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Hemisphere-dependent endocannabinoid system activity in prefrontal cortex and hippocampus of the Flinders Sensitive Line rodent model of depression

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ABSTRACT

Altered endocannabinoid (eCB) signalling is suggested as an important contributor to the pathophysiology of depression. To further elucidate this, we conducted a study using a genetic rat model of depression, the Flinders Sensitive Line (FSL), and their controls, the Flinders Resistant Line (FRL) rats. Plasma, right and left prefrontal cortex, and hippocampus were isolated from FSL and FRL rats. We analyzed each region for the eCB anandamide (AEA) and 2-arachidonoylglycerol (2-AG) levels by liquid chromatography/multiple reaction monitoring (LC/MRM), mRNA and protein levels of the cannabinoid type 1 receptor (CB_1R), fatty acid amide hydrolase (FAAH) and monoacyl glycerol lipase (MAGL) by real time qPCR and Western blotting. Content of 2-AG was lower in the left side of the hippocampus and prefrontal cortex in FSL rats compared to FRL rats. Inversely, levels of AEA were higher in right hippocampus than in left hippocampus. In plasma, AEA levels were increased and 2-AG decreased. Cannabinoid receptor 1 (*Cnr1*), *Faah* and *Magl* mRNA levels were prominently decreased in right prefrontal cortex of FSL rats as compared to FRL rats. Protein expression of CB_1R and FAAH were decreased in left hippocampus. In summary, our data suggest a decreased eCB signalling in the FSL rats, which could contribute to the depressive-like behaviour. Interestingly, the altered eCB system activity appear to be hemisphere-specific in the limbic regions. Our study support the existing literature and showed altered eCB system activity in this particular animal model of depression.

1. Introduction

Major depressive disorder is a common psychiatric condition with a lifetime prevalence of 16% (Kessler et al. 2005). The symptomatology is extensive, reaching from lowered mood to impaired cognition, hedonic processing, and suicidal idealization. The cause appears to be a complex interplay between genetic disposition and environmental factors, such as stress (Dean and Keshavan, 2017). A consistent pathophysiological key mechanism among depressed individuals is still undiscovered. However, disrupted hippocampal and prefrontal cortical neurocircuitry seems as major contributors to the symptomatology (Sampath et al. 2017). The underlying cause of these disruptions appears to be a consequence of neuromodulatory deficiency causing insufficient monoamine neurotransmission, reduced neuroplasticity, and increased cortisol response to stress (de Kloet et al. 2005; Yohn et al. 2017). Accordingly, a growing interest toward neuromodulators, such as the endocannabinoid (eCB) has emerged.

The cannabinoid receptor type 1 (CB_1R) is vastly distributed in the central nervous system (CNS) and is dominantly located at the presynapse, where it inhibits transmitter release when activated (Kano, 2014; Lutz et al. 2015). Primary ligands are N-arachidonoylethanolamine (anandamide; AEA) (Devane et al. 1992) and 2-arachidonoyl glycerol (2-AG) (Sugiura et al. 1995), which are metabolized by their own specific enzymes fatty acid amide hydrolase (FAAH) and monoacyl glycerol lipase (MAGL), respectively (Blankman et al. 2007; Cravatt et al. 1996). Interestingly, preclinical studies demonstrate that activation of prefrontal CB_1R was associated with emotional processing through increased serotonergic and dopaminergic neuronal firing

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Abbreviations

2-AG	2-arachidonoylglycerol
AA	Arachidonic acid
AEA	Anandamide (N-arachidonoylethanolamine)
BDNF	Brain-derived neurotrophic factor
Cnr1	Cannabinoid receptor, mRNA
CB$_1$R	Cannabinoid receptor type 1
eCB	Endocannabinoid
FAAH	Fatty acid amide hydrolase
FSL	Flinders Sensitive Line
FRL	Flinders Resistant Line
MAGL	Monoacyl glycerol lipase
OBX	Olfactory bulbectomized rat
WKY	Wistar Kyoto Rat

(Draycott et al. 2014; Bambico et al. 2007), whereas hippocampal CB$_1$R appeared to be involved in neurogenesis and memory (Puighermanal et al. 2012). Hence, impaired CB$_1$R activation could underlie some of the pathophysiological features of depression. In support of this, mice lacking CB$_1$R possess a depressive-like phenotype including decreased sucrose consumption and increased passive coping behaviour in the forced swim test (Burokas et al. 2014). Notably, the lack of CB$_1$R was found to be associated with hyperactive cortisol response to stress, changes in serotonergic transmission, and deficient neurogenesis (Häring et al. 2013; Zimmermann et al. 2018b; Steiner et al. 2008b). Corroborating preclinical studies exposing rodents to chronic stress revealed depressive-like behaviour associated with decreased eCB functioning (Morena et al. 2016). Notably, drugs increasing endocannabinoid signalling reversed the maladaptive behaviour to chronic stressors (Lim et al. 2016; Griebel et al. 2018), thus suggesting the requirement of a well-functioning eCB system in order to cope with environmental challenges. Human data do support these findings only indirectly, as obese patients, treated with the CB$_1$R inverse agonist rimonabant, showed increased risk of depressed mood and anxiety compared to placebo (Christensen et al. 2007). In line with this, female patients suffering from major depressive disorder revealed decreased circulating levels of 2-AG and AEA in serum (Hill et al. 2009).

However, other studies failed to find any pathological changes in mice lacking CB$_1$R, and other demonstrated antidepressant-like effect when treating animals with the CB$_1$R inverse agonist rimonabant (Rabasa et al. 2015; Häring et al., 2013; Steiner et al. 2008a). These discrepancies could first of all be ascribed to experimental differences between laboratories, but also different endophenotypes of depression with altered endocannabinoid functioning.

To further elucidate the role of the eCB system in the pathophysiology of depression, we conducted a study using the well-validated genetic animal model of depression, the Flinders Sensitive Line (FSL) rats (Overstreet and Wegener, 2013). The Flinders Resistant Line (FRL) rats were included as controls. When comparing the FSL rats to FRL rats under basal conditions, FSL rats show symptoms of depressed individuals in form of psychomotor retardation (Bushnell et al., 1995) and memory deficiency (Thiele et al., 2016). More interestingly, the, FSL rats are highly vulnerable to environmental challenges, as different stress paradigms exacerbate the depressive-like behaviour including exaggerated immobility time in the forced swim test, decreased sucrose preference, and compromised weight gain compared to their inbred littermate the FRL rats (Brand and Harvey, 2017; Pucilowski et al. 1993; Ayensu et al. 1995).

We aimed at characterizing the endocannabinoid system components AEA, 2-AG, CB$_1$R, MAGL and FAAH. This was done peripherally in the plasma, and centrally using both left and right brain hemispheres, since functional changes in depressed patients have been associated to different hippocampal and prefrontal hemispheres (Willner et al. 2013).

2. Methods

2.1. Animals

For mRNA and protein expression studies male Flinders line rats (8 FSL and 8 FRL; 8 weeks of age; 280–350 g) from the colony maintained at Aarhus University (originally derived from the colony at the

Table 1

Characteristics of gene-specific real-time qPCR primers.

Gene Symbol	Gene Name	Accession No.[a]	Primer Sequence	Amplicon size[b]
Reference genes				
18s rRNA	18s subunit ribosomal RNA	M11188	(+) acggaccagagcgaaagcat (−) tgtcaatcctgtccgtgtcc	310
Actb	Beta-actin	NM_031144	(+) tgtcaccaactgggacgata (−) ggggtgttgaaggtctcaaa	165
CycA	Cyclophilin A	XM_345810	(+) agcactggggagaaaggatt (−) agccactcagtcttggcagt	248
Gapd	Glyceraldehyde-3-phosphate dehydrogenase	NM_017008	(+) tcaccaccatggagaaggc (−) gctaagcagttggtggtgca	168
Hmbs	Hydroxy-methylbilane synthase	NM_013168	(+) tcctggctttaccattggag (−) tgaattccaggtgagggaac	176
Hprt1	Hypoxanthine guanine phosphoribosyl transferase 1	NM_012583	(+) gcagactttgctttccttgg (−) cgagaggtccttttcaccag	81
Rpl13A	Ribosomal protein L13A	NM_173340	(+) acaagaaaaagcggatggtg (−) ttccggtaatggatctttgc	167
Ywhaz	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta	BC094305	(+) ttgagcagaagacggaaggt (−) gaagcattggggatcaagaa	136
Target genes				
CnR1	Cannabinoid receptor 1	NM_012784.4	(+) tggggccctctgcttgcgat (−) ggcgtgcttgtgcaggcagt	243
Faah	Fatty acid amide hydrolase	NM_024132.3	(+) gttcaccttggaccctaccg (−) agaagggaatcagcgtgtgg	185
Mgll	Monoglyceride lipase	NM_138502.2	(+) cggaacaagtcggaggttga (−) tgtcctgactcggggatgat	220

[a] Genbank accession number of cDNA and corresponding gene, available at http://www.ncbi.nlm.nih.gov/.

[b] Amplicon length in base pairs.

University of North Carolina, USA) were housed in pairs (Cage 1291H Eurostandard Type III H, 425 × 266 × 185 mm, Techniplast, Buguggiate, Italy) at 20 ± 2 °C on a 12-h light/dark cycle (lights on at 07:00 a.m.). The animals had *ad libitum* access to chow pellets and tap water. For endocannabinoid extraction analysis another cohort of 8 FSL and 8 FRL rats (8 weeks of age; 280–350 g) were used and housed under the same conditions as above. The Danish National Committee for Ethics in Animal Experimentation had approved all animal procedures prior to initiation of the experiments (2012-15-2934-00254).

2.2. Euthanization and tissue dissection

Between 10 and 12 a.m., rats were euthanized by decapitation and the left/right hippocampus and prefrontal cortex were rapidly dissected, frozen on powdered dry ice, and stored at −80 °C until RNA extraction and protein isolation with the PARIS™ kit (Ambion, TX, USA).

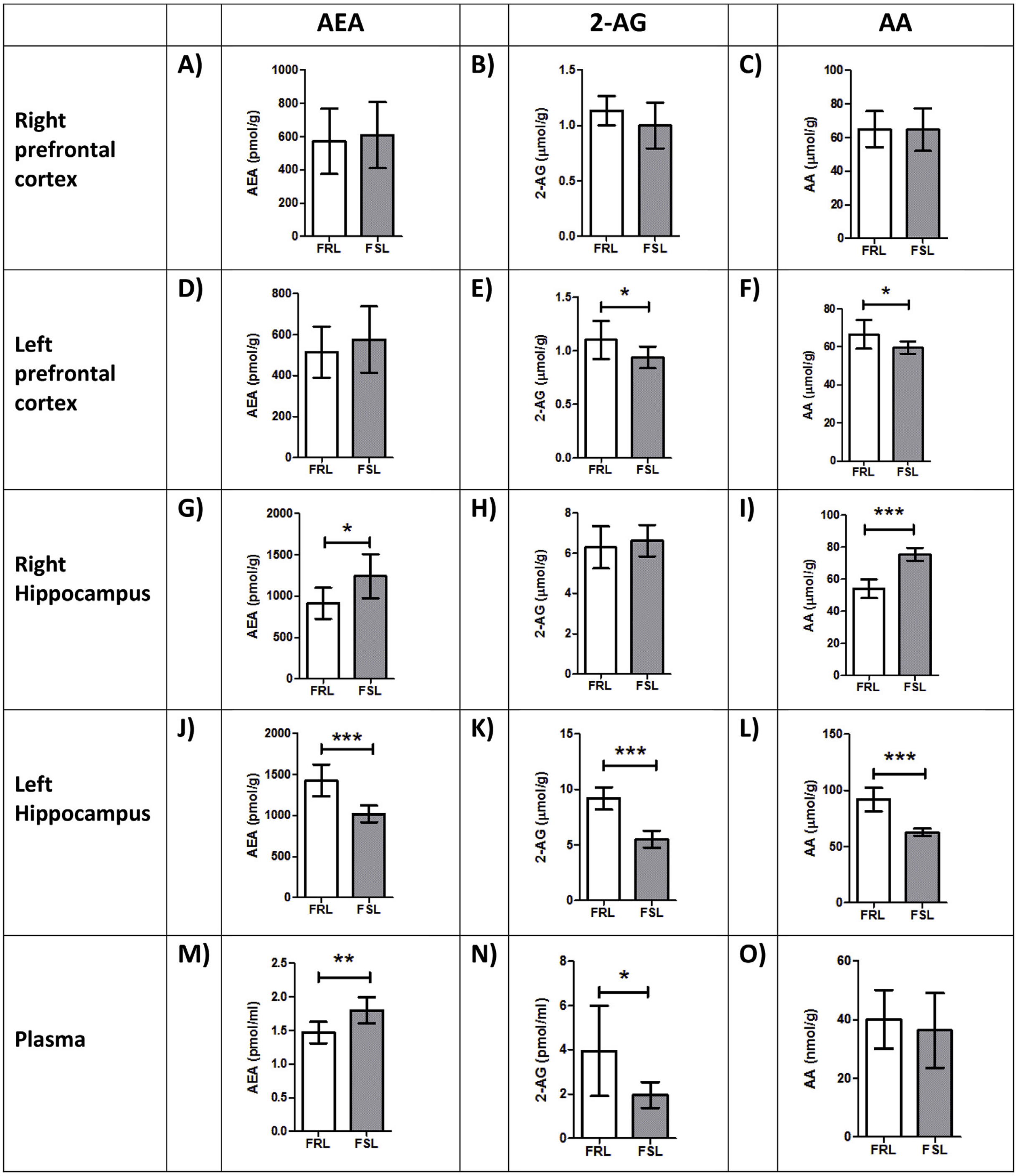

Fig. 1. Basal levels of the endocannabinoids (eCB) anandamide (AEA) and 2-arachidonoylglycerol (2-AG) and their metabolite arachidonic acid (AA) derived from left/right prefrontal cortex and hippocampus and plasma from Flinders Sensitive Line (FSL) compared to control Flinders Resistant Line (FRL) rats. All data are expressed as mean ± SD. N = 7–8 rats/group. Statistical difference between eCB levels of FRL and FSL was analyzed by *t*-test expressing *p < 0.05, **p < 0.01 ***p < 0.001.

2.3. RNA extraction and cDNA synthesis

The isolation procedure with the PARIS™ kit is well-established in our laboratory and was processed as previously described (Müller et al. 2011). The RNA concentration and the purity were determined by a NanoDrop 1000 spectrophotometer (Thermo Fischer Scientific). Before cDNA synthesis, the RNA concentration of the samples was adjusted to match the sample with the lowest concentration. RNA was reversely transcribed using random primers and Superscript IV Reverse Transcriptase (Invitrogen,CA) following manufacturer's instructions and with a RNA input concentration per reaction of 184 ng/μL and 215 ng/μL for prefrontal cortex and hippocampus, respectively. The cDNA samples were stored undiluted at −80 °C until quantitative real-time polymerase chain reaction (real-time qPCR) analysis. Samples from prefrontal cortex were diluted 1:66 and samples from hippocampus 1:78 with DEPC-treated water prior to real-time qPCR analysis.

2.4. Real-time qPCR

Real-time qPCR was carried out on individual samples in duplicate in 96-well PCR-plates using the Mx3005P (Stratagene, La Jolla, CA, USA) and SYBR Green as described previously (Bonefeld et al. 2008; Elfving et al. 2013). The gene expression of eight different reference genes (*18s rRNA, Actb, CycA, Gapd, Hmbs, Hprt1, Rpl13A, Ywhaz) and Faah*, *Magl,* and *Cnr1* were investigated. Essential gene specific data about primer sequences and amplicon sizes are given in Table 1. Briefly, each SYBR Green reaction (10 μl total volume) contained 1 × SYBR Green master mix (Sigma–Aldrich, St. Louis, MO, USA), 0.5 μM primer pairs, and 3 μl of diluted cDNA. The thermal conditions for the PCR were 3 min at 95 °C to activate the hot-start iTaqDNA polymerase, followed by 40 cycles of 10 s denaturation at 95 °C, 30 s annealing at 60 °C, and 60 s extension at 72 °C. Each run was completed by dissociation curve analysis to confirm the amplification specificity and absence of primer dimers. A standard curve performed in duplicate was included on each plate. For data normalization, we first measured mRNA levels

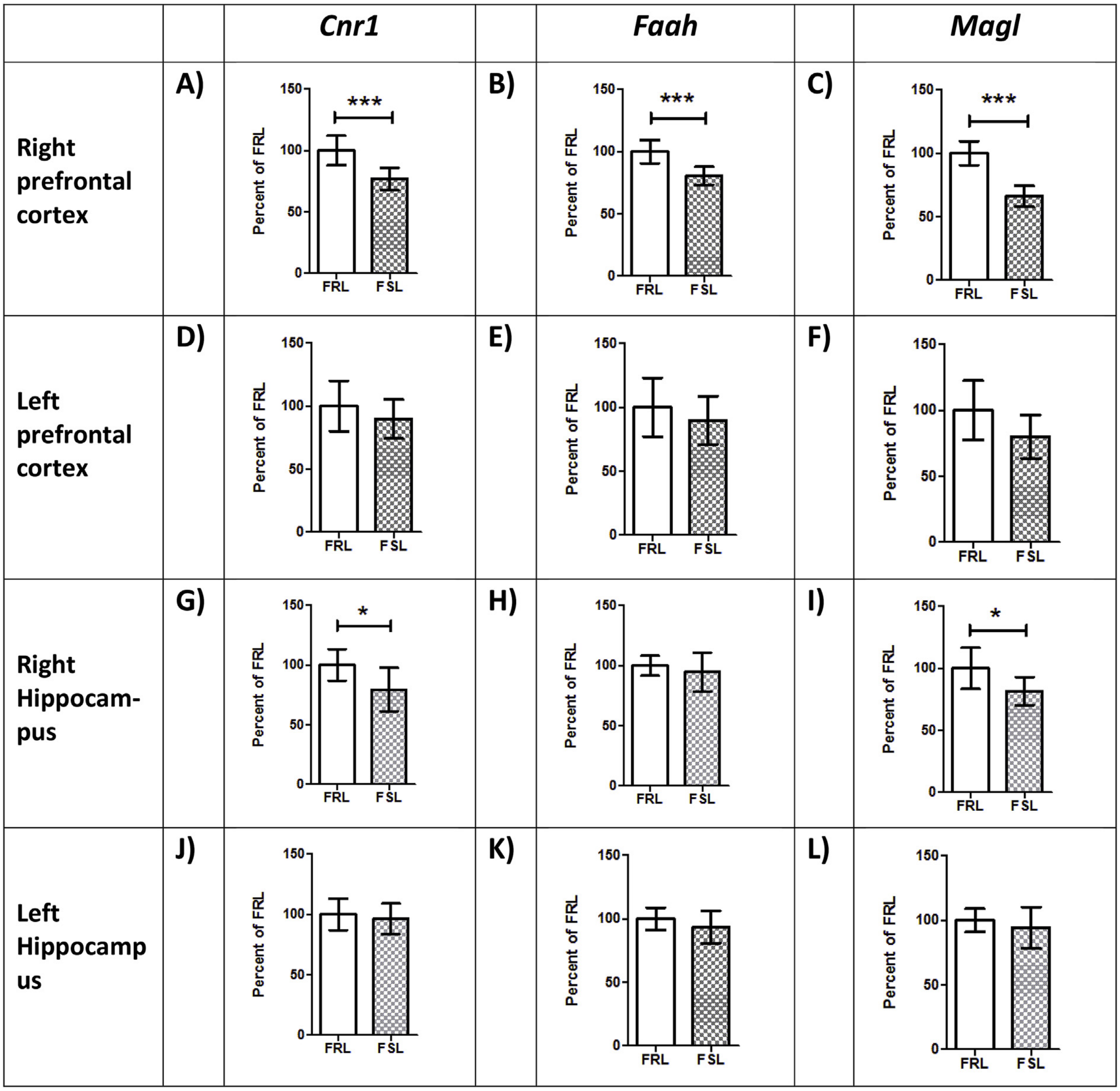

Fig. 2. Basal gene expression levels of the cannabinoid receptor 1 (*Cnr1*), the an adamide hydrolyzing enzyme fatty acid amide hydrolase (*Faah*) and the 2-arachidonoyl glycerol hydrolyzing enzyme monoacyl glycerol lipase (*Magl*) in left/right prefrontal cortex and hippocampus of Flinders Sensitive Line (FSL) and control Flinders Resistant Line (FRL) rats. All data are expressed as mean ± SD. N = 7–8 rats/group. Statistical difference between *Cnr1*, *Faah* and *Mgl* of FRL and FSL was analyzed by Students t-test expressing $^*p < 0.05$, $^{**}p < 0.01$ $^{***}p < 0.001$.

for the reference genes. Stability comparison of the expression of the eight reference genes was conducted with the Norm finder software (http://www.mdl.dk) (Andersen et al., 2004) and the best combination was selected. Values for each individual were normalized with the geometric mean of the reference genes *Hprt1/CycA and Gapd/Rpl13A* in left and right prefrontal cortex, and *Ywhaz/Rpl13A* and *Ywhaz/Actb* in the left and right hippocampus, respectively (Andersen et al. 2004; Elfving et al., 2013; Müller et al., 2011; Bonefeld et al., 2008).

2.5. Determination of FAAH, MAGL and CB_1R protein levels

Aliquots of homogenized lysate (20 μg total protein) obtained using the PARIS kit (see RNA extraction and cDNA synthesis section above) were separated on 10% criterion TGX gels (Bio-Rad), transferred to nitrocellulose membranes using the Trans-Blot Turbo transfer system (Bio-Rad), blocked in Odyssey Blocking Buffer (Licor) and probed with the primary antibodies: rabbit anti- CB_1R (Abcam: ab172970; 1:2000), mouse anti-FAAH (Sigma: WH0002166M7; 1:400), rabbit anti-MAGL (Novus: NBP2-19380; 1:300), and mouse anti-β-actin (Licor 926–42212; 1:3000), overnight at 4 °C followed by incubation with IRDye conjugated secondary antibodies for 1 h at RT: IRDye 800CW goat anti-rabbit IgG or IRDye 680RD goat anti-mouse IgG, at 1:10,000 dilution (Licor). Infrared signals were detected using the Odyssey CLx infrared imaging system, and bands were quantified using Image Studio software (LI-COR Biosciences). Band intensities were normalized to β-actin levels within the same lane.

2.6. Endocannabinoid extraction and analysis

Endocannabinoids were extracted from rat brain regions and plasma

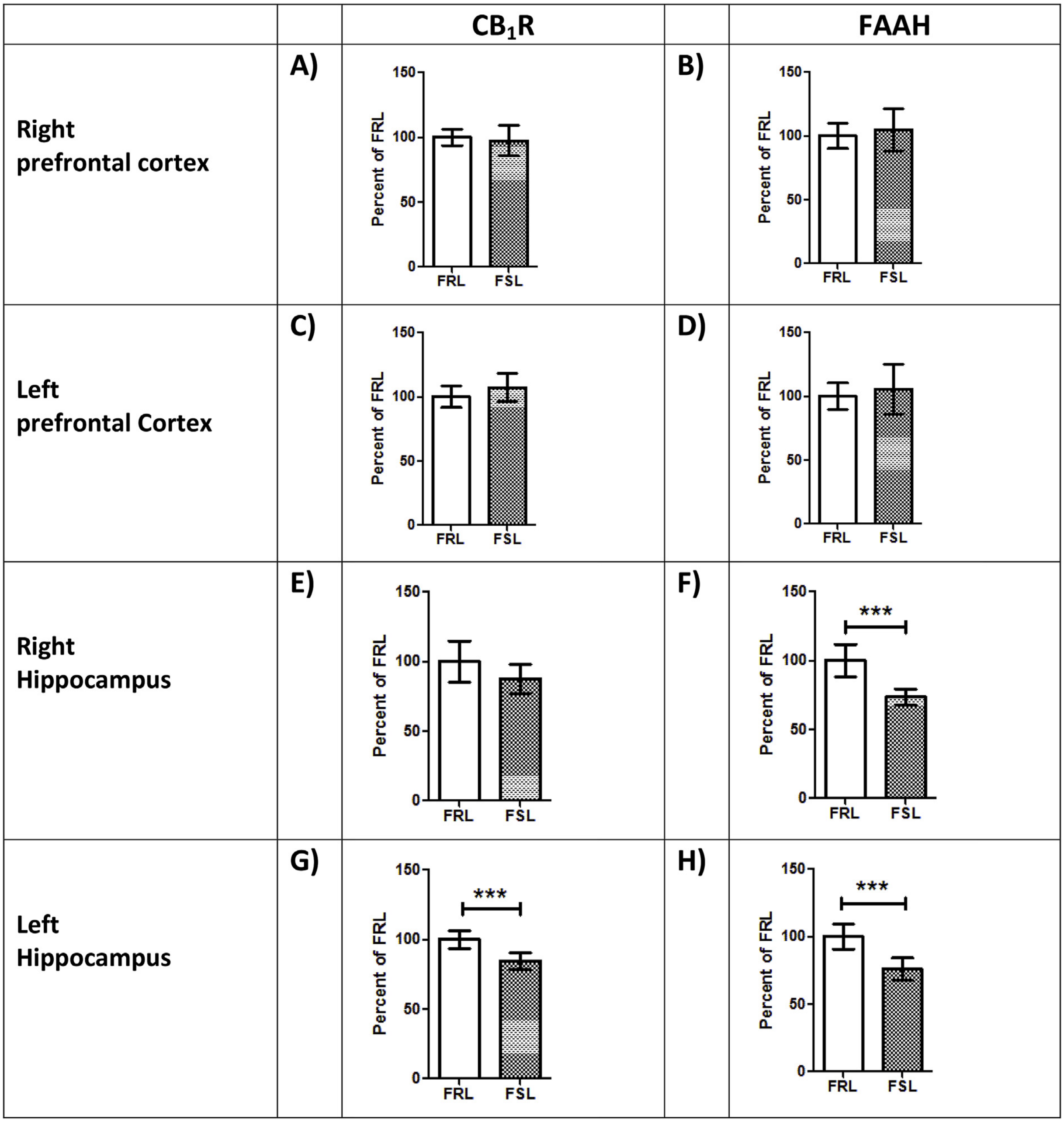

Fig. 3. Basal protein expression levels of the cannabinoid receptor 1 (CB_1R) and the anandamide hydrolyzing enzyme fatty acid amide hydrolase (FAAH) in right/left prefrontal cortex and hippocampus from Flinders Sensitive Line (FSL) compared to control Flinders Resistant Line (FRL) rats. All data are expressed as mean ± SD. N = 7–8 rats/group. Statistical difference between CB_1R and FAAH levels of FRL and FSL was analyzed by *t*-test expressing $^*p < 0.05$, $^{**}p < 0.01$ $^{***}p < 0.001$.

following general protocols previously described (Bindila and Lutz, 2016; Lomazzo et al. 2015) with few modifications: The lipid extract from the tissue was reconstituted in 50 μL acetonitrile: water (1:1, v/v). For hippocampal eCB analysis, lipid solutions were further diluted to 1:20 in acetonitrile: water (1:1, v/v), while for prefrontal cortex and hypothalamus lipid solutions were diluted 1:10 in the same solvent system as for hippocampus. The dilution steps were performed prior to LC/MRM injection. For LC/MRM analysis, 20 μL of the diluted lipid solutions were injected. The eCB tissue values were normalized to the protein amount of tissue determined by BCA analysis (Bindila and Lutz, 2016). For plasma endocannabinoid analysis, 100 μL plasma were used and the eCB values were normalized to the plasma volume.

2.7. Statistical analysis

Statistical analyses were carried out using GraphPad Prism Version 5. Using Student's t-test comparing mRNA, protein and eCB amount of FSL rats compared to control FRL rats. P values < 0.05 were considered statistically significant.

3. Results

3.1. Endocannabinoid tissue and plasma content

In right prefrontal cortex no differences were observed for the levels of either AEA (t (14) = 0.37, p = 0.7153; Fig. 1A), 2-AG (t (14) = 1.554, p = 0.1425; Fig. 1B) or AA (t (14) = 0.0417, p = 0.9673; Fig. 1C) in FSL rats compared to FRL rats. In left prefrontal cortex levels of both 2-AG (t (14) = 2.289, p = 0.0328; Fig. 1E) and AA (t (14) = 2.438, p = 0.028; Fig. 1F) were lower in FSL rats compared to FRL rats, and no differences between and no between-group differences in AEA levels (t (14) = 0.826, p = 0.402; Fig. 1D) were found. In right hippocampus, the levels of AEA (t (14) = 2.848, p = 0.0129; Fig. 1G) and AA (t (14) = 8.560, p < 0.0001; Fig. 1I) were elevated in FSL compared to FRL rats, and no differences in 2-AG (t (14) = 0.728, p = 0.478; Fig. 1H). In left hippocampus, AEA (t (13) = 3.094, p = 0.0085; Fig 1J), 2-AG (t (13) = 7.875, p < 0.0001; Fig. 1K) and AA (t (14) = 5.039, p = 0.0002; Fig. 1L) were lower in FSL rats compared to FRL rats. In plasma elevated level of AEA (t (14) = 3.213, p = 0.0063; Fig. 1M) was found in FSL rats compared to FRL rats, whereas a lower level was observed for 2-AG (t (13) = 2.485, p = 0.0273; Fig. 1N), and no differences between AA levels (t (14) = 0.669, p = 0.514; Fig. 1O).

3.2. Gene expression of Cnr1, Faah and Magl

In right prefrontal cortex, the gene expression levels of *Cnr1* (t (14) = 4.346, p = 0.0007; Fig. 2A), *Faah* (t (14) = 4.676, p = 0.0004; Fig. 2B), and *Magl* (t (14) = 7.610, p < 0.0001; Fig. 2C) were significantly decreased in the FSL rats compared to the FRL rats. In left prefrontal cortex no statistical difference were observed with *Cnr1* (t (14) = 1.1360, p = 0.2750; Fig. 2D), *Faah* (t (14) = 0.9653, p = 0.3508; Fig. 2E), and *Magl* (t (14) = 2.033, p = 0.0614; Fig. 2F) in FSL rats compared to FRL rats. In right hippocampus, the gene expression levels of *Cnr1* (t (14) = 2.587, p = 0.025; Fig. 2G) and *Magl* (t (14) = 2.590, p = 0.0214; Fig. 2I) were significantly decreased in the FSL rats compared to the FRL rats. No difference was observed in the *Faah* mRNA levels (t (14) = 0.8345, p = 0.4180; Fig. 2H). In left hippocampus, no significant differences in mRNA levels of *Cnr1* (t (14) = 0.5680, p = 0.5790; Fig. 2J), *Faah* (t (14) = 1.189, p = 0.2543; Fig. 2K) and *Magl* (t (14) = 0.8789, p = 0.3943; Fig. 2L) were observed between FSL rats and FRL rats.

3.3. Protein expression of CB_1R and FAAH

In both left and right prefrontal cortex of FSL rats and FRL rats no differences in protein expression were observed for CB_1R (left: t (14) = 1.481, p = 0.160; right: t (14) = 0.550, p = 0.590 Fig. 3A,C) and FAAH (left: t (14) = 0.705, p = 0.491; right: t (14) = 0.679, p = 0.507; Fig. 3B,D). In right hippocampus FAAH (t (14) = 5.682, p < 0.0001) but not CB_1R (t (14) = 1.979, p = 0.069; Fig. 3F,E) was significantly decreased in FSL rats compared to FRL rats. In left hippocampus both CB_1R (t (14) = 5.011, p = 0.0002; Fig. 3G) and FAAH (t (14) = 5.501, p < 0.0001; Fig. 3H) were decreased in FSL rats compared to FRL rats. In all regions, MAGL levels were too weak to be eligible for quantification (see Supplementary Materials section 4.1).

4. Discussion

The present study explores, for the first time, the content of AEA and 2-AG as well as CB_1R, FAAH and MAGL at mRNA and protein levels in the FSL rat model of depression. We chose to investigate hippocampus and prefrontal cortex, as these regions are highly associated with depression. In order to extend the perspectives, we analyzed both the right and left parts of these brain regions. Lastly, we also analyzed the peripheral circulating AEA and 2-AG levels to further clarify the correlation between peripheral and cerebral content.

4.1. Hippocampal endocannabinoid signalling

The major finding in the present study is a significantly lower content of AEA and 2-AG in left hippocampus of FSL as compared to their respective controls, probably because of reduced production rather than increased degradation, as their common metabolic product AA was also reduced. Unfortunately, we were not able to measure the protein amount of the 2-AG degrading enzyme MAGL (Supplementary Material section 4.1). However, FSL rats revealed both lower CB_1R and FAAH protein expression in the left hippocampus, whereas the mRNA levels of *Cnr1*, *Faah* and *Magl* were similar between rat strains. Interestingly, correlation analysis in large-scale data sets report approximately 50% correspondence between mRNA and protein levels, thus suggesting differences in protein synthesis and degradation rates (Pradet-Balade et al. 2001; Anderson and Seilhamer, 1997). The right side of hippocampus contained elevated levels of AEA in FSL rats, but no difference in 2-AG level between FSL and FRL rats. Protein expression of FAAH was significantly reduced suggesting decreased turnover of AEA. The protein amount of CB_1R in FSL rats was equal to FRL rats, however mRNA levels of both *Cnr1* and *Magl* were significantly reduced in FSL rats. Taken together, this reveals hemisphere-specific eCB system activity, showing increased signalling in right side and decreased signalling in left side. In support of our findings, decreased hippocampal AEA content has been reported in both the olfactory bulbectomized rat and Wistar Kyoto rat (WKY) model of depression (Smaga et al. 2017; Vinod et al. 2012). Contrary, others fail to find any differences in hippocampal AEA content in WKY rats (Carnevali et al. 2015; Smaga et al., 2017). Beside different technical approaches and origin of breeding colony, this discrepancy could be due to the choice of brain hemisphere or pooling of both hemispheres for analysis, as we demonstrate directly opposite levels of AEA in right and left hippocampus of FSL rats, which in the latter case would mask any differences between study groups. Furthermore, both WKY and olfactory bulbectomized rats showed increased hippocampal FAAH protein expression (Smaga et al., 2017; Vinod et al., 2012) whereas FSL rats revealed consistent decreased FAAH amount in both sides.

Hippocampal eCB signalling is highly involved in neuroplasticity during basal and stressful conditions both through a BDNF mediated pathway (Aso et al. 2008) and HPA axis regulation (Hill and Tasker, 2012). Interestingly, the FSL rats display reduced left hippocampal mRNA and protein levels of BDNF (Elfving et al. 2010). In line with these observations, the WKY rats, also display reduced BDNF levels in both prefrontal cortex and hippocampus that was reversed when pharmacologically enhancing the AEA signalling causing antidepressant-like effect in the FST (Vinod et al., 2012). Hence, the reduced levels of BDNF in FSL rats could be an expression of impaired hippocampal eCB signalling as observed in the left side. Environmental challenges such as acute stress are able to decrease hippocampal AEA levels (Wang et al. 2012) and increase the 2-AG levels. The latter one is dependent on glucocorticoid receptor activation (Wang et al., 2012; Bowles et al. 2012). Given the increased vulnerability of FSL rats to stress (Brand and Harvey, 2017), this underlying mechanism could be a consequence of inadequate ability to mobilize hippocampal 2-AG during stress causing inappropriate response to stress. Corroborating results revealed that rodents exposed to heterotypic chronic stress showed both decreased 2-AG, AEA and CB_1R protein levels associated with depressive-like behaviour (Zhong et al. 2014; Hill et al. 2008a) mirroring our findings in left hippocampus. In contrast, two studies revealed that chronic stress robustly increase the mRNA and protein expression of FAAH (Navarria et al. 2014; Reich et al. 2009), which was not observed in the FSL rats. However, our study was carried out under unstressed conditions, which should be taken into account for further studies. Only few studies make a direct link between depressive-like behaviour and FAAH expression. Pharmacological inhibition of FAAH has been associated with antidepressive-like behaviour and adaptions to environmental challenges through CB_1R mediated stimulation (Danandeh et al. 2018; Griebel et al., 2018), while reduction of anandamide/palmitoylethanolamide signalling by FAAH overexpression in the hippocampus led to increased anxiety and alterations in stress coping (Zimmermann et al. 2018a). Hence, reduced FAAH, CB_1R and AEA contents could underlie a potential mechanism for the increased sensitivity to stress in FSL rats. However, this need further investigation using different stress paradigms and direct inhibition of FAAH.

4.2. Prefrontal cortical endocannabinoid signalling

Prefrontal cortical eCB signalling is highly involved in behavioural active coping strategies through activation of serotonergic neurons in the nucleus raphe (Bambico et al., 2007). The FSL rats only showed lowered 2-AG level in the left side, which appear to be a result of decreased production, since the metabolite AA is also decreased. Right side of the prefrontal cortex revealed decreased mRNA levels of *Cnr1, Faah* and *Magl.* Taken together, we only observed discrete differences in prefrontal cortex of FSL rats. However, preclinical studies imply that eCB changes are strictly confined to subanatomical regions (Aliczki et al. 2016). For instance, frontal cortical tissue derived from WKY and olfactory bulbectomized rat models of depression did not show differences between eCB ligands, whereas in the prefrontal part significantly lower AEA levels in both strains compared to their respective controls were detected (Smaga et al., 2017). Rats undergoing restraint stress only showed increased 2-AG levels and CB_1R binding site in the ventromedial part of prefrontal cortex, which is involved in stress and emotional regulation (Hill et al. 2011; McLaughlin et al. 2013). Therefore, using whole prefrontal tissue could mask some crucial differences between FSL and FRL rats, which has to be taken into account. Nevertheless, our findings do not concur with results from human studies, showing significantly greater CB_1R binding sites in prefrontal cortex of depressed suicide victims compared to controls (Hungund et al. 2004). In line with this, animals undergoing chronic stress demonstrate increased CB_1R at both mRNA and protein levels, but also elevated 2-AG and AEA content (Morena et al., 2016). As mentioned earlier, our study was applied under unstressed conditions. Notably, the common eCB metabolite AA was found to be increased in prefrontal cortex of FSL rats suggesting altered serotonergic transmission because of this (Green et al. 2005). These observations are conflicting with our results showing decreased AA levels in left prefrontal cortex. However, the discrepancy could lie within the use of outbred Sprague Dawley as control rats (Green et al., 2005) suggesting potential differences between inbred and outbred strains. Conflicting genetic studies suggest different polymorphism of the *CNR1* gene as both protective and aggravating factor to depressive disorder. It has been suggested that increased risk might depend on the stability of *CNR1* mRNA; however, this has never been experimentally verified (Hill and Patel, 2013). The FSL rats shows decreased *Cnr1* mRNA levels in right prefrontal cortex, but due to inconsistency in the literature, it is impossible to draw parallels and to make conclusions regarding the significance of these findings. A hypomorphic allele of the *FAAH* genotype gene (leading to decreased FAAH protein stability) in humans has been associated with increased peripheral AEA levels and extinction during stress test. The same study demonstrated that mice with this *Faah* hypomorphic allele was associated with elevated AEA levels during stress (Mayo et al. 2018). This suggests a potential genetic contribution to improved stress coping behaviour, however they did not measure *CNR1* mRNA levels. The FSL rats show decreased mRNA levels of *Faah* but no differences in protein amount nor AEA concentration. Therefore, it is not clear how the alterations of *Cnr1* or *Faah* mRNA contributes to the depressive-like behaviour of FSL rats, and further studies need to detail these issues.

Taken together, we do not observe conspicuous changes in prefrontal cortical eCB signalling in FSL compared to FRL rats.

4.3. Peripheral circulating endocannabinoids

The FSL rats revealed significantly elevated levels of AEA and lowered 2-AG content in plasma compared to FRL rats. Our findings corroborate human studies reporting lowered serum 2-AG content in women suffering from major depression (Hill et al., 2009), whereas AEA levels were elevated among patients with minor depression (Hill et al. 2008b). Healthy volunteers undergoing chronic stress in form of 500 days of isolation exhibited decreased levels of 2-AG that negatively correlated with salivary cortisol (Yi et al. 2016). Even though the role of 2-AG as a negative HPA-axis feedback inhibitor is well established from preclinical models (Hill and Tasker, 2012), the behavioural significance is still not understood. Genetic reduction of 2-AG signalling in mice caused exaggerated anxiety behaviour, increased immobility time in the FST, and reduced weight gain (Jenniches et al. 2016). Indeed, FSL rats exhibit the last two-mentioned features, suggesting that impaired 2-AG signalling could be an important contributor to the depressive-like behaviour. Furthermore, the low circulating levels of 2-AG could also contribute to increased vulnerability to stress in FSL rats, as an impaired ability to mobilize 2-AG, and thereby inhibit HPA-axis activity during environmental challenges which could cause maladaptive behaviour.

Given that the eCB ligands are proposed as potential peripheral biomarkers in depressive disorders, caution must be made, as the source of AEA and 2-AG in serum/plasma is heterogeneous, and their levels depend on additional factors such as food intake, metabolic rate, circadian rhythm, and stress state. Apparently, serum 2-AG was positively correlated with EEG alpha waves (Yi et al., 2016) suggesting that it is to a large extent brain-derived (Hillard, 2018). In agreement with this statement, the FSL rats showed decreased 2-AG levels in both plasma, left prefrontal cortex, and hippocampus indicating that plasma 2-AG in part reflects the cerebral content. However, this interpretation should be handled with caution, and further studies involving pharmacological manipulation and stress paradigms have to be applied in the FSL rats to fully elucidate the neurobehavioral and endocrinological influence of eCB signalling.

5. Conclusion

In the present study, we investigated eCB signalling in the FSL compared to FRL control rats. In general, we observed decreased 2-AG levels and inversely associated levels of AEA which were increased in right hippocampus and lowered in left hippocampus. Protein and mRNA levels of CB_1R, FAAH and MAGL were generally decreased or similar to controls. Correlation between mRNA and protein amount in prefrontal cortex and hippocampus were not found. All together, we demonstrated hemisphere-specific decreased eCB signalling in the FSL rat model of depression. However, our study presents some limitations as discussed above, and further comprehensive research using additional pharmacological approaches, brain regions, and stress protocols should be conducted to further detail the involvement of eCB signalling in mechanisms underlying depression-like behaviour, and its value to plasma/serum monitoring in translational studies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.01.023.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

CK, NL and FAM planned the study design. GW provided the FSL rats and housing facilities. BE made the qPCR and statistical analyses of mRNA expression. HKM made the Western blotting and statistical analysis of protein expression. LB and BL made the eCB measurements and assignment. CK prepared the manuscript with response from the other co-authors. All authors reviewed the manuscript.

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