Research report

Interaction of cannabinoid receptor 2 and social environment modulates chronic alcohol consumption

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HIGHLIGHTS

- CB2 receptors modulate alcohol consumption and reward.
- CB2 knockout mice consumed more alcohol under group-housing conditions.
- CB2 knockout mice showed increased food consumption under group-housing conditions.
- Social environment critically affects CB2 signalling, especially in alcohol intake.

ABSTRACT

Genetic and environmental factors contribute nearly in equal power to the development of alcoholism. Environmental factors, such as negative life events or emotionally disruptive conditions, initiate and promote alcohol drinking and relapse. The endocannabinoid system is involved in hedonic control and modulates stress reactivity. Furthermore, chronic alcohol drinking alters endocannabinoid signalling, which in turn influences the stress reactivity. Recently, it has been shown that CB2 receptor activity influences stress sensitivity and alcohol drinking. We hypothesized that CB2 receptors influence the impact of environmental risk factors on alcohol preference and consumption. Therefore, in this study, we investigated the alcohol-drinking pattern of wild-type and CB2-deficient animals under single- and group-housing conditions using different alcohol-drinking models, such as forced drinking, intermittent forced drinking and two-bottle choice paradigms. Our data showed that CB2 receptor modulates alcohol consumption and reward. Interestingly, we detected that lack of CB2 receptors led to increased alcohol drinking in the intermittent forced drinking paradigm under group-housing conditions. Furthermore, we found that CB2 knockout mice consumed more food and that their body weight gain was modulated by social environment.

On the basis of these data, we conclude that social environment critically affects the modulatory function of CB2 receptors, especially in alcohol intake. These findings suggest that a treatment strategy targeting CB2 receptors may have a beneficial effect on pathological drinking, particularly in situations of social stress and discomfort.

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1. Introduction

There is a substantial risk to inherit alcohol addiction, although environmental factors contribute nearly in equal power to the development of alcoholism [1]. Genetic (G) and environmental (E) influences do not contribute separately to alcoholism, but are considered as a result of G × E interactions [2]. The age of first alcohol use, for example, is largely determined by social environment, whereas the individual response to alcohol is genetically predisposed [3,4]. External factors that may favour the development of addiction are manifold. One of the most important environmental risk factors is stress as any form of negative life events or emotionally disruptive conditions may initiate or promote alcohol drinking and relapse [5]. Moreover, the social environment is critically involved in the development of addiction because family, peers and society determine the level of exposure to social stress as well as to alcohol [6]. Also in rodents, the social environment has a deep impact on animals' behaviour and modulates alcohol addiction [7–9]. A large number of studies show that chronic social isolation as a stress factor enhances alcohol

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consumption in mice, rats and non-human primates [7,10]. Social isolation affects not only ethanol consumption, but also food intake and body weight gain in humans and rodents [11,12]. Beside social stress, another important stress factor is alcohol withdrawal, which leads to the emergence of negative emotional state and finally contributes to relapse [13].

The endocannabinoid system is a modulatory system that alters neural transmission as well as immune function. It consists of at least two G-protein-coupled receptors (cannabinoid receptors (CB) 1 and 2), their endogenous ligands (2-arachidonylethanolamide (2-AG) and anandamide (AEA)), their synthesis and degradation enzymes [14]. CB1 receptors are primarily expressed on neurons, whereas CB2 receptors expressed on immune cells [15,16]. It was thought that the expression of CB2 receptors is very low in the brain, being restricted to glial elements. However, recent publications suggested that neurons also express CB2 receptors in brain areas relevant to addiction and stress [15,17–22]. Zhang et al. [23] recently reported CB2 receptor expression on dopaminergic neurons in the ventral tegmental area that functionally modulates the neuronal activity and dopamine-related behaviours such as hedonic effect of drugs of abuse. Interestingly, latest studies implicated the role of CB2 receptors in models for alcohol, nicotine and cocaine addiction [24–28]. Moreover, a polymorphism in the CB2 receptor gene locus has been associated with alcoholism in a Japanese population [26]. These data suggest that CB2 receptors play an important role in alcohol addiction.

Chronic ethanol exposure induces neuroadaptations in the endocannabinoid system. These alcohol effects are highly dependent on duration, dose and route of alcohol administration as well as on voluntary versus forced alcohol administration [29]. Self-administration of alcohol dose-dependently elevates 2-AG levels without affecting the level of AEA in the nucleus accumbens [30,31].

Pharmacological manipulation of CB2 receptors in the brain modulates stress-related reactions that include neuroinflammatory processes [22]. Ishiguro et al. [26] have also reported the involvement of CB2 receptors in stress modulation. They showed that sub-chronic treatment with CB2 receptor agonist (JWH-015) increased alcohol consumption only after chronic mild stress in BALB/c mice, whereas they observed no effect in unstressed mice. Studies, using specific inhibitors of endocannabinoid metabolizing enzymes (URB597 and JZL184), revealed that potentiation of the AEA and/or 2-AG levels reduces anxiety in mice [32]. These studies suggest that both CB1 and CB2 receptors play an important role in the modulation of anxiety processes. In line with this, Garcia-Gutierrez and Manzanares [17] reported that CB2 receptor overexpressing mice presented reduced anxiety-like behaviour and modified reaction to stress, also suggesting an involvement of CB2 receptors in the regulation of stress responses.

We hypothesized that alcohol exerts reduced reinforcing effect in CB2 knockout animals, particularly when they are exposed to different sorts of stresses at the same time, such as social stress and alcohol withdrawal. The focus of the present study was to investigate the effect of single- and group-housing conditions on alcohol and food consumption as well as on body weight gain in WT and CB2-deficient animals. We also analyzed the role of CB2 receptors in different chronic alcohol-drinking models using forced drinking, intermittent forced drinking with repeated withdrawals and two-bottle choice.

2. Material and methods

2.1. Animals

Male mice (2 months old at the start of the experiments) carrying two truncated alleles of CNR2 (CB2) and wild-type littermates (WT) on a C57BL/6j background (backcrossed for more than eight generations) were used in this study [33]. Animals were kept in a reversed light/dark cycle (light off between 7:00 AM and 7:00 PM) and received food and water ad libitum. The housing conditions were maintained at 21 ± 1°C and 55 ± 10% relative humidity, and animals were housed in single cages or in groups of two to three animals per cage. Experimental procedures complied with all regulations for animal experimentation in Germany and were approved by Landesamt für Natur, Umwelt und Verbraucherschutz in Nordrhein-Westfalen, Germany (84–02.04.2013.A183).

2.2. Experimental setup and groups

In this study, we compared, on the one hand, different housing conditions. On the other hand, we analyzed the effect of different chronic alcohol-drinking models: forced drinking (FD) [34], intermittent forced drinking (IFD) [35,36] and two-bottle choice [34]. We measured alcohol consumption and food intake as well as body weight gain. Water-drinking animals were used as controls; these mice underwent the same handling procedures except for the administration of alcohol. In total, this gives rise to the following experimental groups: 1. WT-forced drinking (FD)-single housed (n = 12), 2. WT-FD-group housed (n = 30), 3. CB2-FD-single housed (n = 10), 4. CB2-FD-group housed (n = 30), 5. WT-intermittent forced drinking (IFD)–single housed (n = 12), 6. WT-IFD-group housed (n = 19), 7. CB2-IFD-single housed (n = 9) and 8. CB2-IFD-group housed (n = 14). The following are water-drinking control groups: WT-single housed (n = 22), WT-group housed (n = 30), CB2-single housed (n = 18) and CB2-group housed (n = 28). Moreover, using different groups of animals, we analyzed WT-two-bottle choice-single housed (n = 14) and CB2-two-bottle choice-single housed (n = 12). Due to the large number of experimental groups, we decided to use only male animals.

2.3. Chronic alcohol models

For chronic alcohol consumption, three drinking paradigms were applied: FD as a model for chronic voluntary alcohol consumption [34], IFD as a model for binge-like alcohol consumption and two-bottle choice as a model for alcohol preference [34,35]. In the FD procedure, animals were supplied with a 16% ethanol solution as the only water source as described previously [34,35]. To familiarize animals with the taste of alcohol, a 4% alcohol solution was given at the beginning of the drinking period for 3 days. Then the alcohol concentration was raised to 8% for a further 4 days. After 1 week, animals got a 16% alcohol solution for the following 5 months. The IFD model was a modification of the FD paradigm sharing similarity (duration of alcohol access) with chronic intermittent ethanol (CIE) exposure protocols [36,37]. We decided to use this paradigm because we aimed at comparing the alcohol self-administration between a chronic continuous and a chronic limited access model. In our setup, 16% alcohol was provided in drinking bottles as only source of liquid and only for 4 days per week. The ethanol consumption was determined twice a week in the FD model and at the end of every drinking session in the IFD model and was calculated as g/kg body weight/day. In the case of group-housing alcohol, food consumption was determined per cage and divided by the number of animals following the procedure described by Lopez et al. [37]. In both models, body weight (g) and food consumption (g) were measured weekly.

Ethanol preference was determined in separate groups of mice using the two-bottle choice paradigm as described previously [34,35]. The drinking paradigm was started with 8–10-week-old animals. In this paradigm, two drinking bottles with 8% v/v alcohol (EtOH) or drinking water were available for the animals ad libitum. In order to avoid the development of side preference, the bottle
positions were changed daily. The consumption of liquid and food and body weight were measured twice a week, and the intake of alcohol was quantified as g/kg body weight per day. The preference is calculated in percent as the ratio of consumed alcohol to total liquid consumption.

2.4. Determination of blood alcohol level

An aliquot of 100 μl of blood was collected from the orbital sinus under short isoflurane anaesthesia, and 10 μl of 0.21 mmol K-EDTA was added to prevent clotting. The blood samples were centrifuged at 4000 rpm for 20 min at 4 ºC. Supernatant was collected and frozen immediately and stored at −20 ºC until use. Plasma alcohol levels were determined using the NAD-NADH Reagent (Sigma). The assay was performed according to the manufacturer’s description. Blood alcohol levels (BALs) were calculated in g/l and indicated as mean ± SEM.

2.5. Statistical data analysis

All statistical analyses were carried out using STATISTICA software package. We evaluated separately the effect of housing conditions and genotype on alcohol consumption and body weight by repeated-measures one-way analysis of variance (ANOVA) (main factor: housing condition or genotype). To determine the effect of interaction between housing conditions, drinking models and genotype on alcohol and food consumption or body weight, we used repeated-measures three-way ANOVA (main factors: genotype, housing condition and drinking model, within effect: time). In the two-bottle choice paradigm, the effect of genotype on alcohol preference was analyzed by repeated-measures one-way ANOVA (main factor: genotype). The ANOVA was considered to be significant at 95% confidence interval; the analyses were followed by a Fisher’s LSD post hoc test.

3. Results

3.1. Effect of housing condition on alcohol consumption

In the FD model, single-housed WT animals drank around 12 g/kg alcohol, approximately 2 g/kg more than their group-housed conspecifics \( F_{(1,39)} = 20.41, p < 0.001 \) (Fig. 1A). This effect was similar in CB2-deficient mice \( F_{(1,36)} = 19.92, p < 0.001 \) (Fig. 1B). Correspondingly, in the IFD model, single-housed WT animals drank more alcohol than did group-housed mice \( F_{(1,29)} = 22.52, p < 0.001 \) (Fig. 1C). Interestingly, the housing conditions did not
3.2. Effect of genotype on alcohol consumption

Surprisingly, group-housed CB2 animals showed increased alcohol consumption in the IFD model compared with WT controls [F(1,21) = 21.60, p < 0.001] (Fig. 1A and B). However, group-housed wild-type and CB2 mice drank similar amounts of alcohol in the FD model [F(1,58) = 0.55, p = 0.46]. Analysis of genotype effect in single-housed animals revealed no difference in FD [F(1,17) = 0.00, p = 0.98] and IFD [F(1,19) = 1.69, p = 0.20] (Fig. 1A and B). The blood alcohol levels were not significantly different between the genotypes (FD single WT: 1.34 ± 0.45, CB2: 1.53 ± 0.33; FD group WT: 0.82 ± 0.15, CB2: 0.71 ± 0.15; IFD single WT: 0.42 ± 0.06, CB2: 0.60 ± 0.21; IFD group WT: 0.71 ± 0.12, CB2: 0.38 ± 0.07). However, as blood was not sampled at the same point of time, it was not possible to compare the BAL of the different alcohol models.

3.3. Effect of housing condition on body weight

In the FD model, single-housed animals showed reduced body weight compared with group-housed mice in both genotypes [WT: F(1,28) = 4.40, p = 0.04; CB2: F(1,41) = 4.49, p = 0.04] (Fig. 1A and B). The body weight was not significantly affected by the housing conditions in the IFD alcohol treatment in none of the genotypes [WT: F(1,29) = 0.15, p = 0.69; CB2: F(1,21) = 0.98, p = 0.33] (Fig. 1C and D). In water-drinking WT animals, housing conditions did not affect the body weight gain [F(1,68) = 0.72, p = 0.40] (Fig. 2E). Interestingly, CB2 animals showed increased body weight when they were group-housed [F(1,62) = 11.14, p = 0.001] (Fig. 2F).

3.4. Effect of genotype on body weight

Deletion of CB2 led to increased body weight compared with WT littermates in group-housed mice in the FD model [F(1,58) = 5.69, p = 0.02] and in the water-drinking group [F(1,93) = 10.31, p = 0.001] (Fig. 2). However, in the IFD model, the body weight gain was not affected by the genotype [F(1,31) = 0.62, p = 0.43] (Fig. 2C and D). On the contrary, single-housed WT and CB2 animals showed similar body weights [FD: F(1,21) = 2.28, p = 0.14; IFD: F(1,19) = 0.00, p = 0.99; H2O: F(1,37) = 0.09, p = 0.75] (Fig. 2).

3.5. Effect of housing conditions on food consumption

As food consumption of the animals was constant during the entire experiments, we calculated mean values for each group (Fig. 3). In general, group-housed animals consumed significantly less food compared with single-housed mice [F(1,262) = 13.08, p = 0.0004]. In WT animals, single-housing led to a significantly elevated food consumption in the FD model from 3.19 to 3.35 g [F(1,39) = 4.08, p = 0.05]. This effect was enhanced in the IFD model (from 3.41 to 3.81 g) [F(1,289) = 10.11, p = 0.003]. Group-housed CB2 animals showed a similar food consumption compared with single-housed mice in both alcohol-drinking paradigms [FD: F(1,41) = 2.44, p = 0.12; IFD: F(1,21) = 3.09, p = 0.09]. In water-drinking mice, food intake was not affected by housing conditions in both genotypes [WT: F(1,60) = 2.24, p = 0.13; CB2: F(1,63) = 1.36, p = 0.24].

3.6. Effect of genotype on food consumption

We found that CB2 mice ate generally more food compared with WT animals [F(1,262) = 7.97, p = 0.005] (Fig. 3). However, single-group comparisons only revealed a significant difference between group-housed CB2 and WT in the IFD model [F(1,21) = 4.2, p = 0.04].

3.7. Effect of gene x environment interaction on alcohol and food consumption as well as body weight

For the statistical analysis of G x E interactions in alcohol and food consumption as well as body weight, we used a repeated-measures three-way ANOVA. The main factors were the following: genotype, drinking model and housing conditions. Due to the complexity of the study, this section only describes results of statistical interactions and does not show the direction of any change, as this has already been addressed in the previous paragraphs. The alcohol intake was significantly affected by the model [F(4,921) = 3.921, p = 0.049] and the housing conditions [F(1,21) = 30.97, p = 0.000], but not by the genotype [F(1,21) = 0.13, p = 0.71] (Table 1). WT and CB2 knockout animals showed a significantly altered alcohol consumption, which was dependent on the housing conditions [F(1,21) = 3.842, p = 0.05] and on the drinking paradigm, together with the social environment [F(1,131) = 1.149, p = 0.001] (Table 1).

The body weight was influenced by all main factors examined [G; F(1,260) = 5.75, p = 0.01; M; F(1,260) = 9.85, p < 0.001; H; F(1,260) = 14.07, p < 0.001] (Table 1). Moreover, the changes were not affected by interactions among genotypes, housing conditions and models. Food intake was influenced by the genotype [F(1,260) = 4.32, p < 0.05], drinking model [F(1,260) = 123.17, p < 0.0001] and housing conditions [F(1,260) = 34.59, p < 0.0001]. Moreover, there was an interaction between drinking model and housing conditions for food consumption [F(1,260) = 4.37, p < 0.05].

Alcohol consumption was significantly affected by two main factors: alcohol-drinking model and housing conditions. Furthermore, we observed significant interactions for gene x housing and gene x model x housing. The body weight and food intake were significantly affected by all main factors analyzed. Moreover, we observed a significant interaction effect on food intake for model x housing. Data were analyzed by repeated-measures three-way ANOVA (main factors: genotype, model and housing condition, within effect: time) and represented as p-value.

3.8. Alcohol preference

In the two-bottle choice test, WT animals showed a constant preference for alcohol around 60% during the entire drinking period (Fig. 4). As the preference values of the individual animals showed a high standard deviation, we analyzed the data using a non-parametric Kolmogorov–Smirnov analysis. We compared the preferences between the first and the last 2 weeks (Fig. 4A and B). In the beginning, alcohol preference of WT and CB2 knockout animals was similar. However, after 10 weeks of the two-bottle choice paradigm, the alcohol preference was significantly reduced in CB2 mice compared with WT control animals (p < 0.05) (Fig. 4B). Moreover, CB2 knockout animals showed a significant reduction in preference over time from 60% to 36% [F(1,120) = 2.95, p = 0.01] (Fig. 4C). However, alcohol consumption was not significantly different between the genotypes [F(1,24) = 1.72, p = 0.2] (Fig. 4D).

4. Discussion

The endocannabinoid system has been implicated in the modulation of neuronal function, stress reactivity and immune responses [38]. Latest studies showed that CB2 receptors modulate not only...
Fig. 2. Body weight of single- and group-housed animals in the FD (A and B), IFD (C and D) and water-drinking mice (E and F). (A and B) Single-housed WT and CB2 mice showed decreased body weight in the forced drinking model. (C and D) In the intermittent forced drinking model, both WT and CB2 animals showed the same body weight, independent of the housing conditions. (E) Similarly, the housing conditions did not alter the body weight in water control WT animals. (F) Interestingly, water-treated, group-housed CB2 animals showed a significant increase in body weight compared with single-housed animals (n: WT FD single = 12, group = 30; WT IFD single = 12, group = 19; WT H2O single = 22, group = 30; CB2 FD single = 10, group = 30; CB2 IFD single = 9, group = 14; CB2 H2O single = 18, group = 28). Data were analyzed by repeated-measures one-way ANOVA (main factor: housing condition, within effect: time) and represented as mean ± SEM. *p < 0.05 and **p < 0.01.

We found that single-housed WT animals showed increased alcohol intake in the FD and IFD models compared with group-housed animals. These results are consistent with the literature as group housing reduced alcohol intake in WT animals [10], thereby further supporting the impact of social environment on the immune responses; but are also involved in the development of drug abuse and stress reactivity [22,24,26,27]. In this study, we investigated the effect of different housing conditions on alcohol and food consumption as well as body weight gain using wild-type and CB2 knockout animals.
alcohol-drinking behaviour. A possible explanation for this is that single housing, particularly in adolescent age, functions as a stress factor that promotes alcohol drinking [37]. Moreover, our data suggest that CB2 receptor signalling plays an important role in the reactivity to different environmental stress factors. Unexpectedly, group-housed CB2 animals increased their alcohol consumption to the level of single-housed animals after repeated alcohol withdrawal. This result is surprising because group housing should reduce alcohol consumption. Our finding suggests that repeated withdrawal-induced stress cannot be relieved by the enrichment of social environment in CB2 animals. Withdrawal from chronic alcohol is known to activate the amygdalar stress response system [13], which is modulated by the endocannabinoid system [39]. Furthermore, Serrano et al. [39] showed that repeated alcohol

![Table 1](http://dx.doi.org/10.1016/j.bbr.2015.03.051)

<table>
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**Interactions**

| G × H | 0.05 | 0.128 | 0.724 |
| G × M | 0.22 | 0.611 | 0.076 |
| M × H | 0.25 | 0.515 | 0.013 |
| G × M × H | 0.001 | 0.476 | 0.368 |

**Preference ratio**

![Fig. 3](http://dx.doi.org/10.1016/j.bbr.2015.03.051)

Fig. 3. Mean food consumption of single- and group-housed animals in the FD, IFD models and water-drinking mice. The food consumption was significantly increased in single-housed WT animals in the FD (●) and IFD (●●) models compared with group-housed mice. Single housing did not affect the food intake in water-treated WT animals. Single-housed CB2 mice ate similar amounts of food in all models compared with group-housed animals. Alcohol treatment in the FD model resulted in reduced food consumption in both genotypes and housing conditions compared with the IFD model and water controls as indicated by asterisks (*). Furthermore, group-housed WT animals in the IFD model ate significantly less compared with water controls (+) and CB2 knockout animals (●) under the same housing condition and model (n: WT FD single = 12, group = 30; WT IFD single = 12, group = 15; WT H2O single = 22, group = 30; CB2 FD single = 10, group = 30; CB2 IFD single = 9, group = 14; CB2 H2O single = 18, group = 28). Data were analyzed by three-way ANOVA (main factors: housing condition, genotype and model) and represented as mean ± SEM. *; #; + p < 0.05; **; &●& < 0.01; ***p < 0.001.

![Fig. 4](http://dx.doi.org/10.1016/j.bbr.2015.03.051)

Fig. 4. Alcohol preference and consumption in the two-bottle choice test. (A) In the first 2 weeks of the experiment, the preference to an 8% ethanol solution was similar in both genotypes, whereas (B) in the end (11th and 12th week), the alcohol preference was significantly reduced in CB2 animals (WT n = 14 and CB2 n = 12). Data were analyzed by the non-parametric Kolmogorov–Smirnov test and represented as mean ± SD *p < 0.05. (C) Alcohol preference in WT animals remained constant, whereas CB2-deficient animals showed reduced alcohol preference over time. (D) The alcohol consumption was significantly reduced over time in CB2-deficient animals (WT n = 14 and CB2 n = 12). Data were analyzed by repeated-measures one-way ANOVA (main factor: genotype, within effect: time) and represented as mean ± SEM. **p < 0.01 compares to the first time point.

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withdrawal led to a downregulation of mRNAs of genes associated with the endocannabinoid system including CB1 and CB2 receptors and MAGL in the amygdala. This suggests a downregulated CB1 and CB2 receptor signalling, probably as a compensation for the excess 2-AG activity during repeated alcohol withdrawal. This implies that CB2 knockout animals may have a disrupted stress response system in the amygdala and consequently disturbed stress reactivity. Thus, CB2 receptors play an important role in the modulation of stress responses that involve multiple factors, such as social environment and repeated alcohol withdrawal.

Interestingly, in 2007, Ishiguro et al. [26] have already considered an involvement of the CB2 receptor in gene × environment interactions. They reported that sub-chronic treatment with the CB2 receptor agonist (JWH-015) increased alcohol consumption only after chronic mild stress using BALB/c mice; whereas it had no effect in unstressed mice. Moreover, they showed that CB2 antagonist (AM630) did not alter the alcohol consumption in chronically stressed or unstressed mice. These findings are contradictory to our results and may be attributed to the use of different mouse strains (BALB/c versus C57BL/6j) and stressors (chronic mild stress versus housing and alcohol withdrawal). In the same study, Ishiguro et al. [26] found a functional single nucleotide polymorphism (SNP) in the CB2 receptor associated with alcoholism in a Japanese cohort. This SNP in the CNR2 gene locus, R63Q, leads to a missense mutation in the first intracellular domain, which results in a decreased cellular response to CB2 receptor ligands [26]. Thus, our results from animals lacking CB2 receptors support the findings from the association study and may have translational value. In line with this, a recent study investigated a novel natural CB2 receptor agonist, beta-caryophyllene (BCP), in alcohol-related behaviours [24]. BCP-treated animals showed consistently low preference for alcohol, whereas vehicle-treated mice showed high preference for alcohol.

In this experiment, animals were daily intraperitoneally injected with BCP or vehicle. These daily injections can also be considered as a repeated stress factor, which is specifically known to promote alcohol consumption [40]. Thus, the effect of BCP may be related to its stress-relieving action as latest results revealed an anxiolytic- and antidepressant-like effect of this compound [41]. In line with this, mice overexpressing CB2 receptors showed reduced hormonal and behavioural stress reactivity [17]. Altogether, these findings suggest that CB2 receptors play an important role in stress reactivity that is associated with alcohol-related behaviours. Due to the increased susceptibility to environmental stress factors, we suppose that CB2 receptors are rather involved in the initiation of drug use and that pharmacological targeting of this receptor might prevent the development of alcohol dependence. However, further investigations are needed to prove this hypothesis in more detail.

In our study, we used the FD and IFD as models for regular, but moderate drinking as BALs were between 0.6 and 1 g/l, respectively. Compared with other chronic models in which alcohol is administered via vapour chambers or intragastric catheters in our study, mice drank voluntarily alcohol [36,37,43]. Although alcohol is the only drinking source, animals show individual differences in the alcohol consumption depending on the mouse line or on the species used for the experiments (rats or mice) [34,44]. By the introduction of factors such as genotype, housing conditions and repeated phases of withdrawal to the FD model, it is possible to analyze the effect of different environmental factors on alcohol drinking.

Acute and chronic alcohol exposure impairs the innate and adaptive immune responses in the periphery and in the brain [45] and increases the release of inflammatory mediators in the brain [46,47]. Activation of CB2 receptors is generally considered to be anti-inflammatory [48], and lack of CB2 signalling should therefore result in an exacerbated immune response. Furthermore, it has been recently published that CB2 deficiency by itself leads to a different activation state of microglia in the brain [49]. We therefore investigated the development of alcohol-induced neuroinflammation in WT and CB2-deficient animals. As expected, lack of CB2 receptors led to an altered immune response, which was dependent on the environmental conditions. Interestingly, we did not detect correlation between the expression of inflammatory cytokines and alcohol consumption in the models employed for this study (unpublished data).

We also detected a difference between genotypes in the alcohol preference test. Single-housed CB2 animals showed a reduced preference for alcohol, which became significant over time. These data indicate that alcohol has no rewarding effect in CB2 animals. We did not detect any difference in BAL levels between the genotypes. This suggests that the alteration in the alcohol preference was not likely due to difference in alcohol metabolism of wild-type and CB2 animals. In contrast to our findings, Ortega-Alvaro et al. [27] found an increase in alcohol preference in the two-bottle choice test using CB2 knockout animals. It is important to mention that these mice were on an outbred (CD1) background, which is known for its alcohol avoidance [50]. Mice used in our study were on a C57BL/6j inbred background that is known for its high alcohol preference [50]. Here, they showed a preference around 60%, which is consistent with earlier findings [35,51]. Thus, the contradictory results between these two reports may be a consequence of background differences, but also indicate that the CB2 receptor may be a bidirectional modulator of alcohol reward.

Next, we detected increased body weight gain in group-housed WT animals with continuous alcohol access compared with single-housed WT mice. This finding is supported by the literature as several works revealed that social housing conditions modulate weight gain and food consumption in mice [12,52,53]. Interestingly, CB2 mice in the FD model were more sensitive to the social environment as group housing led to a pronounced increase in body weight. Furthermore, water-drinking CB2 mice gained more weight compared with WT animals under conditions of group housing, which was accompanied with a slightly increased food intake. These data are in line with previously published studies as we already reported increased body weight and food intake in single-housed female CB2-deficient mice [54]. In line with this, CB2 overexpressing mice appeared to be leaner and also displayed reduced food intake [55]. Furthermore, Agudo et al. [56] showed that only old male CB2-deficient animals displayed increased body weight, which was associated with enhanced food intake. Our data are also consistent with a report by Oanaei et al. [20], in which CB2 receptor antagonist has been shown to increase food intake in fasted single-housed mice. On the contrary, the same study reported a reduction in food intake after CB2 antagonist (AM630) application when animals had food ad libitum. However, this effect was also highly dependent on the mouse line used for the experiment. In the light of the present data, it is possible that the social environment affects the regulatory role of CB2 receptors in body weight gain and food consumption and that CB2 signalling may play an important role in reactivity on social environment or in handling situations with multiple stress factors.

Independent of the genotype, the food consumption was negatively correlated with the amount of alcohol ingested. This effect was the most pronounced in the FD model in which animals drank the largest amount of alcohol and consumed the least food. The relationship between alcohol and food consumption has been little addressed in preclinical studies, but widely investigated in alcoholic patients. A detailed review analysing this interaction was based on a Medline database search for the period from 1984 to 2010. Thirty-one studies were included and selected, depending on relevance and quality of design [57]. Interestingly, they found positive, negative and no correlation between alcohol consumption and weight gain. However, this effect was highly dependent on the drinking pattern of the patients (heavy and light-to-moderate...
drinks) and the type of alcoholic beverages consumed (beer, wine and spirits). Analogous to human studies, our results suggest that the effect of alcohol on food consumption may depend on the genetic background of the mouse line and also on the alcoholic strength of ethanol solutions.

On the basis of these data, we conclude that the social environment critically affects the modulatory function of CB2 receptors. CB2 receptor signalling impacts alcohol drinking and weight gain, depending on social housing conditions. These findings suggest that a treatment targeting CB2 receptors may have a beneficial effect on pathological drinking, particularly in situations of social stress and discomfort, and may prevent the development of addiction.

Author contribution
L.R. and B.P. were responsible for the study concept and drafted the manuscript. E.E. and A.M. contributed to the acquisition of animal data.

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