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Cannabinoid Receptor Activation Prevents the Effects of Chronic Mild Stress on Emotional Learning and LTP in a Rat Model of Depression

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Most psychiatric disorders are characterized by emotional memory or learning disturbances. Chronic mild stress (CMS) is a common animal model for stress-induced depression. Here we examined whether 3 days of treatment using the CB1/2 receptor agonist WIN55,212-2 could ameliorate the effects of CMS on emotional learning (ie, conditioned avoidance and extinction), long-term potentiation (LTP) in the hippocampal-accumbens pathway, and depression-like symptoms (ie, coping with stress behavior, anhedonia, and weight changes). We also examined whether the ameliorating effects of WIN55,212-2 on behavior and physiology after CMS are mediated by CB1 and glucocorticoid receptors (GRs). Rats were exposed to CMS or handled on days 1–21. The agonist WIN55,212-2 or vehicle were administered on days 19–21 (IP; 0.5 mg/kg) and behavioral and electrophysiological measures were taken on days 23 and 28. The CB1 receptor antagonist AM251 (IP; 0.3 mg/kg) or the GR antagonist RU-38486 (IP; 10 mg/kg) were co-administered with WIN55,212-2. Our results show that CMS significantly modified physiological and behavioral reactions, as observed by the impairment in avoidance extinction and LTP in the hippocampal-accumbens pathway, and the alterations in depression-like symptoms, such as coping with stress behavior, weight gain, and sucrose consumption. The most significant effect observed in this study was that 3 days of WIN55,212-2 administration prevented the CMS-induced alterations in emotional memory (ie, extinction) and plasticity. This effect was mediated by CB1 receptors as the CB1 receptor antagonist AM251 prevented the ameliorating effects of WIN55,212-2 on extinction and LTP. The GR antagonist RU-38486 also prevented the CMS-induced alterations in extinction and plasticity, and when co-administered with WIN55,212-2, the preventive effects after CMS were maintained. The findings suggest that enhancing cannabinoid signaling could represent a novel approach to the treatment of cognitive deficits that accompany stress-related depression.

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INTRODUCTION

Most psychiatric disorders are characterized by emotional memory or learning disturbances. These learning and memory alterations are not just secondary symptoms but are key components of these disorders. For example, patients with major depression have a memory bias with preferred storage and retrieval of negative information.

Chronic mild stress (CMS) is a common animal model for stress-induced depression (Willner *et al*, 1992). In CMS, animals are exposed to moderate stressors, such as food or water deprivation, overnight lighting, and changes in housing, for a relatively long time. The behavioral profile of animals that have been exposed to CMS has high face validity. It includes reduction of sucrose intake and impairments in brain stimulation reward as a measure for anhedonia, decreased sexual activity and self-care, and changes in sleep and appetite (Grønli *et al*, 2004; Willner, 2005). The model also has high predictive validity, as the symptoms are reversed with chronic antidepressant treatment but not with acute treatment (Willner, 2005; Willner *et al*, 1992).

Long-term potentiation (LTP) is one of the prime candidates for mediating learning and memory as well as many other forms of experience-dependent plasticity. The successful *vs* unsuccessful induction of LTP can serve as a 'diagnostic' measure with which to assess the functional state of a brain structure (Diamond *et al*, 2007). Growing attention has been focused on plasticity in the ventral Subiculum (vSub)-nucleus accumbens (NAc) pathway (Abush and Akirav, 2012, 2013; O'Donnell and Grace, 1995) as there is growing evidence for a role of the NAc in the regulation of mood and motivation under normal

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conditions and in mediating many of the prominent behavioral abnormalities seen in depression and other mood disorders (for a review, see Nestler and Carlezon, 2006).

The NAc is involved in mediating stress-related dysfunction (Nestler *et al*, 2002; Willner *et al*, 1992), and with input from the vSub, it mediates goal-directed behavior and is important for aspects of cognitive function, such as context-dependent processing (Belujon and Grace, 2008).

The endocannabinoid (eCB) system has recently emerged as a promising therapeutic target for the treatment of stress-related emotional disorders. Generally, facilitation of eCB signaling promotes antidepressant- and anxiolytic-like responses in preclinical animal models, while disruption of this system profoundly affects emotion, cognition, and neuroendocrine functioning (Abush and Akirav, 2010, 2013; Bortolato *et al*, 2007; Ganon-Elazar and Akirav, 2009, 2012, 2013; Hill *et al*, 2010; Lutz, 2009; Marsicano *et al*, 2002; Patel *et al*, 2004; Ramot and Akirav, 2012; Viveros *et al*, 2005).

Recent data suggest that the eCB system could represent a new therapeutic target for the treatment of depression (Bortolato *et al*, 2007; Hill and Gorzalka, 2005; Macrì and Laviola, 2004). CB1-knockout mice show altered hypothalamic–pituitary–adrenal (HPA) axis function and a higher tendency to exhibit depressive-like responses in the chronic unpredictable mild stress procedure (Martin *et al*, 2002). These characteristics together with their heightened anxiety (Haller *et al*, 2002) and deficits in extinction of aversive memories (Marsicano *et al*, 2002) have been proposed to be analogous to certain symptoms of melancholic depression (Hill and Gorzalka, 2005). Moreover, several cannabinoid compounds have been evaluated in the forced swim test (FST), a widely used screening test for antidepressant potential of novel compounds. In the rat FST, administration of the eCB uptake inhibitor AM404 and the potent CB1 receptor agonist HU-210 induced decreases in immobility (indicative of antidepressant activity) that were blocked by pretreatment with the CB1 receptor antagonist AM251 (Hill and Gorzalka, 2005).

We have recently suggested that cannabinoid receptor activation, using the CB1/2 receptor agonist WIN55,212-2, could represent a novel approach to the treatment of cognitive deficits that accompany a variety of stress-related neuropsychiatric disorders (Abush and Akirav, 2013). We found that chronic WIN55,212-2 administration in proximity to chronic (ie, 2 weeks) restraint stress prevented the stress-induced impairment in LTP in the vSub-NAc pathway and performance in a non-aversive spatial task (Abush and Akirav, 2013). In that study (Abush and Akirav, 2013), chronic restraint stress did not result in 'classic' depression-like symptoms such as alterations in anhedonia and coping with stress behavior, and the drug treatment was applied throughout the 2 weeks of the stress period at a high dose (1.2 mg/kg).

Studies indicate a bidirectional, functional relationship between glucocorticoids and eCBs (for a review, see: Akirav, 2013). ECBs have a key role in regulating the HPA axis under basal and stressful conditions (Ganon-Elazar and Akirav, 2009, 2012, 2013; Hill *et al*, 2009; Patel *et al*, 2004). On the other hand, stress and glucocorticoids can trigger eCB synthesis and CB1 receptors signaling to constrain HPA axis activity under acute conditions (Hill *et al*, 2011; Rademacher *et al*, 2008). We have recently found that WIN55,212-2 can prevent the impairing effects of an acute stressful experience on contextual extinction in a rat model for post-traumatic stress disorder and that these ameliorating effects of WIN55,212-2 on extinction were mediated by glucocorticoid receptors (GRs) in the basolateral amygdala (BLA) and hippocampus (Ganon-Elazar and Akirav, 2013).

In the current study, we aimed to examine whether 3 days of treatment with WIN55,212-2 (0.5 mg/kg) could ameliorate the effects of CMS on emotional learning (ie, conditioned avoidance and extinction), LTP in the vSub-NAc pathway, and depression-like symptoms (ie, coping with stress strategies, anhedonia, and weight gain). As our previous results suggest that the preventing effects of WIN55,212-2 on extinction after an intense acute stressor are mediated by CB1 and GRs (Ganon-Elazar and Akirav, 2012, 2013), here we examined whether the preventing effects of WIN55,212-2 after CMS exposure are also mediated by CB1 and GRs.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (45-days old), caged individually at 22 ± 2 °C under 12-h light/dark cycles (lights turned on at 0700 hours). Rats had access to water and laboratory rodent chow *ad libitum*, except when the CMS procedure required deprivation. The experiments were approved by the University of Haifa Ethics and Animal Care Committee, and adequate measures were taken to minimize pain or discomfort.

CMS Protocol

Rats were subjected to handling or 21 days of mild stressors. The procedure included the following: 18 h of food deprivation followed by 1.5 h of limited food access (0.2 g pellet), 21 h of wet cage (300 ml of water added per 100 g of bedding), 18 or 21 h of water deprivation followed by 1.5 h of empty bottle exposure, 2 h of paired caging, 3 h of 45° cage tilting, or 36 h of continuous lighting (based on Grønli *et al*, 2004 with modifications). This schedule of stressors was applied for a 1-week period and repeated over 3 weeks (see Table 1).

Drug Treatment

The CB1/2 receptor agonist WIN55,212-2 (WIN; IP: 0.5 mg/kg; intra-BLA: 5 μg/side) or the CB1 receptor antagonist AM251 (AM; IP: 0.3 mg/kg), or the GR antagonist RU-38486 (RU; IP:10 mg/kg) were initially dissolved in dimethylsulfoxide (DMSO) and further diluted with 1% Tween 80 and 98% saline (0.9% NaCl). Final DMSO concentration was 1%. This DMSO and saline solution was also used as the vehicle. All drugs were from Cayman Chemicals.

Drug concentrations were based on previous results (Abush and Akirav, 2010, 2013; Ganon-Elazar and Akirav, 2009, 2012, 2013; Wulsin *et al*, 2010). No stress was applied for 4 h before and after the injection.

Table 1 The Chronic Mild Stress (CMS) Protocol

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
0700		Oi, 36 h					
0800							Eb, 1.5 h
1000		Fr, 1.5 h			Eb, 1.5 h		
1100				Til, 3 h			
1200					Ph, 2 h		
1300						Wd, 21 h	
1400							
1600	Fd, 18 h						
1700			Wet, 21 h				
1800				Wd, 18 h			

Abbreviations: Eb, exposure to empty bottle; Fd, food deprivation; Fr, food restriction; Oi, overnight illumination; Ph, paired housing; Til, tilted cage; Wd, water deprivation; Wet, wet cage.

Cannulation and Drug Microinjection

Rats were anesthetized with 4.8 ml/kg Equithesin, restrained in a stereotactic apparatus, and implanted bilaterally with a stainless steel guide cannula (23-gauge, thin wall) aimed at the BLA (anteroposterior, −5 mm; lateral, ±3 mm; ventral, −6.7 mm). The cannula was positioned in place with acrylic dental cement and secured by two skull screws. A stylus was placed in the guide cannula to prevent clogging. Animals were allowed 1 week to recuperate before being subjected to experimental manipulations. For microinjection, the stylus was removed from the guide cannula, and a 28-gauge injection cannula, extending 1.0 mm from the tip of the guide cannula, was inserted. The injection cannula was connected via PE20 tubing to a Hamilton microsyringe driven by a microinfusion pump. Microinjection was performed bilaterally with a 0.5-μl volume per side delivered over 1 min.

Inhibitory Avoidance (IA)

Animals were placed in an IA apparatus with a metal grid floor (Ganon-Elazar and Akirav, 2009, 2012). The apparatus was divided into a light side and a dark side, and the rats were placed in the light side, facing the left rear corner of the box.

For conditioning (Cond), when the rats crossed over to the dark side of the box (with four paws on the grid), the opening between the two sides of the box was blocked and they received a 2-s, 0.8-mA scrambled footshock. After administration of the footshock, the rats remained in the dark side for an additional 60 s, after which they were returned back to the home cage.

For extinction, rats were submitted to a non-reinforced test trial every 24 h for 5 days (Ext1–Ext5), beginning 24 h after conditioning. The first extinction trial also indicated fear retrieval (Ret/Ext1). Each rat was placed in the light side of the box, and the time elapsed until it crossed over to the dark side (ie, latency) was measured. If, after 180 s, the rat did not cross over on its own, the experimenter gently guided it to the dark side. The opening between the two sides of the shuttle was then blocked, no footshock was administered, and the rat was allowed to explore the dark side freely for 180 s, after which it was returned back to the home cage.

Electrophysiology

Rats were anesthetized (with 40% urethane, 5% chloral hydrate in saline, injection volume of 4 ml/1 kg, IP) and placed in a stereotaxic frame. Small burr holes were drilled in the skull to allow electrodes to be inserted into the brain. A recording microelectrode (glass, tip diameter of 2–5 μm, filled with 2 M NaCl, resistance of 1–4 M ohm) was inserted into the NAc shell (anteroposterior, +1.6 mm; lateral, ±1.0 mm; ventral, −5.5 mm). A bipolar 125-μm stimulating electrode was positioned in the vSub (anteroposterior, −6.5 mm; lateral, ±5.0 mm; ventral, −6.0 mm). After positioning the electrodes, the rat was left for 60 min before commencing the experiment.

LTP was induced by theta-like high-frequency stimulation (HFS; three sets of 10 trains; each train consisting of 10 pulses at 200 Hz; inter-train interval, 200 ms; inter-set interval, 1 min) to the vSub. Field potentials were recorded from the NAc every 5 min for 60 min after HFS to the vSub. LTP was measured as an increase in the amplitude and slope of the evoked field potentials. Potentiation was measured as a percentage of change from the average of the 30 min baseline before HFS (Abush and Akirav, 2013). The amplitude was measured by peak-to-peak values based on the previous findings of LTP in the vSub-NAc pathway (Abush and Akirav, 2012; Dong *et al*, 2007). However, it is the slope that is considered to reflect LTP (ie, the strengthening of existing synaptic contacts), whereas the amplitude may also reflect the activity of the cells (Abush and Akirav, 2013).

FST

A cylindrical water container made from dark non-transparent plastic (62 cm diameter, 40 cm height, filled with water at temperature of 24 °C). The water level was such that the rat could not touch the bottom with its hind paws. Rats were exposed to the swim tank for 15 min on the first day (pre-test) and 5 min on the second day (test). Video films of the second day of each FST session were

analyzed for passive coping (immobility) or active coping (climbing and swimming) strategies. After each session, water in the cylinders was emptied and replaced with fresh water for the next subject (Abush and Akirav, 2013).

Sucrose Intake

Water bottles were removed before the dark part of the cycle and replaced with bottles containing a 1% sucrose solution. Sucrose consumption was measured during the 12-dark hours of the cycle and was then normalized according to every rat's specific weight. Measurements of sucrose consumption were taken once a week, on days 0 (baseline), 7, 14, 21, and 28 (Abush and Akirav, 2013).

Weight Monitoring

The weight of all animals was monitored once a week, on days 0 (baseline), 7, 14, 21, and 28. Body weight change is presented as the cumulative percentage from baseline.

Statistical Analysis

The results are expressed as means ± SEM. For statistical analysis, mixed-design ANOVA or one-way ANOVA were used. All *post hoc* comparisons were made using the least significant difference multiple-comparison test (LSD).

Research Plan

Each test, except for the sucrose intake and weight gain, was performed on different sets of rats to prevent carryover effects due to multiple tests.

1. Emotional learning (IA): CMS or handling on days 1–21. Vehicle (Veh), WIN, or AM + WIN IP on days 19–21. IA conditioning was tested on day 23 (Figure 1a) or day 28 (Figure 1b). To examine the effects of the drugs on IA without exposure to CMS, rats were injected with Vehicle, WIN, or AM on days 1–3 and tested for IA conditioning on day 5 (Figure 1c) or day 10 (Figure 1d). To test for GR involvement, CMS or handling on days 1–21. Vehicle (Veh), RU, or RU + WIN IP on days 19–21. IA conditioning was tested on day 23 (Figure 1e).
2. LTP: CMS or handling on days 1–21. Vehicle, WIN, or AM + WIN IP on days 19–21. LTP was measured on day 23 (Figure 2a and b) or day 28 (Figure 2c and d). To test for GR involvement, CMS or handling on days 1–21. Vehicle (Veh), RU, or RU + WIN IP on days 19–21. LTP was measured on day 23 (Figure 3a and b). To examine the effects of the drugs on LTP without exposure to CMS, rats were injected with Vehicle, WIN, AM, or RU on days 1–3 and tested for LTP on day 5 (Figure 3c and d).
3. FST: CMS or handling on days 1–21. Vehicle or WIN IP on days 19–21. Coping behavior was measured on day 23 (Figure 4a) or day 28 (Figure 4d). In another experiment, rats were exposed to CMS on days 1–21 and to intra-BLA WIN55,212-2 immediately after the pre-test on day 22 (Figure 4b). To test the effects of systemic WIN55,212-2 on FST without CMS exposure, rats were injected with vehicle or WIN55,212-2 IP immediately after the pre-test on day 1 (Figure 4e).
4. Anhedonia and weight gain: CMS or handling on days 1–21. Vehicle or WIN IP on days 19–21. Sucrose intake (Figure 5a) and weight gain (Figure 5b) were measured once a week on days 0 (baseline), 7, 14, 21, and 28.

RESULTS

The Effects of CMS and WIN55,212-2 on Conditioned Avoidance and Extinction

First, we examined the effects of CMS exposure on conditioned avoidance and extinction. As WIN55,212-2 is a CB1/CB2 agonist, we also examined whether the preventing effects of WIN55,212-2 on avoidance after CMS are mediated by the CB1 receptor by using a combination of WIN55,212-2 and the CB1 receptor antagonist AM251 (Abush and Akirav, 2013).

When conditioned avoidance behavior was tested on day 23, mixed-design ANOVA for group × days (4 × 6) on the latency to enter the dark side revealed a significant difference between the groups ($F_{(3,40)} = 10.2$, $p < 0.001$), the days ($F_{(1,40)} = 6.42$, $p < 0.05$), and a significant interaction effect ($F_{(3,40)} = 9.49$, $p < 0.001$) (Figure 1a).

Post hoc comparison revealed that on Ret/Ext1, the No CMS-Veh group demonstrated decreased latency compared with all the groups (CMS-Veh and CMS-AM + WIN: $p < 0.01$; CMS-WIN: $p < 0.05$). On Ext2, Ext3, and Ext4, the No CMS-Veh and CMS-WIN groups demonstrated decreased latency compared with the CMS-Veh and CMS-AM + WIN groups (Ext2: CMS-Veh: $p < 0.01$; CMS-AM + WIN: $p < 0.05$; Ext3–4: $p < 0.01$). On Ext5, the No CMS-Veh group demonstrated decreased latency compared with the CMS-Veh and CMS-AM + WIN groups ($p < 0.01$). Hence, CMS impaired extinction, and WIN55,212-2 prevented this impairment. The preventing effect of WIN55,212-2 after CMS was mediated via CB1 receptors as rats co-administered with AM251 and WIN after CMS behaved in a similar manner to the CMS vehicle group.

When conditioned avoidance behavior was tested on day 28, mixed-design ANOVA for group × days (4 × 6) on the latency to enter the dark side revealed a significant difference between the groups ($F_{(3,32)} = 8.815$, $p < 0.001$) and a significant interaction effect ($F_{(3,32)} = 8.483$, $p < 0.001$), with no effect on days ($F_{(1,32)} < 1$, NS) (Figure 1b). *Post hoc* comparison revealed that on Ext1, the CMS-WIN group demonstrated decreased latency compared with the CMS-Veh ($p < 0.01$) and CMS-AM + WIN group ($p = 0.05$). On Ext2, the No CMS-Veh group demonstrated decreased latency compared with the CMS-Veh group ($p < 0.05$). On Ext3, Ext4, and Ext5, the No CMS-Veh and CMS-WIN groups demonstrated decreased latency compared with the CMS-Veh ($p < 0.01$) and CMS-AM + WIN (Ext 3: $p < 0.05$; Ext4 and Ext5: $p < 0.001$) groups. Hence, the effects of CMS and WIN55,212-2 on extinction were observed even 1 week after the last stress exposure and the last injection.

To examine the effects of the drugs on extinction without exposure to CMS, rats were injected with Vehicle, WIN, or AM251 on days 1–3 and tested for IA conditioning on day 5 (Figure 1c; equivalent to testing conditioning on day 23 in Figure 1a, after the drugs were injected on days 19–21) or

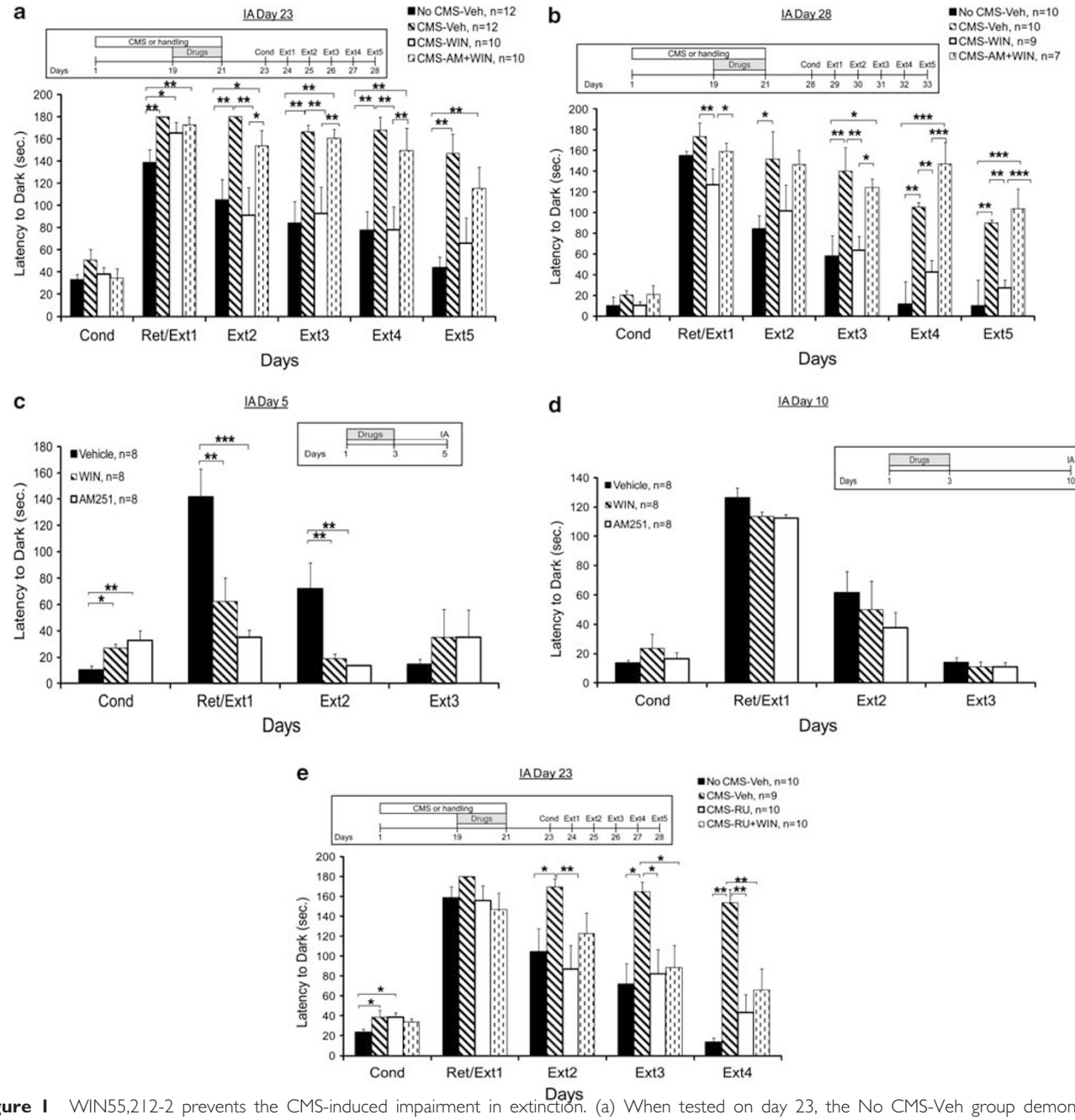

Figure 1 WIN55,212-2 prevents the CMS-induced impairment in extinction. (a) When tested on day 23, the No CMS-Veh group demonstrated decreased latency compared with all the groups on Ext1. On Ext2, Ext3, and Ext4, the No CMS-Veh and CMS-WIN groups demonstrated decreased latency compared with the CMS-Veh and CMS-WIN + AM groups. On Ext5, the No CMS + Veh group demonstrated decreased latency compared with the CMS + Veh and CMS + WIN + AM groups (*$p<0.05$; **$p<0.01$). (b) When tested on day 28, the CMS-WIN group demonstrated decreased latency compared with the CMS-Veh and CMS + WIN + AM group on Ext1. On Ext2, the No CMS + Veh group demonstrated decreased latency compared with the CMS-Veh. On Ext3, Ext4, and Ext5, the No CMS + Veh and CMS + WIN groups demonstrated decreased latency compared with the CMS-Veh and CMS + WIN + AM groups (*$p<0.05$; **$p<0.01$; ***$p<0.001$). (c) When the drugs were injected with no stress exposure 2 days before conditioning, the Vehicle group demonstrated decreased latency compared with the other groups on Cond and increased latency on Ext1 and Ext2 (*$p<0.05$; **$p<0.01$; ***$p<0.001$). (d) When WIN55,212-2 or AM251were injected without stress exposure a week before conditioning, conditioned avoidance and extinction levels were not significantly different from the vehicle-treated rats. (e) When tested on day 23, the No CMS-Veh group demonstrated decreased latency compared with the CMS-Veh and CMS-RU groups on Cond. On Ext2, the CMS-Veh group demonstrated increased latency compared with the No CMS-Veh and CMS-RU groups. On Ext3 and Ext4, the CMS-Veh group demonstrated increased latency compared with all the groups (*$p<0.05$; **$p<0.01$).

day 10 (Figure 1d; equivalent to testing conditioning on day 28 in Figure 1b, after the drugs were injected on days 19–21).

When conditioned avoidance was tested on day 5 (Figure 1c), mixed-design ANOVA on the latency to enter the dark side revealed a significant effect for group ($F_{(2,21)}=3.444$, $p=0.05$) but not for days ($F_{(1,21)}=1.41$, NS) or the interaction between group and days ($F_{(2,21)}<1$, NS). *Post hoc* comparison revealed that the Vehicle group demonstrated decreased latency compared with the other

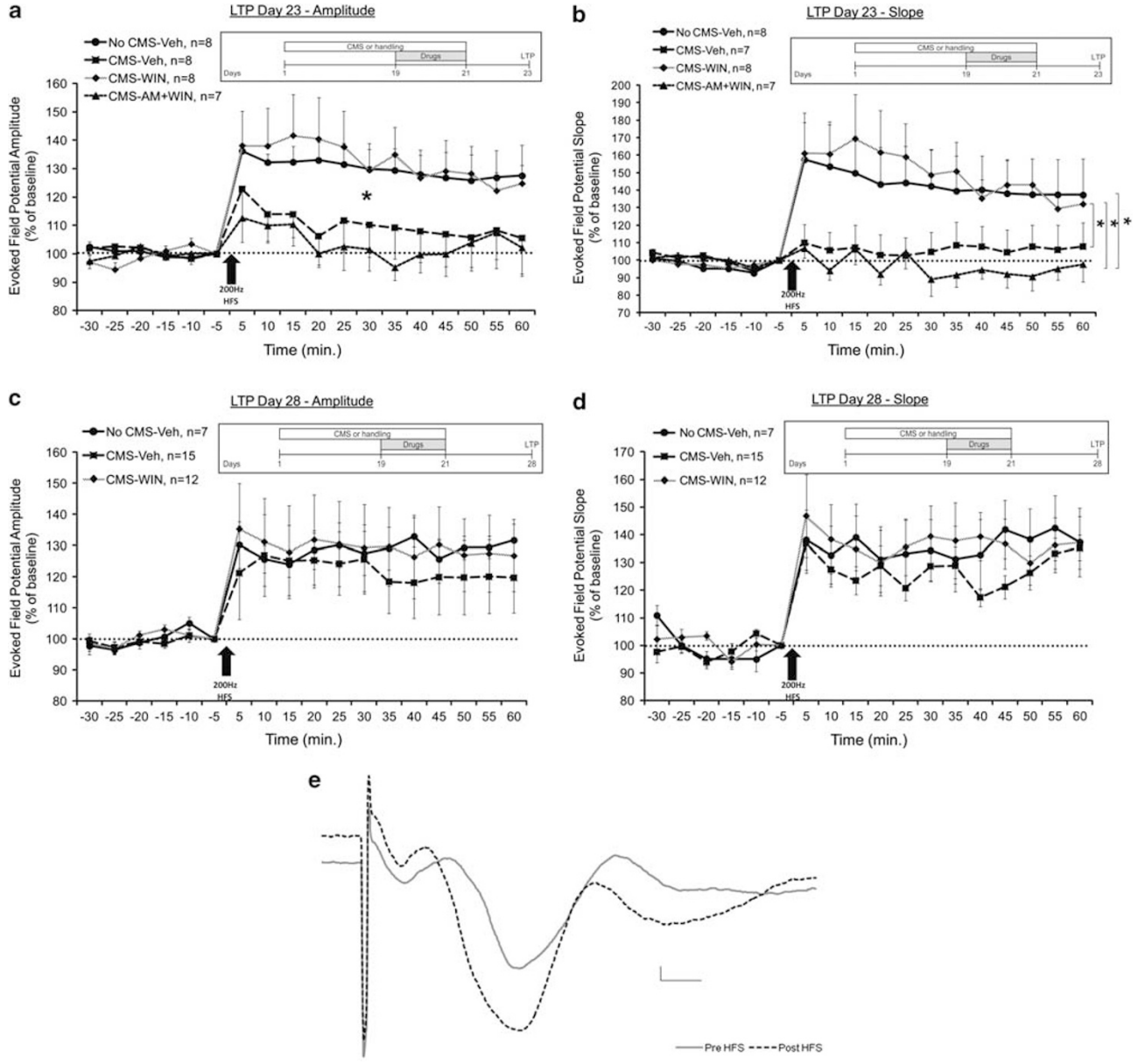

Figure 2 WIN55,212-2 prevents the CMS-induced impairment in LTP in the vSub-NAc pathway. (a) When tested on day 23, the CMS-Veh and CMS-AM + WIN groups demonstrated significantly reduced amplitude compared with the No CMS-Veh and CMS-WIN groups post HFS (*$p<0.05$). (b) When tested on day 23, the CMS-Veh and CMS-AM + WIN groups demonstrated significantly reduced slope compared with the CMS-WIN group post HFS. Also the CMS-WIN + AM showed reduced slope compared with the No CMS-Veh group post HFS (*$p<0.05$). (c) When tested on day 28, all the groups demonstrated similar amplitude, suggesting intact LTP. (d) When tested on day 28, all the groups demonstrated similar slope, suggesting intact LTP. (e) Representative traces in the NAc taken before (continuous line) and 1 h after (broken line) HFS to the vSub (calibration: 0.2 mV, 10 msec).

groups on Cond (WIN: $p<0.05$; AM: $p<0.01$) and increased latency on Ext1 (WIN: $p<0.01$; AM: $p<0.001$) and Ext 2 (WIN and AM: $p<0.01$). Hence, when WIN55,212-2 or AM251 were injected without stress exposure 2 days before IA, rats showed impaired fear retrieval (Figure 1c).

However, when the drugs were injected during the last days of the CMS procedure, they had no effect on fear retrieval (see Figure 1a) as the CMS-WIN group showed increased latency compared with the No CMS-Vehicle group on Ret/Ext1.

When conditioned avoidance was tested on day 10 (Figure 1d), mixed-design ANOVA on the latency to enter the dark side did not reveal a significant effect for group ($F_{(2,21)}<1$, NS) or the interaction between group and days ($F_{(2,21)}<1$, NS). A significant effect was found for days $F_{(1,21)}=10.506$, $p<0.01$). Hence, when WIN55,212-2 or AM251 were injected without stress exposure a week before IA, conditioned avoidance and extinction levels were not significantly different from vehicle-treated rats. A possible explanation for the different effects of WIN55,212-2 and AM251 on avoidance when administered a week before training could be that cannabinoids have delayed effects on acquisition of the avoidance memory.

Although the drugs impaired fear retrieval when administered 2 days, but not 7 days, before conditioning, WIN55,212-2 prevented the CMS-induced impairment in extinction on both occasions.

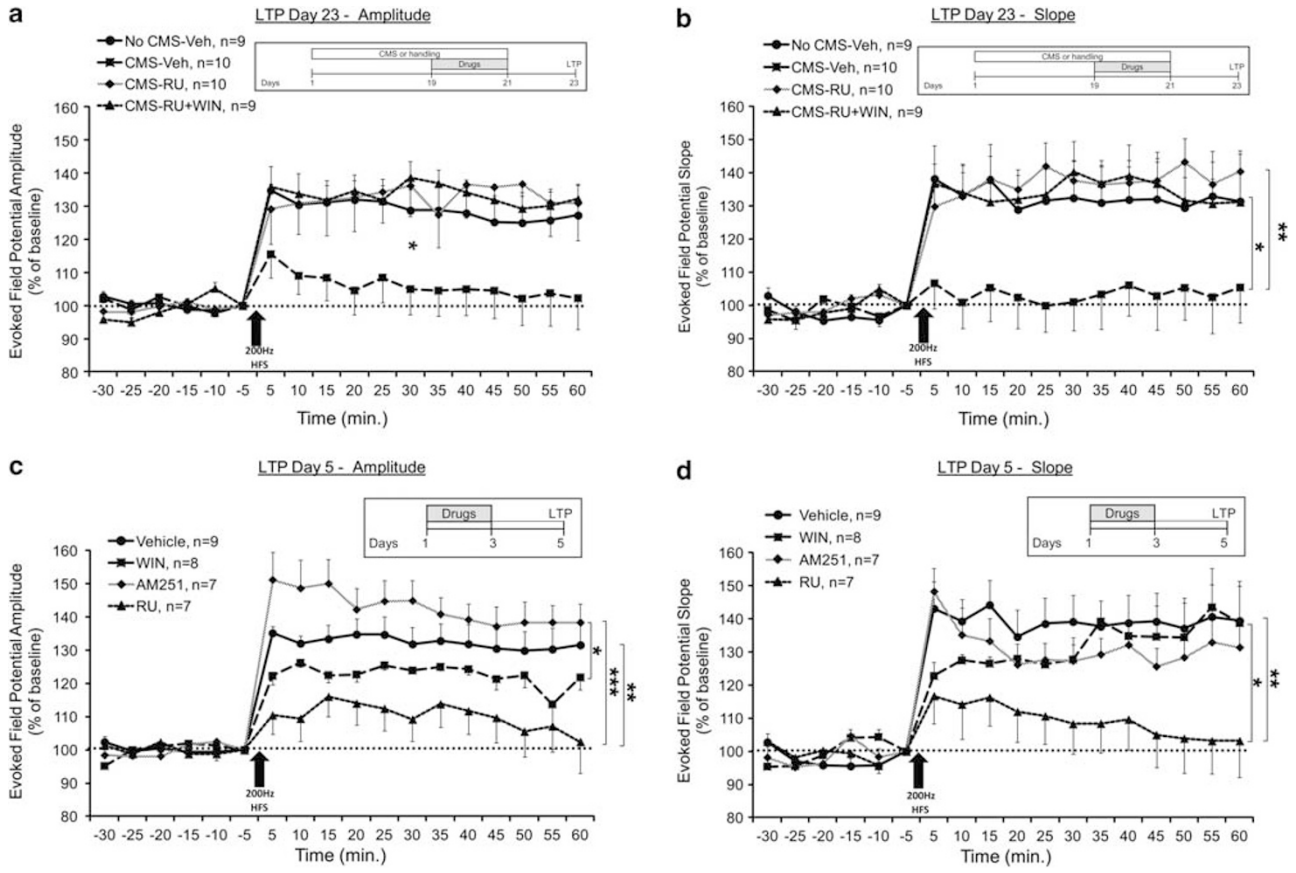

Figure 3 RU-38486 prevents the CMS-induced impairment in LTP in the vSub-NAc pathway. (a) When tested on day 23, the CMS-Veh group demonstrated significantly reduced amplitude compared with all groups post HFS (*$p<0.05$). (b) When tested on day 23, the CMS-Veh group demonstrated significantly reduced slope compared with all the groups post HFS (*$p<0.05$; **$p<0.01$). (c) When the drugs were injected without stress exposure, the RU group demonstrated reduced amplitude compared with the Vehicle and AM groups, and the WIN group demonstrated reduced amplitude compared with the AM group (*$p<0.05$; **$p<0.01$; ***$p<0.001$). (d) When the drugs were injected without stress exposure, the RU group demonstrated reduced slope compared with the Vehicle and WIN groups (*$p<0.05$; **$p<0.01$).

Studies indicate a bidirectional, functional relationship between glucocorticoids and the eCB system (Akirav, 2013). It has been suggested that glucocorticoids recruit eCB signaling in the BLA and hippocampus to modulate aversive memory consolidation (Atsak *et al*, 2012; Campolongo *et al*, 2009). Moreover, de Bitencourt *et al* (2013) suggested that eCBs are recruited by glucocorticoids in the process of extinction of aversive memories.

We have recently found that GRs in the BLA and hippocampus mediate the preventive effects of WIN55,212-2 on contextual extinction after an acute stressful experience. Hence, here we aimed to examine whether GRs also mediate the preventive effects of WIN55,212-2 on avoidance extinction in a rat model of depression. To that end, we examined the effects of the GR antagonist RU-38486 on avoidance after CMS and used a combination of RU-38486 and WIN55,212-2 to examine whether the antagonist would block the preventing effects of WIN55,212-2 on extinction.

When conditioned avoidance behavior was tested on day 23, mixed-design ANOVA for group × days (4 × 5) on the latency to enter the dark side revealed a significant difference between the groups ($F_{(3,35)}=7.309$, $p<0.001$) and a significant interaction effect ($F_{(3,35)}=9.361$, $p<0.001$), with no effect on days ($F_{(1,35)}<1$, NS) (Figure 1e).

Post hoc comparison revealed that on Cond, the No CMS-Veh group demonstrated decreased latency compared with the CMS-Veh and CMS-RU groups ($p<0.05$). On Ext2, the CMS-Veh group demonstrated increased latency compared with the No CMS-Veh ($p<0.05$) and CMS-RU ($p<0.01$) groups. On Ext3 and Ext4, the CMS-Veh group demonstrated increased latency compared with all the groups (Ext3: $p<0.05$; Ext4: $p<0.01$). Hence, the GR antagonist also prevented the effects of CMS on extinction and when co-administered with WIN55,212-2, the preventive effect on extinction was maintained. This suggests that the preventing effects of WIN55,212-2 on extinction after CMS are not mediated by GRs. A higher dose of RU (20 mg/kg) had a similar effect of preventing the effects of CMS on extinction with or without co-administering WIN55,212-2 (data not shown).

The Effects of CMS and WIN55,212-2 on Synaptic Plasticity in the vSub-Nac Pathway

When synaptic plasticity in the vSub-Nac pathway was tested on day 23, mixed-design ANOVA on amplitude

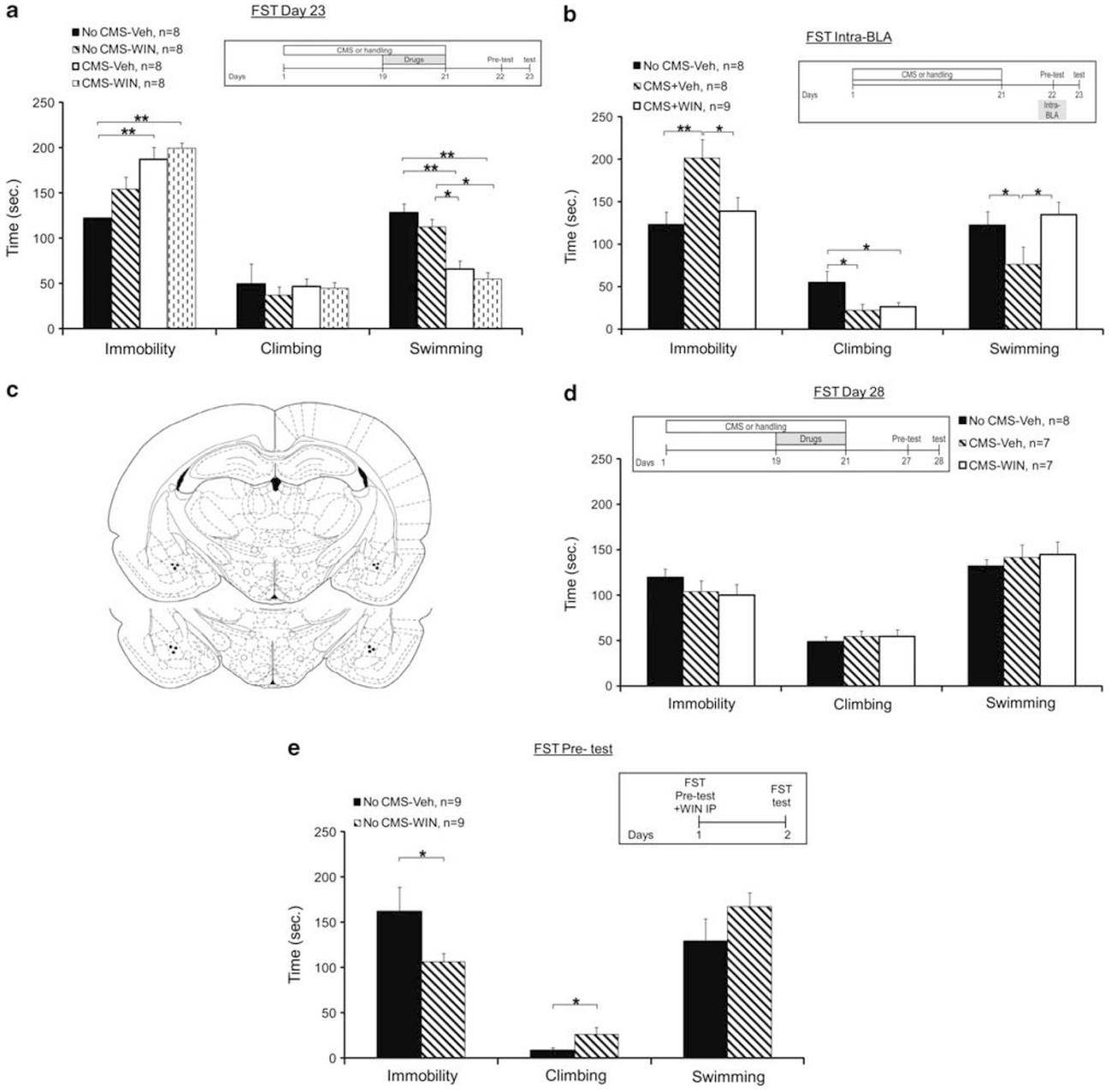

Figure 4 The effects of CMS and WIN55,212-2 on stress-coping behavior in the forced swim test. (a) When tested on day 23, the No CMS-Veh group demonstrated less immobility compared with the CMS-Veh and CMS-WIN groups. Also, the No CMS-Veh and No CMS-WIN groups demonstrated more swimming compared with the CMS-Veh and CMS-WIN groups (*$p<0.05$; **$p<0.01$). (b) When microinjected into the BLA, the CMS-Veh group demonstrated increased immobility and reduced swimming compared with the No CMS-Veh and CMS-WIN groups. Also, the No CMS-Veh group demonstrated more climbing compared with the CMS-Veh and CMS-WIN groups (*$p<0.05$; **$p<0.01$). (c) Representative schematic drawings of cannulae tip positions in the BLA. A coronal view at position 3.14 and 3.30 mm posterior to bregma. (d) When coping behavior was tested on day 28, there were no differences between the groups in immobility, climbing, or swimming. (e) When WIN was injected systemically after pretest with no CMS exposure, the WIN group demonstrated less immobility and more climbing than the Vehicle group (*$p<0.05$).

(Figure 2a) and slope (Figure 2b) post HFS (group × time (4×12)) revealed a significant effect for group (amplitude: $F_{(3,26)}=3.341$, $p<0.05$; slope: $F_{(3,26)}=3.149$, $p<0.05$), time (amplitude: $F_{(1,26)}=4.392$, $p<0.05$; slope: $F_{(3,26)}=6.615$, $p<0.05$) but not the interaction between group and time. *Post hoc* comparison revealed significantly reduced amplitude in the CMS-Veh and the CMS-AM + WIN groups compared with the No CMS-Veh and CMS-WIN groups ($p<0.05$) and reduced slope in the CMS-AM + WIN and CMS-Veh group compared with the CMS-WIN group ($p<0.05$). Also the CMS-WIN + AM group showed reduced slope compared with the No CMS-Veh group ($p<0.05$).

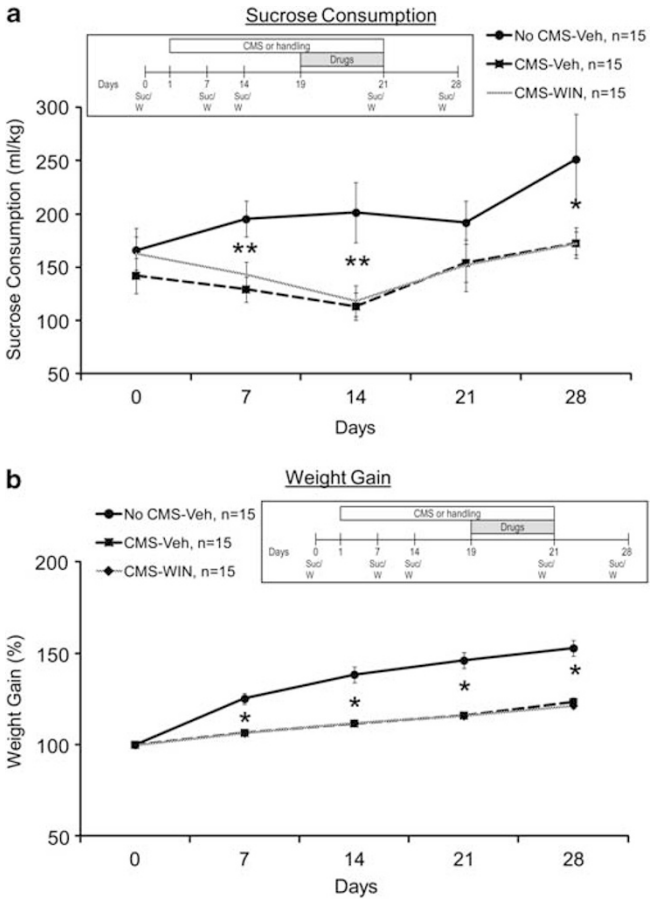

Figure 5 The effects of CMS and WIN55,212-2 on sucrose intake and weight gain. (a) The CMS-Veh and CMS-WIN groups demonstrated reduced sucrose intake compared with the No CMS-Veh group on days 7, 14, and 28 (*$p<0.05$; **$p<0.01$). (b) The CMS-Veh and CMS-WIN groups demonstrated lower weight gain compared with the No CMS-Veh group on days 7, 14, 21, and 28 (*$p<0.05$; averaged baseline weight: 397.37 ± 33.86).

This suggests that CMS impairs LTP, that WIN55,212-2 can prevent this impairment, and that the preventing effect is mediated via CB1 receptors.

When synaptic plasticity was tested on day 28, mixed-design ANOVA on amplitude (Figure 2c) and slope (Figure 2d) post HFS (group × time (3 × 12)) did not reveal a significant effect for group (amplitude: $F_{(2,31)}<1$, NS; slope: $F_{(2,31)}=1.833$, NS), time (amplitude: $F_{(1,31)}=3.1$, NS; slope: $F_{(1,31)}=1.885$, NS), or the interaction between group and time (amplitude: $F_{(2,31)}<1$, NS; $F_{(2,31)}=3.124$, NS). This suggests a significant recovery of LTP in the CMS group 1 week after the stress ended.

We also examined whether GRs mediate the preventive effects of WIN55,212-2 on LTP tested 2 days after CMS. When synaptic plasticity in the vSub-Nac pathway was tested on day 23, mixed-design ANOVA on amplitude (Figure 3a) and slope (Figure 3b) post HFS (group × time (4 × 12)) revealed a significant effect for group (amplitude: $F_{(3,34)}=3.104$, $p=0.039$; slope: $F_{(3,34)}=3.518$, $p<0.025$) but not for time (amplitude: $F_{(1,34)}=3.276$, NS; slope: $F_{(1,34)}<1$, NS) or the interaction between group and time (amplitude: $F_{(3,34)}=1.185$, NS; slope: $F_{(3,34)}<1$, NS). *Post hoc* comparison revealed significantly reduced amplitude in the CMS-Veh group compared with the No CMS-Veh (amplitude and slope: $p<0.05$), CMS-RU (amplitude: $p<0.05$; slope: $p<0.01$), and CMS-RU + WIN (amplitude and slope: $p<0.05$) groups. This suggests that RU-38486 prevented the CMS-induced impairment in LTP, and when co-administered with WIN55,212-2, the preventive effects on LTP after CMS were maintained.

To examine the effects of the drugs on LTP without exposure to CMS, rats were injected with Vehicle, WIN, AM251, or RU on days 1–3 and tested for LTP on day 5 (equivalent to LTP tested on day 23 in Figure 2a, after the drugs were injected on days 19–21).

When synaptic plasticity in the vSub-Nac pathway was tested on day 5, mixed-design ANOVA on amplitude (Figure 3c) and slope (Figure 3d) post HFS (group × time (4 × 12)) revealed a significant effect for group (amplitude: $F_{(3,27)}=6.719$, $p<0.01$; slope: $F_{(3,27)}=2.975$, $p<0.05$), time (amplitude: $F_{(1,27)}=10.494$, $p<0.01$ but not slope), and the interaction between group and time (slope: $F_{(1,27)}=3.089$, $p<0.05$ but not amplitude). *Post hoc* comparison revealed significantly reduced amplitude in the RU group compared with the Vehicle ($p<0.01$) and AM ($p<0.001$) groups and reduced amplitude in the WIN group compared with the AM group ($p<0.05$). The RU group showed reduced slope compared with the Vehicle ($p<0.01$) and WIN ($p<0.05$) groups. Hence, RU-38486 by itself, with no stress exposure, impaired LTP tested 2 days after the last injection.

The Effects of CMS and WIN55,212-2 on Stress-Coping Behavior in the FST

When coping behavior was tested on day 23, one-way ANOVA revealed a significant difference between the groups in immobility ($F_{(3,11)}=4.642$, $p<0.01$) and swimming ($F_{(3,31)}=6.014$, $p<0.01$) (Figure 4a). *Post hoc* comparison revealed that the No CMS-Veh group demonstrated less immobility compared with the CMS-Veh and CMS-WIN groups ($p<0.01$). Also, the No CMS-Veh ($p<0.01$) and No CMS-WIN ($p<0.05$) groups demonstrated more swimming compared with the CMS-Veh and CMS-WIN groups. Hence, CMS caused an increase in passive stress coping and a decrease in active stress coping and the treatment with the agonist WIN55,212-2 did not prevent this effect. Furthermore, WIN55,212-2 by itself (without stress exposure; No CMS-WIN group) had no effect on FST behavior when injected for 3 days on days 19–21 of the experiment.

We have previously demonstrated that intra-BLA WIN55,212-2 can prevent the effects of stress on behavior and physiology (Ganon-Elazar and Akirav, 2009, 2012, 2013; Ramot and Akirav, 2012). Hence, next we examined whether WIN55,212-2 microinjected into the BLA immediately after the pre-test on day 22 would prevent the effects of CMS on coping behavior tested on day 23. The drug was injected specifically into the BLA after the pre-test as previous results from our lab showed that cannabinoid receptor activation in the BLA in proximity to acute stress exposure can prevent the effects of stress on behavior (Ganon-Elazar and Akirav, 2009, 2012).

One-way ANOVA on stress-coping strategy revealed significant differences between the groups in immobility ($F_{(2,22)}=5.236$, $p<0.05$), climbing ($F_{(2,22)}=3.893$, $p<0.05$),

and swimming ($F_{(2,22)} = 3.85$, $p = 0.05$) (Figure 4b). *Post hoc* comparison revealed that the CMS-Veh group demonstrated increased immobility and reduced swimming compared with the No CMS-Veh (immobility: $p < 0.01$; swimming: $p = 0.05$) and CMS-WIN (immobility and swimming: $p < 0.05$) groups. Also, the No CMS-Veh group demonstrated more climbing compared with the CMS-Veh and CMS-WIN groups ($p < 0.05$). Hence, CMS caused an increase in passive stress coping and a decrease in active stress coping, and intra-BLA WIN55,212-2 prevented some of these effects.

When coping behavior was tested on day 28, one-way ANOVA did not reveal a significant difference between the groups in immobility, climbing, or swimming ($F_{(2,27)} < 1$, NS) (Figure 4d). This suggests a significant recovery of coping behavior in the CMS group 1 week after the stress ended.

Other studies have shown that enhancing cannabinoid signaling had an antidepressant-like effect in the FST, when the drugs were injected between test sessions (Bambico *et al*, 2007; Gobbi *et al*, 2005; Hill and Gorzalka, 2005). Hence, we added an experiment in which we injected WIN55,212-2 IP immediately after the pre-test (with no previous CMS exposure) and tested the rats the day after (Figure 4e). ANOVA on stress-coping strategy revealed significant differences between the groups in immobility ($F_{(1,17)} = 4.386$, $p = 0.05$) and climbing ($F_{(1,17)} = 4.605$, $p < 0.05$). Hence, WIN55,212-2 reduced passive stress coping and increased active stress coping when administered systemically after the pre-test. Taken together, the results show that WIN55,212-2 administered during the last 3 days of CMS did not prevent the CMS-induced alterations in coping behavior. However, when administered systemically or locally into the BLA after the pre-test, WIN showed an antidepressant-like effect.

The Effects of CMS and WIN55,212-2 on Sucrose Consumption and Weight Gain

We have recently found that chronic exposure to restraint had no effect on despair-like behavior as measured in the FST and the sucrose consumption test 24 h, 10 d, or 30 d after stress ended (Abush and Akirav, 2013). However, studies demonstrated that animals subjected to CMS show impairments in a variety of tests of rewarded behavior, including decreased intake and preference for sweet fluids (Grønli *et al*, 2004). It has been suggested that changes in sucrose intake may be artifacts related to loss of body weight (Matthews *et al*, 1995). To avoid this problem, we did a correction for body weight and measured sucrose intake/g of body weight. We also measured total fluid intake and found no differences between CMS and control rats in their water consumption throughout the experiment (averaged water consumption ml/kg per day from day 0 to day 28: No CMS group: 78 ± 4; CMS group $= 74.1 \pm 3.9$).

Mixed-design ANOVA (group $\times$ days (3×5)) on sucrose intake revealed a significant difference between the groups ($F_{(2,42)} = 5.17$, $p = 0.01$) and a significant difference across the days ($F_{(1,42)} = 6.14$, $p = 0.017$), with no significant interaction effect ($F_{(2,42)} = 2.41$, NS) (Figure 5a). *Post hoc* comparison indicated that the No CMS-Veh group consumed more sucrose compared with the CMS-Veh and CMS-WIN groups on days 7, 14 ($p < 0.01$), and 28 ($p < 0.05$). Hence, WIN55,212-2 did not revert the CMS-induced effects on sucrose intake.

When testing the effects on body weight gain, mixed-design ANOVA (group $\times$ days (3×5)) revealed a significant difference between the groups ($F_{(2,42)} = 41.57$, $p < 0.001$), a significant within-subject effect for days ($F_{(1,42)} =$, $p < 0.001$), and a significant interaction ($F_{(2,42)} =$, $p < .01$) (Figure 5b). *Post hoc* comparison indicated that the No CMS-Veh group gained more weight compared with the CMS-Veh and CMS-WIN groups on days 7, 14, 21, and 28 ($p < 0.05$). Hence, WIN55,212-2 did not prevent the CMS-induced effects on weight gain.

DISCUSSION

CMS significantly modified physiological and behavioral reactions, as observed by the impairment in avoidance extinction and LTP in the hippocampal-accumbens pathway, and the alterations in depression-like symptoms, such as coping with stress behavior, weight gain, and sucrose consumption. The most significant effect observed in this study was that 3 days administration of the CB1/2 receptor agonist WIN55,212-2 or the GR antagonist RU-38486 prevented the CMS-induced alterations in emotional memory (ie, extinction) and plasticity. The preventive effect of WIN55,212-2 after CMS was found to be CB1 but not GR dependent.

CMS and WIN55,212-2 Effects on Emotional Memory

Exposure to CMS impaired extinction when tested 2 and 7 days after the stress ended. Delay in the extinction of fear memories has also been seen in patients with depressive syndrome and in rodents at high risk of signs of depression (Marsicano *et al*, 2002; Milad *et al*, 2006; Shumake *et al*, 2005). Mice with defects in cannabinoid receptors are characterized by high sensitivity to depressive-like responses in stress, and rats with innate learned helplessness are characterized by impaired extinction of conditioned fear reactions (Marsicano *et al*, 2002; Shumake *et al*, 2005). It has been suggested that 'depressed' rats may show impaired extinction as a result of negative evaluations of the environment and the development of the anhedonia typical of the depressive state (Anisman and Matheson, 2005).

Importantly, 3 days administration of WIN55,212-2 prevented the CMS-induced impairment of extinction tested 2 and 7 days after the last injection. This may have potential implications to developing pharmacological approaches to correct the prolonged retention of memories of negative events in depressive states.

WIN55,212-2 or AM251 administered for 3 days, with no stress exposure, had no effect on conditioning and extinction tested 7 days after injection but impaired fear retrieval when testing was performed 2 days after the last drug injection. Hence, it seems that cannabinoids have a different effect on behavior with or without stress exposure. This corroborates with our previous findings suggesting that the effects of WIN55,212-2 on memory and plasticity are quiet different when administered in proximity to stress exposure or without stress exposure (Abush and Akirav, 2013). We recently found that chronic WIN55,212-2

administration (ie, 2 weeks) can impair object location short-term memory even 75 days after the last injection. However, when administered in proximity to chronic stress exposure, WIN55,212-2 can prevent the effects of stress on performance in this task (Abush and Akirav, 2013). Furthermore, acute intra-BLA WIN55,212-2 can prevent the effects of acute elevated platform stress on performance in an aversive learning task (Ganon-Elazar and Akirav, 2009), but acute intra-BLA WIN55,212-2 could not prevent the effects of the same stressor on the performance in a non-aversive task (Segev *et al*, 2012). In general, the cannabinoid system and the stress system are highly interconnected (Gorzalka *et al*, 2008; Hill and McEwen, 2010; Hill *et al*, 2010; Patel *et al*, 2004; Patel and Hillard, 2008), and it has been suggested that the eCB system might become activated specifically in highly aversive situations but not in non-aversive situations (Harloe *et al*, 2008; Holter *et al*, 2005).

CMS and WIN55,212-2 Effects on Plasticity

Exposure to CMS impaired LTP when tested 2 days, but not when tested 7 days, after the stress ended. This suggests a recovery of LTP one week after CMS ended. Many chronic stress paradigms produce changes that are dynamic and reversible (Conrad *et al*, 1999), suggesting that behavioral recovery is possible. Nevertheless, the exposure to CMS had significant effects on emotional learning and depression-like symptoms that suggest that some of the effects do not go through a habituation process and probably do not recover.

A similar result was found in CA1 slices; Holderbach *et al* (2007) found no effect on LTP tested within 8 days after the end of the CMS protocol. However, they found that CMS exposure facilitated CA1 LTD (Holderbach *et al*, 2007). On the other hand, Li *et al* (2012) exposed rats to 4 weeks of chronic unpredictable stress (CUMS) and 1 week later found impaired LTP in the CA1. The difference between the findings could result from the fact that we measured LTP in the NAc or because the CUMS involves the use of various physical and psychological stressors in a predetermined manner so that the animal is not able to adapt to the stressor. In the CMS model, the animal may develop some adaptation of the HPA axis as the same schedule is repeated for 3 weeks. Hence, some of the effects of CMS exposure may be short-lived.

It has been proposed that long-term synaptic plasticity or its modulation might be disturbed in depressed patients (Garcia, 2002; Stewart and Reid, 2002). Different antidepressants and electroconvulsive therapy have been shown to effectively modulate synaptic plasticity in the dentate gyrus and the CA1 and in the neostriatum (De Murtas *et al*, 2004; Stewart and Reid, 2000). Here we found an impaired ability to induce LTP in a brain circuit that may be crucially involved in the pathophysiology of major depression (Nestler and Carlezon, 2006).

WIN55,212-2 administered for 3 days, with no stress exposure, had no effect on LTP levels tested 2 days after the last injection. Previous studies have shown that WIN55,212-2 administered acutely or chronically before HFS impair LTP in the hippocampus and NAc (Abush and Akirav, 2010, 2013; Terranova *et al*, 1995). When comparing our previous results (Abush and Akirav, 2013) with the results obtained here, different drug administration protocols (3 days *vs* 2 weeks), different testing times (2 days after the last injection *vs* 24 h after the last injection) and different doses (0.5 mg/kg *vs* 1.2 mg/kg) may explain the different effects of WIN55,212-2 on plasticity.

The prefrontal cortex (PFC), hippocampus, and amygdala, which densely innervate the NAc, show moderate-to-very high CB1 receptor levels, whereas in the NAc low levels of CB1 receptors were reported (Egertová and Elphick, 2000; Mailleux and Vanderhaeghen, 1992; Matsuda *et al*, 1993; Tsou *et al*, 1997). GRs, on the other hand, are abundant in the amygdala, hippocampus, NAc, and cerebral cortex (Ahima and Harlan, 1990). A previous study that specifically assessed the effects of stress on plasticity in the vSub-NAc pathway (Dong *et al*, 2007) has shown that exposure to behavioral stress enabled low-frequency stimulation to induce long-term depression (LTD), and this stress-induced LTD was dependent on GRs.

NAc neuronal activation is subject to the competing drive of converging inputs from the PFC, vSub, and BLA (Belujon and Grace, 2008; Goto and Grace, 2005; Mulder *et al*, 1998; O'Donnell and Grace, 1994; O'Donnell and Grace, 1995). NAc lesions have been shown to block the acute memory-enhancing properties of glucocorticoids in the BLA, demonstrating a functional BLA–NAc interaction affecting memory formation (Roozendaal *et al*, 2001). Those properties might be dependent on the rapid non-genomic effects of glucocorticoids in the BLA that have been shown to be CB1 dependent (Campolongo *et al*, 2009). In the case of repeated stress such as CMS exposure, the effects on vSub-NAc plasticity may result from long-term changes in BLA GR and CB1 activity. In support of this, previous studies found that animals exposed to CMS show alterations in the expression of GRs and CB1 receptors in the hippocampus, NAc, and PFC (Bortolato *et al*, 2007; Guidotti *et al*, 2013; Hill *et al*, 2005; Hill *et al*, 2008).

Altered BLA function following exposure to repeated stress may mediate the effects of stress on vSub-NAc plasticity (Gill and Grace, 2011). Activating the amygdala (using HFS) can suppress vSub-evoked responses in the NAc (Gill and Grace, 2011) and repeated but not acute exposure to stress prevented potentiating the vSub-NAc pathway by HFS while causing a depression in the non-tetanized BLA-NAc pathway (Gill and Grace, 2013). Cannabinoid agonists were found to presynaptically modulate GABAergic synaptic transmission in the amygdala and hippocampus and other brain areas (Chan *et al*, 1998; Hoffman and Lupica, 2001; Szabó *et al*, 1998; Takahashi and Linden, 2000; Vaughan *et al*, 1999, 2000). Katona *et al* (2001) found that WIN 55,212-2 significantly reduced the amplitude of $GABA_A$ receptor-mediated evoked IPSCs in the amygdala, which is in agreement with previous findings obtained in the hippocampus, which show presynaptic CB1 receptor localization on GABAergic axon terminals along with the inhibition of GABA release (Hájos *et al*, 2000; Hoffman and Lupica, 2000; Irving *et al*, 2000; Katona *et al*, 1999, 2000). Reich *et al* (2013) have recently shown that WIN 55,212-5 (1 μM) after CMS exposure resulted in a significant increase in excitatory neurotransmission in the hippocampus; however, WIN 55,212-5 significantly decreased excitatory neurotransmission in CMS animals when $GABA_A$ neurotransmission in the hippocampus was

blocked. Taken together, the data suggest that the preventing effects of WIN 55,212-5 on hippocampal-accumbens plasticity after stress may be mediated by its effect on GABAergic terminals in the BLA and hippocampus.

Nevertheless, several studies suggested that CB1-expressing neurons in the NAc, although sparse, are critical for cellular and behavioral alterations induced by cocaine and other drugs of abuse (Morra *et al*, 2010; Ramiro-Fuentes *et al*, 2010). Hence, the role of NAc-CB1 receptors in the preventing effects of WIN 55,212-5 on hippocampal-accumbens plasticity after stress cannot be excluded.

Cannabinoids and Glucocorticoids

The GR antagonist RU-38486 also prevented the CMS-induced alterations in emotional learning and plasticity. This corroborates with other studies showing that RU-38486 prevented stress-induced decreases in neuroplasticity as well as stress-induced increases in depression-like behaviors (de Kloet *et al*, 1988; Oomen *et al*, 2007; Wulsin *et al*, 2010). Taken together, our results suggest that both cannabinoids and GR blockers can be considered as therapeutic candidates for stress-induced conditions.

When RU-38486 was co-administered with WIN55,212-2 during the last 3 days of CMS, the beneficial effects on emotional learning and plasticity were maintained. This corroborates with a previous study in which chronic restraint stress reduced expression levels of GRs in the NAc, BLA, PFC, and hippocampus, and chronic administration of WIN55,212-2 (2 weeks) together with stress exposure did not affect this stress-induced decline in GRs in all the brain areas examined (Abush and Akirav, 2013). This suggests that the beneficial effects of WIN55,212-2 on memory and plasticity after chronic stress were not mediated by alterations in GR levels in the brain areas tested. Hence, the mechanism through which WIN55,212-2 prevents the CMS-induced memory and plasticity impairments is yet to be determined. One possible explanation could be that WIN55,212-2 affects systems that are activated by stress stimulation before the activation of the HPA axis, such as CRH or norepinephrine.

It has been hypothesized that the anxiolytic effects of cannabinoids are mediated via CB1 activation of GABAergic (Katona *et al*, 2001) or glucocorticoid (Rodriguez de Fonseca *et al*, 1996) mechanisms within the amygdala. WIN55,212-2 administered during stress exposure may reduce GABA release in BLA interneurons, thereby reducing their inhibition of the GABAergic neurons of the intercalated nuclei, which, in turn, increases their inhibition of the pyramidal neurons of the central amygdala (Katona *et al*, 2001). The end result may be reduced HPA axis activity and a reduction in the stress-induced increase in glucocorticoid levels. Glucocorticoids easily re-enter the brain to affect GRs in brain areas that are highly involved in memory processes (eg, the hippocampus and NAc). Hence, the reduction in HPA axis activity by cannabinoids or by GRs may prevent the effects of stress on memory and plasticity. In support of this, it has been shown that CB1 agonists decrease the excitability of projection neurons in the rat BLA (Pistis *et al*, 2004).

Several studies have shown that activating CB1 receptors or increasing eCB signaling prevents some of the effects of stress in the amygdala and hippocampus and can reduce stress-induced HPA axis activation (Ganon-Elazar and Akirav, 2009, 2012, 2013; Gorzalka *et al*, 2008; Patel *et al*, 2004). Nevertheless, other possible mechanisms could not be excluded; eg, RU-38486 can affect extinction and plasticity by regulating hippocampal neurogenesis. In support, it has been shown that RU-38486 rapidly reversed a chronic corticosterone-induced reduction of adult neurogenesis in rats (Mayer *et al*, 2006). Another possible explanation is that WIN55,212-2 administration induced long-term changes in endogenous cannabinoid signaling, ie, altering the expression of CB1 receptors in the relevant brain regions, that could have affected directly emotional memory and plasticity. In support of this, it has been demonstrated that CB1 receptors expression is altered in the amygdala and the hippocampus following stress (Hill *et al*, 2005; Zoppi *et al*, 2011). Specifically, exposure to CMS resulted in reduced CB1 receptor binding and protein expression in the hippocampus (Hill *et al*, 2005; Hill *et al*, 2008). CMS was also shown to produce an increase in CB1 receptor mRNA and CB1 receptor binding in the PFC, whereas in NAc, CMS reduced CB1 receptor binding (Bortolato *et al*, 2007; Hill *et al*, 2008).

CMS and WIN55,212-2 Effects on Depression-Like Symptoms

Exposure to CMS impaired coping strategies examined 2 days, but not 7 days, after CMS. The effects of CMS on sucrose consumption and weight gain lasted at least 1 week after the stress ended.

Several studies found alterations in sucrose intake and body weight (reviewed by Willner, 2005), and Willner *et al* (1996) summarized data from different laboratories using the CMS procedure and concluded that decreased hedonic sensitivity following CMS cannot be attributed to loss of body weight. In addition to measurement of responsiveness to rewards using the sucrose test, the FST measures despair and coping in an aversive situation. Several studies found increased immobility in the FST following CMS; however, most studies measured FST 24 h after the last stressor (Molina *et al*, 1994) or CMS exposure duration was significantly longer than in our study (7 weeks; Griebel *et al*, 2005).

The 3 days administration of WIN55,212-2 did not prevent the CMS-induced alterations in depression-like symptoms. Bortolato *et al* (2007) showed that daily administration of URB597 for 5 weeks corrected the reduction in body weight gain and sucrose intake induced by CMS. The difference between the studies could be due to the different drugs used. URB597 is a selective inhibitor of the enzyme fatty-acid amide hydrolase, which catalyzes the intracellular hydrolysis of the eCB anandamide. Another explanation could be the differences in injection protocols (5 weeks for URB597 *vs* 3 days for WIN55,212-2). Nevertheless, we observed an antidepressant like effect when WIN55,212-2 was injected between test sessions, corroborating with previous studies (Bambico *et al*, 2007; Gobbi *et al*, 2005; Hill and Gorzalka, 2005).

There is substantial evidence supporting the involvement of the eCB system in both motivation to feed (hedonic properties) and energy metabolism, and one of the effects of

chronic treatment with WIN 55,212-2 is increased appetite and food consumption (Di Marzo and Matias, 2005). Hence, chronic administration of WIN55,212-2 would probably overcome the CMS-induced decrease in weight gain.

It should be noted that responding to natural and artificial rewards, including sucrose intake, is mediated by the NAc and its dopaminergic inputs (Nestler *et al,* 2002), which is probably different from the neural substrate that mediates hippocampal-accumbens LTP, which is NMDA dependent (Dong *et al*, 2007).

Summary

WIN 55,212-2 can prevent the effects of different stressors on physiology and behavior (Abush and Akirav, 2013; Ganon-Elazar and Akirav, 2009, 2012, 2013; Ramot and Akirav, 2012). The preventing effects of WIN 55,212-2 are mediated by CB1 receptors, and in some of the stress paradigms (see Ganon-Elazar and Akirav, 2013) this effect is mediated by GRs in the amygdala and hippocampus. Here, cannabinoid receptor activation prevented the effects of CMS exposure on emotional learning and LTP in a brain circuit relevant to motivation and emotions. This suggests that cannabinoids could represent a novel approach to the treatment of cognitive deficits that accompany stress-related depression.

FUNDING AND DISCLOSURE

The authors declare no conflict of interest.

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