

Novelty-Induced Emotional Arousal Modulates Cannabinoid Effects on Recognition Memory and Adrenocortical Activity

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Although it is well established that cannabinoid drugs can influence cognitive performance, the findings—describing both enhancing and impairing effects—have been ambiguous. Here, we investigated the effects of posttraining systemic administration of the synthetic cannabinoid agonist WIN55,212-2 (0.1, 0.3, or 1.0 mg/kg) on short- and long-term retention of object recognition memory under two conditions that differed in their training-associated arousal level. In male Sprague-Dawley rats that were not previously habituated to the experimental context, WIN55,212-2 administered immediately after a 3-min training trial, biphasically impaired retention performance at a 1-h interval. In contrast, WIN55,212-2 enhanced 1-h retention of rats that had received extensive prior habituation to the experimental context. Interestingly, immediate posttraining administration of WIN55,212-2 to non-habituated rats, in doses that impaired 1-h retention, enhanced object recognition performance at a 24-h interval. Posttraining WIN55,212-2 administration to habituated rats did not significantly affect 24-h retention. In light of intimate interactions between cannabinoids and the hypothalamic–pituitary–adrenal axis, we further investigated whether cannabinoid administration might differently influence training-induced glucocorticoid activity in rats in these two habituation conditions. WIN55,212-2 administered after object recognition training elevated plasma corticosterone levels in non-habituated rats whereas it decreased corticosterone levels in habituated rats. Most importantly, following pretreatment with the corticosterone-synthesis inhibitor metyrapone, WIN55,212-2 effects on 1- and 24-h retention of non-habituated rats became similar to those seen in the low-aroused habituated animals, indicating that cannabinoid-induced regulation of adrenocortical activity contributes to the environmentally sensitive effects of systemically administered cannabinoids on short- and long-term retention of object recognition memory.

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INTRODUCTION

Extensive evidence indicates that cannabinoids, either administered exogenously or released from endogenous sites, have pronounced effects on learning and memory (Marsicano and Lafenetre, 2009; Wilson and Nicoll, 2002). The cannabinoid system consists of two types of G-protein-coupled receptors (CB1 and CB2 receptors), endogenous ligands, and enzymes involved in their synthesis and inactivation (Piomelli, 2003). Endogenous ligands for

cannabinoid receptors, that is, endocannabinoids, are synthesized on demand in an activity-dependent manner and released from postsynaptic neurons. They travel backward across the synapse to activate presynaptic CB1 receptors and modulate presynaptic functions (Piomelli, 2003). Cannabinoid signaling is crucial for certain forms of short- and long-term plasticity at both excitatory and inhibitory synapses (Deadwyler *et al*, 2007) and thereby contributes to various aspects of brain function, including learning and memory (Marsicano and Lafenetre, 2009). However, evidence indicates that the mnemonic consequence of cannabis exposure in humans does not always correspond to the effects observed in laboratory animals administered with cannabinoid compounds systemically or into discrete brain regions. For example, although it is well appreciated that cannabis use can impair short-term memory and executive function in humans (Pattij *et al*, 2008), not all

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preclinical studies, using WIN55,212-2 or other synthetic cannabinoid agonists, were able to confirm these findings (Baek *et al*, 2009; Clarke *et al*, 2008; Kosiorek *et al*, 2003; Schneider *et al*, 2008; Suenaga and Ichitani, 2008). Cannabinoid effects on long-term memory in humans did not receive much attention and findings of preclinical studies are not unequivocal, independent of the pharmacodynamic properties of the drug used. Whereas some animal studies indicate that systemic or intracranial administration of cannabinoid agonists impairs the encoding and consolidation of long-term memory processing (Barros *et al*, 2004; Robinson *et al*, 2008), enhancing effects are reported as well (De Oliveira Alvares *et al*, 2008). Although such discrepancies are not unusual in memory research, the factors contributing to these conflicting findings are yet poorly understood.

Emerging evidence indicates that cannabinoid drugs can induce distinct and even opposite effects on anxiety and several other behaviors, depending on the aversiveness of the environmental context (Campolongo *et al*, 2012; Carlin *et al*, 1972; Haller *et al*, 2009; Szuster *et al*, 1988; Zanettini *et al*, 2011). Based on these findings, we hypothesized that the level of emotional arousal that is associated with a training experience might also be a primary factor in determining the outcome of cannabinoid administration on learning and memory. Therefore, in the present study we investigated the effects of cannabinoid administration on both short- and long-term object recognition memory under two experimental conditions that differed with respect to their training-associated arousal level. By employing a previously described procedure (Okuda *et al*, 2004), one group of rats was not habituated to the training context, whereas the other group was extensively habituated to the experimental apparatus to decrease its novelty-induced stress response during the training trial. The cannabinoid receptor agonist WIN55,212-2 was administered intraperitoneally to either extensively habituated or not previously habituated rats immediately after the object recognition training trial. In the first experiment, retention was tested 1 h after the training trial to assess possible cannabinoid effects on short-term cognitive performance. In the second experiment, we investigated cannabinoid effects on long-term object recognition memory by assessing retention 24 h after the training trial and drug treatment.

In addition to direct actions in the brain, cannabinoids are crucially involved in regulating hypothalamic–pituitary–adrenocortical (HPA) axis activity (Atsak *et al*, 2012b; Campolongo *et al*, 2009; Di *et al*, 2003; Hill *et al*, 2010b), shaping the corticosterone response to stressful stimulation. As it is well established that glucocorticoids hormones are potent modulators of learning and memory (Campolongo and Roozendaal, 2011; Okuda *et al*, 2004; Roozendaal, 2002; Roozendaal *et al*, 2006b; Schwabe *et al*, 2012), we next investigated whether the neuroendocrine consequence of cannabinoid administration on plasma corticosterone levels contributes to the environmentally sensitive effects of systemically administered WIN55,212-2 on object recognition memory. We first investigated whether WIN55,212-2 administration after object recognition training differentially affected the release of endogenous corticosterone in rats in the two habituation conditions. Further, to assess

whether this cannabinoid-induced shaping of the corticosterone response plays a role in regulating the memory-modulatory influence of WIN55, 212-2, we examined whether pharmacological suppression of corticosterone synthesis with metyrapone altered the effects of WIN55,212-2 administration on both short- and long-term retention of object recognition memory.

MATERIALS AND METHODS

Animals

Male adult Sprague–Dawley rats (350–450 g at the time of training, Charles River Laboratories, Italy) were kept individually in an air-conditioned colony room (temperature: $21 \pm 1^\circ\text{C}$; relative humidity: $60 \pm 10\%$; lights on from 0700 to 1900 hours). Pellet food and water were available *ad libitum*. Training and testing were performed during the light phase of the cycle between 1000 and 1400 hours. All procedures involving animal care and treatments were in accordance with the guidelines released by the Italian Ministry of Health (D.L. 116/92) and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drug Treatment

The cannabinoid receptor agonist WIN55,212-2 (R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl) methyl] pyrrolol [1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl) methanone mesylate; 0.1, 0.3, and 1.0 mg/kg; Sigma-Aldrich) was administered intraperitoneally in a volume of 1.0 ml/kg immediately after the training trial. Prior findings indicate that WIN55,212-2, after intraperitoneal administration, crosses the blood–brain barrier (Saghafi *et al*, 2011). For adrenocortical suppression, the 11β -hydroxylase inhibitor metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone; 35 mg/kg; Sigma-Aldrich) was injected in a volume of 2.0 ml/kg 40 min before the training trial. Metyrapone is a selective inhibitor of glucocorticoid synthesis in animals and humans (Schimmer and Parker, 2001). It blocks the conversion of the corticosterone precursor deoxycorticosterone in the adrenal cortex, thereby preventing the release of endogenous corticosterone into the bloodstream (Strashimirov and Bohus, 1966). All drugs were dissolved in 5% polyethylene glycol, 5% Tween-80, and 90% saline. The vehicle solution contained 5% polyethylene glycol and 5% Tween-80 in saline only.

Object Recognition Task

The experimental apparatus was a gray open-field box (in cm, 40 wide \times 40 deep \times 40 high) with the floor covered with sawdust, positioned in a dimly illuminated room. The objects to be discriminated were transparent glass vials (5.5 cm diameter and 5 cm height) and white glass light bulbs (6 cm diameter and 11 cm length).

All rats were handled twice per day for 1 min each for 7 days preceding the training day. The rats were divided into two groups. One group of rats was not habituated to the experimental apparatus (WITHOUT-habituation condition), whereas the other group was extensively habituated to the experimental context (WITH-habituation condition)

to decrease their novelty stress to the apparatus during the training trial. During habituation, the rats were allowed to explore the apparatus in the absence of objects twice per day for 3 min each for 7 days (Okuda *et al*, 2004).

On the training trial, each rat was individually placed in the experimental apparatus at the opposite end from the objects. The rat was allowed to explore two identical objects (A1 and A2) for 3 min, then was removed from the apparatus and, after drug treatment, returned to its home cage. To avoid the presence of olfactory trails, sawdust was stirred and the objects were cleaned with 70% ethanol after each trial. Rat's behavior was recorded by using a video camera positioned above the experimental apparatus. Exploration of an object was defined as pointing the nose to the object at a distance of <1 cm and/or touching it with the nose. Turning around or sitting on an object was not considered as exploration. The time spent exploring the two objects was taken as a measure of object exploration, and exploratory behavior of the experimental apparatus was analyzed by the total number of rearings and crossings. For crossings, the floor of the apparatus was divided into four imaginary squares and the total number of crossings between squares was determined. Retention was tested either 1 or 24 h after the training trial. On the retention test trial, one copy of the familiar object (A3) and a new object (B) were placed in the same location as stimuli during the training trial. All combinations and locations of objects were used to reduce potential biases due to preference for particular locations or objects. Each rat was placed in the apparatus for 3 min, and its behavior was recorded. Videos were analyzed by a trained observer who was unaware of treatment condition. The time spent exploring each object and the total time spent exploring both objects were recorded. To analyze cognitive performance, a discrimination index was calculated as the difference in time exploring the novel and the familiar object, expressed as the percentage ratio of the total time spent exploring both objects.

Plasma Corticosterone Levels

Corticosterone levels were determined in parallel groups of rats in the WITHOUT-habituation and WITH-habituation condition and in rats that were handled (twice per day for 7 days) but not trained. For the last experiment, the corticosterone-synthesis inhibitor metyrapone was injected 40 min prior to the training trial. As novelty stimulation triggers an HPA-axis response that leads to a corticosterone plasma peak at 15–30 min and returns to baseline by 60–90 min (Grotta *et al*, 1997), rats were killed 30 min after training and WIN55,212-2 administration. Trunk blood was collected after decapitation and samples were centrifuged at 1900 *g* for 20 min at 4 °C. Plasma was stored at –80 °C and analyzed for corticosterone using ELISA kits (Assay designs, Ann Arbor, MI, USA; IDS, Boldon, Tyne and Wear, UK) according to the manufacturer's instructions.

Statistics

All data are expressed as mean \pm SEM. Data were analyzed by one- or two-way ANOVA, followed by Tukey's *post hoc* comparison tests or paired or unpaired Student's *t*-tests,

when appropriate. One-sample *t*-tests were used to determine whether the discrimination index was different from zero. A probability level of <0.05 was accepted as statistically significant. Fourteen rats were removed from statistical analyses because they showed a total exploration time of <10 s on either training or testing. Prior findings indicate that such rats do not adequately acquire the task (Okuda *et al*, 2004).

RESULTS

Posttraining WIN55,212-2 Induces Opposite effects on 1-h Retention of Object Recognition Memory of Rats in the WITHOUT-Habituation and WITH-Habituation Condition

This experiment investigated whether immediate posttraining injection of the cannabinoid receptor agonist WIN55,212-2 altered short-term performance on an object recognition task and whether this WIN55,212-2 effect was influenced by prior habituation to the experimental context.

Training trial. Two-way ANOVA for total exploration time of the two identical objects on the training trial revealed a significant habituation condition effect ($F_{(1,88)} = 11.46$, $P = 0.001$), but no differences between posttraining drug groups or an interaction between habituation condition and later drug treatment. Rats in the WITHOUT-habituation condition showed significantly less total exploration of the two objects than rats in the WITH-habituation condition ($t_{94} = -3.37$, $P = 0.001$; Figure 1a). In contrast, examination of rats' exploratory behavior of the training apparatus during the training trial indicated that the rats in the WITHOUT-habituation condition explored the experimental apparatus more than the rats in the WITH-habituation condition. Figure 1b and c shows that the number of crossings and rearings were significantly higher in rats in the WITHOUT-habituation condition than that in rats in the WITH-habituation condition ($t_{94} = 4.33$, $P < 0.0001$ for crossings, $t_{94} = 3.36$, $P = 0.001$ for rearings).

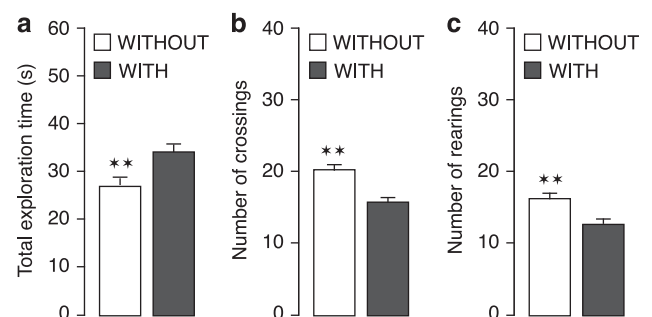


Figure 1 Behavioral effects during object recognition training in rats in the WITHOUT-habituation and WITH-habituation conditions. Rats in the WITHOUT-habituation (WITHOUT) condition spent significantly less time exploring the two identical objects during the training trial as compared with rats in the WITH-habituation (WITH) condition (a). Conversely, rats in the WITHOUT-habituation condition showed a higher number of crossings (b) and rearings (c) during training as compared with rats in the WITH-habituation condition. ** $P < 0.01$ vs the WITH-habituation group. Data are expressed as mean \pm SEM ($n = 48$ per group).

Retention trial. One-sample *t*-tests revealed that the discrimination index of vehicle-treated rats was significantly different from zero in both the WITHOUT-habituation ($t_{11} = 4.27, P = 0.001$) and WITH-habituation condition ($t_{11} = 2.15, P = 0.05$), indicating that rats in both conditions discriminated the novel object at the 1-h retention interval. As shown in Figure 2, WIN55,212-2 administered immediately after the 3-min training trial, induced opposite effects on 1-h retention performance of rats in the WITHOUT-habituation and WITH-habituation conditions. Two-way ANOVA for discrimination index revealed no main effect of either drug treatment or habituation condition, but revealed a statistically significant interaction between these two factors ($F_{(3,88)} = 8.60, P < 0.0001$). *Post hoc* analysis indicated that the 0.3 mg/kg dose of WIN55,212-2, but not lower or higher doses, significantly decreased the discrimination index of rats in the WITHOUT-habituation condition ($P < 0.05$; Figure 2a). In contrast, the same dose of WIN55,212-2 increased the discrimination index of rats in the WITH-habituation condition ($P < 0.05$; Figure 2b). Two-way ANOVA for total exploration time of the two objects during the retention trial revealed no statistically significant effects of habituation condition, drug treatment or of the interaction between these two factors. Rats' exploratory behavior of the apparatus during the test trial also did not differ between rats in the WITHOUT-habituation and WITH-habituation condition (Table 1).

Posttraining WIN55,212-2 Enhances 24-h Retention of Object Recognition Memory of Rats in the WITHOUT-Habituation but not in the WITH-Habituation Condition

This experiment examined, in separate groups of rats, whether immediate posttraining injection of WIN55,212-2 influenced long-term performance on an object recognition task and whether this WIN55,212-2 effect was also influenced by prior habituation to the experimental context.

Training trial. The pattern of effects on the training trial was highly comparable to that observed in the first experiment. Two-way ANOVA for total exploration time

of the two objects on the training trial revealed a significant habituation condition effect ($F_{(1,88)} = 4.04, P = 0.05$), but no differences between posttraining drug groups or an interaction between habituation condition and posttraining drug treatment. Rats in the WITHOUT-habituation condition explored the two objects significantly less (23.7 ± 1.3 s) than did rats in the WITH-habituation condition (28.2 ± 1.9 s) ($t_{94} = -2.05, P < 0.05$). Also, as found in the first experiment, rats in the WITHOUT-habituation condition showed significantly more exploration of the experimental apparatus than did rats in the WITH-habituation condition, as indicated by a higher number of crossings ($t_{94} = 2.89, P = 0.005$) and rearings ($t_{94} = 2.92, P = 0.004$).

Retention trial. As expected, after a 3-min training trial, rats of both vehicle groups did not express long-term retention of the familiar object: One-sample *t*-tests revealed no preference for the novel object in vehicle-treated rats in either the WITHOUT-habituation or WITH-habituation condition. As shown in Figure 3, posttraining WIN55,212-2 induced different effects on object recognition memory of rats in the WITHOUT-habituation and WITH-habituation groups at the 24-h retention interval. Two-way ANOVA for discrimination index indicated no main effect of either drug treatment or habituation condition, but revealed a statistically significant drug treatment \times habituation condition interaction ($F_{(3,88)} = 5.65, P = 0.001$). WIN55,212-2 improved 24-h retention of rats in the WITHOUT-habituation condition in an inverted U-shape relationship ($F_{(3,49)} = 3.81, P = 0.02$; Figure 3a), without significantly affecting 24-h retention of rats in the WITH-habituation condition ($F_{(3,39)} = 2.49, P = 0.07$ Figure 3b). *Post hoc* analysis indicated that the 0.3 mg/kg dose of WIN55,212-2, but not lower or higher doses, administered to rats in the WITHOUT-habituation condition increased the discrimination index as compared with vehicle controls ($P < 0.05$). Two-way ANOVA for total exploration time of the two objects during the retention trial revealed a significant habituation

Table 1 Exploratory Behavior of Rats in the WITHOUT- and WITH-Habituation Conditions at the 1-h Retention Test

	Total object exploration time (s)	Number of crossings	Number of rearings
<i>WITHOUT</i>			
Vehicle	28.2 \pm 3.3	14.0 \pm 1.3	17.5 \pm 2.2
WIN 0.1	21.3 \pm 2.0	13.5 \pm 1.2	15.5 \pm 2.0
WIN 0.3	23.2 \pm 1.8	11.0 \pm 1.5	15.9 \pm 3.2
WIN 1.0	22.8 \pm 3.7	13.4 \pm 2.4	14.9 \pm 3.0
<i>WITH</i>			
Vehicle	25.9 \pm 3.3	15.0 \pm 1.9	15.9 \pm 1.5
WIN 0.1	25.2 \pm 3.3	14.6 \pm 1.5	19.9 \pm 3.2
WIN 0.3	30.8 \pm 4.0	14.3 \pm 1.7	15.3 \pm 1.7
WIN 1.0	30.9 \pm 3.9	12.6 \pm 1.5	15.1 \pm 2.2

Total time spent exploring the two objects (in seconds) and the number of crossings and rearings of all groups in the WITHOUT- and WITH-habituation conditions. Results are expressed as mean \pm SEM ($n = 12$ per group).

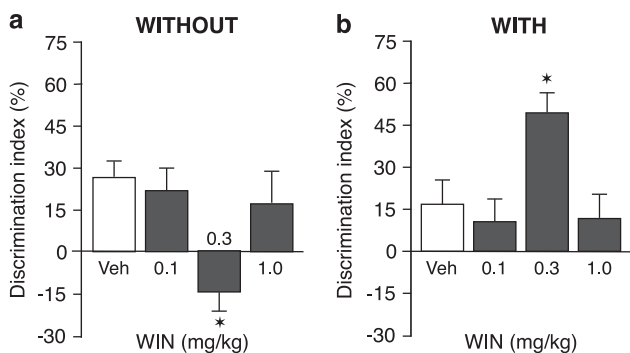


Figure 2 Effect of posttraining administration of WIN55,212-2 on 1-h retention of rats in the WITHOUT- and WITH-habituation conditions. Posttraining administration of WIN55,212-2 (WIN, 0.3 mg/kg, i.p.) impaired 1-h retention of object recognition memory of rats in the WITHOUT-habituation condition (a) but enhanced 1-h retention of rats in the WITH-habituation condition (b). * $P < 0.05$ vs the corresponding vehicle control group. Data are expressed as mean \pm SEM ($n = 12$ per group).

condition effect ($F_{(1,88)} = 6.67$, $P = 0.01$), but no drug treatment effect or interaction between both factors (Table 2). Comparable to the findings of the first experiment, rats' exploratory behavior of the experimental apparatus during the retention test trial did not differ between rats in the WITHOUT-habituation and the WITH-habituation conditions (Table 2).

Posttraining WIN55,212-2 Induces Opposite Effects on Plasma Corticosterone Levels in Rats in the WITHOUT-Habituation and WITH-Habituation Condition

Figure 4 shows plasma corticosterone levels of parallel groups of trained rats in the WITHOUT-habituation or WITH-habituation condition, as assessed 30 min after the training trial and WIN55,212-2 injection. Another group of

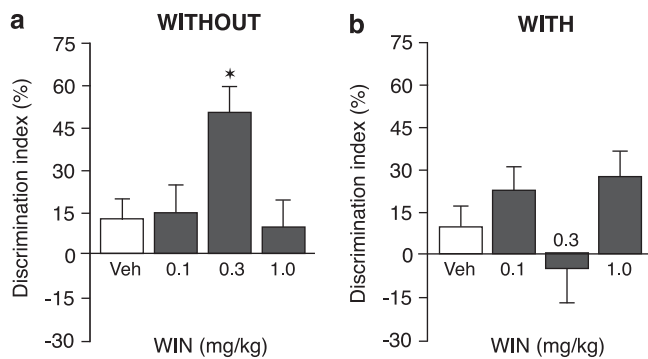


Figure 3 Effect of posttraining administration of WIN55,212-2 on 24-h retention of rats in the WITHOUT- and WITH-habituation conditions. Posttraining administration of WIN55,212-2 (WIN, 0.3 mg/kg, i.p.) enhanced 24-h retention of object recognition memory of rats in the WITHOUT-habituation condition (a) and did not significantly affect 24-h retention of rats in the WITH-habituation condition (b). * $P < 0.05$ vs the corresponding vehicle control group. Data are expressed as mean \pm SEM ($n = 10$ –14 per group).

Table 2 Exploratory Behavior of Rats in the WITHOUT- and WITH-Habituation Conditions at the 24-h Retention Test

	Total object exploration time (s)	Number of crossings	Number of rearings
<i>WITHOUT</i>			
Vehicle	18.0 \pm 1.7	13.2 \pm 1.4	12.3 \pm 1.2
WIN 0.1	19.9 \pm 2.1	13.4 \pm 1.2	12.2 \pm 0.9
WIN 0.3	15.5 \pm 0.7	13.8 \pm 1.6	14.3 \pm 1.1
WIN 1.0	22.6 \pm 2.3	10.5 \pm 0.9	11.8 \pm 1.2
<i>WITH</i>			
Vehicle	25.0 \pm 3.8	13.9 \pm 1.9	12.9 \pm 1.2
WIN 0.1	18.8 \pm 1.7	11.0 \pm 1.1	11.1 \pm 1.5
WIN 0.3	26.0 \pm 3.9	12.9 \pm 2.6	13.3 \pm 2.6
WIN 1.0	24.1 \pm 2.3	10.3 \pm 1.1	12.3 \pm 2.2

Total time spent exploring the two objects (in seconds) and the number of crossings and rearings of all groups in the WITHOUT- and WITH-habituation conditions. Results are expressed as mean \pm SEM ($n = 10$ –14 per group).

rats was administered WIN55,212-2, but was only handled and not trained (home cage). Concerning plasma corticosterone levels in rats treated with vehicle only, one-way ANOVA revealed a significant habituation condition effect ($F_{(2,14)} = 30.75$, $P < 0.0001$). *Post hoc* comparison tests showed that plasma corticosterone levels in rats in the WITHOUT-habituation condition treated with vehicle were significantly higher than those in vehicle-injected rats in the WITH-habituation condition ($P < 0.05$), supporting the view that the habituation procedure efficiently suppressed the level of emotional arousal during the training trial. Further, plasma corticosterone levels in vehicle-treated trained rats in both the WITHOUT- and WITH-habituation conditions were significantly higher than those in non-trained vehicle-treated rats ($P < 0.01$ for both comparisons).

WIN55,212-2 induced opposite effects on plasma corticosterone levels in rats after object recognition training, depending on the level of emotional arousal at the time of drug administration. Two-way ANOVA revealed a significant habituation condition effect ($F_{(2,56)} = 41.19$, $P < 0.0001$), drug treatment effect ($F_{(3,56)} = 9.58$, $P < 0.0001$) and interaction between both factors ($F_{(6,56)} = 4.78$, $P = 0.0005$). *Post hoc* analysis indicated that the 1.0 mg/kg dose of WIN55,212-2 elevated plasma corticosterone levels in rats in the WITHOUT-habituation condition ($P < 0.01$). In contrast, the 0.3 mg/kg dose of WIN55,212-2, but not any of the other doses, decreased plasma corticosterone levels when administered to rats in the WITH-habituation condition ($P < 0.05$). WIN55,212-2 administration did not significantly alter plasma corticosterone levels in non-trained control rats.

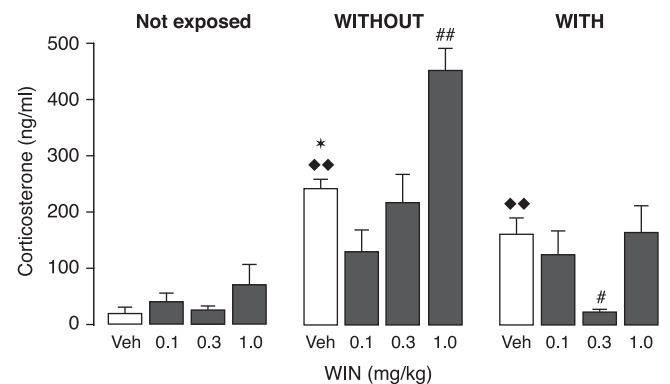


Figure 4 Effect of object recognition training and posttraining administration of WIN55,212-2 on plasma corticosterone levels in rats in the WITHOUT- and WITH-habituation conditions. Plasma corticosterone levels in rats in the WITHOUT-habituation condition treated with vehicle posttraining were significantly higher than those in vehicle-injected rats in the WITH-habituation condition, as assessed 30 min after training; * $P < 0.05$ vs vehicle-treated rats in the WITH-habituation condition. Further, plasma corticosterone levels in vehicle-treated trained rats in both the WITHOUT- and WITH-habituation conditions were significantly higher than those in non-trained vehicle-treated rats; ♦♦ $P < 0.01$ vs vehicle-treated not exposed rats. WIN55,212,2 (WIN, 1 mg/kg, i.p.) administered immediately after object recognition training increased plasma corticosterone levels in rats in the WITHOUT-habituation condition; ## $P < 0.01$ vs the corresponding vehicle-treated control group. In contrast, WIN55,212-2 (0.3 mg/kg, i.p.) decreased plasma corticosterone levels in habituated rats; # $P < 0.05$ vs the corresponding vehicle-treated control group. Data are expressed as mean \pm SEM ($n = 4$ –7 per group).

Adrenocortical Suppression in Rats in the WITHOUT-Habituation Condition Modifies the Effect of Posttraining WIN55,212-2 on Short- and Long-Term Object Recognition Memory

The findings described above indicate that WIN55,212-2 administered to rats in the two habituation conditions not only induces opposite effects on short- and long-term retention of object recognition memory but also on plasma corticosterone levels. To determine whether the WIN55,212-2 effect on the corticosterone response contributes to how WIN55,212-2 influences object recognition memory, in the last experiment we investigated whether pharmacological suppression of adrenocortical activity with metyrapone (35 mg/kg, i.p.), administered to non-habituated rats 40 min prior to the training trial, altered the effects of posttraining WIN55,212-2 administration on short- and long-term object recognition memory.

Training trial. Two-way ANOVA for total exploration time of the two identical objects on the training trial revealed no significant metyrapone effect, no difference between posttraining WIN55,212-2 treatment groups or interaction between these two parameters. Two-way ANOVAs for the number of crossings and rearings on the training trial also did not reveal any significant metyrapone or later WIN55,212-2 treatment effect.

One-hour retention. As shown in Figure 5a, pretreatment of rats in the WITHOUT-habituation condition with metyrapone transformed the effect of posttraining WIN55,212-2 administration on 1-h retention performance into that of rats in the WITH-habituation condition. Two-way ANOVA for discrimination index indicated no main effect of metyrapone or WIN55,212-2 treatment, but revealed a significant metyrapone \times WIN55,212-2 interaction effect ($F_{(1,35)} = 11.83$, $P = 0.02$). Comparable to the

findings shown in Figure 2a, WIN55,212-2 (0.3 mg/kg) administered alone to non-habituated rats immediately after the training trial significantly decreased the discrimination index relative to that of vehicle-treated rats ($P < 0.05$). However, in non-habituated rats pretreated with metyrapone, the same dose of WIN55,212-2 significantly increased the discrimination index ($P < 0.05$ vs vehicle) and thus highly resembled the WIN55,212-2 effect on 1-h retention as described above for rats in the WITH-habituation condition (see, Figure 2b). Metyrapone pretreatment by itself did not significantly alter the discrimination index. Total exploration time of the two objects and rats' exploratory behavior of the apparatus on the 1-h retention test did not differ between drug treatment groups (Table 3).

Twenty-four-hour retention. As shown in Figure 5b, pretreatment of non-habituated rats with metyrapone also transformed the effect of posttraining WIN55,212-2 on 24-h retention performance into that of rats in the WITH-habituation condition. Two-way ANOVA for discrimination index indicated no main effect of either metyrapone or WIN55,212-2 treatment, but revealed a significant metyrapone \times WIN55,212-2 interaction effect ($F_{(1,40)} = 4.94$, $P = 0.03$). Comparable to the findings shown in Figure 3a, WIN55,212-2 (0.3 mg/kg) administration alone significantly increased the discrimination index of rats in the WITH-OUT-habituation condition ($P < 0.01$). However, in rats pretreated with metyrapone, posttraining WIN55,212-2 administration did not significantly alter retention performance on the 24-h test trial, thus resembling the findings described above for rats in the WITH-habituation condition (see, Figure 3b). Metyrapone pretreatment alone did not significantly alter the discrimination index. Total exploration time of the two objects during the 24-h retention trial and rats' exploratory behavior of the apparatus did not differ between drug treatment groups (Table 3).

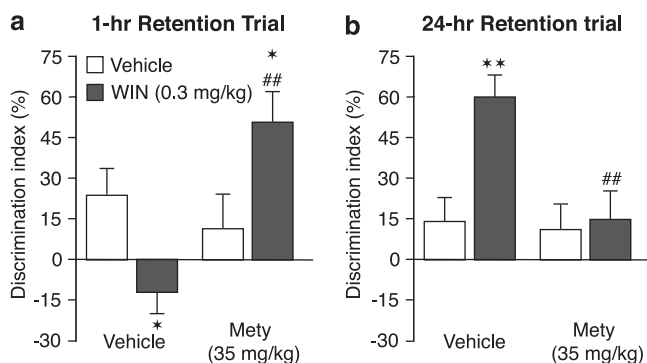


Figure 5 Effect of pretreatment with metyrapone on the effect of posttraining administration of WIN55,212-2 on 1- and 24-h retention of rats in the WITHOUT-habituation condition. Metyrapone (Mety, 35 mg/kg, i.p.) administered to rats in the WITHOUT-habituation condition 40 min before object recognition training reverted the impairing effect of posttraining WIN55,212-2 (WIN, 0.3 mg/kg, i.p.) on 1-h retention (a) and the enhancing effect of WIN55,212-2 (0.3 mg/kg, i.p.) on 24-h retention (b). Both 1- and 24-h retention performance became very similar to those observed in low-aroused rats in the WITH-habituation condition (see Figures 2b and 3b). * $P < 0.05$; ** $P < 0.01$ vs the corresponding vehicle group, ### $P < 0.01$ vs the WIN55,212-2 alone group. Data are expressed as mean \pm SEM ($n = 9$ –12 per group).

Table 3 Exploratory Behavior of Rats in the WITHOUT-Habituation Condition Injected with Metyrapone and WIN55,212-2 at the 1- and 24-h Retention Tests

	Treatment		Total object exploration time (s)	Number of crossings	Number of rearings
1-h Retention trial	Vehicle	Vehicle	24.9 \pm 2.3	12.7 \pm 1.5	15.9 \pm 3.0
	Vehicle	WIN 0.3	23.1 \pm 1.8	11.6 \pm 1.5	14.9 \pm 3.6
	Mety 35	Vehicle	19.3 \pm 2.2	11.0 \pm 1.8	17.4 \pm 2.8
	Mety 35	WIN 0.3	21.4 \pm 3.0	11.1 \pm 2.3	13.5 \pm 3.0
24-h Retention trial	Vehicle	Vehicle	21.3 \pm 3.5	14.5 \pm 1.5	8.3 \pm 0.9
	Vehicle	WIN 0.3	16.5 \pm 0.8	15.5 \pm 0.8	12.1 \pm 1.2
	Mety 35	Vehicle	25.4 \pm 3.0	15.3 \pm 2.3	12.1 \pm 1.9
	Mety 35	WIN 0.3	20.3 \pm 3.1	14.2 \pm 2.4	11.6 \pm 2.5

Total time spent exploring the two objects (in seconds) and the number of crossings and rearings of rats in the WITHOUT-habituation condition administered metyrapone (Mety, mg/kg, i.p.) 40 min prior to training and WIN55,212-2 (WIN, mg/kg, i.p.) immediately posttraining. Results are expressed as mean \pm SEM ($n = 10$ –12 per group).

Plasma corticosterone levels. Table 4 shows the effect of metyrapone pretreatment and posttraining WIN55,212-2 administration on plasma corticosterone levels in rats in the WITHOUT-habituation condition, as assessed 30 min after object recognition training and WIN55,212-2 administration. Rats treated with metyrapone 40 min prior to the training trial had significantly lower plasma corticosterone levels than rats administered vehicle before training ($t_8 = 3.44$, $P = 0.009$). In rats pretreated with metyrapone, posttraining WIN55,212-2 administration did not significantly elevate plasma corticosterone levels.

DISCUSSION

Using a previously described habituation procedure, we show that the synthetic cannabinoid agonist WIN55,212-2 interacts with training-associated emotional arousal in influencing both short- and long-term object recognition memory. Furthermore, our findings indicate that the cannabinoid system shapes the response of the HPA-axis to emotional arousal and that this neuroendocrine action is importantly involved in determining how WIN55,212-2 might affect object recognition memory. WIN55,212-2, administered systemically immediately after object recognition training, biphasically impaired retention at a 1-h interval in rats that were not habituated to the experimental context. In contrast, the same dose of WIN55,212-2 enhanced 1-h retention of rats that had reduced novelty-induced emotional arousal because of extensive prior habituation to the experimental context. Additionally, immediate posttraining administration of WIN55,212-2 to non-habituated rats, in a dose that impaired 1-h retention, enhanced object recognition performance at a 24-h interval. Posttraining WIN55,212-2 administration did not significantly affect 24-h retention of habituated rats. This habituation procedure also produced opposite cannabinoid effects on training-induced HPA-axis activity. WIN55,212-2 administration after object recognition training elevated plasma corticosterone levels in rats that were not previously habituated to the experimental context, but decreased levels in habituated rats. Most importantly, as non-habituated rats administered the corticosterone-synthesis inhibitor metyrapone prior to training and WIN55,212-2

injection performed equally to extensively habituated rats on short- and long-term retention tests, these findings indicate that cannabinoid-induced regulation of adrenocortical activity contributes to the environmentally sensitive effects of systemically administered cannabinoids on short- and long-term retention of object recognition memory. Table 5 summarizes our findings of WIN55,212-2 on both short- and long-term recognition memory and on adrenocortical activity.

Clinical evidence indicates that cannabis use, either chronic consumption or acute intoxication, can impair both short-term memory and executive function (Pattij *et al*, 2008). Although some preclinical studies were able to confirm these findings (Baek *et al*, 2009; Kosiorek *et al*, 2003), the literature appears highly ambiguous (Clarke *et al*, 2008; Suenaga and Ichitani, 2008). In a previous study, we demonstrated that pretraining enhancement of endocannabinoid tone biphasically modified short-term retention of a recognition task in a spatial open field, and that this effect was likely influenced by the emotional state of the rat during the training trial (Campolongo *et al*, 2012). Here, we employed posttraining drug administration in order to more properly investigate the cognitive processes involved and found that the level of emotional arousal at encoding influenced the outcome of later administration of the cannabinoid agonist WIN55,212-2 on both short- and long-term retention of object recognition memory. As vehicle-control rats in both habituation conditions showed a similar preference for the novel object at the 1-h retention interval, it is unlikely that any difference in acquisition (ie, total exploration of the objects during the training trial) between the two groups underlies the differential effects. It seems most likely that a difference in the arousal level induced by the habituation procedure was critical. The finding that non-habituated rats displayed significantly higher levels of locomotion and rearing behavior during the training trial than habituated rats is consistent with previous evidence that exposure of rats to novel contexts induces changes in behavioral responses, including hyperlocomotion and increased rearing behavior (Okuda *et al*, 2004; van den Buuse *et al*, 2001). Novelty-induced arousal also activates stress hormone systems, including glucocorticoids (Emmert and Herman, 1999; Handa *et al*, 1994) and epinephrine (Feenstra *et al*, 2000; McQuade *et al*, 1999). Corroborating previous findings by Okuda *et al* (2004), we found that non-habituated rats had a significantly more

Table 4 Plasma Corticosterone Levels in Rats in the WITHOUT-Habituation Condition Injected with Metyrapone and WIN55,212-2

Mety	WIN	Cort, ng/ml
Vehicle	Vehicle	245.3 ± 12.7
Mety 35	Vehicle	158.1 ± 22.0 **
Mety 35	WIN 0.1	190.0 ± 47.8
Mety 35	WIN 0.3	222.8 ± 40.4
Mety 35	WIN 1.0	180.1 ± 20.3

Plasma corticosterone (Cort) levels (mean ± SEM) in ng/ml in rats in the WITHOUT-habituation condition as assessed 30 min after the training trial. Metyrapone (Mety, mg/kg, i.p.), injected 40 min prior to training, reduced training-induced plasma corticosterone levels and prevented the increase in corticosterone plasma levels induced by posttraining WIN55,212-2 (WIN, mg/kg, i.p.) administration. ** $P < 0.01$ vs the vehicle control group ($n = 4-5$ per group).

Table 5 Schematic Representation of the Effects of Posttraining WIN55,212-2 on Recognition Memory

Habituation condition	Treatment	Short-term memory effect	Long-term memory effect
WITHOUT	WIN	↓	↑
WITH	WIN	↑	—
WITHOUT	WIN + Mety	↑	—

Schematic representation of the effects of posttraining WIN55,212-2 (WIN, 0.3 mg/kg, i.p.) either alone or together with the corticosterone-synthesis inhibitor metyrapone (Mety, 35 mg/kg, i.p.) on 1- and 24-h retention performance in rats in the WITHOUT-habituation and WITH-habituation conditions.

pronounced training-induced increase in plasma corticosterone levels than habituated rats. Although these behavioral and endocrine measures clearly indicate that our habituation procedure efficiently reduced the level of emotional arousal during the training session, we cannot exclude the possibility that it is the stress history and not the level of emotional arousal *per se* that may be influencing cannabinoid effects on memory. Repeated exposure to the testing arena might be mildly stressful to the animals. Prior evidence indicated that a history of repeated (restraint) stress reduces CB1 receptor function on GABAergic terminals within several brain regions involved in learning and memory (Hu *et al*, 2011; Patel *et al*, 2009). As such, the divergent effects of WIN55,212-2 on short-term retention of rats in the WITHOUT-habituation and WITH-habituation groups may be due to a different ability of CB1 receptor activation to suppress GABAergic inhibitory control because of the stress history of the animals and not the immediate levels of arousal during the training session. In both scenarios, however, our findings show that the cannabinoid system exerts an environmentally sensitive modulation of short-term memory. As WIN55,212-2 levels are probably still elevated at the time of the 1-h retention test, it is most likely that WIN55,212-2 affected short-term retention performance via direct influences on the retrieval of memory processing (Atsak *et al*, 2012a).

Whereas clinical studies mainly focused on the potentially disruptive effects of cannabinoid drugs on short-term cognitive performance, findings of animal experiments provide extensive evidence that cannabinoid treatments also influence long-term memory. However, findings of these experiments have been conflicting as well. Some studies reported that cannabinoid agonists impair the consolidation of long-term memory of training on several tasks (Barros *et al*, 2004; Robinson *et al*, 2008), whereas others found enhancing effects (De Oliveira Alvares *et al*, 2008). Differences in dosage or drug administration regimen (eg, pretraining *vs* posttraining administration) could have contributed to these opposite findings. Here we show that, similar to WIN55,212-2 effects on short-term memory, the level of emotional arousal at encoding, or alternatively the stress history of the animals, is another important factor modulating cannabinoid effect on long-term memory. WIN55,212-2 enhanced 24-h retention of rats that were not previously habituated to the experimental context, but failed to significantly alter performance of well-habituated rats. As WIN55,212-2 was administered immediately after the training trial, the effect of WIN55,212-2 on long-term retention is likely mediated by a selective influence on the consolidation of memory (McGaugh and Roozendaal, 2002). Moreover, because retention testing took place 24 h after the training and drug treatment, it is unlikely that the effects are mediated by any residual pharmacological effects on directly influencing behavior during retention testing (Cahill *et al*, 2001).

Clinical evidence supports the view that cannabis consumption can induce opposite effects on a variety of behaviors and subjective feelings in different individuals or even in the same subject (Green *et al*, 2003). Apart from genetic vulnerability (Xian *et al*, 2008), environmental factors such as stress-induced alterations could contribute to these differential effects (Viveros *et al*, 2012; Xian *et al*,

2008). Preclinical models have confirmed opposite effects of cannabinoid drugs on motor activity (Fonseca *et al*, 1998), positive incentive and/or motivational processes (Chaperon and Thiebot, 1999), anxiety (Haller *et al*, 2009) and fear-related behaviors (Moreira and Wotjak, 2011). Although differences in dose, route of administration, and timing of exposure are typically listed as being responsible for the opposite behavioral effects, recent findings indicate that variations in the stressfulness of the experimental conditions employed in the different studies are implicated as well. The general assumption that enhancement of cannabinoid levels via an inhibition of the anandamide-degrading enzyme fatty acid amide hydrolase (FAAH) results in anxiolytic- and antidepressant-like effects in rodents (Gobbi *et al*, 2005; Kathuria *et al*, 2003) has recently been revisited. Haller *et al* (2009) found robust anxiolytic-like effects of the FAAH inhibitor URB597 when rats were tested without prior habituation to the experimental room. However, URB597 administration did not induce any anxiolytic-like effect in rats that were habituated to the testing environment. Similarly, both low and high doses of delta9-tetrahydrocannabinol, the main psychoactive constituent of cannabis preparations, exerted anxiolytic-like effects in non-stressed animals (Fokos and Panagis, 2010). In stressed animals, however, only the higher dose of delta9-tetrahydrocannabinol induced an anxiolytic-like response whereas the lower dose induced an anxiogenic effect. Our present findings are thus very reminiscent of these other behaviors and indicate that the level of emotional arousal that is associated with the training procedure is also a key regulator of cannabinoid effects on both short- and long-term recognition memory.

Considering the extensive evidence indicating a close relationship between the cannabinoid system and HPA-axis activity (Armario, 2010; Barna *et al*, 2004), we explored the possibility that the divergent effects of systemic WIN55,212-2 administration on object recognition memory might be related to differential effects of WIN55,212-2 on training-induced glucocorticoid levels in rats in these two habituation conditions. Our findings indicate that WIN55,212-2 administration produced opposite effects on training-induced plasma corticosterone levels in habituated *vs* non-habituated rats. Generally, these findings are thus consistent with the evidence that the cannabinoid system is an important regulator of HPA-axis activity and consequently of the release of glucocorticoid hormones (Cota *et al*, 2007; Ganon-Elazar and Akirav, 2009). Our finding that WIN55,212-2 induced opposite effects on HPA-axis activity in rats in the two habituation conditions has a twofold interest. First, these findings might help to reconcile earlier observations that cannabinoid administration can both activate and inhibit the HPA-axis (Cota *et al*, 2007; Ganon-Elazar *et al*, 2009). Second, as extensive evidence indicates that glucocorticoid hormones influence both short- and long-term memory of emotionally arousing experiences (Roozendaal *et al*, 2009; Schwabe *et al*, 2012), these findings suggest the intriguing possibility that this arousal-dependent influence of WIN55,212-2 on the HPA-axis might contribute to the observed opposite effects of WIN55,212-2 on both short- and long-term memory. Highly comparable to our present findings with WIN55,212-2, it has been previously reported that corticosterone

administration after object recognition training impaired short-term performance in emotionally aroused non-habituated rats (Okuda *et al*, 2004; Roozendaal *et al*, 2006b). Moreover, corticosterone enhanced 24-h retention performance of rats that were not previously habituated to the experimental context. Corticosterone administration to well-habituated rats failed to affect either short- or long-term object recognition performance. Even though the dose of WIN55,212-2 that affected memory function in the non-habituated rats did not overlap entirely with its efficacious dose on corticosterone levels, our assumption was confirmed by the finding that adrenocortical suppression with the corticosterone-synthesis inhibitor metyrapone in non-habituated rats altered the effect of posttraining WIN55,212-2 administration on both short- and long-term recognition memory in such a way that performance became similar to that seen in habituated animals. Thus, these findings provide strong support for the view that a WIN55,212-2-induced potentiation of glucocorticoid secretion plays an important role in determining the pattern of cannabinoid effects on memory in these animals.

Previously, we showed that the cannabinoid and glucocorticoid effects on memory might converge within the basolateral amygdala (BLA). The possible interaction of these two systems was originally investigated for memory consolidation of inhibitory avoidance training (Campolongo *et al*, 2009) but similar mechanisms might exist in other brain regions for regulating other memory functions (Atsak *et al*, 2012a). Extensive evidence indicates that the BLA preferentially modulates memory of emotionally arousing training experiences (McGaugh, 2000) and that arousal-induced activation of noradrenergic signaling plays an important role herein (Quirarte *et al*, 1997; Roozendaal *et al*, 2002, 2004, 2006a). In prior studies, we showed that training-induced noradrenergic activity within the BLA is required for enabling the modulatory influence of glucocorticoids on memory (Quirarte *et al*, 1997). As habituation to the object recognition apparatus is known to attenuate training-induced increases in noradrenergic activity (Roozendaal *et al*, 2006b), we previously hypothesized that glucocorticoids might not modulate memory of object recognition training in habituated rats because of inadequate levels of arousal-induced norepinephrine. In support of this interpretation, we found that stimulating noradrenergic activity with the α_2 -adrenoceptor antagonist yohimbine was sufficient to enable glucocorticoid-induced memory enhancement in habituated rats (Roozendaal *et al*, 2006b). Recent findings indicate that the endocannabinoid system within the BLA might be importantly involved in regulating glucocorticoid effects on memory (Campolongo *et al*, 2009). Systemically administered corticosterone rapidly elevates endocannabinoid levels in the amygdala (Hill *et al*, 2010a), whereas a blockade of CB1 receptor activity in the BLA prevents corticosterone-induced memory enhancement (Campolongo *et al*, 2009). On the basis of these findings, we previously proposed a model in which glucocorticoids bind to a membrane-bound receptor in the BLA that activates a G-protein signaling cascade to stimulate the synthesis of endocannabinoids. The ensuing release of endocannabinoid ligands could diffuse to local GABAergic terminals and inhibit GABA release onto noradrenergic terminals in the BLA (Campolongo *et al*,

2009; Hill and McEwen, 2009). Importantly, as recent findings indicate that the β -adrenoceptor antagonist propranolol infused into the BLA also prevented the memory-enhancing effect of WIN55,212-2 (Hauer *et al*, 2010), it is thus possible that, comparable to the effects of glucocorticoids, cannabinoid effects on memory are also dependent on the availability of sufficient levels of arousal-induced noradrenergic activity within the BLA. On top of this direct interaction of WIN55,212-2 with arousal-induced noradrenergic mechanisms within the brain, the opposite effects of WIN55,212-2 on circulating corticosterone levels might add to the environmentally sensitive effects of cannabinoids on both short- and long-term recognition memory.

In summary, we provide evidence that cannabinoid effects on short- and long-term retention of recognition memory depend on the level of novelty-induced emotional arousal. These cannabinoid effects likely involve, at least in part, peripheral actions on modulating HPA-axis activity. These findings shed light on the contrasting effects of cannabinoid drugs on memory processing, thus providing new evidence that cannabinoid compounds can be either beneficial or detrimental to memory processes depending on the affective state of the individual at the time of drug consumption.

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DISCLOSURE

The authors declare no conflict of interest.

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