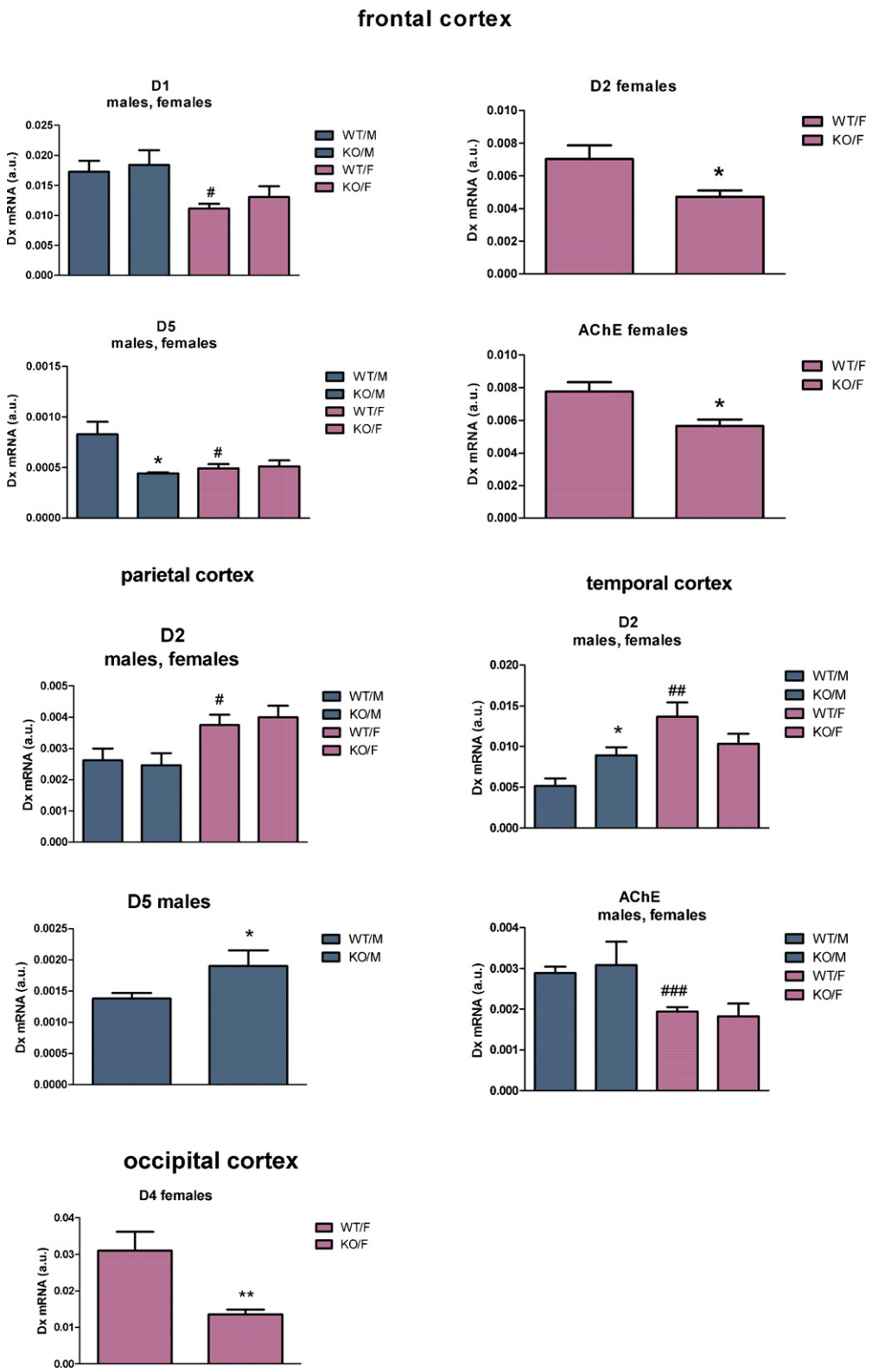

Fig. 2. The changes of gene expression in the frontal, parietal, temporal and occipital cortex, comparison of changes in males/females and between WT and KO animals. WT/M males of wild type mice, KO/M males of c-fos KO mice, WT/F females of wild type mice, KO/F females of c-fos KO mice. See legend below for explanation, $^{*}p < 0.05$, different from appropriate WT, $^{**}p < 0.01$, different from appropriate WT, $^{\#}p < 0.05$, different from another sex, $^{\#\#\#}p < 0.001$, different from another sex. a.u., arbitrary units. Significant changes of dopamine receptors gene expression in respective brain areas are shown only, when there was no difference, the appropriate figure is not shown.

radioligands used were: ^{3}H-SCH 23990 (specific for D_1-like dopamine receptors), and ^{3}H-spiperon (specific for D_2-like dopamine receptors). Afterwards, simplified saturation binding experiments with one saturating concentration of radioligand were used in order to determine the receptor density (B_{max}). Following formula was used: $B_{max} = B \times ([L] + K_L)/[L]$, where B = bound of radioligand [fmol/mg of protein], L = radioligand concentration [fmol/l], and K_L = Kd [fmol/l] of the radioligand. Homogenates were incubated in duplicates in Tris-EDTA buffer (Tris–HCl 50 mmol/l, EDTA 2 mmol/l, pH adjusted to 7.4) with following single fully saturating concentration of the radioligand: 4000 pmol/l of ^{3}H-SCH23390 for D_1-like dopamine receptors and 4000 pmol/l of ^{3}H-spiperon for D_2-like dopamine receptors.

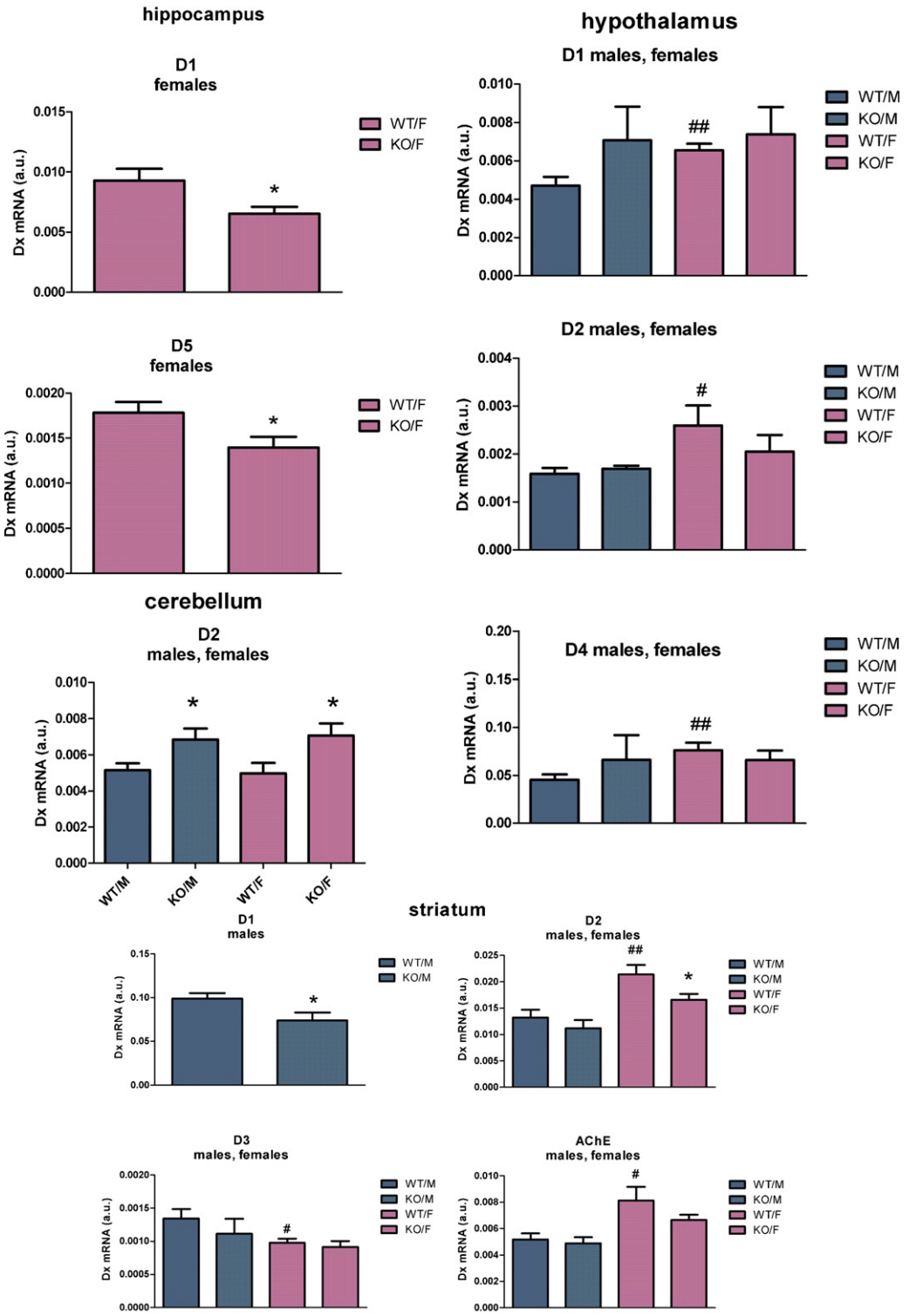

Fig. 3. The changes of gene expression in subcortical structures (hippocampus, hypothalamus, striatum) and cerebellum, comparison of changes in males/females and between WT and KO animals. WT/M males of wild type mice, KO/M males of c-fos KO mice, WT/F females of wild type mice, KO/F females of c-fos KO mice. See legend in Fig. 2 for explanation, $^{*}p < 0.05$, different from appropriate WT, $^{**}p < 0.01$, different from appropriate WT, $^{\#\#\#}p < 0.001$, different from another sex. a.u., arbitrary units. Significant changes of dopamine receptors and acetylcholinesterase gene expressions in respective areas are shown only, when there was no difference, the appropriate figure is not shown.

2.3. Real-time PCR

Total mRNA was isolated using chloroform–isopropanol (RNA Bee) method according to manufacturer's instructions. RNA yield and integrity was evaluated spectrophotometrically at A_1 = 260 nm and A_1/A_2 = 260/280 nm, respectively. Samples with A_1/A_2 between 1.6 and 1.9 were used for downstream procedures. Total RNA was purified to eliminate potentially contaminating genomic DNA using recombinant DNAse. Purified mRNA was subsequently transcribed into cDNA using Ready-To-Go You-Prime First-Strand Beads. qPCR was performed using pre-mixed SYBRGreen MasterMix in a final volume of 25 μL on a CFX-96 real-Time PCR apparatus (Bio-Rad). All reactions were performed in triplicates with a no-reverse transcription control. Each qPCR reaction was followed by a subsequent melting-curve analysis to ensure specificity. The following protocol was used: 3 min for initial denaturation at 95.0 °C followed by 40 cycles consisting of 1 min at 94.0 °C for denaturation, 30 s for annealing at temperatures that were optimized before for each primer-pair and 30 s for elongation at 72.0 °C. The annealing temperatures were: 53.3 °C for D_1 receptors, 58.7 °C for D_2 receptors, 60.5 °C for D_3 receptors, 52.9 °C for D_4 receptors, 61.5 °C for D_5 receptors, 56 °C for AChE and 60.5 °C for GAPDH. The primers were the same as described in [27], [41] and [23]. In detail, the primer sequences were as follows: D_1 (sense): 5′-GTAGCCATTATGATCGTCAC-3′, D1 (antisense): 5′-GATCACAGACAGTGTCTTCAG-3′, D_2 (sense): 5′-GGGATGTTGCAGTCACAGTG-3′, D2 (antisense): 5′-GAGGGCTCCACTAAAGGAGG-3′, D_3 (sense): 5′-AGGTTTCTGTCAGATGCC-3′, D_3 (antisense): 5′-GTTGCTGAGTTTTCGAACC-3′, D_4 (sense): 5′-CACCAACTACTTCATCGTGA-3′, D_4 (antisense): 5′-AAGGAGCAGACGGACGAGTA-3′, D_5 (sense): 5′-CTACGAGCGCAAGATGACC-3′, D_5 (antisense): 5′-CTCTGAGCATGCTCAGCTG-3′, AChE (sense) 5′-GCAGCAATATGTGAGCCTGA-3′, AChE (antisense) 5′-GGTCGAACTGGTTCTTCCAG-3′, GAPDH (sense): 5′-CGTCTTCACCACCATGGAGA-3′, GAPDH (antisense): 5′-CGGCCATCACGCCACAGCTT-3′. In order to determine the relative gene expression, the Delta–Delta-Ct (ddCt) method was used. The levels of analyzed transcripts were normalized to the level of the reference gene transcript (glyceraldehyde-3-phosphate dehydrogenase, GAPDH, which level was practically the same in WT and KO). As another control, we have employed also different internal control (18SrRNA) with another dopamine receptor probe (TaqMan, Applied Biosystems) and the results were similar to those with GAPDH. Then, for each sample the following formula was used: normalized ratio = $2\exp^{CT1-CT2}$, where CT1 is CT of reference transcript and CT2 is CT of target transcript, where CT is the cycle threshold. After the qPCR reaction, melting curve analysis was performed to confirm the specificity of the analyzed transcript. No-template controls (NTC) and non-reverse transcription reactions (NRT) were performed as negative controls.

2.4. Material

^{3}H-SCH23390, [N-methyl-3H] (3.15 TBq/mmol), ^{3}H-spiperon (8-[4-(p-fluorophenyl)-4-oxo[2,3(n)-butyl]-1-phenyl 1,3,8-triazospiro[4,5]decan-4-one) [benzene ring-3H] (0.56 TBq/mmol) were purchased from Perkin-Elmer (Boston, MA, USA), RNA Bee was purchased from TelTest (TX, USA), DNAse was purchased from Ambion (United Kingdom) pre-mixed SYBRGreen MasterMix was purchased from Top-Bio (Czech Republic), and Ready-To-Go You-Prime First-Strand Beads were from GE Healthcare (Czech Republic). Other chemicals were purchased from Sigma–Aldrich (Czech Republic).

2.5. Data treatment and statistical analysis

Radioligand binding and dopamine receptor subtype gene expression (D_1R-mRNA–D_5R-mRNA) normalized to housekeeper gene (GAPDH) was evaluated using GraphPad software (San Diego, USA). Statistical significance was determined with one-way, two-way ANOVA with SNK (Student–Newman–Keuls) post hoc test or with Student t-test, when appropriate. Subsequently, the relative amounts of each receptor subtype mRNA (D_1R-mRNA–D_5R-mRNA) were plotted against each other. Correlations were analyzed by calculating Pearson's correlation coefficient (r), coefficient of determination (r^2), with corresponding p-values (using Statistica software) for all correlations. $p < 0.05$ were considered statistically significant.

3. Results

3.1. Receptor densities

Receptor densities were determined in male cortex and cerebella. There was marked increase in D_1-like receptor (279% of WT, t-test, $p = 0.0048$) and D_2-like receptor (345% of WT, t-test, $p = 0.0375$) binding in the cortex of c-fos KO males while no change in receptor binding in cerebella (see Fig. 1).

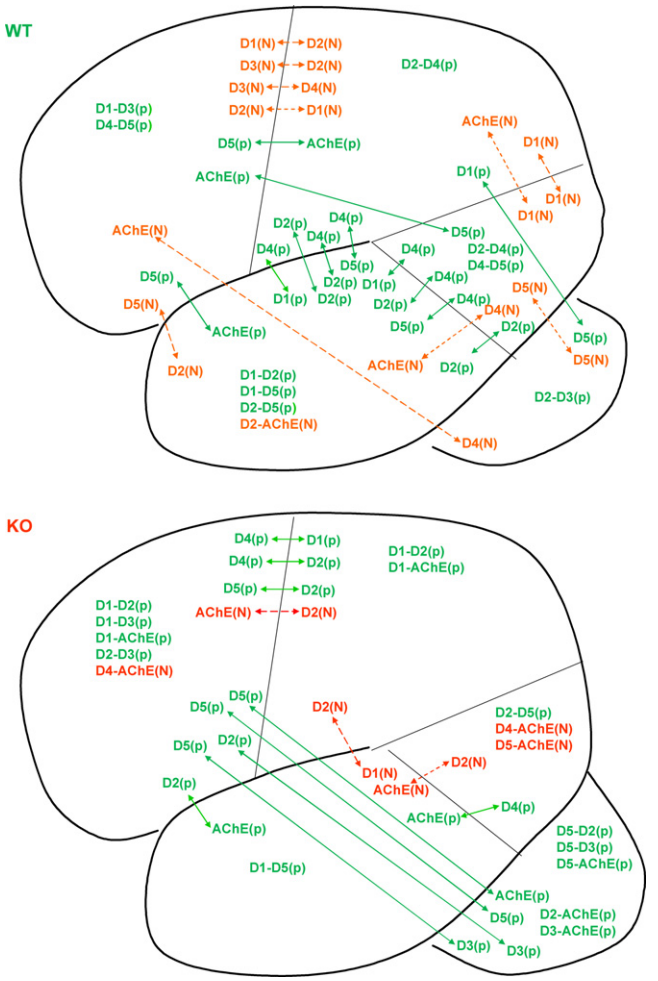

Fig. 4. Schematic representation of dopamine subtype receptor and AChE interactions in cerebral cortex of WT mice (top) and KO mice (bottom). N, negative interaction, p, positive interaction, connecting lines: continuous line: positive interaction, dashed line: negative interaction, dotted line: both interactions. D1–D5 dopamine receptors, AChE, acetylcholinesterase.

3.2. The regions without gene expression of specific dopamine receptor subtypes

In some brain regions, there was no gene expression of certain dopamine receptor subtypes found. These regions and subtypes are as follows: no D_3R-mRNA was found in the parietal cortex, occipital cortex and temporal cortex. D_4R-mRNA was not found in the temporal cortex and no D_1R-mRNA was found in the cerebellum.

3.3. Comparison of gene expression in males and females (in wild type animals)

In some brain regions and subcortical areas, there were differences in gene expression of specific dopamine receptor subtypes in males and females. When compared males and females, females had a lower level of D_1R-mRNA (0.64) and D_5R-mRNA (0.6) in the frontal cortex (D_1R-mRNA: 1-way ANOVA, $p = 0.0095$, $F = 5.004$, degrees of freedom (df) = 23, D_5R-mRNA: 1-way ANOVA, $p = 0.0091$, $F = 4.924$, df = 25), higher level of D_2R-mRNA (1.43) in the parietal cortex (1-way ANOVA, $p = 0.018$, $F = 4.146$, df = 25) and higher level of D_2R-mRNA (2.64) and lower level of AChE-mRNA (0.67) in the temporal cortex (D_2R-mRNA: 1-way ANOVA, $p = 0.002$, $F = 6.833$, df = 25, AChE-mRNA: 1-way ANOVA, $p = 0.0098$, $F = 4.791$, df = 26) as well (Fig. 2). In the striatum (Fig. 3), D_2R-mRNA and

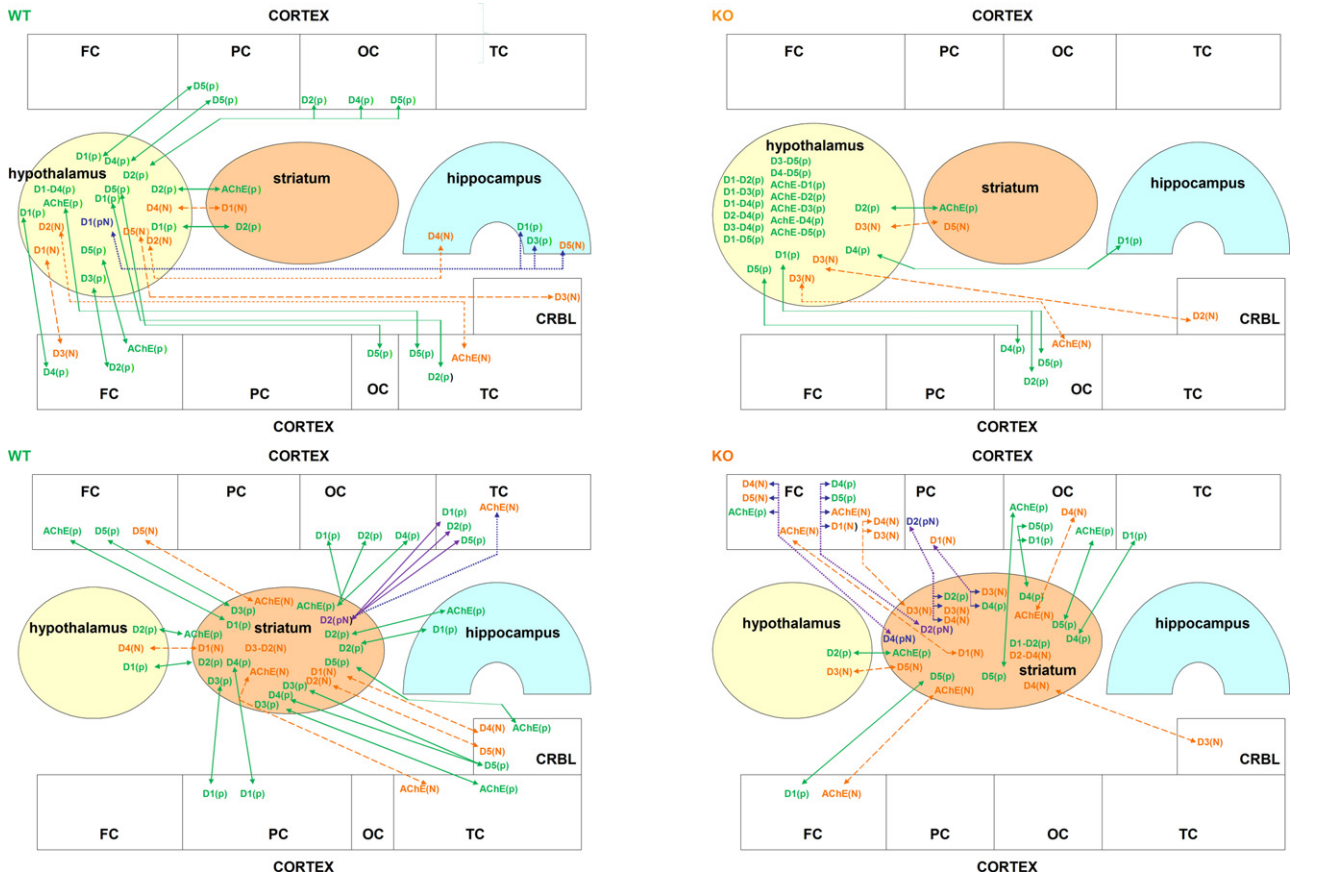

Fig. 5. Schematic representation of dopamine subtype receptor and AChE interactions in subcortical structures (hypothalamus and striatum) and with cerebral cortex of WT and KO mice. N, negative interaction, p, positive interaction, connecting lines: continuous line: positive interaction, dashed line: negative interaction, dotted line: both interactions. D1–D5 dopamine receptors, AChE, acetylcholinesterase. FC, frontal cortex, PC, parietal cortex, OC, occipital cortex, TC, temporal cortex, CRBL cerebellum. The interactions are schematized as comparison of interaction in WT (left side) and KO (right side) in hypothalamus, and striatum, respectively (from top to bottom). You can note the decrease of interregional interactions in KO animals in favor of intraregional interactions what is mainly detectable in hypothalamus and general loss of interactions in KO animals.

AChE-mRNA in females were significantly higher – 1.62 times and 1.57 times, respectively mRNA (D_2R-mRNA: 1-way ANOVA, $p = 0.0011$, $F = 7.556$, df = 26, AChE-mRNA: 1-way ANOVA, $p = 0.0117$, $F = 4.588$, df = 26) and D_3R- was significantly lower (0.73, *t*-test, $p = 0.0265$). In the hypothalamus (Fig. 3) we have found a significantly higher level D_1R-mRNA (1.38), D_2R-mRNA (1.63) and D_4R-mRNA (1.68) in females compared to males (D_1R-mRNA: *t*-test, $p = 0.0071$, D_2R-mRNAt-test, $p = 0.0401$, D_4R-mRNA: *t*-test, $p = 0.0087$).

3.4. Changes in gene expression in c-fos KO (comparison to wild type animals)

In mice, in which c-fos gene was disrupted, there were changes in dopamine receptor subtypes gene expression and also in AChE gene expression. In the frontal cortex (Fig. 2), we have found a decrease in D_2R-mRNA (0.67, *t*-test, $p = 0.0177$) and AchE-mRNA (0.73, 1-way ANOVA, $p = 0.0083$, $F = 5.042$, df = 25) in females and D_5R-mRNA in males (0.53, 1-way ANOVA, $p = 0.0091$, $F = 4.924$, df = 25). In the parietal cortex (Fig. 2), there was an increase in D_5R-mRNA in males (1.31, *t*-test, $p = 0.0416$). In the occipital cortex (Fig. 2), there was a decrease in D_4R-mRNA (0.44) in females (1-way ANOVA, $p = 0.0079$, $F = 5.031$, df = 26). In the temporal cortex (Fig. 2), there was an increase in D_2R-mRNA (1.72) in males (1-way ANOVA, $p = 0.002$, $F = 6.833$, df = 25). In the cerebellum (Fig. 3), we found an increase in D_2R-mRNA (1-way ANOVA, $p = 0.0341$, $F = 3.422$, df = 26) in both males (1.43) and females (1.42). On the contrary, D_1R-mRNA was decreased (*t*-test, $p = 0.0461$) in males (0.75) in the striatum (Fig. 3) and also in females (*t*-test, $p = 0.0311$) in the hippocampus (0.7). In hippocampus (Fig. 3), we also found a decrease in D_5R-mRNA (*t*-test, $p = 0.0409$) in females (0.78).

3.5. Restructuring of dopamine receptor subtypes gene transcripts

Correlation analysis was performed and only the interactions (negative and positive) with $p < 0.05$ were considered as significant. The interactions are then schematically driven in Figs. 4 and 5. Usually, the *p* was less than 0.01. The example of interactions between gene expressions is shown in Fig. 7.

As it is apparent from Figs. 4–6, it is possible to describe main interactions in wild type animals as interregional. This is mainly true for cortico–cortical interactions (see Fig. 4 above), but also for cortico–subcortical interactions (see Figs. 5 and 6, left). When c-fos gene is disrupted the dopamine receptor gene transcripts undergoes an extensive restructuring, which can be characterized as favoring both intraregional interactions in cortical structures (see Fig. 4, bottom) and intraregional interactions in subcortical structures (see Figs. 5 and 6, right). This dramatic increase in intraregional interactions can be mainly seen in the hypothalamus (see Fig. 5, top right), frontal cortex (see Fig. 4, bottom) and cerebellum (see Fig. 4, bottom). Moreover, the loss of inter-regional interactions that is seen in KO animals (Figs. 5 and 6, right) is a

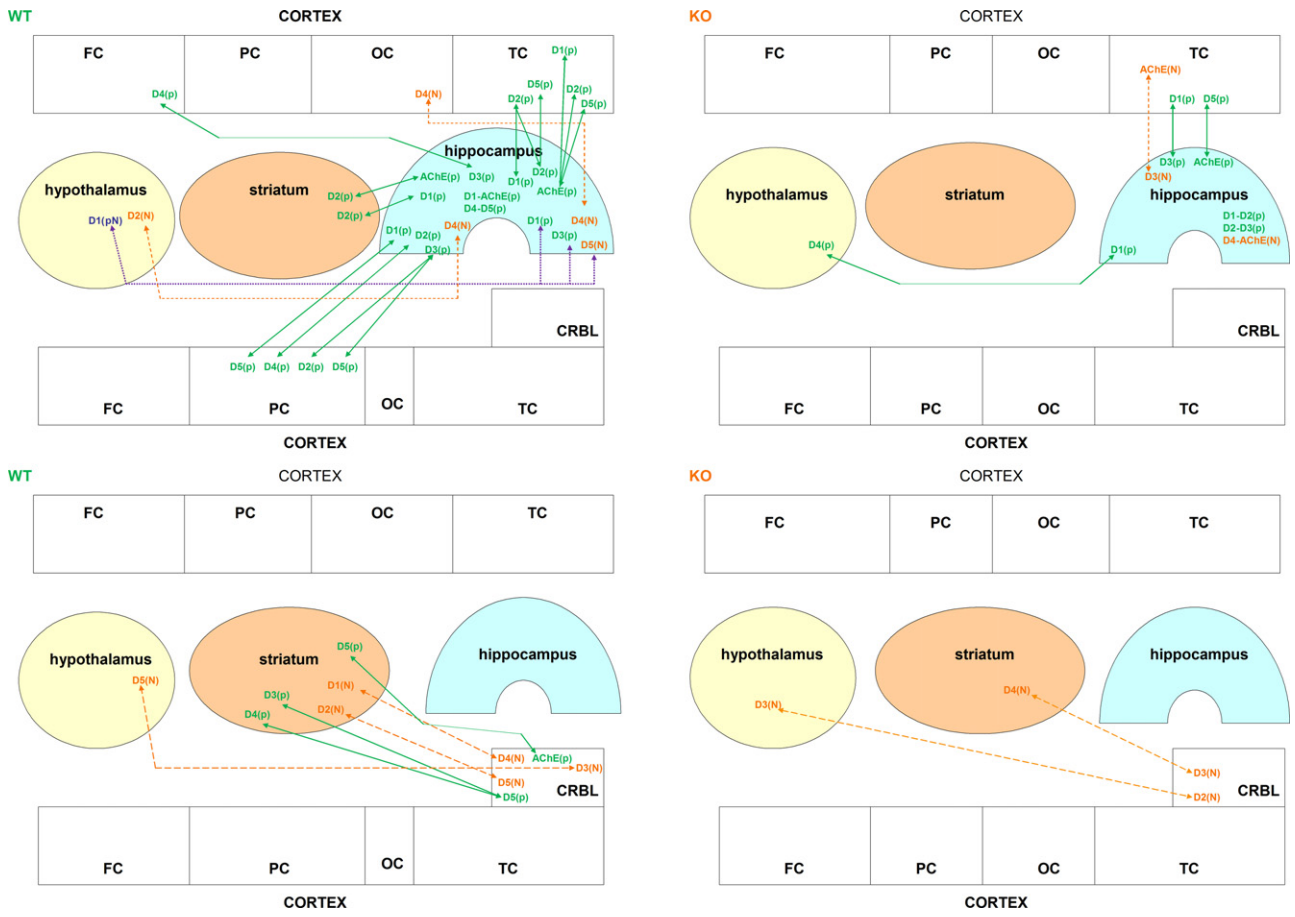

Fig. 6. Schematic representation of interactions among dopamine receptor subtypes mRNA and AChE mRNA in subcortical structures (hippocampus and cerebellum) and with cerebral cortex of WT and KO mice. For legend, see Fig. 5.

feature that can be observed in all subcortical structures (i.e. in cerebellum, hippocampus, striatum and hypothalamus). On the other hand, the decrease in intraregional interactions can be seen in the temporal cortex of KO animals (see Fig. 4, bottom). In addition to that, frontocortical–cerebellar interactions that are almost missing in WT animals become suddenly present in KO mice (see Fig. 4).

Moreover, it can be observed in some regions that the reversal of the correlation occurs. This is true for fronto–striatal interaction between frontocortical AChE and striatal D_1 dopamine receptors, which is positive in WT animals but becomes negative when no c-fos is present (Fig. 5). Similarly, parieto–striatal interaction (Fig. 5) also reverts when no c-fos is present. While parietocortical D_1R-mRNA correlates positively with striatal D_3R-mRNA in WT, this correlation becomes negative in KO. Also, striato–occipitocortical interaction between striatal AChE-mRNA and occipitocortical D_4R-mRNA also reveals reversal in the correlation type. From the positive correlation in WT it becomes negative in KO (see Fig. 5). The last reversal of correlation was found between temporocortical AChE-mRNA and occipitocortical D_4R-mRNA (Fig. 4). We have observed a negative correlation between this pair of transcripts in WT animals but positive in KO animals.

4. Discussion

In our experiments, we have found that disruption of c-fos increased selectively D_5R-mRNA (1.31) in male parietal cortex, D_2R-mRNA (1.72) in male temporal cortex, and cerebellar D_2R-mRNA in both males (1.43) and females (1.42), respectively. This increase is partially in accordance with changes in receptor densities determined in male whole cortex and cerebella (see Fig. 1). There was marked increase in D_1-like (i.e. D_1R and/or D_5R) receptor (279% of WT) and D_2-like (D_2R, and/or D_3R, and/or D_4R) receptor (345% of WT) binding in the cortex of c-fos KO males. Although, we were not able to find more brain areas in which the increase in mRNA level correspond to the increase in receptor binding, there is clear connection between increased D_5R-mRNA in parietal cortex and increased D_1-like receptors in the whole cortex. Similarly, there is correlation between increased D2R-mRNA in the temporal cortex and increased D2-like receptor binding. As there is no selective ligand for D_1–D_5 receptor binding determination, measurement of mRNA levels could give us the better picture of receptor changes than binding that should be considered as general pattern of changes. The number of binding sites (in fact the number of receptors) is believed to be determined by the level of protein translation, posttranslational modifications, protein degradation processes and/or changes of their membrane surface presentation [36]. Therefore, increased amount of mRNA together with unchanged number of binding sites give evidence about decreased level of protein translation and/or increased level of protein degradation. The other possibility (change in membrane surface presentation) seems to be less probable.

The detail analysis of the gene expression revealed that there are region specific changes in the gene expression of dopamine receptor subtypes that in some regions the gene expression differs between males and females and that it is possible to find specific interactions in dopamine receptor subtype gene transcripts. These interactions are mainly interregional in wild type animals but extensively restructuralized in KO mice where the majority of

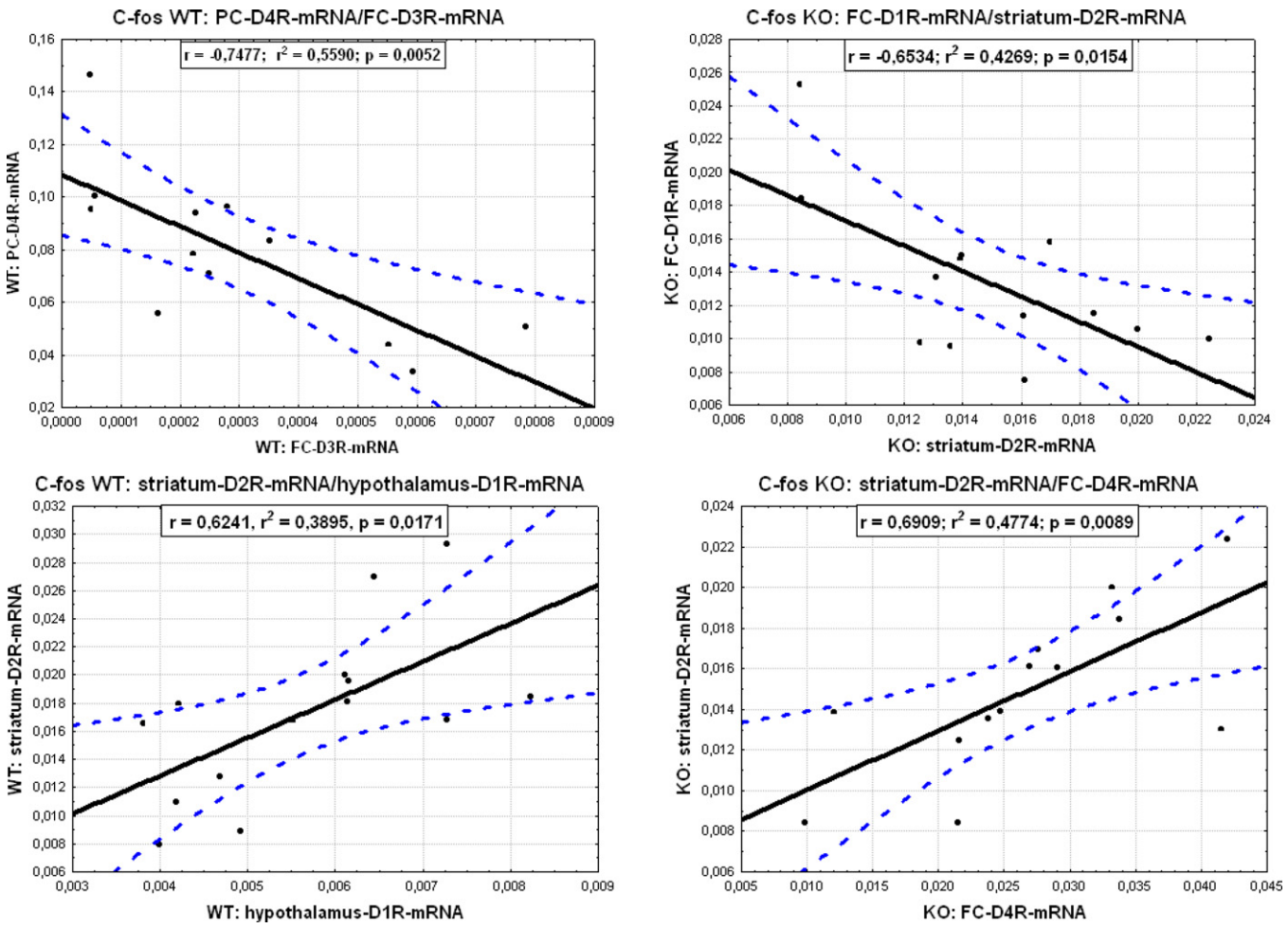

Fig. 7. The examples of regression analysis for WT frontocortical–parietocortical interaction (top, left), WT hypothalamo–striatal interaction (bottom, left), KO striato–frontocortical interaction (top, right) and KO striato–striatal interaction (bottom, right). R, correlation coefficient, R2, coefficient of determination.

interaction is represented by intraregional interactions between dopamine receptor subtype gene transcripts and AChE gene transcripts. Moreover, in four cases interregional interactions revert as a result of c-fos knockout. From that point of view, the data about changes of receptor binding supports the idea of restructuring of dopamine receptor subtypes in central nervous system neurons as a tool for plasticity that plays its role in coping with the deletion of the important gene (c-fos). More specifically, Fos family proteins (c-Fos, FosB, Fra-1, Fra-2) are part of the transcription factor AP-1 [22]. G-protein coupled receptors are able to affect Fos family proteins via proteinkinase C (both diacylglycerol and Ca^{2+} increase) [4], via activation of D_1-like dopamine receptors and D_2-like dopamine receptors [32]. Similarly, D_1 agonists are able to change c-fos gene expression [20]. It is possible to hypothesize that lack of the downstream signaling protein (c-Fos) affects the mRNAs for D_1–D_5 dopamine receptors. Regional specificity of changed gene expression can give evidence about importance of these changes to maintaining the neuronal function.

4.1. Gender differences

Gender differences have been found to be important in dopaminergic function in striatum [2] on the level of receptor binding sites. Shortly, D_1-like and D_2-like receptor densities depend on the presence of estrogens and gestagens: ovariectomy changes these numbers and also estrogen treatment down-regulates D_2-like dopamine receptor density. On the other hand, castration do not change dopaminergic function [2]. It has been shown as well that striatal densities of D_1-like dopamine receptors are increased in male rats [15] while D_2-like dopamine receptors remain unchanged [28].

The data describing gender differences in dopamine receptor gene expression are sparse. Significantly higher D_5 dopamine receptor gene expression in female over male young have been demonstrated in the striatum (in study of CB1-cannabinoid-deficient mouse) despite of genotype [10] which was, however, not found in our study (we did not find any difference in D_1 gene expression between males and females). We have also found striatal D_3 dopamine receptor gene expression to be lower in females than in males. Gerald et al. have found no gender differences for D_3 dopamine receptors. Also, no gender differences have been demonstrated for D_2 dopamine receptor in the striatum [19], which also does not concur with our results. We have found a higher (1.62 times) expression of D_2 dopamine receptor subtype in females than in males. It is possible that strain, age and/or species differences may play a role as it has been demonstrated for other physiological/pathophysiological parameters (e.g. reactivity of airways [18] and many others). In addition to changes in striatum, we have identified other regions with different gene expression in males and females. We have found lower D_1 and D_5 dopamine receptor gene expression in frontal cortex of females. In parietal and temporal cortex, D_2 dopamine receptor gene expression was higher in females

than in males. Hypothalamic gene expressions of D_1, D_2 and D_4 dopamine receptors were also higher in females.

Dopaminergic system gender differences are considered as possible mechanisms relevant to variation in drug abuse [17] as well as in anxiety-like behavior [33]. Our data about differences in dopamine receptor subtype gene expression that are brain area specific could be one of the factors contributing to these differences. From that point of view it is interesting to note that the main differences between males and females were recorded in hypothalamus and striatum. The differences in the hypothalamus as a part of limbic system could be relevant to such variances in female and male drug abuse or anxiety-like behavior. In the striatum, we have found a higher level of D_2R-mRNA and AChE-mRNA, while D_3R-mRNA was in females actually lower. Although one could hypothesize that motor skills differences can be found between genders, data both for mice [1] and for humans [26] do not confirm such differences.

4.2. The effect of c-fos knockout

There are some data describing the plasticity of dopamine receptors under different conditions. For example, it has been shown recently that dopamine receptors undergo plastic changes in mesolimbic system as a result of drug-induced neuroadaptations in excitatory transmission in the ventral tegmental area and the nucleus accumbens [3]. Similarly, an exacerbation of methamphetamine-induced neurotoxicity in c-fos knockout mice was shown [5,24]. Also, D_1- and D_2- dopamine receptor mesolimbic and mesostriatal plasticity was described after perinatal asfyxia in rats [12]. On the other hand, according to our knowledge, this is the first report describing eight central nervous system areas undergo a gene transcripts restructuring after the c-fos is knocked out (in mice).

How the restructuring can maintain relatively preserved CNS functions is not clear but some previously published data could explain the adaptive role of dopamine restructuring in maintaining CNS function in c-fos knockout mice. Fos family proteins (c-Fos, FosB, Fra-1, Fra-2) are part of the transcription factor AP-1 [22] and are involved in a large variety of biological processes. It has been shown previously [7] that a component of AP-1, Fra-1, can functionally substitute for the lack of c-Fos in Fosl1 knock-in mice, suggesting that both Fra-1 and c-Fos would exert their functions as adaptors for other transcription factors or even act as transcriptional repressors. In the light of these results, the dopamine restructuring could result as adaptive mechanism in CNS function that copes with changed transcription factors milieu.

We can therefore conclude that there exist interactions on the level of dopamine receptor subtype gene transcripts between eight specific central nervous system regions (i.e. brain areas: frontal, parietal, temporal and occipital cortex, cerebellum, and subcortical structures: hypothalamus, striatum and hippocampus) in wild type mice and that these interactions are changed when c-fos gene is knocked out. While main interactions in wild type animals are interregional, in c-fos KO mice the central nervous system undergoes an extensive restructuring resulting in an establishment of both intraregional interactions in cortical structures (frontal cortex) and intraregional interactions in subcortical structures (hypothalamus and cerebellum). On the other hand, the decrease in intraregional interactions can be seen in temporal cortex. In addition to that, frontocortical–cerebellar interactions, that are almost missing in WT animals reveal suddenly in KO mice. Thus, the restructuring on the level of dopamine receptor subtypes gene transcripts may play a role in helping to maintain relatively preserved CNS functions in c-fos KO mice.

Acknowledgements

Supported by Grant GACR 309/090/406 from Grant Agency of the Czech Republic. Excellent technical help of Miss Petra Svatosova is greatly acknowledged.

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