

Carles Sanchis-Segura · Brandon H. Cline ·
Giovanni Marsicano · Beat Lutz · Rainer Spanagel

Reduced sensitivity to reward in CB1 knockout mice

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Abstract *Rationale:* Previous studies have demonstrated that the activation and blockade of the cannabinoid type 1 receptor (CB1) leads to an enhancement and decrease of the consumption of food and other orally ingested reinforcers, respectively. *Objective:* To gain further knowledge about the role of CB1 in sucrose/saccharin reinforcing efficacy and intake, we tested CB1 knockout (CB1-KO) and littermate wild-type (WT) control mice in several self-administration experimental protocols. *Methods:* Operant (fixed or progressive ratio schedule) and non-operant conditioning procedures were used. In addition, a choice analysis based on the “matching law” as well as a microstructural analysis of the intra-session pattern of self-administration was performed. *Results:* CB1-KO mice consume less sucrose under operant conditions or when using a two-bottle free choice procedure. Moreover, as revealed by additional behavioural analysis, CB1-KO mice exhibit a decreased sensitivity to the rewarding properties of sucrose. In agreement with this finding, the differences between WT and CB1-KO mice faded away when the palatability of sucrose was devaluated by adding quinine, but not when a non-caloric sweetener, saccharin, was available. *Conclusions:* These results demonstrate a modulatory role of CB1 in the determination of the rewarding properties of sucrose and probably, as suggested by previous studies, other reinforcers.

Keywords Cannabinoid receptor 1 · Sucrose · Reinforcing efficacy · Reward · Matching law

Introduction

It has been shown that the activation of the endogenous cannabinoid system leads to an increase of ingestive behaviour in humans and in animal models. Thus, in addition to the well-known association between marijuana consumption and hyperphagia (reviewed in Cota et al. 2003a; Marsicano et al. 2003) several animal studies have established that the administration of exogenous (Koch 2001) or endogenous (Williams and Kirkham 1999) cannabinoids results in an enhanced food intake. Moreover, these effects, as well as food intake in cannabinoid-naive animals, can be reduced by the administration of CB1 receptor antagonists (Arnone et al. 1997; Kirkham and Williams 2001). Taken together, it seems clear that the endogenous cannabinoid system, and more specifically the activation of CB1, are involved in the promotion of ingestive behaviours (reviewed in Cota et al. 2003a; Marsicano et al. 2003).

However, the exact role of CB1 in the promotion of ingestive behaviours remains unclear. In fact, the hyperphagic/anorectic actions of CB1 agonists/antagonists could result from changes in appetitive or consummatory processes or even both. Thus, CB1 agonists energize ingestive behaviour, advancing the onset of eating (Williams and Kirkham 2002) and increasing the motivation of rats to obtain reinforcing fluids when their availability is limited by progressive ratio (PR) schedules (Gallate et al. 1999). These data suggest that CB1 activation can facilitate the appetitive processes of ingestive behaviour such as incentive motivation. Alternatively, CB1 activation could be more related to consummatory processes such as palatability (orosensory rewarding properties). Thus, it has been proposed that CB1 antagonists produce a higher reduction in the ingestion of palatable foods or reinforcing fluids such as sucrose or alcoholic solutions (Arnone et al. 1997; Freedland et al. 2001; Perio et al.

C. Sanchis-Segura · B. H. Cline · R. Spanagel (✉)
Department of Psychopharmacology, Central Institute for
Mental Health, CIMH, University of Heidelberg,
68159 Mannheim, Germany
e-mail: spanagel@zi-mannheim.de
Tel.: +49-621-1703833
Fax: +49-621-1703837

C. Sanchis-Segura
Area de Psicobiologia, Universitat Jaume I,
Castello, Spain

G. Marsicano · B. Lutz
Molecular Genetics of Behaviour, Max Planck Institute of
Psychiatry,
Munich, Germany

2001; Higgs et al. 2003) rather than chow or tap water. The fact that the CB1 antagonist SR141716 reduces sucrose consumption by increasing the length of the post-reinforcement pauses has also been interpreted as a consequence of the reduction in the perceived palatability after a CB1 blockade (Perio et al. 2001).

The present study was designed to identify a possible role of CB1 in the determination of the rewarding properties of sucrose. For this purpose, we have evaluated sucrose consumption over a wide range of experimental conditions in CB1 knockout (CB1-KO) and wild-type (WT) littermate mice. (i) Sucrose self-administration under a fixed ratio (FR1) schedule following water deprivation as well as under non-deprived conditions was assessed. (ii) We used two different ratio schedules (FR1 versus progressive ratio; PR). (iii) We characterised the micro-structural pattern of self-administration by analysing the temporal distribution of the operant response over time. This procedure specifically assesses the contribution of palatability to ingestive behaviour (Higgs et al. 2003). (iv) We also assessed the consumption of sucrose at several concentrations in a two-bottle free choice experimental protocol, but in this case the traditional analysis based on total consumption and preference has been replaced by a detailed quantitative choice evaluation described by the “matching law” (Herrnstein 1970). This procedure studies the relationship between an organism’s choices among reinforcers available according to the magnitude of the reinforcers, and has been used in the study of choice for drug and non-drug reinforcers (Mazur 1991; Anderson and Woolverton 2000; Martinetti et al. 2000). The observed relationship between reinforcement and behaviour was quantified in the so-called “generalised matching equation” from which the individual sensitivity to reward and the bias for one or the other alternatives can be, respectively, estimated (Anderson and Woolverton 2000; Martinetti et al. 2000). To our knowledge, this is the first study using this procedure to unravel differences in sensitivity to reward in transgenic animals. (v) Finally, to identify which aspects of sucrose reinforcing efficacy are modulated by CB1, we included two additional experiments. In the first one, we assessed how the devaluation of sucrose palatability by adding quinine modifies its consumption. In the second one, we assessed saccharin (a non-caloric sweetener) voluntary consumption. The comparison of the results of these two experiments provides information about a possible role of CB1 mediating palatability, caloric value or both.

Materials and methods

Animals

Eighteen homozygote, nine CB1-KO and nine WT, male mice were generated and genotyped as described in Marsicano et al. (2002). Animals were bred in our colony at the Max Planck Institute of Psychiatry.

Following their arrival at the CIMH facilities in Mannheim, mice were singly housed at 21°C and 50% relative humidity, with water

and food ad libitum, except otherwise noted. Lights were programmed on a 12 h light/dark cycle with lights on at 7 a.m. The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body and performed following the German Law on the Protection of Animals.

One of the CB1-KO mice died in the course of the experiments; for this reason, the data depicted in Figs 3, 4, 5 refer to nine WT and eight CB1-KO mice. At the end of the experiments, mouse genotype was verified using tail-tip DNA via PCR.

Apparatus

The operant self-administration experiments were performed by using eight commercially available skinner boxes (53×37×70 cm; TSE; Bad-Homburg, Germany) controlled by the OBS software (TSE). Each box had two ultra-sensitive levers (required force ≤1 g), one of each designed as “active” and the other as “inactive”. When the programmed ratio requirements were met on the active lever, 10 µl of the sucrose solution was delivered into a micro-reservoir. The delivery was accomplished within 1 s and a yellow light located above the micro-reservoir was turned on during the delivery time.

Behavioural procedure

One week after their arrival in the colony room, mice were subjected to three magazine-training sessions which consisted of a 30-min exposure to the operant conditioning cages used in the following self-administration sessions. These sessions were introduced to familiarize the mice with the experimental settings used in the following experiments. During this training phase, each lever press resulted in the delivery of 10 µl of tap water (fixed ratio 1, FR1).

In the following 3 weeks, animals were 22 h/day water deprived and exposed to five weekly sessions (Monday to Friday) identical to those described in the habituation phase, except that pressing the lever (FR1) then resulted in the delivery of 10 µl of a 5% (w/v) sucrose solution. Each day, 2–4 h after the end of the sucrose self-administration session, mice had access in their home cage to a bottle containing tap water for 1.5 h. Over a 3-week period, animals performed in total 15 self-administration sessions. Free access to water was immediately resumed after the 15th session.

Over the following 3 weeks, mice completed a total number of 15 sessions identical to those described above. Sessions were also daily completed from Monday to Friday and the only difference was that animals had ad libitum availability to water in their home cages. After the last of these sessions, mice completed an additional session but in this case the ratio requirement was progressively increased (progressive ratio schedule; PR) by a step of one ($R_i = 1 + R_{i-1}$). The session lasted for 30 min and breakpoint was defined as the highest accomplished rate to obtain a single reinforcer.

From that day on, animals remained in their home cages and no additional manipulations were introduced till 2 weeks later. Then for five consecutive sessions (Monday to Friday), 1 h per day (11:00–12:00 a.m.), the normal water bottles were replaced by others, as described in Spanagel et al. (2002), containing either tap water or a 5% (w/v) sucrose solution. The position of each bottle was randomised. This phase is considered as necessary training, so that the animals get used to the restricted availability of sucrose in the drinking bottles. In the following 8 weeks, a two-bottle choice test adapted from that used of Martinetti et al. (2000) was used. Again, five test sessions were conducted per week. Thus, all animals received a series of 15 choice conditions, each lasting for 2 or 3 days, 1 h per day. During these choice conditions (summarised in Table 1), two solutions were concurrently available and the consumption of each one was calculated by weighing the bottles after each session using an electronic scale accurate to 0.1 g.

After the completion of this choice procedure, a wash-out period of 11 days was included. During these days animals remained in their home cages with water and food ad libitum. After the completion of this intervening phase, again five consecutive

Table 1 Summary of the experimental conditions tested for sucrose choice analysis. A total of 15 different choice situations were implemented to compare the relative preference for different sucrose concentrations in a pseudo-random order. All sessions were conducted over a total period of 8 weeks, five sessions per week. As shown in this table, each condition lasted for two or three consecutive sessions (session length: 1 h). The data derived from these measurements were used to calculate the consumption of a sucrose (0, 1, 2.5 or 5%) solution (Fig. 2) as well as to estimate the matching behaviour (Fig. 3)

Condition	Sucrose concentration (left bottle)	Sucrose concentration (right bottle)	Measurement days
1	0	2.5	3
2	2.5	0	2
3	2.5	5	3
4	5	2.5	2
5	0	0	3
6	5	0	2
7	0	5	3
8	1	0	2
9	0	1	3
10	1	2.5	2
11	2.5	1	3
12	5	1	2
13	1	5	3
14	5	5	2
15	2.5	2.5	3

(Monday to Friday; 11:00–12:00 a.m.) sessions were conducted. In all these sessions, water and a 5% (w/v) sucrose solution were available in two different bottles. The same experimental conditions were maintained for two more sessions in the following week and then, in the third session, 0.02 mM quinine was added to the 5% (w/v) sucrose solution. The consumption of this new solution was then compared to the preceding days, as described in the data analysis section.

Finally, after another wash-out period of 2 weeks, saccharin consumption was evaluated. Again, a two-bottle free choice procedure was used to test the consumption and preference of WT and CB1-KO mice for a sweet, but devoid of caloric value, solution. Thus, as in previous experiments, a total of 15 self-administration sessions were conducted over 3 weeks (five sessions per week, Monday to Friday). The duration of these sessions was 1 h and three different saccharin concentrations (0.01, 0.1 or 1.0%; w/v) were tested with water always concurrently available.

Data analysis

All analyses, except otherwise noted, were performed using the STATISTICA 4.1 software (Statsoft, Inc., 1991–1994). Correlation studies were performed by using Pearson's index and regression lines were calculated by using the least squares method. Mean differences were analysed by means of Student's *t*-test or ANOVAs, including a repeated measures factor when necessary. When a significant interaction between factors was found, post hoc analyses were conducted using the Newman–Keuls test.

In addition, the data on sucrose consumption in the two-bottle free choice experimental protocol were analysed by using the “generalised matching law equation”. This equation states that $\log B_1/B_2 = a(\log r_1/r_2) + \log c$, where B represents the relative allocation of the behavioural responses to alternatives 1 and 2, r represents the rate or relative reinforcing magnitude of the two alternatives, and a

and c are empirically obtained parameters which illustrate the individual sensitivity to reward and bias for one or the other alternatives, respectively. When applying this equation to a two-bottle free choice procedure, the consumed volume (V_X) of each bottle provides the index of the relative behavioural allocation, whereas the concentration of the available solution (C_X) represents the magnitude of the reinforcer. Thus, for each mouse, the ratio of the consumed volume from the bottle located in the left over that located in the right (V_L/V_R) as well as the concentration of the respective solutions (C_L/C_R) were calculated and then averaged for the 2 or 3 days of identical treatment conditions. When any of the terms was 0, 0.1 substituted this value. The logarithms (base 10) of these ratios were then plotted on arithmetic co-ordinates and, by using the method of the least squares, the best-fit of regression line was estimated. Wellness of fit was assessed using coefficient of determination, r^2 . From the equation, defining this regression line, the free (empirically obtained) parameters of the “generalised matching law equation” were calculated. Thus, the intercept represents the bias ($\log c$) and the slope of the line (a) defines the sensitivity to the changes in the magnitude of the reinforcer as expressed by a change in the relative consumption or the so-called “sensitivity to reward” (Anderson and Woolverton 2000; Martinetti et al. 2000).

Finally, from the data of the last FR1 self-administration session (non-deprived condition), a microstructural analysis was performed according to the procedure described by Higgs et al. (2003). In brief, cumulative responses were derived by summing consecutive reinforced lever presses into 1-min bins and cumulating over the 30-min session. Then, the function $y=a(1-e^{-bt})$ was fitted to these curves by using Sigma Plot (Jandel Corporation, 1992–1994). Results are expressed as the value of the asymptote and the exponent of the cumulative response curve. In addition, from these data the individual number of “empty bins” (1-min bins without any reinforced response), number of “bouts” and “bout size” were estimated. The “bout size” was calculated by averaging the ratio of reinforced responses per minute in one or more consecutive bins preceding/following an “empty bin”. In a second step, the different “bouts” of each subject were averaged and then differences between genotypes were compared by using a Student's *t*-test for independent samples.

Results

Operant conditioning training phase

To introduce and habituate the mice to the operant boxes and general experimental handling procedures, three training sessions were conducted in which, under an FR1 reinforcement schedule, water could be obtained. A two-way repeated measures ANOVA (genotype \times session) revealed that none of the factors, or their interaction, achieved statistical significance, showing that mice, regardless of their genotype, performed a similar number of responses across the three sessions of this experimental phase (data not shown).

Operant (FR1) sucrose self-administration and breakpoint estimation

As described in [Materials and methods](#), 30 sessions of operant (FR1) sucrose self-administration sessions were completed in two different conditions (water deprived versus non-deprived). Figure 1a,b depicts the mean \pm SEM of reinforced lever presses observed in each condition,

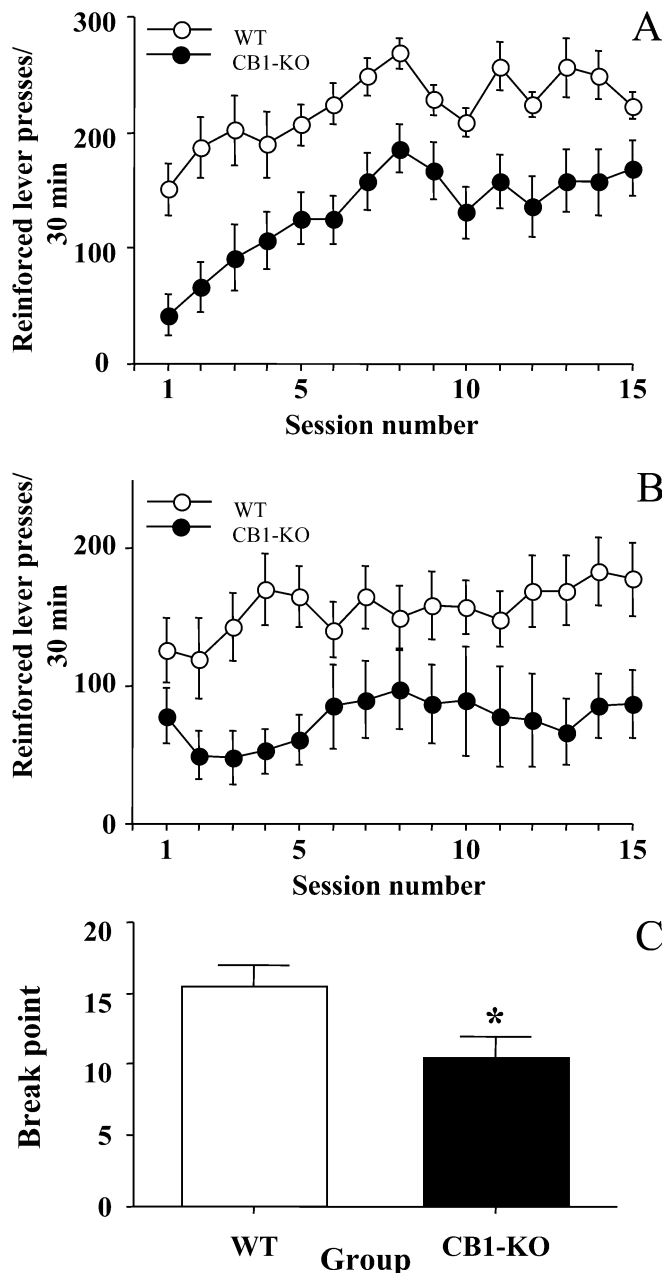


Fig. 1 Operant sucrose self-administration in WT (open circles) and CB1-KO (filled circles) mice. Data depict mean \pm SEM of sucrose (5%) reinforced lever presses per session when mice were 22/h day water deprived (A) or when they had ad libitum access to water (B). In both panels, ANOVA indicated an effect of group and days factors but not a significant interaction between them. C Mean \pm SEM of breakpoint for WT (open bar) and CB1-KO (filled bar) mice, estimated as described in Materials and methods, under a PR schedule. These data were compared by using a Student's *t*-test for independent samples (* P <0.05). In all graphs, the number of subjects was nine per group

respectively. A two-way repeated measures ANOVA (genotype \times session) revealed that, when mice were 22 h/day water deprived, both main factors, genotype [$F(1,16)=11.23$; P <0.01] as well as session number [$F(2,224)=17.08$; P <0.0001], reached statistical significance. The interaction between both factors failed to yield a signif-

icant effect, indicating that the differences between genotypes remained constant through the initial rise and posterior stabilisation of the number of reinforced lever presses observed across sessions. Interestingly, for both groups the number of responses peaked in the eighth session and the slopes of the regression lines defining this ascending (learning) phase were not different between genotypes (means were 16.02 ± 2.43 and 18.85 ± 1.68 for WT and CB1-KO mice, respectively).

A similar pattern of results was observed when animals had ad libitum water access. Thus, again both main factors, genotype [$F(1,16)=6.8$; P <0.05] and session number [$F(2,224)=17.08$; P <0.05], reached statistical significance whereas their interaction did not. Therefore, it seems that decreased operant sucrose self-administration in CB1-KO mice does not depend on water deprivation. On the contrary, as revealed by Student's *t*-test for paired samples, the percentual differences between groups in sucrose self-administration during this phase (calculated from its last five sessions) were bigger [$t(4)=4.61$; P <0.01] than those observed during the last five sessions of sucrose self-administration under water deprivation (means were $217.7\pm 10.8\%$ and $157.7\pm 5.27\%$, respectively).

Finally, an additional session of operant sucrose self-administration was conducted but in this case, instead of an FR1, a progressive ratio (step size: 1) reinforcement schedule was used. Breakpoint, defined as the maximum numbers of responses accomplished to obtain a 10 μ l drop of the sucrose solution, was calculated. The obtained results (depicted in Fig. 1c) were analysed by means of a Student's *t*-test for independent samples, which revealed that breakpoint was statistically higher in WT than in CB1-KO mice [$t(16)=2.25$; P <0.05]. More specifically, WT showed a 150% increase in their breakpoint. Thus WT mice displayed a higher number of reinforced responses across all experimental conditions (fluid deprivation or schedule of reinforcement) imposed and the differences between groups were bigger when both genotypes were tested in a primary consummatory experimental protocol (FR1, non-deprived).

Non-operant sucrose consumption and choice analysis

As described in Materials and methods, sucrose consumption in a two-bottle free choice procedure was also assessed. Figure 2 depicts the average volume of a sucrose solution (0, 1, 2.5 or 5% w/v) consumed. A two-way ANOVA (genotype \times concentration) revealed that both main factors, genotype [$F(1,61)=18.57$, P <0.0001] and sucrose concentration [$F(3,61)=32.99$, P <0.0001], as well as their interaction [$F(3,61)=2.80$, P <0.05], yielded a significant effect. Newman-Keuls post hoc comparisons revealed that WT consumed higher amounts of a 1, 2.5 or 5%, but not 0%, sucrose solution. It is important to note that, although in the case of the 0% sucrose solution one of the bottles was arbitrarily selected as the "solution bottle", this fact did not change the results. Thus, because the observed preference was 49.31 ± 1.58 and 54.02 ± 1.22 for

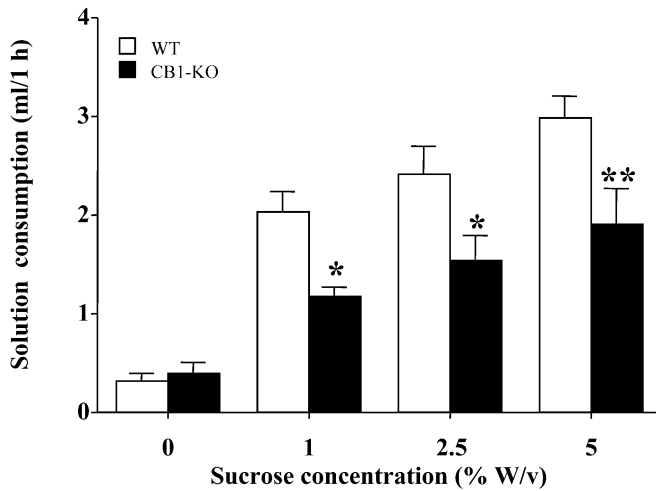


Fig. 2 WT (open bars) and CB1-KO (filled bars) sucrose consumption in a two-bottle free choice paradigm. Data depict mean±SEM ($n=9$ per group) of consumed millilitre of a sucrose solution. Tests were performed as described in Table 1 (conditions 1, 2, 5, 6, 7, 8 and 9). Sucrose consumption was assessed for two or three consecutive sessions. ANOVA yielded main effects for the group, concentration and their interaction. Post hoc comparisons were carried out using Newman–Keuls test and revealed that whereas water (0%) consumption was not different, sucrose consumption was significantly reduced in CB1-KO mice (* $P<0.05$, ** $P<0.01$)

WT and CB1-KO mice, respectively, similar results were obtained when consumption of the other bottle or total water consumption was considered (data not shown). Therefore, from those data it is apparent that CB1-KO drank less sucrose, but not water, than WT mice and that the higher the concentration of sucrose, the greater the amount drunk for both genotypes.

As described in the previous experiment, the direct proportional relationship between sucrose concentration and ingestion fulfils required *a priori* criteria to apply the choice analysis based on the “matching law”. Therefore, a detailed choice analysis was applied to the data obtained across the 15 experimental conditions shown in Table 1. Individual analysis of the preference was conducted for each animal according with the generalised matching law equation ($\log B_1/B_2 = a(\log r_1/r_2) + \log c$) described in Materials and methods. The individual regression equations and goodness of the fitting procedure (r^2) are included in Fig. 3. In a second step, the calculated individual intercepts and slopes were averaged by genotype (Fig. 3) and then compared by two independent Student’s *t*-tests. These tests show that whereas the average of the intercepts (an index of side bias) was not different [$t(15)=-0.43$, $P>0.05$], the average of the slopes (sensitivity to reward) was significantly lower in the CB1-KO than in the WT mice [$t(15)=-3.47$; $P<0.01$]. Thus, across a wide variety of experimental conditions and in a procedure whose results are independent of the volume consumed, a decreased sensitivity for the rewarding properties of sucrose was found in CB1-KO mice.

A corollary derived from this conclusion is that if genotype differences arise from differential sensitivities to

the rewarding properties of sucrose, the manipulation of those rewarding properties should change the observed group differences. To confirm this hypothesis, two additional experiments were conducted. First, after resuming 5% sucrose self-administration as described in Materials and methods, the effect of the addition of quinine (0.02 mM) was evaluated. Figure 4 depicts the mean±SEM of the volume (ml) consumed in the last day of the baseline period and the day in which quinine was added to the 5% sucrose solution. A two-way repeated measures ANOVA (genotype×session) revealed a significant effect of the genotype [$F(1,15)=7.85$, $P<0.05$] and session [$F(1,15)=47.02$, $P<0.001$] factors, as well as of their interaction [$F(1,15)=5.68$, $P<0.05$]. Newman–Keuls post hoc test revealed that as expected, WT consumed higher amounts of sucrose during the baseline ($P<0.001$). Moreover, the mean comparisons revealed that after the addition of quinine both groups reduced their solution’s intake ($P<0.05$) and the differences between groups vanished ($P>0.05$). Therefore, the results of this experiment revealed that both groups of mice were able to react to the addition of quinine, suggesting that the differences observed in sucrose consumption between WT and CB1-KO mice are not the result of a general taste impairment. Even more interestingly, the differences between groups faded away when the rewarding properties of sucrose were devaluated by quinine addition. This finding confirms that the differences between genotypes in sucrose consumption arise from differences in their sensitivity to the palatability that is the orosensory rewarding properties of sucrose.

To assess the possible role of the caloric value as a determinant of the differences between groups observed in sucrose consumption, saccharin consumption was also assessed as described in Materials and methods. A two-way repeated measures ANOVA (genotype×saccharin concentration) revealed that both main factors genotype [$F(1,15)=5.35$; $P<0.05$] and saccharin concentration [$F(2,30)=41.50$; $P<0.0001$], but not their interaction [$F(2,30)=1.39$; $P>0.05$], yielded a significant effect, showing that WT drink more saccharin than CB1-KO mice at all concentrations tested. These results are depicted in Fig. 5. Identical results were obtained when saccharin preference was considered. Thus, the genotype [$F(1,15)=5.67$; $P<0.05$] and the saccharin concentration [$F(2,30)=47.80$; $P<0.0001$] factors resulted in a significant effect, whereas their interaction did not [$F(2,30)=0.78$; $P>0.05$]. These results confirm a decreased consumption of sweet solutions in CB1-KO mice regardless of their caloric value.

To specifically assess the contribution of palatability to the differences in sucrose consumption between WT and CB1-KO mice, the microstructural (within session) temporal pattern of self-administration was analysed. For this purpose, the data of the last session of operant sucrose self-administration under an FR1 schedule were used as described in Materials and methods. The individual parameters of the fitting procedure (asymptote and exponential value) were then averaged and compared by means of two independent Student’s *t*-tests. Thus, it was observed

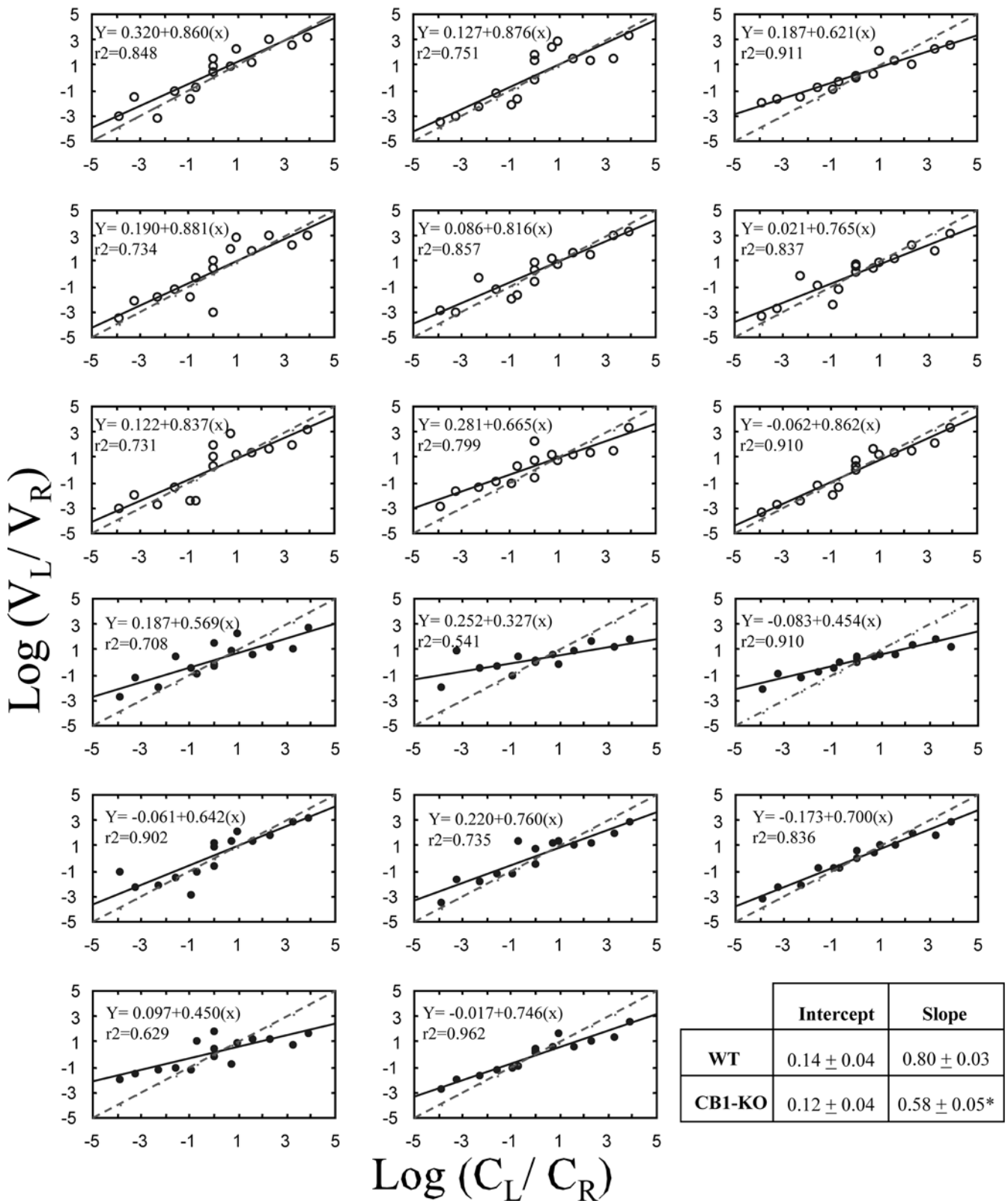


Fig. 3 Individual analysis of the sucrose choice in WT (open circles; $n=9$) and CB1-KO (filled circles; $n=8$) mice. The details of this analysis are described in Materials and methods. Individual parameters of the empirical regression lines (plain line) and wellness of fitting are included in the corresponding scattergrams. For a better

comparison, the theoretical perfect matching behaviour is shown (dashed line). In a second step, these individual values were averaged by genotype and compared by means of Student's t -test for independent samples ($*P<0.01$)

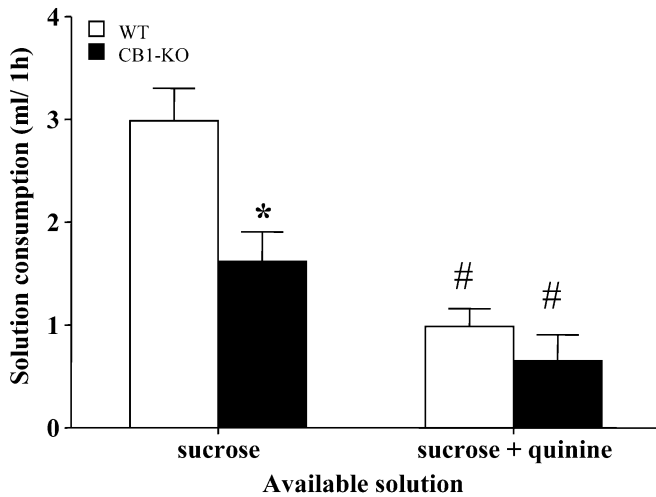


Fig. 4 Effects of the devaluation of sucrose palatability on its consumption in WT (*open bars*; $n=9$) and CB1-KO (*filled bars*; $n=8$) mice. Both groups of mice were re-introduced to self-administer sucrose (5%) in a two-bottle free choice procedure. After the completion of seven sessions, in the eighth session, 0.02 mM of quinine was added to the sucrose solution. Data depict mean \pm SEM ml consumed of the sucrose solution available in the seventh and eighth self-administration session. A two-way ANOVA revealed a significant effect for group, session and their interaction. Newman-Keuls post hoc comparisons revealed that WT and CB1-KO mice drink different amounts of a 5% sucrose solution ($*P<0.001$), but not when this solution was altered with quinine. Moreover, the same comparisons revealed that after the addition of quinine, both groups significantly ($\#P<0.01$) reduced their intake of the available solution in the eighth session

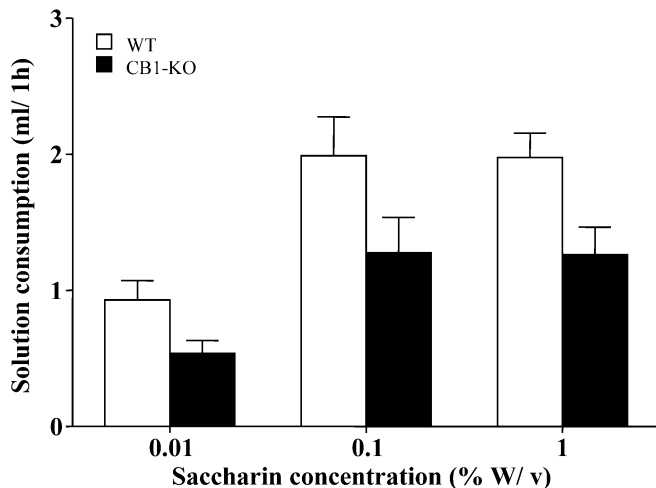


Fig. 5 WT (*open bars*; $n=9$) and CB1-KO (*filled bars*; $n=8$) saccharin consumption in a two-bottle free-choice paradigm. Data depict mean \pm SEM of consumed ml of a saccharin (0.01, 0.1 or 1%) solution, with water concurrently available. ANOVA yielded main effects for the group and concentration but not for their interaction confirming that CB1-KO mice drank less saccharin, regardless of the saccharin concentration

that although the exponential values were similar in both groups, it was found that the predicted asymptotic value was significantly lower [$t(16)=-2.29$, $P<0.05$] in CB1-KO than in WT mice. Moreover, the “bout size”, but not the number of bouts or “empty bins”, was significantly bigger

Table 2 Microstructural analysis of sucrose self-administration in WT and CB1-KO mice. Analysis of the temporal distribution of operant responding for sucrose. This table shows the average (mean \pm SEM, $n=9$ per group) of empty bins, “bout size”, and the estimated parameters of the exponential function, which defines the individual cumulative response over time of the last operant (FR1) sucrose self-administration session (session 15, Fig. 1b). As in the table inserted in Fig. 3, these parameters were first individually calculated and then averaged by genotype and compared by using a Student’s *t*-test for unpaired samples.

	WT	CB1-KO
Empty bins	5.55 \pm 1.44	7.44 \pm 1.55
Number of “bouts”	3.44 \pm 0.58	4.37 \pm 0.78
Bout size	5.43 \pm 0.53	3.48 \pm 0.35**
Asymptotic value	301.6 \pm 54.6	149.7 \pm 33.8*
Exponent value	0.033 \pm 0.007	0.025 \pm 0.005

* $P<0.05$, ** $P<0.01$

in WT than in CB1-KO mice [$t(16)=3.07$, $P<0.01$]. These results are presented in Table 2 and confirm the conclusion that decreased palatability is responsible for the reduced reinforcing efficacy of sucrose and saccharin in CB1-KO mice.

Finally, a significant correlation ($r=0.74$, $P<0.01$) between the individual breakpoint and “sensitivity to reward” values was found. This finding suggests that the individual and group variations in approximatory and consummatory behaviour are not independent (i.e. changes in incentive motivation, measured as performance in the PR schedule, could result from differences in the rewarding value of sucrose).

Discussion

The main finding of the present report is the observation that the genetic deletion of CB1 results in a reduction of the sensitivity to the rewarding properties of sucrose, and therefore profoundly affects the consummatory aspects of sucrose self-administration. This finding is in line with the previously proposed role of the endogenous cannabinoid system in the control of sucrose consumption based on pharmacological studies. Thus, it has been demonstrated that the administration of endogenous or exogenous cannabinoid agonists enhances sucrose voluntary consumption (Kirkham and Williams 2001; Koch 2001), whereas the administration of CB1 antagonists decreases sucrose consumption (Freedland et al. 2001; Perio et al. 2001; Higgs et al. 2003). Therefore, it is clear that CB1 activation promotes the consumption of sucrose and, as shown by similar studies, other reinforcers (Rodriguez de Fonseca et al. 1999; Freedland et al. 2001; Hungund et al. 2003; Racz et al. 2003).

In our first set of experiments, we have observed that, regardless of the level of fluid deprivation, CB1-KO mice consume less sucrose under operant conditions than littermate controls. This effect was observed under an FR1 as well as under a PR schedule. These data are in

agreement with previous reports showing that the number of sucrose reinforced lever presses as well as breakpoint values are increased/decreased by the administration of CB1 agonists/antagonists, respectively (Gallate et al. 1999; Freedland et al. 2001; Perio et al. 2001). Decreased operant sucrose self-administration observed in CB1-KO mice does not seem to be related to a learning deficit. Thus, when analysing the data depicted in Fig. 1a, it is apparent that WT and CB1-KO mice display a parallel initial rise in the number of reinforced lever presses until both groups reached a peak value in the eighth session. Moreover, the slopes of the regression lines defining this rising phase were not different between genotypes, suggesting that WT and CB1-KO mice learnt the operant contingencies in a similar way. This is coincident with the results of previous reports which have shown no changes in initial acquisition of memory in CB1-KO mice (Marsicano et al. 2002; Varvel and Lichtman 2002). Furthermore, our results do not seem to be explained by a motor deficit either. Thus, during the three initial training sessions, no differences between groups in the number of water reinforced lever presses were observed. This result seems coincide with previous data showing normal locomotion in CB1-KO mice (Marsicano et al. 2002; Cota et al. 2003a,b). In fact, similar differences between genotypes in sucrose, but not water, consumption were also observed in a two-bottle free choice experimental protocol that is far less sensitive to motor differences and does not require the learning of operant contingencies. In summary, it can be concluded that the observed differences between genotypes seem to be related to a differential reinforcing efficacy of sucrose rather than to learning or motor deficits.

However, reinforcing efficacy is not a homogenous construct (Bickel et al. 2000). In this respect, it has been suggested that the analysis of the microstructural temporal pattern of self-administration may specifically assess the contribution of palatability to ingestive behaviour (Higgs et al. 2003). This microstructural analysis revealed that CB1-KO mice responded fewer times (i.e. they reached a lower asymptote), but their decline in rate of responding over the course of the session was similar to WT mice. We also observed that a decrease in sucrose self-administration in CB1-KO mice arises from a reduction in the “bout size” rather than in the number of bouts, suggesting that the reduced operant sucrose consumption in CB1-KO mice results from a longer inter-response interval rather than from differences in the frequency of the self-administration episodes (“bouts”). Although we had to adapt the parameters of the microstructural analysis to our specific operant experimental set-up (i.e. lever responses), we finally obtained results identical to those reported after the administration of CB1 antagonists in a lickometer set-up (Higgs et al. 2003). In conclusion, these results can be interpreted as a reflection of a decrease of sucrose palatability rather than a consequence of post-ingestive feed-back factors such as satiety (Davis and Levine 1977; Higgs et al. 2003).

This notion is further supported when choice, instead of raw consumption, is considered. In the present study, sucrose preference of both groups were tested using a procedure based on the “matching law” (Herrnstein 1970). This behavioural law states that when two reinforcing alternatives are available at the same cost, an organism’s choice between them will be related to their relative magnitude. Therefore, the individual choice behaviour can be described by the generalised matching equation which illustrates the individual sensitivity to reward and bias for one or the other alternatives, respectively. Obviously, this law can only be applied if the reinforcer’s magnitude is within a linear relationship with its consumption, a condition satisfied in our experiment. Thus, when tested across 15 different experimental conditions, CB1-KO mice showed no difference in their intercept values, meaning that bias (side preference) was similar in both groups and that CB1-KO mice do not exhibit impaired discrimination for different sucrose concentrations. Conversely, CB1-KO showed a flatter slope than WT mice, indicating a decreased capacity to allocate their behaviour accordingly with the differential rewarding magnitude (sucrose concentration) of the two concurrently available alternatives. According to the theoretical considerations of the matching law, this fact can be understood as reflection of a reduced “sensitivity to reward” (Martinetti et al. 2000), leading to the conclusion that CB1 deletion results in a reduced “sucrose liking”.

Therefore, the conclusion so far is that genetic CB1 deletion leads to a decrease in the sensitivity to the rewarding properties of sucrose and affects consummatory aspects of sucrose self-administration. In agreement with this hypothesis, the differences between groups in sucrose consumption could be altered by manipulating the palatability of the sucrose solution. Thus, when the rewarding value of sucrose was reduced by adding quinine, the differences between groups in sucrose consumption vanished. Moreover, both WT and CB1-KO mice were able to sense taste changes and properly react to them, thus discarding general taste impairment as a possibility of their different levels of sucrose consumption. In addition, since the sucrose solution altered with quinine contains the same caloric value as the original one, the results of the present study also point to the perceived hedonic quality of sucrose (sweetness) as the major determinant of the differences between genotypes. This conclusion is additionally supported by reduced consumption of saccharin (a non-caloric sweetener) in our knockout mice and by a recent report that showed less consumption of sweet tasting fluids in another line of CB1-KO mice (Poncelet et al. 2003).

However, it can be argued that changes in appetitive behaviour (i.e. incentive motivation; Wyvell and Berridge 2001; Salamone and Correa 2002) also would result in reduced self-administration without implying any change in the rewarding properties of sucrose. This explanation, however, seems to be unlikely, since changes in incentive motivation and their behavioural expression are clearly related to the experimental requirements that determine the

availability to the reinforcer (Salamone and Correa 2002; Salamone et al. 2003). However, CB1-KO mice displayed reduced sucrose consumption across a wide range of self-administration conditions and group differences were smaller under those experimental conditions which enhance the participation of motivational components (PR performance and fluid deprivation) than under those that mainly reflect the consummatory aspects of reinforced behaviour (FR1, non-deprived). On the other hand, a reduction in incentive motivation is accompanied by compensatory behavioural re-allocation and the selection of alternatives of reinforcement with lower costs (Salamone et al. 2003), whereas the deletion of CB1 specifically blunted the choice of reinforcement sources with different relative rewarding properties but identical costs. Thus, in the analysis of the matching behaviour, the effort requirements to access one or the alternate solution were identical but CB1-KO mice showed a decreased ability to allocate their preference accordingly to the magnitude of the reinforcer (sucrose concentration). Yet, it can be argued that incentive motivation depends on the deprivation state of the individual (Balleine 1994) and that the observed differences between genotypes could be a consequence of a differential responsiveness to the initial water deprivation. However, this explanation seems unlikely, since (i) differences between genotypes in operant sucrose self-administration were bigger when the animals were not deprived than under water deprivation; (ii) the slopes of the learning curves of the operant contingencies were not different between genotypes; (iii) the selective alteration of the asymptote of the cumulative responding curve is the result of manipulations that change palatability but not the nutritive or osmotic properties of ingested fluids (Davis and Levine 1977; Higgs et al. 2003). Therefore, CB1 deletion seems to impair fundamental aspects of reinforced behaviour which are preserved after incentive motivation changes, but results in a reduction of the rewarding properties of sucrose.

This conclusion does not necessarily imply that the effects of CB1 deletion are restricted to consummatory behaviours. In fact, although our PR study presents some limitations (i.e. only one session was implemented) we found that CB1-KO reached a lower breakpoint. Interestingly, these individual breakpoint values were significantly correlated to the estimated sensitivity of the rewarding properties of sucrose (slope observed in the analysis of matching behaviour), suggesting that both phenomena are not independent. Therefore, our results and conclusions are similar to those of Freedland et al. (2001), who suggested that CB1 antagonism affects most profoundly consummatory than appetitive components of sucrose self-administration. More specifically, we propose that CB1 could play a major role in the determination of the hedonic value of sucrose, saccharin and probably other reinforcers.

In agreement with this proposal, it should be noted that CB1 deletion or pharmacological blockade results in a reduction of the reinforcing effects of drugs of abuse such as morphine (Ledent et al. 1999; Martin et al. 2000),

heroin (De Vries et al. 2003; Solinas et al. 2003), or ethanol (Rodriguez de Fonseca et al. 1999; Freedland et al. 2001; Hungund et al. 2003; Racz et al. 2003; Wang et al. 2003). Furthermore, the activity of this receptor is also associated with a reduction in the reinforcing effects of electrical medial forebrain bundle (MFB) self-stimulation (Deroche-Gamonet et al. 2001) as well as with enhanced susceptibility to develop anhedonia (Martin et al. 2002). In this respect, we also propose that the role of CB1 is a modulatory one, as CB1 deletion does not completely abolish the rewarding properties of sucrose or other stimuli. Thus, for example, CB1-KO mice showed flatter slopes in the analysis of the matching behaviour but their consumption still showed some degree of relationship with the relative magnitude of the available reinforcers. In fact, most of the studies assessing CB1 deletion or blockade on reinforced behaviour have found an attenuation of reinforcing efficacy rather than a total suppression of reinforcing efficacy.

In summary, the results of the present study revealed that the genetic deletion of CB1 results in a reduction of the sensitivity to the rewarding properties of sucrose that leads to its reduced consumption. These results are in agreement with the previously demonstrated involvement of the CB1 receptor in the reinforced behaviours maintained by sucrose and other reinforcers.

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