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Gene expression signature in brain regions exposed to long-term psychosocial stress following acute challenge with cannabinoid drugs

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ABSTRACT

Repeated exposure to life stressors can overwhelm the body's capacity to restore homeostasis and result in severe negative consequences. Cannabinoid CB_1 receptors are highly expressed in the Central Nervous System (CNS) and regulate both glucocorticoid signalling and neurotransmitter release. In rodents, WIN55212.2 is a full agonist at the cannabinoid receptor type-1, while Rimonabant is a potent and selective cannabinoid inverse agonist at this receptor. This study aims to investigate the effect of long-term psychosocial stress following acute challenge with cannabinoid drugs on gene expression in distinct brain regions; this is done by employing digital multiplexed gene expression analysis.

We found that repeated stress increased cortical mRNA levels of dopamine receptor D2, while the expression of neuregulin-1 decreased in both the prefrontal cortex and cerebellum. Further, we found that the acute injection of the agonist WIN55212.2 reduced striatal levels of dopamine receptor D2, while the use of inverse agonist Rimonabant acted in the opposite direction. The analysis of the interaction between the drugs and repeated stress revealed that defeat mice treated with WIN55212.2 showed lower expression of a set of myelin-related genes, as did the expression of SRY-box 10 and dopamine receptors-D1 and -D2 in the prefrontal cortex when compared to vehicle. In addition, in the hippocampus of stressed mice treated with WIN55212.2, we found an elevated expression of oligodendrocyte transcription factor-1, -2 and zinc finger protein 488 when compared to vehicle. In comparison to vehicle, an increase in 2′,3′-Cyclic nucleotide 3′-phosphodiesterase and oligodendrocyte transcription factor-1 occurred in the cerebellum of stressed animals treated with the agonist. Moreover, treatment with Rimonabant under the influence of stress induced an overexpression of a set of myelin-related genes in the prefrontal cortex when compared to WIN-treated animals.

In conclusion, repeated stress interfered with the dopaminergic system in the prefrontal cortex. We demonstrated that the expression of dopamine receptor D2 in the striatum was mediated by the CB_1 receptor. Stressed mice exposed to either WIN55212.2 or Rimonabant displayed pronounced deficits in CNS myelination. In addition, the pharmacological blockage of CB_1 receptor in stressed mice deregulated the expression of dopamine receptors and might lead to dysfunctions in dopamine metabolism.

1. Introduction

When a living organism is subjected to a life stressor, a cellular machinery is activated to recover homeostasis (Sapolsky, 2003) by regulating distinct physiological features, such as the hypothalamo–pituitary–adrenal (HPA) axis and cardiovascular functions, which, per se, do not result in a pathological condition. However, when a long-term and constant stress exposure stimulation overwhelms the body's capacity to maintain homeostasis, it can result in severe consequences in numerous brain regions, including the prefrontal cortex (Tomas-Roig et al., 2018), the dorsal striatum (Tomas-Roig et al., 2018), the hippocampus (Conrad, 2008) and the cerebellum (Tomas-Roig et al., 2016a). Under normal conditions, the HPA axis is mainly affected by two factors: stress (either physical or psychological) and the circadian

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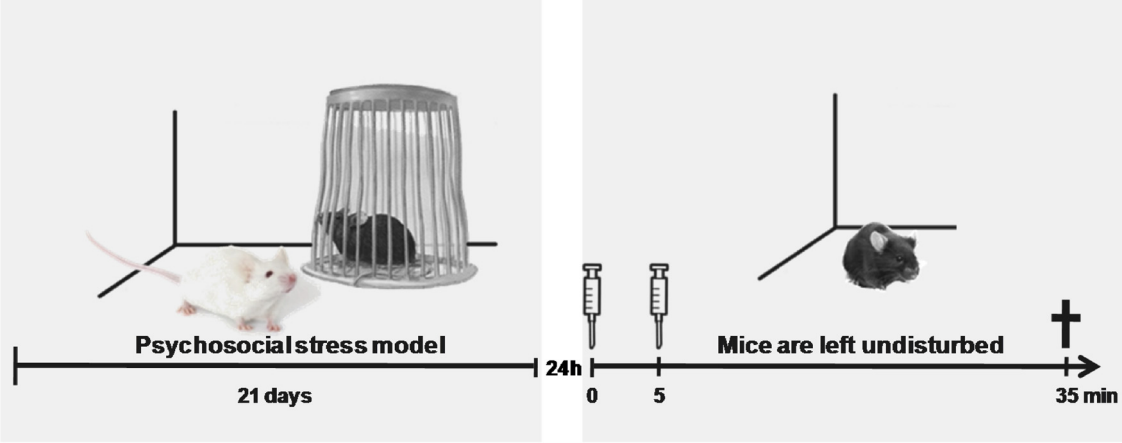

Fig. 1. Schematic drawing of the experiment. Mice were exposed daily to social stress for one hour (stress) or were left undisturbed (control). After 22 days, the mice were injected with the appropriate drug (Rim and/or WIN) or the vehicle, after which the mouse brains were surgically removed and finally analysed using digital multiplexed gene expression analysis.

rhythm (de Kloet, 2000). Cannabinoid CB_1 receptors are highly expressed in the Central Nervous System (CNS) (Herkenham et al., 1991) and can regulate both glucocorticoid signalling and neurotransmitter release (Scerif et al., 2013; Boychuk et al., 2013). Specifically, the endogenous cannabinoid system is implicated in a broad variety of pathophysiological states, such as psychosis and drug addiction, which are associated with alterations in dopaminergic neurotransmission (Bloomfield et al., 2016). In rodents, WIN55212.2 is a full agonist at the cannabinoid receptor type-1 (Ki = 1.9 nM), while Rimonabant is a potent and selective cannabinoid inverse agonist at this receptor with a Ki of 1.89 nM and low affinity for the cannabinoid receptor type-2 (Pozzi et al., 2003). The main endocannabinoid ligands for CB_1 receptors are N-arachidonylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG). *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) is responsible for AEA synthesis, while 2-AG is mostly produced by diacylglycerol lipase (DAGL-α). After activating cannabinoid receptors, these ligands are hydrolysed by the fatty acid amide hydrolase (FAAH) or the monoacylglycerol lipase (MAGL), respectively (Di Marzo, 2008).

With the rationale that repeated stress induces marked alterations in the brain (Tomas-Roig et al., 2018) and that the CB_1 receptor regulates both glucocorticoid signalling and dopamine neurotransmission (Scerif et al., 2013; Bloomfield et al., 2016), we aimed to study the influence of acute cannabinoid administration in the context of prolonged psychosocial stress. For this purpose, we analysed the expression levels of a set of genes involved in dopamine neurotransmission, CNS myelination and the risk of developing pathophysiological brain states.

2. Material and methods

The investigation presented here was approved by the Göttingen University Institutional Animal Care and Use Committee and is in accordance with the National Institutes of Health (NIH) guidelines for the use of animals in research and the European Communities Council Directive (2010/63/EU).

2.1. Animals

In this study, 32 C57Bl6/J male mice aged 7–8 weeks were purchased from Charles River Laboratories (Sulzfeld, Germany). Upon arrival, they were housed in groups of four per cage and kept under standard conditions (12 h light/dark cycle with 6:00/18:00 lights on/off, a room temperature of 21 ± 2 °C and food and water *ad libitum*). Experiments began after one week of habituation. We used one-year-old FVB/N male mice (Charles River Laboratories, Sulzfeld, Germany) as residents in the resident-intruder paradigm. FVB/N mice were housed individually and selected as residents, because they were more aggressive than the C57Bl6/J strain. FVB/N mice were kept under the same conditions as the C57Bl6/J mice, but were housed in a different room to avoid the habituation of C57Bl6/J mice to the odour of residents.

2.2. Drugs

The cannabinoid agonist WIN55212.2 (Sigma–Aldrich, Seelze, Germany) and the selective cannabinoid inverse agonist Rimonabant (Sequoia Research Products Ltd, Pangbourne, UK) were mixed in a buffer solution containing 10% DMSO (Sigma–Aldrich, Seelze, Germany) and 0.1% Tween80 (Sigma–Aldrich, Seelze, Germany) in 0.9% saline (vehicle). A volume of 200 µl of drug and/or vehicle was applied intraperitoneally (either WIN55212.2 or Rimonabant were administered at a concentration of 3 mg/kg) on the day following the experimentally induced psychosocially stressed model (day 22). We employed the same dosage for either WIN55212.2 or Rimonabant, since the lowest Ki for CB_1 receptor was similar for both drugs (Pozzi et al., 2003). An elevated dose of either WIN55212.2 or Rimonabant was applied because high-dose cannabinoid users displayed a greater risk for developing psychosis and addiction phenotypes (Bloomfield et al., 2016).

2.3. Experimental design and experimental groups

The C57Bl6/J male mice were divided into two groups: one group that was exposed daily to 1h-psychosocial stress (stress) and another group which was left undisturbed (control). The resident-intruder paradigm was applied daily for a period of three weeks. On day 22, the animals were subjected to either drug or vehicle administration and sorted into four subgroups each (four animals per subgroup) in the following manner: mice treated twice with vehicle (Veh), mice subjected to vehicle first and then WIN55212.2 (WIN), mice treated first with Rimonabant and then with WIN55212.2 (Rim + WIN) and those exposed to Rimonabant first and then with vehicle (Rim). Finally, the animals were deeply anesthetized and appropriately sacrificed. The psychosocial stress paradigm and the treatment with either vehicle or cannabinoid drugs was done during the dark phase, in which the rodents were active (i.e. after 9 p.m.). Fig. 1 depicts a schematic drawing of the experiment.

2.4. Social stress procedure

The resident-intruder paradigm has been extensively used as a

psychosocial stress model in mice (Tomas-Roig et al., 2018). To summarize, an intruder (C57Bl6/J mouse) was placed in contact with a resident (FVB/N mouse) and then left to interact freely. At the first sign of aggression, the intruder was protected against new direct attacks by a small plastic wire-mesh cage within the resident's home cage, but continued to be subjected to olfactory, visual and limited vibrissae contact with residents. After 1 h, the intruder was put back into its home cage. To prevent habituation, intruders were exposed to different residents every day. The experiment was conducted daily at a similar time to increase the effects of stress by threat expectation in intruders. In parallel, controls were placed in an empty cage every day for 1 h and tested using the same protocol, except exposure to residents.

2.5. Brain sample collection and tissue processing

Animals were sacrificed 30 min after the last injection. In brief, all mice were deeply anesthetized by intraperitoneal injection of 2,2,2-tribromo-ethanol (Sigma-Aldrich, Hamburg, Germany) and transcardially perfused with cool 0.1% saline phosphate buffer (PBS). Mouse brains were surgically removed and the prefrontal cortex (PFC), the dorsal striatum (dorsal CPu), the hippocampus (HIPP) and the cerebellum (CB) were freshly isolated and frozen in liquid nitrogen. Total RNA was purified from 128 samples (control and stress; Veh, WIN, Rim + WIN and Rim; PFC, dorsal CPu, HIPP, CB; n = 4 animals/group) using the TRIzol protocol (Invitrogen, NY, USA). The RNA was digested with RNase-Free DNase (Qiagen, Düsseldorf, Germany) and checked for integrity using capillary gel electrophoresis (Bioanalyzer, Agilent Technologies, Santa Clara, USA).

2.6. Focused gene signature profiling

The prognostic 35-gene signature was conducted in the digital transcript counting (nCounter) assay (NanoString). The total RNA (200–400 ng) was assayed according to the manufacturer's instructions. Data were normalized by scaling with the geometric mean of built-in control gene probes after log transformation (base 2) for each sample. Candidate genes were selected according to the following criteria: genes involved in neurotransmission, psychiatric disorders and myelin-related processes. The NanoStringnCounter™ code set was assigned in the following manner: galactosylceramidase (*Galc*; NM_008079.3), 2′,3′-Cyclic nucleotide 3′-phosphodiesterase (*Cnp*; NM_001146318.1), myelin basic protein (*Mbp*; NM_001025251.2), myelin-associated glycoprotein (*Mag*; NM_010758.2), myelin oligodendrocyte glycoprotein (*Mog*; NM_010814.2), oligodendrocyte transcription factor 1 (*Olig1*; NM_016968.4), oligodendrocyte transcription factor 2 (*Olig2*; NM_016967.2), zinc finger protein 488 (*Zfp488*; NM_001013777.2), SRY-box 10 (*Sox10*; NM_011437.1), discoidin domain receptor 1 (*Ddr1*; NM_001198831.1), ryanodine receptor 3 (*Ryr3*; NM_177652.2), special AT-rich sequence-binding protein-2 (*Satb2*; NM_139146.2), ski proto-oncogene (*Ski*; NM_011385.2), cannabinoid receptor 1 (*Cnr1*; NM_007726.3), cannabinoid receptor 2 (*Cnr2*; NM_009924.3), *N*-acyl phosphatidylethanolamine-specific phospholipase D (*Nape-pld*; NM_178728.5), diacylglycerol lipase (*Dagl-α*; NM_198114.2), dopamine receptor D1 (*Drd1*; NM_010076.3), dopamine receptor D2 (*Drd2*; NM_010077.2), dopamine receptor D3 (*Drd3*; NM_007877.1), dopamine receptor D4 (*Drd4*; NM_007878.2), dopamine receptor D5 (*Drd5*; NM_013503.2), neuregulin 1 (*Nrg1*; NM_178591.2), reticulon 4 receptor (*Rtn4r*; NM_022982.2), cub and sushi multiple domains 1 (*Csmd1*; NM_053171.2), dystrobrevin binding protein 1 (*Dtnbp1*; NM_025772.4), apolipoprotein E (*ApoE*; NM_009696.3), calreticulin (*Calr*; NM_007591.3), C-X-C motif chemokine 12 (*CxCl12-Y*; NM_001012477.2), retinoid X receptor alpha (*Rxra*; NM_011305.3), nerve growth factor inducible (*Vgf*; NM_001039385.1), zinc finger protein GLI1 (*Gli1*; NM_010296.2), low density lipoprotein receptor (*Ldlr*; NM_001252658.1), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*; NM_008084.2) and actin beta (*β-actin*; NM_007393.3). The mean value of the expression levels of both *Gapdh* and *β-actin* was used as standard control. Further, Venn-diagram analyses were used to examine the different and overlapping gene sets among the experimental groups (Figure S1).

2.7. Statistical analysis

Gene expression analysis was conducted, as a two-tailed *t*-test on the log-transformed normalized data that assumed unequal variance, using nSolver™ analysis software version 4.0. The distribution of the t-statistic was calculated using the Welch-Satterthwaite equation for the degrees of freedom in the estimation of the 95% confidence limits for observed differential expression between the two groups. The significance threshold was set at $p \leq 0.05$. In all figures and text, data are represented as mean ± SEM.

3. Results

The results from this study can be summarized in three main categories: 1) Effects of stress; 2) Effects of drugs; 3) Effects of stress under drug influence.

3.1. Effects of stress

Long-term exposure to repeated stress increased *Drd2* expression in the PFC ($p = 0.021$) and decreased levels of *Nrg1* in both PFC ($p = 0.008$) and CB ($p = 0.043$) (Table 1).

3.2. Effects of drugs

In the PFC, treatment with Rim + WIN enhanced the expression of *Cnr1* ($p = 0.041$), *Galc* ($p = 0.014$), *Dagl-α* ($p = 0.041$) and *Csmd1* ($p = 0.05$), but decreased the expression of *Mog* ($p = 0.05$) (Table 2, PFC). In the dorsal CPu, the cannabinoid agonist WIN increased the expression of *Vgf* ($p = 0.024$) and reduced *Drd2* ($p = 0.047$) in relation to the vehicle (Table 2, dorsal CPu). Accordingly, in comparison to WIN-treated animals, acute injection of the inverse agonist Rim resulted in an elevation in the expressions of *Drd1* ($p = 0.022$), *Drd2* ($p = 0.027$) and *Ryr3* ($p = 0.044$), while the amount of *Satb2* ($p = 0.025$) decreased (Table 2, dorsal CPu). Moreover, in comparison to Rim + WIN-treated animals, the administration of Rim alone reduced the levels of *Drd3* ($p < 0.001$) and *GalC* ($p = 0.032$) (Table 2, dorsal CPu). Further, co-administration of Rim + WIN resulted in higher expression levels of *Vgf* when compared to vehicle ($p = 0.015$), as well as higher expression levels of *Calr* ($p = 0.05$), *Drd1* ($p = 0.05$) and *Drd2* ($p = 0.023$), while levels of *Rxra* decreased in comparison to WIN-treated animals ($p = 0.019$) (Table 2, dorsal CPu). In the HIPP, animals exposed to either WIN, Rim or Rim + WIN underwent a significant reduction of *Ski* transcripts ($p = 0.045$, $p = 0.008$, $p = 0.036$; respectively) (Table 2, HIPP). Similarly, animals subjected to

Table 1
Differentially regulated genes in response to long-term psychosocial stress exposure. Long-term exposure to repeated stress increased the expression of *Drd2* in the PFC ($p = 0.021$) and decreased the levels of *Nrg1* in both PFC ($p = 0.008$) and CB ($p = 0.043$). Significance values were set to $p \leq 0.05$. *Drd2*, dopamine receptor D2; *Nrg1*, neuregulin 1. N = 4 mice/subgroup.

Effects of long-term exposure to psychosocial stress				
PFC	Gene name	t-value	Sig.	FC
	Drd2	2.47	0.021	2.18
	Ngr1	−2.87	0.008	−1.21
CB				
	Ngr1	−2.19	0.043	−2.06

Table 2
Differentially expressed genes after acute cannabinoid drug administration. In the dorsal CPu, the cannabinoid agonist WIN increased the expression of *Vgf* (p = 0.024) and reduced *Drd2* (p = 0.047) in relation to the vehicle. Accordingly, in comparison to WIN-treated animals, acute injection of the inverse agonist Rim resulted in an elevation in *Drd2* (p = 0.027). In the HIPP, mice exposed to either WIN, Rim or Rim + WIN underwent a significant reduction of *Ski* transcripts (p = 0.045, p = 0.008, p = 0.036; respectively). The remaining comparisons are depicted in the table. Significance values were set to p ≤ 0.05. *Cnr1*, cannabinoid receptor 1; *Mog*, myelin oligodendrocyte glycoprotein; *Galc*, galactosylceramidase; *Dagl-α*, diacylglycerol lipase; *Csmd1*, cub and sushi multiple domains 1; *Vgf*, nerve growth factor inducible; *Drd2*, dopamine receptor D2; *Drd1*, dopamine receptor D1; *Rxra*, retinoid X receptor alpha; *Ski*, skiproto-oncogene; *ApoE*, apolipoprotein E; *Ryr3*, ryanodine receptor 3; *Olig1*, oligodendrocyte transcription factor 1; *Sox10*, SRY-box 10; *Calr*, calreticulin. N = 4 mice/subgroup.

Effects of drug administration					
PFC					
	Comparison	Gene name	t-value	Sig.	FC
	Rim + WIN vs Veh	*Cnr1*	2.24	0.041	1.2
		Galc	2.96	0.014	1.43
		Dagl-α	2.24	0.041	1.2
		Csmd1	2.06	0.05	1.11
		Mog	−2.17	0.05	−1.48
Dorsal CPu					
	WIN vs Veh	*Vgf*	2.54	0.024	1.18
		Drd2	−2.21	0.047	−1.49
	Rim + WIN vs Veh	*Vgf*	2.84	0.015	1.25
	Rim vs WIN	*Drd1*	3.69	0.022	1.79
		Drd2	3.66	0.027	1.43
		Ryr3	2.67	0.044	1.18
		Satb2	−2.51	0.025	−1.52
	Rim vs Rim + WIN	*Drd3*	−4.64	< 0.001	−1.81
		GalC	−3.02	0.032	−2.5
	Rim + WIN vs WIN	*Calr*	2.14	0.05	1.37
		Drd1	2.09	0.05	1.52
		Drd2	2.56	0.023	1.8
		Rxra	−2.74	0.019	−1.74
HIPP					
	WIN vs Veh	*Ski*	−2.20	0.045	−1.3
	Rim vs Veh	*Ski*	−2.87	0.008	−1.21
	Rim + WIN vs Veh	*Ski*	−2.53	0.036	−1.34
	Rim vs WIN	*Vgf*	3.54	0.003	1.25
		ApoE	−2.77	0.015	−1.28
	Rim vs Rim + WIN	*Ryr3*	−3.63	0.004	−1.27
		Ski	−2.37	0.049	−1.52
	Rim + WIN vs WIN	*Ski*	−2.95	0.012	−1.81
CB					
	Rim + WIN vs Veh	*Olig1*	2.85	0.012	1.25
		Sox10	2.68	0.024	1.5
		Calr	3.17	0.009	1.44
		Drd2	2.93	0.012	2.15
		Rxra	4.21	0.001	1.24
		Vgf	3.17	0.009	1.44
		Dagl-α	−2.71	0.017	−1.12

Rimonabant had a higher expression of *Vgf* (p = 0.003) and a lower expression of *ApoE* (p = 0.015) than WIN-treated animals (Table 2, HIPP). mRNA levels for *Ryr3* and *Ski* were lower in response to an acute injection with Rimonabant than Rim + WIN-treated animals (p = 0.004, p = 0.049; respectively) (Table 2, HIPP). Further, we reported lower expression of *Ski* following Rim + WIN administration in comparison to WIN-treated mice (p = 0.012) (Table 2, HIPP). The pharmacological manipulation of CB_1 receptor by administration of Rim + WIN led to elevated levels of *Olig1* (p = 0.012), *Sox10* (p = 0.024), *Calr* (p = 0.009), *Drd2* (p = 0.012), *Rxra* (p = 0.001), *Vgf* (p = 0.009) and lower *Dagl-α* (p = 0.017) mRNA levels than in the vehicle group (Table 2, CB).

Table 3
Repeated stress following acute challenge with cannabinoid drugs interfere with gene expression. The activation of CB_1 through the cannabinoid WIN under the influence of repeated stress increased the levels of *Calr* as compared to their matched controls in the dorsal CPu (p = 0.033) and the HIPP (p = 0.031). The administration of Rimonabant in stressed mice resulted in lower expression of *CxCl12-Y* in the PFC, the dorsal CPu and the HIPP when compared to defeat mice subjected to vehicle (p = 0.024, p < 0.001, p = 0.005, respectively). Upon repeated stress, administration with Rim + WIN in the PFC and the HIPP reduced the expression of *ApoE* in comparison to WIN-treated animals (p = 0.044 and p = 0.034, respectively). Daily defeat mice that received an acute injection of WIN displayed higher levels of *ApoE* than the vehicle in both PFC (p = 0.007) and HIPP (p = 0.029). The remaining comparisons are depicted in the table. Significance values were set to p ≤ 0.05. *Cnp*, 2′,3′-Cyclic nucleotide 3′-phosphodiesterase; *Mbp*, myelin basic protein; *Mag*, myelin-associated glycoprotein; *Mog*, myelin oligodendrocyte glycoprotein; *Olig1*, oligodendrocyte transcription factor 1; *Olig2*, oligodendrocyte transcription factor 2; *Zfp488*, zinc finger protein 488; *Sox10*, SRY-box 10; *Ddr1*, discoidin domain receptor 1; *Ryr3*, ryanodine receptor 3; *Satb2*, special AT-rich sequence-binding protein-2; *Ski*, ski proto-oncogene; *Cnr1*, cannabinoid receptor 1; *Nape-pld*, *N*-acyl phosphatidylethanolamine-specific phospholipase D; *Dagl-α*, diacylglycerol lipase; *Drd1*, dopamine receptor D1; *Drd2*, dopamine receptor D2; *Drd3*, dopamine receptor D3; *Nrg1*, neuregulin 1; *Csmd1*, cub and sushi multiple domains 1; *Dtnbp1*, dystrobrevin binding protein 1; *ApoE*, apolipoprotein E; *Calr*, calreticulin; *CxCl12-Y*, C-X-C motif chemokine 12; *Rxra*, retinoid X receptor alpha; *Gli1*, zinc finger protein GLI1. N = 4 mice/subgroup.

Effects of drug administration under stress influence					
HIPP					
	Comparison	Gene name	t-value	Sig.	FC
	WIN (STS vs CTR)	*Olig1*	3.46	0.013	1.43
		Olig2	3.91	0.009	1.48
		Calr	2.79	0.031	1.28
		Nrg1	2.63	0.039	1.62
	Rim + WIN (STS vs CTR)	*Csmd1*	2.59	0.049	1.17
	WIN vs Veh (STS)	*Olig1*	3.82	0.012	1.69
		Olig2	4.12	0.012	1.78
		Zfp488	3.03	0.041	1.62
		ApoE	3.62	0.029	1.28
		Rxra	2.74	0.038	1.27
		Cnr1	−2.91	0.045	−1.57
	Rim vs Veh (STS)	*CxCl12-Y*	−4.96	0.005	−1.23
	Rim + WIN vs Veh (STS)	*Dtnbp1*	2.91	0.027	7.72
	Rim vs WIN (STS)	*Olig1*	−2.68	0.039	−1.36
	Rim + WIN vs WIN (STS)	*Cnr1*	2.68	0.038	1.36
		Sox10	−3.04	0.023	−8.61
		ApoE	−2.73	0.034	−1.29
CB					
	WIN (STS vs CTR)	*Olig2*	3.89	0.009	1.52
		CxCl12-Y	−4.37	0.015	−1.29
		Ngr1	−2.83	0.031	−1.01
		Gli1	−2.75	0.047	−1.24
	Rim + WIN (STS vs CTR)	*Dagl-α*	3.49	0.009	1.12
		Calr	3.11	0.029	1.54
		Ddr1	−2.61	0.033	−1.75
	WIN vs Veh (STS)	*Cnp*	3.17	0.026	1.2
		Olig1	2.65	0.044	1.52
	Rim + WIN vs Veh (STS)	*Calr*	3.51	0.015	1.69
		Ddr1	−2.73	0.034	−1.11
	Rim + WIN vs WIN (STS)	*Ski*	4.01	0.006	1.65
		Cnp	−2.74	0.047	−1.19

Effects of drug administration under stress influence					
HIPP					
	Comparison	Gene name	t-value	Sig.	FC
	WIN (STS vs CTR)	*Olig1*	3.46	0.013	1.43
		Olig2	3.91	0.009	1.48
		Calr	2.79	0.031	1.28
		Nrg1	2.63	0.039	1.62
	Rim + WIN (STS vs CTR)	*Csmd1*	2.59	0.049	1.17

(*continued on next page*)

Table 3 (*continued*)

Effects of drug administration under stress influence					
HIPP					
	Comparison	Gene name	t-value	Sig.	FC
	WIN vs Veh (STS)	*Olig1*	3.82	0.012	1.69
		Olig2	4.12	0.012	1.78
		Zfp488	3.03	0.041	1.62
		ApoE	3.62	0.029	1.28
		Rxra	2.74	0.038	1.27
		Cnr1	−2.91	0.045	−1.57
	Rim vs Veh (STS)	*CxCl12-Y*	−4.96	0.005	−1.23
	Rim + WIN vs Veh (STS)	*Dtnbp1*	2.91	0.027	7.72
	Rim vs WIN (STS)	*Olig1*	−2.68	0.039	−1.36
	Rim + WIN vs WIN (STS)	*Cnr1*	2.68	0.038	1.36
		Sox10	−3.04	0.023	−8.61
		ApoE	−2.73	0.034	−1.29
CB					
	WIN (STS vs CTR)	*Olig2*	3.89	0.009	1.52
		CxCl12-Y	−4.37	0.015	−1.29
		Ngr1	−2.83	0.031	−1.01
		Gli1	−2.75	0.047	−1.24
	Rim + WIN (STS vs CTR)	*Dagl-α*	3.49	0.009	1.12
		Calr	3.11	0.029	1.54
		Ddr1	−2.61	0.033	−1.75
	WIN vs Veh (STS)	*Cnp*	3.17	0.026	1.2
		Olig1	2.65	0.044	1.52
	Rim + WIN vs Veh (STS)	*Calr*	3.51	0.015	1.69
		Ddr1	−2.73	0.034	−1.11
	Rim + WIN vs WIN (STS)	*Ski*	4.01	0.006	1.65
		Cnp	−2.74	0.047	−1.19

3.3. Modulation of stress effects by cannabinoid drugs

In the PFC of stressed animals, administration of WIN lowered the expression of *Mog* as compared to non-stressed controls (p = 0.008) (Table 3, PFC). Social defeat mice treated with the drug WIN showed higher mRNA levels of *ApoE* (p = 0.007), while levels of *Mag* (p = 0.01), *Mbp* (p = 0.003), *Mog* (p = 0.007), *Cnp* (p = 0.017), *Olig1* (p = 0.019), *Sox10* (p = 0.023), *Drd1* (p = 0.032) and *Drd2* (p = 0.042) were lower than in social defeat mice treated twice with vehicle (Table 3, PFC). Moreover, upon Rimonabant exposure, stressed mice displayed a greater amount of *ApoE* than their matched controls (p = 0.043) (Table 3, PFC). The administration of the inverse agonist Rim in stressed mice led to a less prominent increase in *CxCl12-Y* expression than in long-term stressed mice subjected to vehicle (p = 0.024) (Table 3, PFC). Animals subjected to daily social defeat and treated with Rimonabant showed an over-expression of *Mag* (p = 0.002), *Mbp* (p = 0.007), *Cnp* (p = 0.011), *Mog* (p = 0.012), *Sox10* (p = 0.014) and *Olig1* (p = 0.034) when compared to WIN-treated stressed animals (Table 3, PFC). When Rim + WIN were administered to defeat animals, an increase in *Mag* (p = 0.026), *Mbp* (p = 0.022) and *Mog* (p = 0.044) was measured in comparison to their control counterparts (Table 3, PFC). The co-administration of Rim + WIN in stressed mice resulted in higher levels of *Mag* (p = 0.003), *Mbp* (p = 0.010), *Mog* (p = 0.022) and *Cnp* (p = 0.027) and were accompanied by a lower expression of *ApoE* (p = 0.044) than defeat mice treated solely with the drug WIN (Table 3, PFC).

In comparison to controls, use of the cannabinoid agonist under the influence of stress increased the striatal levels of *Calr* (p = 0.033) (Table 3, DS). In contrast, in relation to unstressed controls, the blockage of the CB_1 receptor induced an elevation in the expression of *Satb2* (p = 0.05), while it lowered the mRNA concentration of *CxCl12-Y* (p = 0.001), *RyR3* (p = 0.011), *Drd1* (p = 0.034) and *Drd3* (p = 0.05) (Table 3, DS). In comparison to vehicle, the inverse agonist attenuated the expression of both *CxCl12-Y* (p < 0.001) and *Rxra* (p = 0.05) in social defeat mice (Table 3, DS). In long-term stressed mice, an acute challenge with Rimonabant induced higher transcription levels of *Dtnbp1* (p = 0.049) and lower mRNA levels of *CxCl12-Y* (p = 0.001) and *RyR3* (p = 0.031) than in social defeat mice treated with WIN (Table 3, DS). Upon acute administration of Rimonabant, defeat animals exhibited an upregulation of *Mag* (p = 0.006), *Ryr3* (p = 0.006), *Cnp* (p = 0.008), *Sox10* (p = 0.009) and *Mbp* (p = 0.011) in contrast to stressed mice treated with Rim + WIN (Table 3, DS). In addition, chronically stressed mice treated with Rim + WIN showed an elevated *Drd3* (p = 0.040) and a lower expression of both *Dagl-α* (p = 0.032) and *Nape-pld* (p = 0.034) than their non-stressed counterparts (Table 3, DS).

In response to repeated stress, there was higher expression of *Olig1* (p = 0.013), *Olig2* (p = 0.009), *Calr* (p = 0.031) and *Nrg1* (p = 0.039) following the activation of CB_1 receptor in the HIPP (Table 3, HIPP). Chronically stressed mice treated with WIN showed higher levels of *Olig1* (p = 0.012), *Olig2* (p = 0.012), *ApoE* (p = 0.029), *Zfp488* (p = 0.041) and *Rxra* (p = 0.038), while the expression of *Cnr1* decreased (p = 0.045) in comparison to defeat mice subjected to vehicle (Table 3, HIPP). In comparison to vehicle, the inverse agonist decreased the expression of *CxCl12-Y* in repeatedly stressed mice (p = 0.005) and also the expression of *Olig1* in comparison to defeat mice treated with the drug WIN (p = 0.039) (Table 3, HIPP). In response to Rim + WIN exposure, an over-expression of *Csmd1* was detected in stressed mice when compared to their non-stressed counterparts (p = 0.049), as was the expression of *Dtnbp1* in contrast to defeat animals subjected to vehicle (p = 0.027) (Table 3, HIPP). The administration of Rim + WIN enhanced the expression of *Cnr1* (p = 0.038) and lowered the mRNA expression of *Sox10* (p = 0.023) and *ApoE* (p = 0.034) in comparison with stressed animals treated with WIN (Table 3, HIPP).

The CB subjected to daily psychosocial stress following single cannabinoid agonist injection showed an increase in *Olig2* (p = 0.009) as well as a lower expression of *CxCl12-Y*, *Nrg1* and *Gli1* (p = 0.015, p = 0.031, p = 0.047, respectively) when compared to their paired controls (Table 3, CB). An upregulation of *Cnp* and *Olig1* was found in stressed animals treated with WIN in contrast to those treated with vehicle under stress (p = 0.026 and p = 0.044, respectively) (Table 3, CB). Treatment with Rim + WIN combined with repeated stress increased the mRNA levels of *Dagl-α* (p = 0.009) and *Calr* (p = 0.029) and lowered the expression of *Ddr1* (p = 0.033) in comparison to their paired controls (Table 3, CB). Further, the co-administration of Rim + WIN under stress resulted in a higher expression of *Calr* (p = 0.015) and *Ski* (p = 0.006) in comparison to vehicle and WIN-treated animals, respectively (Table 3, CB). In addition, stressed animals treated with Rim + WIN had lower levels of *Ddr1* (p = 0.034) and *Cnp* (p = 0.047) when compared to vehicle and WIN-treated mice, respectively (Table 3, CB).

4. Discussion

4.1. Long-term effects of psychosocial stress

Repeated exposure to stressors or traumatic life experiences could lead to severe health consequences. The present investigation revealed that chronic exposure to psychosocial stress increased the expression of *Drd2* in the PFC (Bagalkot et al., 2015), which might confer vulnerability to developing psychiatric disorders in humans (for a review, see Misiak et al., 2017). Stressed mice showed a lower expression of *Ngr1* in both PFC and CB, which suggests dysfunctions in synaptic formation, dendritic plasticity (Petratos et al., 2010), neuronal circuitries consolidation and learning skills (Tomas-Roig et al., 2016a; van Gaalen et al., 2012).

4.2. Effects of acute injection with synthetic cannabinoid drugs

In comparison to vehicle, we found significant effects in the PFC upon administration of Rim + WIN. Indeed, the co-administration of Rim + WIN reduced the mRNA levels of *Mog* (Tomas-Roig et al., 2016b) and, concomitantly, a higher expression of *Cnr1*, *Dagl-α*

(Laprairie et al., 2012), *GalC* (Tomas-Roig et al., 2016b) and *Csmd1* was found. The *Csmd1* gene has been related to addiction phenotypes, particularly with cannabis dependence (Selner et al., 2014); however, the precise role of the cannabinoid drugs in this context remains unknown and requires further investigation.

Further, the expression of *Vgf* peptide in the striatum was elevated following WIN treatment (Velasco et al., 2001), while this drug reduced the expression of *Drd2*; Rimonabant acted in the opposite direction (Crunelle et al., 2013). The administration of Rimonabant activated the expression of *Drd1* (Crunelle et al., 2013) and *Ryr3*, and simultaneously decreased levels of *Satb2* when compared to WIN-treated animals. In contrast to the present findings, Zhuang et al. (2005) reported that an inverse agonist for CB_1 inhibited the expression of *Ryr*-coupled calcium channels. In addition, CB_1 receptor knockout animals showed elevated levels of *Satb2*, while Rimonabant administration induced the opposite effect (Diaz-Alonso et al., 2012). It is well known that CB_1 receptors and their lipid ligands regulate dopaminergic neurotransmission (Herkenham et al., 1991). Here, we found a lower expression of *Drd3* upon a single Rimonabant exposure in contrast to the combination of Rim + WIN (Bloomfield et al., 2016). Our results reported that upon Rimonabant administration, the expression of *GalC* was lower than that in Rim + WIN-treated mice, which in turn is consistent with earlier work (Tomas-Roig et al., 2016b; Xapelli et al., 2014). Elevated levels of *Vgf* were found following Rim + WIN treatment when compared to vehicle, which is in keeping with Velasco et al. (2001), since Rimonabant partially antagonizes the effects of the drug WIN (Tomas-Roig et al., 2018). We reported that the administration of Rim + WIN resulted in a higher expression of *Calr* than that in WIN-treated animals, most likely due to the fact that the CB_1 receptor is involved in the protein assembly and maturation of calreticulin (Lim et al., 2011). Upon Rim + WIN treatment, the levels of *Drd1* and *Drd2* were higher than those in WIN-treated animals; however, these findings are not supported by earlier research (Bloomfield et al., 2016) and thus require further investigation. We hypothesize that cholesterol metabolism and oligodendrogenesis could be compromised after the administration of Rim + WIN as a result of a decrease in *Rxra* expression (Huang et al., 2011).

Gene expression analysis of the HIPP revealed a decrease in *Sky* levels when each of the drugs or both drugs were administered, which could favour an impairment of newly projecting neurons (Baranek et al., 2012). Mice subjected to Rimonabant displayed a higher expression of *Vgf* than WIN-treated animals; however, this finding differs from a previous report (Velasco et al., 2001). This discrepancy is attributable to the experimental model (*in vitro vs* in vivo), the methods or the sampling time applied. In addition, the administration of the inverse agonist reduced the expression of *ApoE* when compared to WIN-treated mice, which is in keeping with Russell et al. (2010). Further, in agreement with Zhuang et al. (2005), we found that animals treated with Rimonabant expressed lower mRNA of *Ryr3* when compared to Rim + WIN-treated animals. In particular, they reported that the use of cannabinoid agonist activates the *Ryr*-coupled calcium channels, while the administration of cannabinoid antagonist blocks their activity.

Further, exposure to Rim + WIN in the CB resulted in a higher expression of *Olig1*, *Sox10* (Xapelli et al., 2014), *Rxra* (Mukhopadhyay et al., 2010), *Vgf* (Velasco et al., 2001), *Calr* (Lim et al., 2011) and *Drd2* (Crunelle et al., 2013). This fact occurred in parallel with a decrease in *Dagl-α* (Laprairie et al., 2012).

4.3. Effects of cannabinoid drugs under the influence of repeated stress

The activation of CB_1 by use of WIN in defeat mice induced higher levels of *Calr* than their paired controls in both dorsal CPu and HIPP. In line with these results, Tomas-Roig et al. (2016a) reported an increase in this endoplasmic reticulum-chaperone after chronic psychosocial stress. Defeat mice that received an acute injection of WIN exhibited higher levels of *ApoE* than vehicle exposed to stress in the PFC and the HIPP. Despite this, it is well known that a dysregulation of *ApoE* activity is associated with changes in the endocannabinoid system (Bartelt et al., 2011; Russell et al., 2010). In contrast to vehicle, the administration of Rimonabant to defeat mice inhibited the expression of *CxCl12-Y* in the PFC, dorsal CPu and HIPP. Repeated stress diminished *Cxcl12-Y* levels (Heidt et al., 2014), as did WIN (Ghosh et al., 2006). This indicates a stress-mediated effect. Remarkably, a decrease in *CxCl12-Y* has been associated with dysfunctions in neurogenesis (Lu et al., 2002), axonal guidance and neurite outgrowth (Pujol (2005)). Defeat mice treated with Rim + WIN displayed lower expression of *ApoE* in both PFC and HIPP, which could be explained by a Rimonabant-mediated effect instead of stress (Babenko et al., 2017) or WIN-related effect (Russell et al., 2010).

When compared to their paired controls, a lower expression of *Mog* could lead to loss of myelin compaction in the PFC of stressed mice subjected to WIN. This fact might be attributable to either stress (Miyata et al., 2016) or a WIN-induced effect (Tomas-Roig et al., 2016b). *ApoE* and corticosterone levels followed an inverse relationship (Raber et al., 2000), while treatment with Rimonabant reduced the expression of this apolipoprotein (Russell et al., 2010); thus, the results that were observed following Rimonabant treatment under stress warrant further investigation. A set of myelin-related genes—including *Mag*, *Mbp*, *Mog* and *Cnp* (Tomas-Roig et al., 2016b)—and certain oligodendrocyte transcription factors such as *Olig1* (Maire et al., 2010) and *Sox10* (Hornig et al., 2013), were found to be downregulated in stressed mice upon WIN treatment when compared to vehicle under the influence of stress, thereby suggesting plausible dysfunctions in myelination (for a review, see Baumann and Pham-Dinh, 2001). Further, repeated stress reduced the cortical levels of *Drd1* and *Drd2* after WIN administration when compared to stressed mice exposed to vehicle (Crunelle et al., 2013). Defeat mice that received an acute injection with Rimonabant displayed a higher expression of *Mag, Mbp, Cnp, Mog* (Tomas-Roig et al., 2016b), *Sox10* (Hornig et al., 2013) and *Olig1* (Maire et al., 2010) than those treated with WIN alone, which could go along with defects in cortico-cortical myelination (for a review, see Baumann and Pham-Dinh, 2001). Exposure to Rim + WIN after chronic stress resulted in greater expression of *Mag*, *Mbp* and *Mog* in comparison to either their paired controls or to stressed mice treated with WIN; thus, this indicates plausible changes in myelin architecture mediated by Rimonabant instead of stress or the drug WIN (Tomas-Roig et al., 2016b).

The pharmacological blockage of CB_1 receptor in the striatum of stressed mice enhanced the expression of *Satb2* when compared to their non-stress counterparts (Diaz-Alonso et al., 2012), an alteration that might confer vulnerability for psychiatric disorders (Jaitner et al., 2016). The application of Rimonabant under stress conditions induced lower expression of *Drd1*, *Drd3* (Howes et al., 2017) and *Ryr3* when compared to their paired controls. However, the effects observed on *Ryr3* expression went in the opposite direction of what Liu et al. (2012) reported. This could be a result of the experimental methods used, a circadian-mediated effect or that of the target brain structure analysed (HIPP vs dorsal CPu). Further, repeated stress following exposure to Rimonabant reduced mRNA levels for *Rxra* when compared to vehicle subjected to the same stress protocol (Mukhopadhyay et al., 2010). Moreover, treatment with the inverse agonist increased the expression of *Dtnbp1* in comparison to WIN-treated animals under stress, consequently causing a risk to synaptic homeostasis and plasticity (Fu et al., 2015). Daily psychosocial stress following Rimonabant administration reduced the expression of *Ryr*-coupled calcium channels when compared to single treatment with WIN (Zhuang et al., 2005). An increase in the set of myelin-related genes encoding for *Mag*, *Cnp*, *Mbp* (Tomas-Roig et al., 2016b) and *Sox10* protein (Hornig et al., 2013) was found following Rimonabant treatment in defeat mice when compared to stressed mice subjected to Rim + WIN. This fact probably indicates an impairment in myelination and oligodendrocyte differentiation (for a review, see Baumann and Pham-Dinh, 2001). Exposure to the inverse

agonist in stressed mice activated the expression of *Ryr3* when compared to defeat mice treated with Rim + WIN as a result of stress rather than the use of cannabinoids (Zhuang et al., 2005). The use of Rimonabant in defeat mice led to a lower expression of *CxCl12-Y* when compared to either their paired controls (Heidt et al., 2014) or stressed mice treated with WIN (Ghosh et al., 2006). The administration of Rim + WIN under repeated stress reduced the levels of *Dagl-α* and *Nape-pld* (Laprairie et al., 2012) and, concomitantly, an upregulation of *Drd3* (Crunelle et al., 2013; Howes et al., 2017) was measured in comparison to their paired non-stress mice.

The administration of WIN increased the expression of *Olig1*, *Olig2* and *Nrg1* in the HIPP of defeat animals as compared to their non-stressed counterparts; this might, in turn, be attributable to a stress-induced effect (Brydges (2016); Chetty et al., 2014). The differentiation of oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes in the HIPP is coordinated by a complex mechanism involving distinct transcription factors, such as *Olig1*, *Olig2* and *Zfp488* (Maire et al., 2010). In particular, we found all of them to be highly expressed in stressed mice following WIN treatment as compared to vehicle animals (Xapelli et al., 2014). The data presented herein reveal elevated levels of *Rxra* in stressed mice treated with WIN as compared to vehicle animals under stress. We speculate that this might indicate alterations in oligodendrocyte proliferation (Huang et al., 2011). Lower mRNA levels of *Cnr1* were identified in stressed mice following WIN treatment as compared to those subjected to vehicle (Tomas-Roig et al., 2016a). Further, in stressed mice, an acute challenge with the inverse agonist diminished the expression of *Olig1* as compared to WIN-treated mice, and this fact might support dysfunctions in oligodendrocyte proliferation (Maire et al., 2010). Defeat mice exposed to Rim + WIN showed higher mRNA levels of *Csmd1* (Selner et al., 2014) and *Dtnbp1* when compared to their control counterparts and to the vehicle group, respectively. However, the underlying mechanisms mediated by the cannabinoid drugs on *Dtnbp1* expression remain unclear and warrant further investigation. The simultaneous use of both cannabinoids under stress was followed by higher levels of *Cnr1* in relation to stressed WIN-treated animals (Mukhopadhyay et al., 2010). Oligodendrocyte differentiation might be impaired, as indicated by a decrease in *Sox10* expression, when stressed mice were subjected to Rim + WIN versus Rimonabant-treated stressed mice (Hornig et al., 2013).

Further, gene expression analysis of the CB revealed an over-expression of *Olig2* in defeat mice treated with WIN as compared to their paired controls (Chetty et al., 2014). The action of WIN under stress lowered the expression of *CxCl12-Y* (Heidt et al., 2014) and *Nrg1* in comparison to their non-stressed counterparts. These differences in *Nrg1* expression are in contrast to a previous report (Dang et al., 2016), which presumably might be related to the species studied (rats vs mice), the method applied, the sampling time or the target brain structure analysed (PFC vs CB). The *Gli1* levels were lower in defeat mice upon WIN treatment, as compared to their non-stress counterparts (Patel et al., 2016), which might indicate dysfunctions in oligodendrocyte differentiation (Zahreddine et al., 2014). We speculate that the differentiation of oligodendrocytes as well as the myelination are compromised in stressed mice treated with WIN due to the fact that both *Cnp* and *Olig1* were found to be upregulated in comparison to vehicle-treated stressed animals (Kasama-Yoshida et al., 1997; Maire et al., 2010). In addition, co-administration of Rim + WIN increased the levels of *Calr* (Lim et al., 2011), while the expression of *Ddr1* diminished when compared to vehicle and their paired controls. A reduction in *Ddr1* expression could be interpreted as an impairment in myelination. Indeed, an over-expression of *Ddr1* has been reported in myelinating oligodendrocytes (Franco-Pons et al., 2006) as well as during oligodendrocyte proliferation (Franco-Pons et al., 2009). The expression of *Dagl-α* increased in defeat animals treated with Rim + WIN-treated defeat when compared to Rim + WIN-treated non-stressed mice (Laprairie et al., 2012). Moreover, the exposure to Rim + WIN in the CB of stressed mice resulted in a higher expression of *Ski* in contrast to the WIN-treated group exposed to stress; thus, this might favour the projection of newly formed cerebellar neurons (Baranek et al., 2012). An acute challenge with Rim + WIN under stress led to a decrease in *Cnp* mRNA levels in relation to defeat mice treated with the agonist, thereby alluding to deficits in myelin integrity (Kasama-Yoshida et al., 1997).

5. Conclusions

In summary, we found that exposure to environmental factors such as psychosocial stress and an acute challenge with synthetic cannabinoid drugs both interfered with distinct biological processes in the mouse brain. Specifically, repeated stress increased levels of *Drd2* in the PFC, while the expression of *Nrg1* diminished concomitantly in both PFC and CB. The pharmacological manipulation of CB_1 receptor differentially affected the expression of *Drd2* in the dorsal CPu. Further, the administration of either WIN55212.2 or Rimonabant in social defeat mice resulted in lower expression of myelin-related genes, and the use of the inverse agonist had a deleterious effect on the expression of dopamine receptors. Based on the transcriptomic data reported here, further research that includes electrophysiology and liquid chromatography-mass spectrometry (LC–MS) analysis must be conducted in order to characterize the contribution of the dopaminergic system in this model.

Conflict of interests

The authors report no potential conflict of interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.11.023.

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