Cannabis has long been known to produce cognitive and emotional effects. Research has shown that cannabinoid drugs produce these effects by driving the brain's endogenous cannabinoid system and that this system plays a modulatory role in many cognitive and emotional processes. This review focuses on the effects of endocannabinoid system modulation in animal models of cognition (learning and memory) and emotion (anxiety and depression). We review studies in which natural or synthetic cannabinoid agonists were administered to directly stimulate cannabinoid receptors or, conversely, where cannabinoid antagonists were administered to inhibit the activity of cannabinoid receptors. In addition, studies are reviewed that involved genetic disruption of cannabinoid receptors or genetic or pharmacological manipulation of the endocannabinoid-degrading enzyme, fatty acid amide hydrolase (FAAH). Endocannabinoids affect the function of many neurotransmitter systems, some of which play opposing roles. The diversity of cannabinoid roles and the complexity of task-dependent activation of neuronal circuits may lead to the effects of endocannabinoid system modulation being strongly dependent on environmental conditions. Recent findings are reviewed that raise the possibility that endocannabinoid signaling may change the impact of environmental influences on emotional and cognitive behavior rather than selectively affecting any specific behavior.

**Keywords:** endocannabinoids, cognition, anxiety, depression, learning, memory, animal models

For example, if blocking cannabinoid CB1 receptors with an antagonist such as rimonabant prevents the treatment from having a certain effect, that effect of the treatment is said to be mediated by CB1 receptors. (3) Ongoing endocannabinoid signaling can be blocked by administering a cannabinoid receptor antagonist alone. When endocannabinoid signaling is blocked, behaviors that are modulated by this signaling should increases or decrease, depending on whether the modulation is negative or positive. This approach assumes that the antagonist blocks endogenously released endocannabinoids, but does not otherwise affect signaling. Unfortunately, the antagonists that have been used most frequently for this purpose (rimonabant and AM251) also function as inverse agonists and may affect neuronal functions even in the absence of the release of endocannabinoid agonists. (4) Endocannabinoid signaling can be enhanced by administering an enzyme inhibitor that prevents the breakdown of endocannabinoids that have been released. Endocannabinoids are synthesized “on demand” when synaptic neurotransmission surpasses a certain threshold. Treatments that prevent the breakdown of endocannabinoids should mainly affect cells in the immediate areas where an endocannabinoid is being released. In contrast, exogenous agonists affect synapses wherever the receptors are expressed. Thus, treatments that prevent...
endocannabinoid breakdown should magnify the ongoing effects of endocannabinoids and might provide better insight into normal function. Anandamide, the most frequently studied endocannabinoid, is degraded by the enzyme fatty acid amid hydrolase (FAAH). Inhibitors of FAAH first became available about 7 years ago (e.g., Tarzia et al., 2003; Mor et al., 2004), and there is now a large amount of information concerning the effects of FAAH inhibitors on cognitive and emotional behavior. Inhibitors of the degradation of 2-AG, the other major endocannabinoid that has been identified, have also been recently developed (e.g., Long et al., 2009), but information on their cognitive or emotional impact is too scarce to be reviewed at this time.

In addition to these four pharmacological approaches, the role of endocannabinoids in cognitive and emotional processes can be investigated with genetically modified strains of rodents. This has been accomplished in two general ways: deleting a specific cannabinoid receptor subtype (i.e., CB1), which excludes cannabinoid signaling; and deleting a metabolizing enzyme (i.e., FAAH), which enhances endogenous endocannabinoid signaling.

Each of these pharmacological and genetic approaches has advantages and disadvantages. For example, manipulating FAAH affects not only endocannabinoids but related fatty acids that bind at non-cannabinoid sites, such as peroxisome proliferator-activated receptors and transient receptor-potential vanilloid receptors. When a receptor or enzyme is genetically deleted, other mechanisms may be affected by their absence. Therefore, the best understanding is gained through convergence, comparing the results obtained with different approaches.

EFFECTS OF ENDOCANNABINOID SYSTEM MODULATION ON LEARNING AND MEMORY

The memory-impairing effects of marijuana in humans have been widely recognized since at least the 1970s (Tart, 1970). Interest in the role played by endocannabinoids in cognitive processes has been stimulated by evidence that CB1 receptors are highly expressed (Herkenham et al., 1991) – and endocannabinoids (anandamide and 2-AG) occur in high concentrations (Di Marzo et al., 2000) – in the hippocampus, a brain area that plays a critical role in learning and memory. Animal models have been used extensively to assess the effects of cannabinoid manipulations on various stages of learning and memory, including acquisition, consolidation, and retrieval (Riedel and Davies, 2005; Vanvler et al., 2009). Most of these studies have involved spatial learning. In general, the findings are that exogenous and endogenous cannabinoid agonists impair working memory and the acquisition of long-term memory, while cannabinoid antagonists/inverse agonists or genetic deletion of cannabinoid receptors are sometimes found to enhance learning and memory.

Endocannabinoid signaling can affect many behavioral and physiological processes, including locomotion, feeding, anxiety, reward, and nociception. Therefore, to confidently attribute the effects of cannabinoid manipulations to learning and memory processes per se, as opposed to motivational, emotional, or motor processes, it is important to consider complementary models. For example, some memory models involve aversive motivation (e.g., escape from a water-filled pool), while others involve appetitive motivation (e.g., food-reinforced behavior in delayed matching tasks); finding similar effects of a drug in both aversive and appetitive models would suggest an effect on memory rather than motivation. It can also be informative to test the effects of a treatment in both a memory model and a more general, non-cognitive behavioral assay, such as spontaneous locomotor activity in an open field. In the following sections, we will consider the findings obtained with specific models of long-term memory (see Effects of Endocannabinoid System Modulation on Learning and Memory) and working memory (see Working Memory).

EFFECTS OF CANNABINOID CB1 RECEPTOR AGONISTS AND ANTAGONISTS ON MEMORY ACQUISITION AND LONG-TERM MEMORY

Water maze

Much of the evidence that activating cannabinoid receptors can impair learning comes from studies using water maze procedures, which focus on spatial memory. In these tests the animals are trained to find a submerged platform in a tank filled with opaque water. Memory acquisition becomes evident over trials as successive reductions in the path length or the latency to reach the platform. In mice, acute systemic administration of Δ9-THC (8 mg/kg, IP) before the training session disrupts acquisition in the water maze test without affecting locomotion; this effect is prevented by the CB1 antagonist/inverse agonist rimonabant (DaSilva and Takahashi, 2002). Deficits in place-learning have also been reported in rats treated repeatedly with Δ9-THC (Moore et al., 2010) or acutely with Δ9-THC (Diana et al., 2003) or synthetic CB1 agonists such as HU-210 (Ferrari et al., 1999), but not with the synthetic agonist nabilone (Diana et al., 2003). However, in these experiments, the effects of CB1-receptor blockade were not tested. Another synthetic cannabinoid, WIN55212-2 (1 and 3 mg/kg), has been found to impair acquisition in the water maze, but its effect was not blocked by CB1 antagonists, suggesting WIN55212-2 may impair learning by more than one mechanism (Robinson et al., 2010).

Water maze procedures have also been used to study the effects of Δ9-THC on memory retrieval. For this purpose, rats that have already reached a criterion level of performance in the task are injected with the drug prior to a test session. Two laboratories have reported that – at doses known to impair memory acquisition – Δ9-THC did not impair memory retrieval in the water maze (Mishima et al., 2001; Vanvler et al., 2001, 2007). These findings suggest that, once established, reference memory is not susceptible to modulation by cannabinoid compounds.

Contextual fear conditioning

CB1 agonists can also impair acquisition in another model of spatial memory, contextual fear conditioning. In this test, rodents are briefly exposed to footshock in a distinctive context, then tested by re-exposing them to the context. Immobility (freezing) during the test provides a measure of memory. The synthetic CB1 agonist WIN55212-2 (2.5 and 5.0 mg/kg), given 30 min before the conditioning phase, impaired acquisition of contextual fear conditioning, but not conditioning to a discrete auditory cue (tone), which unlike contextual conditioning is believed to be independent of hippocampal function (Pamplona and Takahashi, 2006).
This finding is consistent with an impairment of hippocampal functioning, since the hippocampus mediates acquisition of fear conditioning involving contextual cues but not discrete cues (Phillips and LeDoux, 1992). Rimonabant (1 mg/kg) blocked the impairing effects of WIN55212-2, demonstrating the involvement of CB1 receptors (Pamplona and Takahashi, 2006). Sink et al. (2010) showed that administration of CB1 inverse agonists during the acquisition phase improves the retention of the contextual fear, consistent with endogenous cannabinoids having a negative modulatory effect on memory acquisition.

Object recognition and social recognition

In a typical object recognition task, animals are exposed to an object during one session, and then exposed to the same object plus a novel object in a subsequent test session. The relative amount of time spent exploring the novel object provides an index of memory. Systemic or intra-hippocampal administration of Δ9-THC or WIN55212-2, either acute or repeated, impaired object recognition in rats (Barna et al., 2007; Quinn et al., 2008; Schneider et al., 2008). This impairment is associated with differential expression of proteins in the hippocampus (Quinn et al., 2008). However, in another study acute systemic administration of Δ9-THC before the task failed to affect object recognition in adult rats (Ciccocioppo et al., 2002). Enhanced memory performance was observed in CB1-knockout in this task (Maccarrone et al., 2002).

The roles of hippocampal functioning and spatial learning in the conventional object recognition procedure are still controversial (Ainge et al., 2006; Heuer and Bachevalier, 2011). It is possible to modify the procedure to focus on spatial memory by presenting objects during the exposure phase, then presenting the same objects during the test but with one placed in a different position. Suenaga and Ichitani (2008) found that microinjection of WIN55212-2 (1–2 μg/side in the hippocampus 10 min before the initial exposure to the objects) did not affect memory in the conventional procedure but impaired in a CB1 dependent fashion the ability to recognize a new spatial configuration of objects.

The social recognition test is similar to the object recognition test but uses conspecifics instead of objects as the stimuli. Using long delays (15–30 min) it has been shown that the administration of WIN55212-2 impairs the performance of rats in a CB1 dependent fashion (Schneider and Koch, 2002; Schneider et al., 2008). Rimonabant has been found to enhance recognition memory in this test (Terranova et al., 1996).

Radial maze

The effects of CB1 compounds on the acquisition and recall of spatial memory in rodents have also been studied using a modified version of the radial maze test. In the conventional version of the test a food pellet is available at the end of each of the eight arms of the maze, and re-entering the same arm more than once indicates a working-memory error. In the modified version, to manipulate the mnemonic demand of the test, the rat is removed from the maze after it enters the seventh arm of the maze, and then it is placed back in the maze after a delay (Lichtman, 2000). With long delays, this test provides a test of long-term memory. Rimonabant (3 mg/kg), given to rats before the first placement in the maze, reduces the number of errors after a 6-h delay (Lichtman, 2000). Rimonabant had no effect when administered immediately after the first placement (Wise et al., 2007) or before the test placement (Lichtman, 2000), suggesting rimonabant enhances memory acquisition but not consolidation or retrieval. However, in other studies, the facilitating effects of CB1 antagonism have been observed not only for acquisition, but also for consolidation (Wolf and Leander, 2003; Wise et al., 2008).

Passive avoidance

Data obtained with passive-avoidance procedures suggest a modulatory action of the endocannabinoids system on all phases of memory. In a widely used, hippocampal-dependent version of this test, rodents are allowed to explore a apparatus with two compartments, one lighted and one dark (Isaacson and Wickelgren, 1962). Entrance into the dark compartment is paired with a foot shock during a training session, and increased latency to enter the dark compartment during a subsequent test session is used as an index of conditioning. Systemic injections of Δ9-THC or anandamide or intra-hippocampal injections of WIN55212-2 impair memory acquisition, consolidation, and recall in rats and mice (Castellano et al., 1997; Mishima et al., 2001; Costanzi et al., 2004; Nasehi et al., 2010). However it has been shown that the effects of anandamide on passive-avoidance performance can vary depending on the strain of the animals and on the protocol used (e.g., whether subjects are pre-exposed to the testing apparatus; Castellano et al., 1999; Costanzi et al., 2004).

Caveats

Taken together, the findings with these various animal models of long-term memory suggest a modulatory role of the endocannabinoid system during the acquisition phase of a place memory (see Table 1). Generally, CB1 agonists have been found to impair acquisition, and antagonism or deletion of CB receptors has been found to enhance it. However, there are some caveats to this conclusion. For example, neither the CB1 inverse agonist/antagonist rimonabant at different doses (1, 3 mg/kg) nor the genetic disruption of CB1 receptors facilitated acquisition in the water maze (DaSilva and Takahashi, 2002; Varvel and Lichtman, 2002; Varvel et al., 2007). Several reports have indicated that the effects of CB1 agonists are not limited to acquisition in passive avoidance and delayed radial maze procedures.

In some cases, discrepant results in models of memory may be attributable to cannabinoid effects on other processes. For example, Mikics et al. (2006) reported an enhancement of fear conditioning, rather than an impairment, after administration of WIN55212-2, and tests employing genetic disruption or pharmacological blockade of CB1 receptors indicated that this enhancement of fear conditioning was due to actions of WIN55212-2 at CB1 receptors. Although this finding is inconsistent with the more common finding that CB1 activation impairs memory acquisition, in this case it is possible that WIN55212-2 may have increased anxiety. It is possible that some of the effects of CB1 agonists on water maze behavior are due to thigmotaxis, an anxiety-related tendency to maintain close proximity to the wall of the maze. When Acheson et al. (2011) controlled for thigmotaxis, the impairing effects of WIN55212-2 were no longer detectable.

Another issue to consider is that endocannabinoid receptors localized in different brain structures may modulate distinct
Table 1 | Summary of studies investigating the effects of cannabinoid receptor agonists, cannabinoid receptor antagonists, FAAH inhibitors, or genetic deletion of cannabinoid receptors on learning and memory in rodents.

| Authors             | Animals          | Drug               | Doses and route | Test                        | Administered before | Effects on memory |
|---------------------|------------------|--------------------|-----------------|-----------------------------|----------------------|-------------------|
| Harloe et al. (2008)| C57BL/6J         | Rimonabant        | 3 mg/kg, IP     | Appetitive Barnes maze tasks | Extinction           | =                 |
|                     |                  |                    |                 | Aversive Barnes maze tasks  |                      | ↓                 |
| Pamplona and Takahashi (2006) | Wistar rat | AM404              | 10 mg/kg, IP    | Contextual fear conditioning |                      | ↑                 |
| Pamplona and Takahashi (2006) | Wistar rat | WIN55,212-2       | 0.25 mg/kg, IP  | Contextual fear conditioning |                      | ↑                 |
| Bitencourt et al. (2008) | Wistar rats | AM404              | 1.0 μg/μL, i.c.v. | Contextual fear conditioning |                      | ↑                 |
| Suzuki et al. (2004) | C57BL/6          | Rimonabant        | 1–3–10 mg/kg, IP| Contextual fear conditioning |                      | ↓                 |
| Niyuhire et al. (2007) | C57BL/6J        | Rimonabant        | 3 mg/kg, IP     | Contextual fear conditioning |                      | ↓                 |
| Pamplona and Takahashi (2006) | Wistar rat | Rimonabant        | 1 mg/kg, IP     | Contextual fear conditioning |                      | ↓                 |
| Ganon-Elazar and Akirav (2009) | Sprague-Dawley rats | WIN55,212-2   | 2.5 μg/0.5 μL, IC (basolateral amygdala) | Extinction | ↑           |
| Mikics et al. (2006) | CD1 mice         | WIN55,212-2       | 3 mg/kg, IP     | Contextual fear conditioning |                      | ↑                 |
| Mikics et al. (2006) | CD1 mice         | AM251              | 3 mg/kg, IP     | Contextual fear conditioning |                      | ↓                 |
| Mikics et al. (2006) | CB1 KO           | N/A                | N/A             | Contextual fear conditioning |                      | ↓                 |
| Pamplona and Takahashi (2006) | Wistar rats | WIN55,212-2       | 2.5 and, IP 5.0 mg/kg | Contextual fear conditioning |                      | ↓                 |
| Sink et al. (2010)  | Sprague-Dawley rats | AM251              | 4.0 or 8.0 mg/kg, IP | Contextual fear conditioning |                      | ↑                 |
| Pamplona and Takahashi (2006) | Wistar rats | Rimonabant        | 1 mg/kg, IP     | Contextual fear conditioning |                      | =                 |
| Pamplona and Takahashi (2006) | Wistar rats | WIN55,212-2       | 2.5 and 5.0 mg/kg, IP | Cue fear conditioning |                      | =                 |
| Marsicano et al. (2002) | CB1 KO           | N/A                | N/A             | Cue fear conditioning |                      | ↓                 |
| Kamprath et al. (2006) | CB1 KO           | N/A                | N/A             | Cue fear conditioning |                      | ↓                 |
| Wise et al. (2008)  | Sprague-Dawley rats | CE                 | 0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg, IP | Delay radial maze |                      | ↑                 |
| Wise et al. (2008)  | Sprague-Dawley rats | CE                 | 0.1 mg/kg, IP  | Delay radial maze |                      | ▲                 |
| Wise et al. (2008)  | Sprague-Dawley rats | CE                 | 0.1 mg/kg, IP  | Delay radial maze |                      | ▲                 |

(Continued)
| Authors                     | Animals                                      | Drug           | Doses and route | Test                      | Administered before | Effects on memory |
|----------------------------|----------------------------------------------|----------------|-----------------|---------------------------|---------------------|-------------------|
| Nakamura et al. (1991)     | Wistar rats                                  | Δ 9-THC        | 1.25 mg/kg, IP  | Delay radial maze         | Recall              | =                 |
| Lichtman (2000)            | Sprague-Dawley rats                          | Rimonabant    | 3 mg/kg, IP     | Delay radial maze         | Acquisition         | ↑                 |
| Wise et al. (2007)         | Sprague-Dawley rats                          | Rimonabant    | 1 mg/kg, IP     | Delay radial maze         | Acquisition         | ↑                 |
| Wise et al. (2007)         | Sprague-Dawley rats                          | Rimonabant    | 1 mg/kg, IP     | Delay radial maze         | Consolidation       | =                 |
| Wolff and Leander (2003)   | Sprague-Dawley rats                          | Rimonabant    | 1 mg/kg, IP     | Delay radial maze         | Consolidation       | ↑                 |
| Lichtman (2000)            | Sprague-Dawley rats                          | Rimonabant    | 3 mg/kg, IP     | Delay radial maze         | Recall              | =                 |
| Wise et al. (2007)         | Sprague-Dawley rats                          | Rimonabant    | 1 mg/kg, IP     | Delay radial maze         | Recall              | =                 |
| Hampson and Deadwyler (2000)| Long–Evans rats                           | WIN55,212-2   | 0.25–0.75 mg/kg, IP | DNMTTP                  | Working-memory test | ↓                 |
| Deadwyler et al. (2007)    | Long–Evans rats                              | WIN55,212-2   | 0.35 mg/kg, IP  | DNMTTP                    | Working-memory test | ↓                 |
| Hampson and Deadwyler (2000)| Long–Evans rats                          | Δ 9-THC        | 0.5, 1.0, 1.5, and 2.0 mg/kg, IP | DNMTTP                  | Working-memory test | ↓                 |
| Heyser et al. (1993)       | Sprague-Dawley rats                          | Cannabidiol   | 2 mg/kg, IP     | DNMTTP                    | Working-memory test | ↓                 |
| Heyser et al. (1993)       | Sprague-Dawley rats                          | Δ 9-THC        | 2 mg/kg, IP     | DNMTTP                    | Working-memory test | ↓                 |
| Panilio et al. (2011)      | Sprague-Dawley and Long–Evans hooded rats   | Δ 9-THC        | 1–5.6 mg/kg, IP | DNMTTP                    | Working-memory test |                   |
| Mallet and Beninger (1998) | Wistar rats                                  | Anandamide     | 2 mg/kg, IP     | DNMTTP                    | Working-memory test | ↓                 |
| Deadwyler et al. (2007)    | Long–Evans rats                              | Rimonabant    | 2 mg/kg, IP     | DNMTTP                    | Working-memory test | ↑                 |
| Mallet and Beninger (1998) | Wistar rats                                  | Rimonabant    | 2 mg/kg, IP     | DNMTTP                    | Working-memory test | =                 |
| Chhatwal et al. (2005)     | Sprague-Dawley rats                          | WIN 55,212-2  | 5 mg/kg, IP     | Fear potentiated startle response | Extinction         | =                 |
| Chhatwal et al. (2005)     | Sprague-Dawley rats                          | AM404          | 10 mg/kg, IP    | Fear potentiated startle response | Extinction         | ↑                 |

(Continued)
Table 1 | Continued

| Authors                      | Animals            | Drug          | Doses and route | Test                  | Administered before | Effects on memory |
|------------------------------|--------------------|---------------|-----------------|-----------------------|---------------------|-------------------|
| Chhatwal et al. (2005)       | Sprague-Dawley rats | Rimonabant    | 1.5 and 5 mg/kg, IP | Fear potentiated startle response | Extinction          | ↓                 |
| Niyuhire et al. (2007)       | C57BL/6J           | Rimonabant    | 3 mg/kg         | Food Self administration | Extinction          | =                 |
| Höltter et al. (2005)        | CB1 KO             | N/A           | N/A             | Food Self administration | Extinction          | =                 |
| Varvel et al. (2007)         | C57BL/6 mice       | Δ9-THC        | 3 mg/kg, IP     | Modified water maze    | Recall              | ↓                 |
| Varvel et al. (2007)         | C57BL/6 mice       | Δ9-THC        | 10 mg/kg, IP    | Modified water maze    | Recall              | ↓                 |
| Varvel et al. (2007)         | FAAH KO            | N/A           | N/A             | Modified water maze    | ↑                   |
| Varvel et al. (2007)         | FAAH KO            | N/A           | N/A             | Modified water maze    | ↑                   |
| Schneider et al. (2008)      | Sprague-Dawley rats | WIN55,212-2  | 1.2 mg/kg, IP   | Object recognition test | Acquisition         | ↓                 |
| Ciccocioppo et al. (2002)    | Wistar rats        | Δ9-THC        | 2 or 5 mg/kg, IP | Object recognition test | Acquisition         | =                 |
| Barna et al. (2007)          | Wistar rats        | WIN55,212-2  | Osmotic pump 0.13TBq/mmol, IC (hip-pocampus) | Object recognition test | Acquisition         | ↓                 |
| Quinn et al. (2008)          | Wistar rats        | Δ9-THC        | 5 mg/kg, IP     | Object recognition test | Acquisition         | ↓                 |
| Suenaga and Ichitani (2008)  | Wistar-Imamichi rats | WIN55,212-2  | 1–2 μg/side, IC (hip-pocampus) | Object recognition test | Acquisition         | =                 |
| Maccarrone et al. (2002)     | CB1 KO             | N/A           | N/A             | Object recognition test | ↑                   |
| Costanzi et al. (2004)       | CD1 mice           | Anandamide    | 0.3 and 0.5 mg/kg, IP | Passive avoidance | Consolidation       | ↓                 |
| Castellano et al. (1999)     | CD1 mice           | Anandamide    | 1.5, 3, 6 mg/kg, IP | Passive avoidance | Consolidation       | ↓                 |
| Nasehi et al. (2010)         | NMRI mice          | WIN55,212-2  | 0.25, 0.5, and 1 μg/mouse, IC (hip-pocampus) | Passive avoidance | Recall              | ↓                 |
| Mazzola et al. (2009)        | Sprague-Dawley rats | URB597       | 0.1–0.3–1 mg/kg, IP | Passive avoidance | Acquisition         | ↑                 |
| Authors                          | Animals                  | Drug        | Doses and route | Test                | Administered before | Effects on memory |
|---------------------------------|--------------------------|-------------|-----------------|---------------------|---------------------|-------------------|
| Mazzola et al. (2009)           | Sprague-Dawley          | WY14643     | 10 20 10 mg/kg, | Passive avoidance   | Acquisition ↑       |
|                                 |                          |             | 40 mg/kg, IP    |                     |                     |                   |
| Mazzola et al. (2009)           | Sprague-Dawley          | URB597      | 0.1–0.3–1 mg/kg,| Passive avoidance   | Consolidation =     |
|                                 |                          |             | 1 mg/kg, IP     |                     |                     |                   |
| Mazzola et al. (2009)           | Sprague-Dawley          | URB597      | 0.1–0.3–1 mg/kg,| Passive avoidance   | Recall =            |
|                                 |                          |             | 1 mg/kg, IP     |                     |                     |                   |
| Mishima et al. (2001)           | Wistar rats              | Δ 9-THC     | 10 mg/kg, IP    | Passive avoidance   | Acquisition ↓       |
| Murillo-Rodríguez et al. (2001) | Wistar rats              | OEA         | 30 mg/kg        | Passive avoidance   | Extinction ↑        |
| Mishima et al. (2001)           | Wistar rats              | Δ 9-THC     | 6 mg/kg, IP     | Passive avoidance   | Recall ↓            |
| Campolongo et al. (2009b)       | Sprague-Dawley          | WIN55,212-2 | 50 ng, intra BLA| Passive avoidance   | Recall ↑            |
| Campolongo et al. (2009b)       | Sprague-Dawley          | AM251       | 0.28 ng, IC (basolateral amygdala) | Passive avoidance   | Recall ↓            |
| Niyuhire et al. (2007)          | C57BL/6J                | Rimonabant  | 3 mg/kg, IP     | Passive avoidance   | Extinction ↓        |
| Suenaga and Ichitani (2008)     | Wistar-Imamichi rats     | WIN55,212-2 | 1–2 μg/side, IC (hippocampus) | Place recognition test | Acquisition ↓ |
| Inui et al. (2004)              | Wistar rats              | Δ 9-THC     | 6 mg/kg, IP     | Radial maze         | Working-memory test ↓|
| Lichtman et al. (1995)          | Sprague-Dawley          | CP-55,940   | 0.13 mg/kg, IP  | Radial maze         | Working-memory test ↓|
| Lichtman et al. (1995)          | Sprague-Dawley          | CP-55,940   | 8 μg/rat, IC (hippocampus) | Radial maze         | Working-memory test ↓|
| Lichtman et al. (1995)          | Sprague-Dawley          | WIN55,212-2 | 2.1 and 2.2 mg/kg, IP | Radial maze         | Working-memory test ↓|
| Lichtman et al. (1995)          | Sprague-Dawley          | Δ 9-THC     | 2.1 and 2.2 mg/kg, IP | Radial maze         | Working-memory test ↓|
| Lichtman and Martin (1996)      | Sprague-Dawley          | Δ 9-THC     | 3 mg/kg, IP     | Radial maze         | Working-memory test ↓|
| Wise et al. (2009b)             | Sprague-Dawley rats     | CP-55,940   | 10 μg/rat, IC (hippocampus) | Radial maze         | Working-memory test ↓|
| Wise et al. (2009b)             | Sprague-Dawley rats     | Δ 9-THC     | 5.6 mg/kg, IP   | Radial maze         | Working-memory test ↓|
| Rubino et al. (2009)            | Sprague-Dawley rats     | Δ 9-THC     | 2.5 to 10 mg/kg in 10 days, IP | Radial maze         | Working-memory test ↓|

(Continued)
Table 1 | Continued

| Authors                        | Animals               | Drug     | Doses and route | Test               | Administered before | Effects on memory |
|-------------------------------|-----------------------|----------|-----------------|--------------------|----------------------|-------------------|
| Egashira et al. (2002)        | Wistar rats           | Δ 9-THC  | 20 μg/3 d, side, IC (hip-pocampus) | Radial maze       | Working-memory test  | ↓                 |
| Egashira et al. (2008)        | Wistar rats           | Δ 9-THC  | 6 mg/kg, IP     | Radial maze        | Working-memory test  | ↓                 |
| Molina-Holgado et al. (1993)  | Wistar rats           | Δ 9-THC  | 5 mg/kg, PO     | Radial maze        | Working-memory test  | ↓                 |
| Nakamura et al. (1991)        | Wistar rats           | Δ 9-THC  | 1.25 mg/kg, IP  | Radial maze        | Working-memory test  | ↓                 |
| Rodrigues et al. (2011)       | Wistar rats           | Δ 9-THC  | 0.5 μL, IC (medial prefrontal cortex) | Radial maze       | Working-memory test  | ↓                 |
| Mishima et al. (2001)         |                       | Δ 9-THC  | 4–6 mg/kg, IP   | Radial maze        | Working-memory test  | ↓                 |
| Varvel et al. (2005b)         | C57BL/6 mice          | Δ 9-THC  | 10 mg/kg        | T-maze             | Working-memory test  | ↓                 |
| Nava et al. (2001)            | Sprague-Dawley        | Δ 9-THC  | 2.5 and 5 mg/kg, IP | T-maze             | Working-memory test  | ↓                 |
| Jentsch et al. (1998)         | Sprague-Dawley        | Δ 9-THC  | 5 mg/kg, IP     | T-maze             | Working-memory test  | ↓                 |
| Varvel et al. (2007)          | C57BL/6 mice          | Δ 9-THC  | 0.1, 0.3, 1, or 10 mg/kg, IP | Water maze       | Extinction =         |
| Varvel et al. (2001)          | C57Bl/6 mice          | Δ 9-THC  | 3, 10, and 30 mg/kg, IP | Water maze       | Recall =             |
| Varvel et al. (2007)          | C57BL/6J              | DL-135   | 30 mg/kg, IP    | Water maze         | Extinction ↑         |
| Robinson et al. (2010)        | Lister Hooded rats    | WIN55,212-2 | 1 and 3 mg/kg, IP | Water maze        | Acquisition ↓        |
| Moore et al. (2010)           | Sprague-Dawley        | Δ 9-THC  | 10 mg/kg, IP    | Water maze         | Acquisition ↓        |
| Diana et al. (2003)           | Sprague-Dawley rats   | Nabilone | 0.1, 0.5, and 1.0 mg/kg, IP | Water maze       | Acquisition =        |
| Acheson et al. (2011)         | Sprague-Dawley rats   | WIN55,212-2 | 1 mg/kg, IP     | Water maze         | Acquisition =        |
| Diana et al. (2003)           | Sprague-Dawley rats   | Δ 8-THC  | 5 mg/kg, IP     | Water maze         | Acquisition ↓        |
| DaSilva and Takahashi (2002)  | Swiss albino          | Δ 9-THC  | 8 mg/kg, IP     | Water maze         | Acquisition ↓        |

(Continued)
Table 1 | Continued

| Authors               | Animals                        | Drug          | Doses and route | Test               | Administered before | Effects on memory |
|-----------------------|--------------------------------|---------------|-----------------|--------------------|----------------------|-------------------|
| Ferrari et al. (1999) | Wistar                         | HU-210        | 50 and 100 μg/kg, IP | Water maze         | Acquisition          | ↓                 |
| Mishima et al. (2001) | Wistar rats                     | Δ 9-THC       | 6 and 10 mg/kg, IP | Water maze         | Recall               | =                 |
| Varvel et al. (2007)  | C57BL/6 mice                    | Rimonabant    | 3 mg/kg, IP     | Water maze         | Acquisition          | =                 |
| Varvel et al. (2005a) | C57BL/6J Swiss albino mice      | Rimonabant    | 3 mg/kg, IP     | Water maze         | Acquisition          | =                 |
| DaSilva and Takahashi (2002) |                     | N/A            |                 | Water maze         | Acquisition          | =                 |
| Varvel et al. (2005a) | CB1 KO                          | N/A            |                 | Water maze         | (Extinction)         | ↓                 |
| Varvel et al. (2007)  | FAAH KO                         | N/A            |                 | Water maze         | (Extinction)         | ↑                 |
| Varvel et al. (2006)  | FAAH KO                         | N/A            |                 | Water maze         | Working-memory test  | ↑                 |
| Varvel et al. (2007)  | FAAH KO                         | N/A            |                 | Water maze         | reversal learning    | ↑                 |
| Varvel and Lichtman (2002) |                     | CB1 KO         | N/A            | Water maze         | =                   |                   |

DNMTP, delayed non-matching to position; KO, knockout; WT, wild type. For effects on memory, ↓ indicates impairment; ↑ indicates enhancement; = indicates no effect.

memory process. This may explain cases where microinfusion of cannabinoid compounds into specific areas produces effects opposite to those usually seen with systemic administration. For example, Campolongo et al. (2009b) found that micro-injections of WIN55212-2 into the basolateral amygdala enhanced memory retention and the CB1 antagonist AM251 caused impairments in a passive-avoidance test.

WORKING MEMORY

Working memory involves the temporary storage and manipulation of information. The memory impairments induced by cannabis and Δ9-THC in humans are most robust in tests of short-term episodic and working memory (Ranganathan and D'Souza, 2006). In animal models, the effects of cannabinoids on working memory have received much attention (see Table 1), and the data appear more congruent than in the models of long-term reference memory discussed above. Some of the procedures used to study working memory are adapted from procedures used to study acquisition of long-term memory.

Water maze

The basic water maze procedure can be modified to test working memory by changing the location of the platform each day and testing with only a brief delay between acquisition and a test trial. Varvel et al. (2001) have demonstrated that Δ9-THC administered before the testing session impairs memory in a CB1 dependent manner without affecting locomotion.

Radial maze

The findings obtained with the working-memory version of the water maze procedure agree with those obtained with the conventional version of the radial maze, which focuses on working memory. In rodents, systemic administration of Δ9-THC or CB1 agonists like WIN55212 or CP-55,940 increase the number of errors (Molina-Holgado et al., 1993; Lichtman et al., 1995; Lichtman and Martin, 1996; Mishima et al., 2001). Interestingly, Nakamura et al. (1991) found that Δ9-THC (given 30 min before the task) impaired performance in the test when a short delay of 5 s was introduced between entering the fourth and fifth arms, but not when the delay was longer (1 h); this suggests a more prominent effect of Δ9-THC on working memory than on long-term reference memory. However, under a similar task Silva de Melo et al. (2005) obtained opposite results, with systemic or intra medial prefrontal cortex administration of THC selectively impairing memory in the long-delay condition. A series of experiments exploring the brain structure involved in cannabinoid-induced impairments of working memory in the radial maze have shown that both the hippocampus and prefrontal cortex are involved (Egashira et al., 2002; Silva de Melo et al., 2005; Suenaga et al., 2008; Rubino et al., 2009; Wise et al., 2009b; Rodrigues et al., 2011) and that CB1 and D1,-2 receptors play critical roles (Wise et al., 2009b; Rodrigues et al., 2011).

T-maze

T-maze procedures also provide a test of spatial working memory. There are two goal arms, and rodents obtain food by entering
the goal arm that was not entered on the previous trial. Systemic administration of Δ⁹-THC (Jentsch et al., 1998; Nava et al., 2001; Varvel et al., 2005b) or intra-hippocampal administration of WIN55212 (Suenaga et al., 2008) impairs the performance of and rats, and CB₁ antagonists reverse these effects. Several lines of evidence indicate the involvement of acetylcholine systems in the effects of Δ⁹-THC on working memory in task such as the T-maze and radial maze. Extracellular levels of hippocampal acetylcholine have been shown to decrease after Δ⁹-THC administration (Mishima et al., 2002), and drugs that reestablish levels of this neurotransmitter can reverse the impairing effects of Δ⁹-THC (Nava et al., 2000, 2001; Mishima et al., 2002; Inui et al., 2004; Wise et al., 2007; Egashira et al., 2008).

**Delayed spatial matching**

Extensive studies of working memory have been performed by Hampton, Deadwyler, and associates, using the delayed non-matching to position task in rats. In this task, one of two retractable levers is extended as a sample. After the rat presses the sample lever, the lever is retracted. After a delay period, both levers are extended and the rat receives food or water if it presses the non-matching lever (i.e., the one that was not presented as a sample; Deadwyler et al., 1996; Mallet and Beninger, 1998). Many such trials can be conducted during a daily session, with the length of the delay varied across trials. Administration of Δ⁹-THC, anandamide, or WIN55212-2 before the session impairs performance (Heyser et al., 1993; Mallet and Beninger, 1998; Hampson and Deadwyler, 2000; Deadwyler et al., 2007; Goonawardena et al., 2010; Panilillo et al., 2011). This effect is associated with a drug-induced decrease in the firing rate of hippocampal pyramidal neurons during the initiation of the trial; preadministration of rimonabant (IP 1.5 mg/kg) reestablishes a normal level of hippocampal neuronal activity and blocks the memory-imparing effects of Δ⁹-THC and WIN55,212-2 (Hampson and Deadwyler, 2000; Goonawardena et al., 2010). Under some conditions, the administration of a higher concentration (IP 2 mg/mL) of rimonabant alone can enhance performance in this working-memory task (Deadwyler and Hampson, 2008). However, this result has not been reported consistently by the Deadwyler lab and was not obtained with the same dose of rimonabant in a study by Mallet and Beninger (1998). Possibly, the enhancing effect is sometimes prevented by ceiling effects and requires modifications of the procedure (e.g., longer delay periods) to be observed.

**ENHANCED ANANDAMIDE SIGNALING AND PPAR-α ACTIVATION**

Compounds that inhibit the activity of the fatty acid amide hydrolase enzyme (FAAH) prevent the degradation of endocannabinoid anandamide and thereby magnify and prolong anandamide’s actions (Kathuria et al., 2003). FAAH inhibition also increases levels of several other fatty acids – oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) – that constitute endocannabinoïd-like systems in the brain (Fegley et al., 2005). OEA and PEA do not bind to cannabinoid receptors, but are ligands for alpha-type peroxisome proliferator-activated nuclear receptors (PPAR-α). PPAR-α is well known to be involved in a number of physiological processes, but is just beginning to received attention for having cognitive and other behavioral effects (Mazzola et al., 2009; Melis et al., 2010; Mascia et al., 2011).

Surprisingly, given the preponderance of findings that cannabinoid agonists impair memory and the fact that FAAH inhibitors increase levels of the endogenous cannabinoid agonist anandamide, FAAH inhibitors have been found to enhance learning in several procedures. The FAAH inhibitor OL-135 (30 mg/kg) enhanced the acquisition rate in a working-memory version of the water maze test or in the conventional fixed-platform test (Varvel et al., 2007); however, the same dose of OL-135 did not have such effects in an earlier study (Varvel et al., 2006). Another FAAH inhibitor, URB597 (0.1–1 mg/kg), enhanced the acquisition of passive-avoidance learning, but was not found to affect consolidation or retrieval (Mazzola et al., 2009). In genetically modified FAAH-compromised mice, acquisition was enhanced in the working-memory water maze test, but not in the conventional, fixed-platform test (Varvel et al., 2007; Wise et al., 2009a).

Although both Varvel et al. (2007) and Mazzola et al. (2009) found that rimonabant (1 mg/kg in rats; 3 mg/kg in mice) was able to block the facilitating effects of FAAH inhibition on memory acquisition, suggesting mediation by CB₁ receptors, there is evidence that non-cannabinoid effects of FAAH inhibition also can enhance learning and memory. Mazzola et al. (2009) found that the enhancing effects of FAAH inhibition on passive-avoidance learning could be blocked not only by rimonabant but by the PPAR-α antagonist MK886. This suggests that FAAH inhibition might enhance memory by increasing the levels of the endogenous PPAR-α ligands OEA and PEA. Consistent with this hypothesis, the PPAR-α agonist WY14643 produced effects similar to those of the FAAH inhibitor URB597 on acquisition of passive-avoidance test, and this effect of WY14643 was also blocked by the PPAR-α antagonist MK886. In both a passive avoidance and a fixed-platform water maze procedure in rats, administration of exogenous OEA enhanced memory (Campolongo et al., 2009a). However, it should be noted that in the study where the FAAH inhibitor OL-135 did not enhance water maze learning (Varvel et al., 2006), OEA (50 mg/kg) and PEA (50 mg/kg) also failed to affect working memory in the water maze. In addition, while Mazzola et al. (2009) found that FAAH inhibition or administration of the PPAR-α agonist WY14643 specifically affected acquisition of passive avoidance, Campolongo et al. (2009a) found that OEA had effects when given immediately post-training, indicating an effect on consolidation.

The finding that FAAH inhibition has memory effects opposite to those of cannabinoid agonists might be at least partially explained by differences in the brain areas affected by these two kinds of treatment. As mentioned above, systemic injection of a drug such as Δ⁹-THC affects CB₁ receptors throughout the brain. Systemic injection of a FAAH inhibitor selectively increasing anandamide levels in areas where it is being released. It is likely that different brain areas subserve different mnemonic processes; for example, endocannabinoid signaling in the hippocampus might be more involved in acquisition, while endocannabinoid signaling in the amygdala might be more involved in consolidation and forgetting (Riedel and Davies, 2005).

**EXTINCTION AND FORGETTING**

While the fact that exogenous cannabinoids impair memory has been studied in humans and animals for decades, it has only
recently been recognized that endocannabinoid systems might be involved in extinction learning. Extinction learning refers to the cessation of a learned response when the conditions that induced the learning no longer hold. For example, after the initial exposure to shock in contextual fear conditioning, the conditioned freezing response will gradually decrease if the subject is repeatedly exposed to the context but no longer shocked. This loss of the learned response might be described as forgetting, or as the establishment of new learning appropriate to the current situation.

Using fear conditioning with a discrete cue, Marsicano et al. (2002) were the first to report compromised extinction learning in CB₁-knockout mice and in wild type mice given rimonabant (3 mg/kg). Interestingly, the behavioral patterns observed in CB₁-knockout and rimonabant-treated mice were associated with decreased long-term depression of neurons in the amygdala, a structure known to play a critical role in extinction learning (Quirk and Mueller, 2008). Moreover, presentation of the shock-associated tone during extinction was followed by increased release of anandamide in the basolateral amygdala of wild type mice, suggesting the involvement of endocannabinoid neurotransmission in extinction learning (Marsicano et al., 2002). It has been proposed that endocannabinoids modulate fear-related extinction learning by regulating the activity of kinases and phosphatases in regions involved in fear and memory processing (Cannich et al., 2004).

The impairing effects of genetic and pharmacological blockade of CB₁ receptors on extinction learning, but not on the acquisition of long-term and short-term fear-related memory, have been replicated in many laboratories with rats and mice (Suzuki et al., 2004; Chhatwal et al., 2005; Kamprath et al., 2006; Niyuhire et al., 2007; Pamplona et al., 2008). Also consistent with the hypothesis that CB₁ dependent mechanisms modulate extinction learning, activation of CB₁ receptors has been shown to facilitate fear conditioning, producing effects opposite to those of CB₁ antagonists. Administration of the anandamide uptake inhibitor AM404 (IP: 10 mg/kg; 1.0 μg/μL, i.c.v.) during extinction training facilitated the extinction of startle or freezing elicited by a shock-associated context (Chhatwal et al., 2005; Bitencourt et al., 2008; Pamplona et al., 2008); this effect was CB₁ dependent, since it is blocked by a dose of rimonabant that was ineffective by itself (Bitencourt et al., 2008). Low doses (0.25 mg/kg, IP) of the CB₁ agonist WIN55,212-2 impaired contextual fear conditioning under the same conditions where rimonabant enhanced it (Chhatwal et al., 2005; Pamplona and Takahashi, 2006). Moreover Ganon-Elazar and Akirav (2009) have shown that micro-injection of a low dose of WIN55,212-2 in the basolateral amygdala has no effect by itself but can reverse the disrupting effect of a stressor on extinction of passive avoidance.

The effects of cannabinoid compounds on extinction learning have also been confirmed with another aversively motivated test, the water maze. In this test, Varvel et al. (2005b, 2007) found that rimonabant (3 mg/kg) treatment or genetic CB₁ disruption impaired extinction learning, but THC did not affect extinction (Varvel et al., 2007). Surprisingly, pharmacological and genetic manipulations of CB₁ have not been found to affect extinction learning in tasks based on appetitive conditioning (Höller et al., 2005; Niyuhire et al., 2007; Harloe et al., 2008).

It has been suggested that the effects of CB₁ antagonism in extinction procedures may depend on perseverance. For example, rimonabant-treated or CB₂-knockout mice show deficits in learning when the platform is moved to a new location in the water maze test (Varvel and Lichtman, 2002; Pamplona et al., 2006). However this view is not supported by another study in which certain doses of CB₂ agonists and antagonists facilitated or impaired, respectively, flexibility between different strategies (Hill et al., 2006). In this experiment, separate groups of rats were trained to use either a visual cue or a spatial (left vs. right) strategy to locate food in one arm of a plus-maze. Flexibility was then measured as the number of perseverative errors when the opposite strategy was required. Administration of the CB₁ antagonist AM251 (2 mg/kg) 20 min before testing reduced perseverative errors, whereas the CB₁ agonist HU-210 (20 μg/kg, IP) increased them.

The effects of FAAH inhibition on extinction learning have also been studied. FAAH null mice and mice treated with the FAAH inhibitor OL-135 show enhanced extinction learning in the water maze test (Varvel et al., 2007). Therefore FAAH inhibitors have unique effects among the endocannabinoid-related compounds, facilitating both acquisition and extinction processes. This characteristic may be due, as previously mentioned, to the ability of FAAH inhibitors to increase not only brain levels of anandamide but also of PEA and OEA. Indeed it has been shown that OEA administration (30 mg/kg) can facilitate extinction of passive avoidance in rats (Murillo-Rodríguez et al., 2001).

### CONCLUSION – ENDOCANNABINOID SIGNALING AND COGNITION

Most of the evidence indicates that activating the endocannabinoid system interferes with situation-dependent working memory and the acquisition of long-term memory (see Table 1). Inhibiting the endocannabinoid system, on the other hand, can enhance learning and memory. Surprisingly, increasing endogenous levels of anandamide and facilitating endocannabinoid signaling with a FAAH inhibitor can enhance learning; but, this probably occurs through the endocannabinoid-related PPAR-α system and the fatty acids OEA and PEA. There is accumulating evidence that the endocannabinoid system plays a special role in extinction learning related to aversive conditioning. This role, along with its role in emotion, suggests cannabinoid-related medications might be developed for treating phobias.

### EFFECTS OF ENDOCANNABINOID SYSTEM MODULATION ON EMOTIONAL BEHAVIOR

The effects of cannabinoid agonists and antagonists on emotional behavior have recently been reviewed elsewhere (Bambico et al., 2009; Moreira and Wotjak, 2010) and will only be discussed briefly here. Instead, we will focus on studies that involve genetic disruption of CB₁ receptors or genetic or pharmacological manipulation of the anandamide-degrading enzyme FAAH. These methods generally provide more direct information about endocannabinoid function because they exclude or enhance cannabinoid signaling, rather than directly stimulating cannabinoid receptors.

The animal models of anxiety that have been used with these endocannabinoid manipulations generally measure changes in rodents’ tendency to avoid certain inherently aversive situations;
increased avoidance implies increased anxiety (an anxiogenic effect), and decreased avoidance indicates decreased anxiety (an anxiolytic effect). The avoided situations include brightly lit areas (light/dark test), open areas (open field test), open elevated areas (elevated plus-maze and O-maze tests), social interaction with unfamiliar conspecifics (social interaction test), and pain-associated stimuli (Vogel conflict test, shock prod burying test). In most of these tests, locomotor activity can also be monitored to assess the possibility that a drug or dose is causing non-specific sedation or motor depression, rather than affecting emotional behavior. Animal tests of depression generally model specific depression-like symptoms. For example, some tests measure changes in rodents’ tendency to eventually becoming immobile when it is not possible to escape from water (forced swim test) or being suspended by the tail (tail suspension test). A decrease in the duration of immobility is considered an antidepressant-like effect and an increase in duration a depressant-like effect. Increased immobility is believed to be a sign of “behavioral despair” that putatively models the depression symptoms “loss of energy” and/or “feelings of hopelessness.” A depression-like state can be induced in laboratory rodents by exposing them to mild but recurrent and unpredictable stressors (chronic mild stress model). In this model, a decrease in consumption of sucrose is believed to model anhedonia (loss of pleasure) another important symptom of depression in humans.

**EFFECTS OF CB1 RECEPTOR AGONISTS AND ANTAGONISTS ON EMOTIONAL BEHAVIOR**

Cannabinoid receptor agonists decrease depression-like behaviors in a variety of species and models (Bambico et al., 2009). For example, in the forced swim test the CB1 agonists anandamide, Δ9-THC, CP-55,940, HU-210, and WIN55,212-2 decrease immobility in the forced swim paradigm in BALB/C and CD1 mice and in Long–Evans, Sprague-Dawley, and Wistar rats, effects that are blocked by the CB1 antagonists rimonabant and AM251. Although these findings suggest that cannabinoid receptor agonists hold promise as targets for the treatment of depression, these drugs have significant side effects (e.g., psychosis and panic) that preclude their clinical use (Moreira et al., 2009). Similarly, CB1 antagonists also been found to have both therapeutic potential and unacceptable side effects; the antagonist rimonabant, which showed promise as a treatment for obesity, was recalled from the market because of emotional, depression-like side effects.

The effects of cannabinoid agonists are somewhat more complex in animal models of anxiety than in animal models of depression. High and low doses of cannabinoid agonists often have opposite effects (Moreira and Wotjak, 2010), with low doses inducing anxiolytic effects, while high doses induce anxiogenic effects. Both effects can be inhibited by CB1 antagonists, although paradoxical agonist/antagonist interactions have also been reported (Haller et al., 2007).

**Caveats**

Discrepant findings with CB1 receptor ligands are usually attributed to differences in dosage and treatment duration, experimental conditions, and species (Bambico et al., 2009; Moreira and Wotjak, 2010). However, these factors have rarely been studied systematically, and the reasons for discrepant findings are actually poorly understood. One possible explanation lies in the fact that CB1 receptors are expressed on both glutamatergic and GABAergic synapses and these neurotransmitter systems often have opposite effects on emotions, especially on anxiety. We have shown that the relative cannabinoid sensitivity of GABA and glutamate neurotransmission differs between CD1 and Wistar rats and that these differences are likely responsible for the differential effects of cannabinoids on anxiety in these two species (Haller et al., 2007). Similar differences in cannabinoid function might be present in different strains of the same species, or even individual subjects. Thus, discrepant findings could be due to differences in the expression, distribution, and functional characteristics of CB1 receptors.

**GENETIC DELETION OF CB1 RECEPTORS**

The impact of genetic deletion of CB1 in animal models of anxiety and depression was demonstrated in three studies published in 2002 (see Table 2). Maccarrone et al. (2002) showed that CB1-knockout mice were more anxious than wild type in the open field and light/dark tests; however, this effect was present in young mice but not in 4-month-old mice. Martin et al. (2002) reported that deletion of the CB1 gene induced signs of anxiety in the light/dark test and depression-like symptoms in the sucrose consumption test after chronic mild stress. Finally, Haller et al. (2002) showed that CB1-knockout mice robustly express anxiety in the elevated plus-maze, but this kind of effect was not induced by the CB1 antagonist rimonabant in wildtype mice. A later study by the same group found that, unlike rimonabant but like CB1 deletion, the CB1 antagonist AM251 did increase anxiety (Haller et al., 2004b). Subsequent studies have also replicated the depression-like phenotype of CB1-knockout mice in the forced swim test (Fride et al., 2005), but others have not (Steiner et al., 2008a,b). Conditional mutants lacking CB1 receptors at their cortical glutamatergic neurons showed decreased immobility in the forced swim test, suggesting an antidepressant effect of this more targeted genetic manipulation (Steiner et al., 2008b).

Like the depressant effects, the anxiogenic effects of CB1 deletion have been replicated in a number of studies using a variety of procedures (see Table 2). These include the elevated plus-maze, social interaction, and light/dark tests (Urigüen et al., 2004; Mikics et al., 2009; Hill et al., 2011). In other cases, however, the effect was weak. For example, risk assessment was decreased in the elevated plus-maze, but open arm exploration (the main measure of anxiety in this test) was not affected (Jacob et al., 2009). Also, mutant mice lacking the CB1 receptor at their glutamatergic synapses showed no changes in anxiety (Jacob et al., 2009). The effects of gene disruption were also weak in the mouse defense test battery, a less commonly used but behaviorally valid model of anxiety that measures responses to an unconditioned predator-related stimulus (Griebel et al., 2005). In one study, the anxiolytic effects of ethanol were not diminished in CB1-knockout in the elevated plus-maze (Houchi et al., 2005). In another experiment that used the shock prod burying test, CB1 deletion itself had anxiolytic effects (Degroot and Nomikos, 2004).

Some of the inconsistency in the effects of CB1 on anxiety- and depression-like behavior might be due to changes in
Table 2 | Summary of studies investigating anxiety-like and depression-like behavior in knockout with cannabinoid CB1 with deleted.

| Authors                  | Animals                             | Test                                | Anxiety | Depression |
|--------------------------|-------------------------------------|-------------------------------------|---------|------------|
| Maccarrone et al. (2002) | CB1 KO adolescents (CD1)            | Open field (bright light)           | ↑       |            |
| Maccarrone et al. (2002) | CB1 KO adults (CD1)                 | Open field (bright light)           | =       |            |
| Maccarrone et al. (2002) | CB1 KO adolescents (CD1)            | Light–dark test                     | ↑       |            |
| Maccarrone et al. (2002) | CB1 KO adults (CD1)                 | Light–dark test                     | ↑       |            |
| Martin et al. (2002)     | CB1 KO (CD1)                        | Light–dark test                     | ↑       |            |
| Martin et al. (2002)     | CB1 KO (CD1)                        | Active avoidance                    | ↑       |            |
| Haller et al. (2002)     | CB1 KO (CD1)                        | EPM                                 | ↑       |            |
| Fride et al. (2005)      | CB1 KO (C57BL/6J)                   | Forced swim test                    | ↑       |            |
| Steiner et al. (2008a)   | CB1 KO (C57BL)                      | Forced swim test                    | =       |            |
| Steiner et al. (2008b)   | Glu-CB1 KO (C57BL/6N)               | Forced swim test                    | ↓       |            |
| Steiner et al. (2008b)   | CaMK-CB1 KO (C57BL/6N)              | Forced swim test                    | =       |            |
| Steiner et al. (2008b)   | GABA-CB1 KO (C57BL/6N)              | Forced swim test                    | =       |            |
| Jacob et al. (2009)      | CB1 KO (C57BL/6N)                   | Light–dark test (high illumination) | ↑       |            |
| Jacob et al. (2009)      | CB1 KO (C57BL/6N)                   | EPM                                 | ↑       |            |
| Jacob et al. (2009)      | Glu-CB1 KO (C57BL/6N)               | Light–dark test                     | =       |            |
| Jacob et al. (2009)      | Glu-CB1 KO (C57BL/6N)               | EPM                                 | =       |            |
| Griebel et al. (2005)    | CB1 KO (C57BL)                      | Mouse defense test battery          | =       |            |
| Houchi et al. (2005)     | CB1 KO (CD1)                        | EPM                                 | =       |            |
| Houchi et al. (2005)     | CB1 KO (CD1) treated with 1.5 mg/kg ethanol, IF | EPM KO=WT                           | ↑       | (under some parameters) |
| Degroot and Nomikos (2004)| CB1 KO (C57BL/6J)                  | Shock-probe burying test            | ↑       |            |
| Haller et al. (2004a)    | CB1 KO (CD1)                        | EPM (high illumination)             | ↑       |            |
| Hill et al. (2011)       | CB1 KO (ICR)                        | EPM                                 | ↑       |            |

EPM, elevated plus-maze; KO, knockout; WT, wild type. For effects in models of anxiety and depression, ↓ indicates impairment; ↑ indicates enhancement; = indicates no effect.

Responsiveness to environmental stimuli. In a recent study, Jacob et al. (2009) showed that behavioral differences between wild type and CB1-knockout mice were strongly influenced by the level of illumination under which the test was performed; in models of anxiety, such as the open field and the elevated plus-maze the behavior of CB1-knockout differed markedly depending on light intensity. The impact of light intensity was also studied by Haller et al. (2004a), who reported that the anxiogenic effects of CB1 gene disruption are evident when mice are tested in light, but not when they are tested in darkness. The same study suggested that the impact of CB1 gene deletion on social behaviors depends on the level of familiarity with the testing environment; opposite effects were obtained in the home-cage and in an unfamiliar cage. Even the study where CB1 deletion had an anxiolytic effect (Degroot and Nomikos, 2004) can be perceived as a particular case of the interaction between environmental stimuli and CB1-knockout behavior, as the shock prod burying test examines the immediate behavioral response to electric shocks.

Taken together, these findings suggest that deletion of endogenous CB1 signaling generally produces an anxious phenotype, but this effect is strongly dependent on environmental conditions. Intriguingly, Hill et al. (2011) recently demonstrated that the behavioral and neural changes associated with CB1 gene disruption are very similar to those seen in chronically stressed wild type mice. This suggests that CB1 deletion produces a chronic stress state that might contribute to altered responsiveness to environmental stimuli.

Enhancement of anandamide signaling through inhibition of FAAH

The first study demonstrating the impact of FAAH inhibitors on emotional behavior was published by Kathuria et al. (2003). They showed that the FAAH inhibitor URB597 robustly increases brain levels of anandamide but not 2-AG, and it has the anxiolytic effects of decreasing pup ultrasonic vocalizations and promoting exploration of the open section of the elevated O-maze. The authors concluded that their “results indicate that anandamide participates in the modulation of emotional states and point to fatty acid amide hydrolase inhibition as an innovative approach to anti-anxiety therapy.” In a later publication, an overlapping group of authors demonstrated that URB597 decreases depression-like behaviors in both the forced swim and tail suspension models of depression, findings that “support a role for anandamide in mood regulation and point to fatty acid amide hydrolase as a previously uncharacterized target for antidepressant drugs” (Gobbi et al., 2005). Piomelli et al. (2006) concluded that URB597 does not evoke classical cannabinoid-like effects, but enhances the tonic actions of anandamide on a subset of CB1 receptors that are normally engaged in controlling emotion and pain. As such, FAAH inhibition in general and URB597 in particular show promise as treatments for anxiety and depression.

Effects of FAAH inhibition in models of depression

These early publications on the antidepressant- and anxiolytic-like effects of FAAH inhibition were supported by a series of
Table 3 | Summary of studies investigating the effects of the FAAH inhibitor URB597 or genetic deletion of FAAH on anxiety-like and depression-like behavior in rodents.

| Authors               | Animals                      | Drug      | Doses and route | Test                                | Anxiety | Depression |
|-----------------------|------------------------------|-----------|-----------------|-------------------------------------|---------|------------|
| Kathuria et al. (2003)| Sprague-Dawley rats         | URB597   | 0.1 mg/kg, IP   | Elevated 0 maze                     | ↓       |            |
| Kathuria et al. (2003)| Sprague-Dawley rats (pups)  | URB597   | 0.1 mg/kg, IP   | Isolation induced USVs              | ↓       |            |
| Gobbi et al. (2005)   | Sprague-Dawley rats         | URB597   | 0.1 mg/kg, IP   | Tail suspension test                | ↓       |            |
| Gobbi et al. (2005)   | Sprague-Dawley rats         | URB597   | 0.1 mg/kg, IP   | Forced swim test                    | ↓       |            |
| Gobbi et al. (2005)   | Sprague-Dawley rats         | URB597   | 0.1 mg/kg, IP (repeated 4 days) | Forced swim test | ↓       |            |
| Adamczyk et al. (2008)| Wistar rats                 | URB597   | 0.1–0.3 mg/kg, IP | Forced swim test                  | ↓       |            |
| Bambico et al. (2010)| FAAH KO mice (C57BL/6J)     | N/A       | N/A             | Tail suspension test                | ↓       |            |
| Bambico et al. (2010)| Mice, FAAH KO (C57BL/6J)   | N/A       | N/A             | Forced swim test                    | ↓       |            |
| Bortolato et al. (2007)| Wistar rats              | URB597   | 0.3 mg/kg IP (repeated 5 weeks) | Sucrose consumption after chronic mild stress | ↓       |            |
| Hill et al. (2007)    | Long–Evans rats (ovariectomized female + estradiol treatment) | URB597 | 0.1 mg/kg, IP | Forced swim test                  | ↓       |            |
| Realini et al. (2011)| Sprague-Dawley rats (females + 10 days THC treatment) | URB597 | 0.3 mg/kg IP (repeated 30 days) | Forced swim test | ↓       |            |
| Realini et al. (2011)| Sprague-Dawley rats (females + 10 days THC treatment) | URB597 | 0.3 mg/kg, IP (repeated 30 days) | Sucrose consumption | ↓       |            |
| Wright et al. (2010) | Sprague-Dawley rats (DFP treated) | URB597 | 3 mg/kg, IP | Forced swim test | =       |            |
| McLaughlin et al. (2007)| Sprague-Dawley rats       | URB597   | 0.5 and 1 μg (hippo campus) | Forced swim test | =       |            |
| Manna and Jain (2011)| Swiss mice                  | URB597   | 0.05–10 μg/mouse, ICV | Forced swim test | ↓       |            |
| Moise et al. (2008)   | Syrian hamsters             | URB597   | 0.1–0.3 mg/kg, IP | EPM                              | ↓       |            |
| Moise et al. (2008)   | Syrian hamsters             | URB597   | 0.3–3 mg/kg, IP | Conditioned and unconditioned social defeat test | =       |            |
| Moreira et al. (2008) | C57BL/6N mice              | URB597   | 10 mg/kg, IP   | EPM                               | ↓       |            |
| Patel and Hillard (2006)| ICR mice                  | URB597   | 0.1–0.3 mg/kg, IP | EPM                              | ↓       |            |
| Lisboa et al. (2008)  | Wistar rats                 | URB597   | 0.01 nmol, IC (dorsal periaqueductal gray) | Vogel conflict test | ↓       |            |
| Rubino et al. (2008)  | Sprague-Dawley rats        | URB597   | 0.1 μg/rat     | EPM                               | ↓       |            |
| Scherma et al. (2008) | Sprague-Dawley rats        | URB597   | 0.1–0.3 mg/kg, IP | Light–dark test | ↓       |            |
| Naderi et al. (2008)  | NMRI mice                  | AM404     | 1–2 mg/kg, IP  | EPM                               | ↓       |            |
| Naderi et al. (2008)  | NMRI mice                  | URB597   | 0.03–0.3 mg/kg, IP | EPM                              | =       |            |
| Micale et al. (2009)  | C57BL/6J mice              | URB597   | 1 mg/kg, IP    | EPM                               | ↓       |            |
| Micale et al. (2009)  | C57BL/6J mice              | URB597   | 0.1–0.5 mg/kg, IP | EPM                              | =       |            |
| Naidu et al. (2007)   | C57BL/6J-ICR mice          | URB597   | 0.3–1–3 mg/kg, IP | EPM                              | =       |            |
| Naidu et al. (2007)   | C57BL/6J-ICR mice          | URB597   | 10 mg/kg, IP   | EPM                               | =       |            |
| Naidu et al. (2007)   | C57BL/6J-ICR mice          | URB597   | 0.1 mg/kg, IP  | Modified EPM                       | ↓       |            |
| Naidu et al. (2007)   | FAAH KO mice (C57BL/6J)    | N/A       | N/A            | Tail suspension test              | =       |            |
| Naidu et al. (2007)   | FAAH KO mice (C57BL/6J)    | N/A       | N/A            | Tail suspension test              | =       |            |
| Naidu et al. (2007)   | C57BL/6J mice              | URB597   | 0.1–10 mg/kg, IP | Tail suspension test              | =       |            |

(Continued)
subsequent findings (see Table 3). In models of depression, systemic, and i.c.v. treatments with URB597, as well as genetic deletion of FAAH, decreased immobility in the forced swim, and tail suspension tests (Adamczyk et al., 2008; Bambico et al., 2010; Manna and Jain, 2011; Umathe et al., 2011), while systemic URB597 administration counteracted the deleterious effects of chronic mild stress (Bortolato et al., 2007), abolished estrogen deficiency-induced depression in female rats (Hill et al., 2007), and reversed depression-like symptoms induced by THC in adolescent female rats (Realini et al., 2011). In the forced swim test, URB597 reversed depression-like effects in rats 29 days (but not 8 days) after exposure to diisopropylfluorophosphate (Wright et al., 2010). The CB1 dependence of these effects was verified in most of the cited studies, confirming that they were due to FAAH-induced enhancement of anandamide signaling at CB1 receptors. The role of anandamide in these antidepressant effects is further supported by the finding that the anandamide-transport inhibitor AM404 exerted similar effects in some studies (Adamczyk et al., 2008; Umathe et al., 2011).

However, conflicting findings also exist. URB597 had no effect when infused into the dentate gyrus of the hippocampus, despite the fact that the direct CB1 agonist HU-210 administered in the same way produced antidepressant effects in the forced swim test (McLaughlin et al., 2007). This finding suggests that depression-like behavior is affected by anandamide-independent cannabinoid mechanisms in certain cases and in certain brain areas. Naidu et al. (2007) found that the FAAH inhibitors URB597 and OL-135 only affected depression-like behavior in the forced swim and tail suspension tests when the tests were performed under modified lighting conditions and when large sample sizes were used.

### Effects of FAAH inhibition in models of anxiety

URB597 decreased anxiety in the elevated plus-maze when given systemically (Patel and Hillard, 2006; Moise et al., 2008; Moreira et al., 2008) or when injected into the medial prefrontal cortex or dorsolateral periaqueductal gray, two regions that play important roles in the control of anxiety (Lisboa et al., 2008; Rubino et al., 2008). URB597 also abolished the anxiogenic response measured in the elevated plus-maze during withdrawal after an acute administration of alcohol (Gippitelli et al., 2008). Anxiolytic effects of URB597 were also shown in the Vogel conflict test (injected into dorsolateral periaqueductal gray; Lisboa et al., 2008) and light–dark test (injected systemically; Scherma et al., 2008). Like FAAH inhibition, anandamide-transport inhibition decreased anxiety (Lisboa et al., 2008; Naderi et al., 2008), suggesting that the enhancement of endogenous anandamide release decreases anxiety irrespective of the method by which it was achieved. Mice with FAAH genetically deleted showed reduced emotionality in both the social interaction test and the open field test, and these differences were abolished by treatment with rimonabant (Cassano et al., 2011).

However, there are also a number of conflicting findings regarding the effects of FAAH inhibition on anxiety (see Table 3). Some of these contradictions can be considered negligible. For example, acute or chronic treatment with URB597 doses that were very effective at producing anxiolytic effects in other studies (0.1, and 0.5 mg/kg) did not affect anxiety in the elevated plus-maze in the study by Micale et al. (2009), but a higher dose (1 mg/kg) did. In another study, URB597 had no effect on anxiety in the mouse defense test battery, but had an anxiolytic effect in a more conventional model, the elevated plus-maze (Moise et al., 2008). To a certain extent, the findings by Scherma et al. (2008) are also at variance with the assumption that enhanced anandamide signaling decreases anxiety. Although these authors did show an anxiolytic effect with URB597, co-administration of anandamide reversed this effect. This finding might be explained by the fact that FAAH inhibition selectively affects areas where endogenous anandamide is being released, while exogenous administration of anandamide (the effects of which are prolonged by FAAH inhibition) would affect cannabinoid receptors throughout the brain.

Harder to explain are the findings of Naderi et al. (2008), Naidu et al. (2007), and Seillier and Giuffrida (2011), who failed to detect any anxiolytic effect of URB597 in the elevated plus-maze (i.e., the test in which FAAH inhibition was first found to be anxiolytic). Haller et al. (2009) reported that URB597 did not decrease anxiety when the elevated plus-maze test was performed under mildly aversive conditions (e.g., in a familiar room or under low light). In contrast, the benzodiazepine anxiolytic chlordiazepoxide decreased anxiety under all conditions. In the case of genetic deletion of FAAH, mutant mice showed evidence of decreased anxiety relative to wild type mice under both bright and dim lighting conditions in the social interaction and open field tests; but, when the mutant mice received rimonabant under dim lighting conditions in the open field test (i.e., under less aversive conditions), their behavior suggested hypersensitivity to anxiogenic effects of CB1 blockade (Cassano et al., 2011). After carefully reviewing published

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**Table 3 | Continued**

| Authors | Animals | Drug | Doses and route | Test | Anxiety | Depression |
|---------|---------|------|----------------|------|---------|------------|
| Naidu et al. (2007) | FAAH KO mice (C57BL/6J) | N/A | N/A | Modified tail suspension test | ↓ |  |
| Naidu et al. (2007) | C57BL/6J mice | URB597 | 0.1 mg/kg, IP | Modified tail suspension test | ↓ |  |
| Seillier and Giuffrida (2011) | Wistar rats | URB597 | 0.1, 0.3, 1 mg/kg, IP | EPM |  | = |
| Haller et al. (2009) | Sprague-Dawley rats | URB597 | 0.1–0.3 mg/kg, IP | EPM (low aversiveness) |  | = |
| Haller et al. (2009) | Sprague-Dawley rats | URB597 | 0.1–0.3 mg/kg, IP | EPM (high aversiveness) | ↓ |  |

EPM, elevated plus-maze. For effects in models of anxiety and depression, ↓ indicates impairment; ↑ indicates enhancement; = indicates no effect.
As such, the main role of endocannabinoid signaling appears to be the blockade of excessive neuronal activation. The CB₁ receptor is strongly expressed in limbic structures (Herkenham et al., 1991), suggesting that cannabinoid signaling has a particularly important role in the control of neuronal responses induced by environmental challenges that often involve an emotional dimension. As brain anandamide levels are strongly increased by aversive stimuli (Walker et al., 1999; Kirkham et al., 2002; Marsicano et al., 2002; Hohmann et al., 2005), one can hypothesize that the activity dependent release of endocannabinoids serves as a feedback mechanism that reduces the amplitude of challenge-induced neuronal excitations (Gerdean and Lovinger, 2001; Adermark and Lovinger, 2007; Straiker and Mackie, 2009). This mechanism may be one that explains the strong impact of environmental conditions on the behavioral consequences of FAAH inhibition. Particularly, enhanced dampening of aversion-induced neuronal activations may lessen the behavioral impact of aversive stimuli.

In most cases the cognitive and emotional consequences of FAAH inhibition have been demonstrated to be CB₁-mediated.

CONCLUSION – ENDOCANNABINOID SIGNALING AND EMOTIONAL BEHAVIOR

Cannabinoid signaling appears to decrease depression-like and anxiety-like behaviors in laboratory models. These effects were observed using a variety of means to affect cannabinoid signaling, a variety of animal models, and a variety of species. The reasons for discrepancies are multiple, but an increasing number of publications suggest that the emotional effects of enhanced endocannabinoid signaling largely depend on environmental influences. These findings suggest that the anxiolytic effects, and possibly the antidepressant effects, of endocannabinoid signaling are enhanced under aversive conditions, which strengthens, rather than weakens, the putative usefulness of medications that enhance endocannabinoid signaling in the treatment of emotional disorders.

CONTEXT DEPENDENCE OF ENDOCANNABINOID MODULATION OF COGNITIVE AND EMOTIONAL BEHAVIOR

BRAIN FUNCTIONS AND ASSUMPTIONS FOR BEHAVIOR

Uniquely, endocannabinoids signal “backward”: they are released from the post-synaptic membrane and inhibit the synaptic neurotransmission that triggered their release (Wilson and Nicoll, 2001). Although a certain, probably low, level of tonic activation cannot be excluded, the endocannabinoid signal occurs phasically by demand, i.e., when the intensity of anterograde synaptic communication reaches certain levels (Di Marzo et al., 1999; Marsicano et al., 2003; Lutz, 2004; Adermark and Lovinger, 2007).

As such, the main role of endocannabinoid signaling appears to be the blockade of excessive neuronal activation. The CB₁ receptor is strongly expressed in limbic structures (Herkenham et al., 1991), suggesting that cannabinoid signaling has a particularly important role in the control of neuronal responses induced by environmental challenges that often involve an emotional dimension. As brain anandamide levels are strongly increased by aversive stimuli (Walker et al., 1999; Kirkham et al., 2002; Marsicano et al., 2002; Hohmann et al., 2005), one can hypothesize that the activity dependent release of endocannabinoids serves as a feedback mechanism that reduces the amplitude of challenge-induced neuronal excitations (Gerdean and Lovinger, 2001; Adermark and Lovinger, 2007; Straiker and Mackie, 2009). This mechanism may be one that explains the strong impact of environmental conditions on the behavioral consequences of FAAH inhibition. Particularly, enhanced dampening of aversion-induced neuronal activations may lessen the behavioral impact of aversive stimuli.

In most cases the cognitive and emotional consequences of FAAH inhibition have been demonstrated to be CB₁-mediated.

The broad effects of anandamide signaling may offer an alternative explanation for the impact of environmental conditions on the behavioral consequences of FAAH inhibition. CB₁ receptors occur on GABAergic and glutamatergic synapses, and activation of these receptors can inhibit the release of several neurotransmitters, including glycine, acetylcholine, norepinephrine, dopamine, serotonin, and cholecystokinin (Gifford and Ashby, 1996; Ishac et al., 1996; Cadogan et al., 1997; Katona et al., 1999, 2001; Nakazi et al., 2000; Beinfeld and Connolly, 2001; Hájos and Freund, 2002; Fernández-Ruiz et al., 2010). Thus, endocannabinoids affect the function of many neurotransmitter systems, some of which play opposing roles. For example, glutamatergic mechanisms appear to promote anxiety while GABAergic mechanisms appear to inhibit it (Millan, 2003). This diversity of cannabinoid roles and the complexity of task-dependent activation of neuronal circuits may inherently lead to the effects of endocannabinoid activation being strongly dependent on environmental conditions.

Presumably, each environmental challenge and behavioral response is bound to the activation of particular neuronal circuits. The effects of cannabinoid signaling probably depend on the ratio, brain location, and neurochemical nature of those neurons that express cannabinoid receptors and are activated in the particular situation. A small change in the environment might recruit new neurons in the situation-dependent circuit, changing the share, location, and neurochemical nature of the cannabinoid-controlled synapses that were activated. Thus, each effect of cannabinoids would be specific to the situation.

The hypothesis presented here has two parts: that cannabinoid signaling has an important role in dampening excessive neuronal responses induced by environmental challenges that often involve an emotional dimension, and that the function of endocannabinoid neuronal circuits is situation-dependent. Endocannabinoid signaling is activated when there is a relatively high level of synaptic activity, as would be triggered by environmental challenges that require prompt behavioral responses. Retrograde signaling by cannabinoids would affect only those neurons that: (1) are highly activated by the perception or interpretation of the challenging information and by the behavioral response; and (2) also express CB₁ receptors on their axon terminals. These conditions are likely to be met by neurons that have opposing roles overall (e.g., glutamatergic and GABAergic neurons) or have wide ranging behavioral effects (e.g., monoaminergic neurotransmission). As a result, cannabinoids selectively affect a mosaic of widely heterogeneous neurons that may have convergent, divergent, or independent effects on the development of the behavioral response, and leave many neurons unaffected, or affected only indirectly. Interfering with such a complex regulatory process naturally leads to complex and situation-dependent effects. Under such conditions, the relative consistency of available findings may be due to the fact that scientific studies are highly standardized. Even small deviations from experimental protocols (e.g., directing the light on the tail of rats in the tail suspension test; Naidu et al., 2007) may bring about surprising findings. More surprising findings can be expected after more dramatic changes in experimental conditions, for example by varying the aversiveness of environmental conditions (Haller et al., 2009).
One possible argument against this hypothesis is that anandamide may not be directly involved in CB1-mediated retrograde endocannabinoid signaling, because the post-synaptic localization of its synthesizing enzymes is at variance with the pre-synaptic localization of the CB1 receptor (Katona and Freund, 2008). One has to note, however, that cannabinoids were shown to affect extra-synaptic (volumetric) neurotransmission (Lau and Schloss, 2008; Morgese et al., 2009), and endocannabinoids, especially anandamide, are able to exert effects via the putative CB3 (non-CB1/non-CB2) cannabinoid receptor (De Petrocellis and Di Marzo, 2010). One also has to note that discrepancies between functional and morphological findings may be fairly common in the case of cannabinoid signaling (see e.g., Kawamura et al., 2006).

CONCLUSION AND PRACTICAL IMPLICATIONS

Conflicting findings are not rare in behavioral pharmacology. Yet, the enhancement or blockade of endocannabinoid signaling has provided inconsistent findings even within the same laboratory; moreover, deliberate changes in environmental conditions have resulted in marked changes in the effects of the same manipulations within the same series of experiments. Taken together, the findings reviewed here raise the possibility that endocannabinoid signaling may change the impact of environmental influences on behavior rather than affecting one or another specific behavior. This assumption may be especially valid for emotional behaviors, but it may indirectly affect findings obtained in tests where emotions are not the focus, such as learning and memory. Further research in this respect appears warranted.

From a practical point of view, the assumption formulated above may not necessarily invalidate cannabinoid neurotransmission as a pharmaceutical target. Altered responses to environmental stimuli are at the core of emotional disorders, and also appertain to disorders related to learning and memory. Thus, the ability of cannabinoid-related treatments to modulate the impact of challenging environmental conditions on emotional and cognitive behavior could be a productive focus for medications development.

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