Role of Endogenous Cannabinoids in Cognition and Emotionality

Carsten T. Wotjak*

Max-Planck-Institut für Psychiatrie, AG Neuronale Plastizität, Kraepelinstr. 2, D-80804 Munich, Germany

Abstract: Novel pharmacological tools and the generation of null-mutants enabled the elucidation of the role of endocannabinoids in cognition and emotionality of rats and mice. Endocannabinoids seem to limit memory retention, to facilitate memory extinction and to ensure adequate coping with stressful situations. A selective potentiation of these actions may lead to novel pharmacotherapies for human anxiety disorders.

Keywords: Learning, memory, review, anxiety, fear, stress, FAAH, CB1, TRPV1, VR1, anandamide, 2-AG, SR141716A, AM404, AM251, URB532, URB597, UCM707.

INTRODUCTION

For over 4000 years, *Cannabis sativa* extracts have been used for therapeutic and recreational purposes [85]. However, besides its putative euphoric and therapeutic effects, marijuana or hashish consumption causes disturbances in various aspects of learning and memory (for reviews see [3, 71, 92]). It has, furthermore, ambivalent effects on the mood of the consumers, as marijuana is known to both alleviate and exacerbate anxiety in humans [121, 130]. Initially it was generally assumed that the hydrophobic psychoactive constituents of marijuana, including Δ^9-tetrahydrocannabinol (Δ^9-THC), mediate their effects on cognition and mood in an unspecific manner, by nonselective modification of the fluidity of cell membranes (for review see [88]). This concept had to be revised after the discovery and cloning of specific binding sites for Δ^9-THC (for reviews see [59, 74]). With cannabinoid receptor type 1 (CB1; [80]) and type 2 (CB2; [94]), two receptor subtypes were characterized that mediate central (CB1) and peripheral effects (CB2) of cannabinoids. Both CB1 and CB2 are G-protein-coupled receptors containing seven transmembrane domains. The action of cannabinoids *via* CB1 are mediated by Gi/o proteins to inhibit adenylyl cyclase, Ca^{2+}-channels and K^+-channels (for reviews see [61, 62, 74, 99]). A number of selective agonists of CB1 have been synthesized (for reviews see [37, 62]; Table **1**) that together with Δ^9-THC helped to elucidate cognitive effects of cannabinoids (for review see [71]).

The discovery of CB1 and CB2 raised the question as to the existence of endogenous ligands (endocannabinoids). Indeed, with *N*-arachidonoyl ethanolamide (anandamide; [33]) and 2-arachidonoyl glycerol (2-AG; [87, 116, 117]) two ligands of CB1 could be isolated and characterized. The class of endogenous ligands of cannabinoid receptors is steadily expanding. Today, we know at least five different arachidonoyl derivates, which can activate CB1 as endocannabinoids (for review see [126]). Both anandamide and 2-AG belong to classes of natural lipids, the fatty acid amides and monoacylglycerols, respectively. By now it is generally accepted that endocannabinoids are synthesized on demand and released from cells through a mechanism that does not require vesicular secretion. Termination of endocannabinoid signaling is achieved by intracellular degradation ([34], for reviews see [89, 99]). To this end, anandamide and 2-AG are internalized most likely through a selective process of facilitated diffusion (for reviews see [40, 71]), although the existence of a carrier responsible for this uptake has been questioned by pharmacological means [48]. Fatty acid amide hydrolase (FAAH) mediates degradation of anandamide, whereas 2-AG is primarily degraded by monoglyceride lipase, a serine hydrolase (for reviews see [23, 40]).

Anandamide may interact not only with CB1 but also with the vanilloid type 1 receptor (VR1, TRPV1). This heat- and proton-activated, ligand-gated nonselective cation channel belongs to the TRP family of ion channels (for reviews see [9, 64]) and represents the site of action of the pungent component of 'hot' red chili peppers, capsaicin [16]. The physiological relevance of the expression of TRPV1 in discrete brain areas [91] is still unknown. However, several reports suggest that anandamide (in addition to other putative "endovanilloids") interact with TRPV1 (for review and discussion see [35, 113, 118, 119, 137]). Moreover, this interaction can by dynamically regulated *via* phosphorylation of TRPV1 [103]. There is some evidence that anandamide activates two antagonistic regulatory systems *via* binding to both CB1 and TRPV1 (for review see [35]).

The physiological relevance of the interaction between the endocannabinoids and their receptors remained enigmatic for a long time. However, novel pharmacological tools and the generation of null-mutants that lack expression of CB1, TRPV1 or FAAH helped to characterize the function of endocannabinoids. This review will summarize the progress in this field. It largely concentrates on the role of endocannabinoids in cognition and emotionality of rats and mice. The first part of the review explains the general strategies used for characterizing the involvement of endocannabinoids in memory processes and in anxiety. The second part briefly describes the behavioral tests employed. The third, fourth and fifth parts summarize our current knowledge about the involvement of endocannabinoids in locomotion, cognition and emotionality. The final part merges the different effects of endocannabinoids into a hypothesis of endocannabinoid action. This review ends

*Address correspondence to this author at the Max Planck Institute of Psychiatry, RG Neuronal Plasticity / Mouse Behavior, Kraepelinstr. 2, D-80804 Munich, Germany; Tel: +49-89-30622-652; Fax: +49-89-30622-610; E-mail: wotjak@mpipsykl.mpg.de

1389-5575/05 $50.00+.00

with a list of open questions and an outlook for the clinical relevance of the findings.

1. GENERAL STRATEGIES FOR CHARACTERIZING THE PHYSIOLOGICAL RELEVANCE OF ENDOCANNABINOIDS

CB1 is one of the most abundant G protein-coupled receptor of the brain [57, 58]. It is expressed not only in brain structures implicated in cognition (e.g., prefrontal cortex and hippocampus) and emotionality (e.g., basolateral amygdala), but also in basal ganglia, hypothalamus and cerebellum that control motor behaviors and hormonal systems of the organism. Exogenous agonists bind to CB1 independently of whether or not the endocannabinoid system of the respective brain structure has been activated. They cause a complex set of motor, autonomous, analgesic, cognitive and emotional responses (for reviews see [3, 18]), provide little information about the physiological relevance of endocannabinoids. Therefore, studies have rather to selectively interfere with the endocannabinoid system on its activation by a given test situation. This can be achieved by blocking receptor binding or degradation of endocannabinoids. In behavioral experiments, CB1 was inactivated by administration of receptor antagonists, such as SR141716A [105] (Table **1**) and AM251 [44] (Table **1**), or by genetical ablation of CB1 (Table **2**). Despite the proposed specificity of SR141716A as an antagonist of CB1, the drug seems to bind to other receptors as well, as it was biologically active in mice deficient for CB1 [12, 41, 51, 52, 53, 100]. Furthermore, SR141716A seems to partially act as an inverse agonist of CB1 [5, 68]. However, SR141716A is 3 to 4 magnitudes more potent as a CB1 antagonist than as an inverse agonist. This suggests that the drug may have limited inverse agonist activity *in vivo* (for detailed discussion see [71]). Consequently, behavioral effects of SR141716A treatment would provide evidence for a constitutively active or an acutely activated endocannabinoid tone that regulates locomotion, cognition or emotionality.

To circumvent the shortcomings of SR141716A treatment, namely that the drug mediates parts of its effects as an inverse agonist or *via* receptors other than CB1, four different mouse lines with null-mutation of the CB1 gene were generated independently from each other by homologous recombination [69, 76, 106, 134]. Mice with null-mutation of the CB1 gene on both alleles are called $CB1^{-/-}$, control animals with intact alleles $CB1^{+/+}$. To differentiate between the four lines that differ in their genetic background, an additional suffix is introduced (Table **2**). So far, only three of the four lines were used for behavioral studies. There are, furthermore, two different mouse lines with null-mutation of the TRPV1 gene [17, 27] (Table **2**).

Conventional mutants have the general disadvantage that a given gene product is ablated throughout ontogeny in all cells of the body. Thus, the phenotype for instance of $CB1^{-/-}$ might, at least in part, relate to developmental effects of CB1 deficiency and cannot be ascribed to a distinct cell type. The situation appears to be different in conditional mutants, in which the gene of interest is ablated in a cell-type specific manner using the *Cre-loxP* system (for review see [93]). For instance, a recently generated mouse line lacks expression of CB1 receptors in principal neurons of the forebrain at late stages of development, but leaves the expression of CB1 in GABAergic interneurons intact [77]. Conditional mutants will be certainly of high value for further dissecting the physiological relevance of the endocannabinoid system.

The physiological relevance of the endocannabinoid system can be assessed not only by an inactivation of respective binding sites, but also by an amplification of endocannabinoid signaling. Table **1** summarizes some of the most prominent compounds used in behavioral experiments for pharmacological blockade of endocannabinoid uptake and degradation. Moreover, a conventional mouse mutant has been generated with null-mutation of the FAAH gene (Table **2**), which exhibits constitutively increased brain levels of anandamide [22].

2. GENERAL STRATEGIES FOR MEASURING COGNITION AND EMOTIONALITY IN MICE AND RATS

The terms cognition and emotionality are frequently employed to describe certain alterations in the behavioral performance of mice and rats. They refer to the classical distinction between *ratio* and *emotio*. It is not in the scope of this article to provide a comprehensive definition of either term. In this article, cognition is used as a superordinate concept of learning and memory. Emotionality, in contrast, stands for arousal and motivational states that affect the animals' innate reactions to a given test situation.

In its broadest sense, learning describes a process of adaptation to the environment, based on information transfer and subsequent changes in interneuronal communication. Memory refers to the relative persistence of these changes. We differentiate between short-term and long-term memory, depending on the duration of memory retention and the involvement of protein biosynthesis in memory consolidation [82]. The persistence of a memory is limited either by its decay or by a loss in the ability to retrieve / recall it. In addition, memories can be extinguished by training, whereby the original memory trace will be actively suppressed by an inhibitory learning process (extinction; [95]). Behavioral paradigms for the study of learning and memory can be classified into appetitive and aversive learning task, depending on whether positive (e.g., food reward) or negative reinforcers (e.g., electric footshock) are employed. Recognition learning represents a third category, which is solely based on the innate motivation of the animals to explore unfamiliar conspecifics, odors or objects.

Human studies on adverse effect of cannabinoids suggest a role of the endocannabinoid system in fear and anxiety [121, 130]. In animal experiments fear- and anxiety-related behavior is typically assessed by measuring the avoidance of a potential threat. On the one hand, mice and rats have an innate drive to explore novel environments. On the other hand, they try to avoid brightly lit and open areas. The resulting inner conflict between exploration and avoidance defines the behavioral performance [8], for instance in the light-dark test [10, 56] or on the elevated plusmaze [107, 128].

Table 1. Endogenous Ligands, Agonist and Antagonists of CB1 and Inhibitors of Endocannabinoid Uptake and Degradation

Class	Name	Comments	Refs
Endogenous ligands of CB1 and VR1	Anandamide	· *N*-arachidonoyl ethanolamide; · Endogenous ligand of CB1 (and TRPV1)	[33, 114, 135]
	2-AG	· 2-arachidonoyl glycerol · Endogenous ligand of CB1	[87, 117]
	Noladin ether	· 2-arachidonoylglyceryl ether · Endogenous ligand of CB1	[55]
	Virodhamine	· *O*-arachidonoyl ethanolamine · Endogenous ligand of CB1	[102]
	NADA	· *N*-arachidonoyl dopamine · Endogenous ligand of TRPV1	[63]
Agonists of CB1	Δ^9-THC	· Δ^9-tetrahydrocannabinol · Psychoactive constituent of marijuana, agonist of CB1	[43, 84]
	CP-55,940	· (-)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl) phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexan-1-ol	[32, 36]
	HU210	· (6a*R*)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol	[36, 60, 86]
	WIN55,212-2	· *R*-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate	[26]
Antagonists of CB1	SR141716A	· *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide · May act as inverse agonist · May bind to receptors other than CB1	[105] [68] [12, 41, 51, 52, 53, 100]
	AM251	· *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide	[44, 67, 52]
Irreversible inhibitors of FAAH	URB532	· n-butylcarbamic acid 4-benzyloxy phenyl ester · Irreversible inhibitor of FAAH	[65]
	URB597	· cyclohexylcarbamic acid 3'-carbamoylbiphenyl-3-yl ester · Irreversible inhibitor of FAAH	[65]
Re-uptake inhibitors	AM404	· *N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide · Inhibitor of endocannabinoid uptake · Agonist of TRPV1	[6] [30, 136]
	VDM11	· α-[3-[[2-(3,4-dimethoxyphenyl) ethyl]methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl) benzeneacetonitrile · Inhibitor of endocannabinoid uptake	[30]
	UCM707	· *N*-(3-furylmethyl)eicosa-5,8,11,14-tetraenamide, · Inhibitor of endocannabinoid uptake	[73]

Notably, both learning and memory tasks and tests of emotionality measure changes in locomotion. As cannabinoids exert strong effects on locomotion (for reviews see [3, 18, 126]), pharmacological studies targeting the endocannabinoid system have to carefully dissect unspecific effects of the drugs on locomotion from specific effects on cognition and emotionality. The following sections will briefly review current data about the role of endocannabinoids in locomotion, followed by a compilation of their involvement in cognitive processes and emotionality.

3. ROLE OF ENDOCANNABINOIDS IN LOCOMOTION

Data about an involvement of endocannabinoids in locomotion are inconsistent (Tables **3.1** and **3.2**). In the majority of the studies, blockade of CB1 receptors by intermediate doses of SR141716A [4, 46, 47, 97] and AM251 [112] had no consequences on horizontal and vertical ambulation. High doses of SR141716A, in contrast, caused an increase in locomotion [5, 20]. This effect seems to depend on the genetic background of the animals [20, 122] and likely relates to mechanisms different from an interaction with CB1 receptors [5]. Null-mutation of the CB1 gene had no consequences on horizontal locomotion in $CB1^{CL}$ ([75, 123] but see [69, 125]) and $CB1^{GM}$ mice [76]. $CB1^{AZ}$ mice, in contrast, showed a strong reduction in locomotion [115], what likely relates to differences in the genetic background (Table **2**) and the experimental procedure.

Potentiation of endocannabinoid signaling had either no effect or decreased locomotion (Table **3.2**). Administration of AM404 (Table **1**) reduced locomotion in independent studies [7, 47, 49]. URB532 had similar consequences [65].

Table 2. Mouse Models with Genetical Inactivation of CB1, VR1 and FAAH

Target	Name	Description	Genetic Background	Refs
CB1	$CB1^{CL}$	Null-mutant	CD1 (F5-F14)	[69]
	$CB1^{AZ}$	Null-mutant	C57BL/6J (F3)	[134]
	$CB1^{GM}$	Null-mutant	C57BL/6N (F6)	[76]
	$CB1^{DR}$	Null-mutant	C57BL/6? (F2-3?)	[106]
	$CB1^{f/fCaMKII\alpha Cre}$	Conditional mutation of CB1 in prinicpal neurons of the forebrain; expression in interneurons is intact	C57BL/6N (F6)	[77]
FAAH	FAAH	Null-mutant	C57BL/6? (F2-3)	[22]
TRPV1	$TRPV1^{MC}$	Null-mutant	C57BL/6? (F2-4)	[17]
(VR1)	$TRPV1^{JD}$	Null-mutant	C57BL/6J (F2-3)	[27]

A suffix was added to the name of the mutants in order to facilitate the differentiation between the different lines; ? - exact mouse strain not known; Note that the individual lines differ in their genetic background

In contrast, neither UCM707 [29], as another inhibitor of anandamide and 2-AG uptake (Table **1**), nor null-mutation of the FAAH gene [22] affected locomotion. It remains to be shown whether the effects of AM404 on locomotion are at least partially mediated *via* its agonistic action on TRPV1 receptors [30]. Upon the first glance, such a scenario appears to be unlikely, as null-mutation of the TRPV1 gene had no consequences on locomotion [17, 27] (Table **3.1**). However, AM404 would act as an exogenous agonist, whereas the test situation might not sufficiently activate the endogenous ligands of TRPV1 to cause alterations in locomotion. Taken together the majority of the data argue against a significant involvement of endocannabinoids in the regulation of locomotion in naive, untreated animals. The situation might be different under pathological conditions, as low doses of AM404 alleviated hyperactivity in spontaneous hypertensive rats (SHR; [7]).

4. ROLE OF ENDOCANNABINOIDS IN COGNITION

There is a vast literature on the effects of cannabinoids on learning and memory in animals and humans. The most prominent among the various consequences of CB1 receptor activation by exogenous agonists are disruptive effects on working memory, i.e. on processes necessary to learn and react to new information that differs from session to session. Reference memory (i.e. processes that enable consolidation and recall of information that remain stable from session to session), in contrast, remains largely unaffected (for reviews see [71, 92]). The situation appears to be different for endocannabinoids. As illustrated in Tables **4.1** and **4.2**, the involvement of endocannabinoids in cognition seems to predominantly relate to the temporal limitation of recognition and reference memory, with little consequences on working memory [54, 69, 96, 127]. Blockade of the CB1 receptor with SR141716A prolonged the retention of juvenile recognition in adult mice and rats, restored juvenile recognition in aged mice and rats and disrupted the amnesic consequences of retroactive interference [120]. Accordingly, CB1-deficient mice showed prolonged recognition of a familiar object [75, 104]. Interestingly, the effects of SR141716A treatment on juvenile recognition were limited to a rather narrow time window after the sampling period. Injection of SR141716A 0 min and 5 min but not 15 min or 90 min after sampling improved memory performance, assessed 2h after sampling [120]. Taking into consideration data about pharmacokinetics and pharmacodynamics of the drug [98], it is very likely that, for each time point of the treatment, SR141716A still occupied the CB1 receptors during memory recall. Therefore, endocannabinoids seem to affect early consolidation rather than recall of recognition memory. Similar data were obtained for reference memory in an appetitively-motivated spatial-learning task (radial maze), where SR141716A improved memory performance only if administered before [70] or immediately after training [132], but not 20 min later [70]. The observation that drug administration before training improved reference memory suggests a role of endocannabinoids in memory acquisition. This would be in line with observations that both pharmacological and genetical inactivation of CB1 facilitates induction and maintenance of hippocampal long-term potentiation (e.g., [11, 15]), a cellular model of memory [79].

Contrary to the consequences of pharmacological inactivation of CB1, genetical ablation of the receptor revealed that the interaction of endocannabinoids with CB1 is dispensable for both acquisition and consolidation of reference memory, at least in aversively-motivated learning tasks [76, 127]. The experiments of Varvel and Lichtman [127] suggest a specific involvement of endocannabinoids in memory extinction, as CB1-deficient mice were impaired in relearning a new platform position in a water-maze task. The notion of an involvement of endocannabinoids and CB1 in memory extinction is strongly supported by the experiments of Marsicano and co-workers [76]. The authors could show that $CB1^{-/-GM}$ mice were severely impaired in short-term and long-term extinction of auditory-cued fear memory. This phenotype could be confirmed by treating wild-type mice with SR141716A. In these animals, the CB1 antagonist attenuated short-term and long-term extinction if administered 30 min before memory recall, but had no effects if administered 2 min or 10 min afterwards. Importantly, SR141716A failed to affect memory performance if administered 30 min before conditioning, indicating that endocannabinoids do not interfere with acquisition and consolidation but specifically with extinction of fear memories. Recall of fear memory was accompanied by an activation of the endocannabinoid system

Table 3.1. Role of Endocannabinoids in Locomotion: Inactivation of CB1 and TRPV1 by Pharmacological and Genetical Means

Interference	Dose range	When	Animal (Strain)	Task	Locomotion	Comment	Ref.
SR141716A	0.1 – 30 mg/kg	0 min to 6 h before testing	Mouse (ICR)	OF (10 min, ? lux)	↑	· Biphasic response, with 10 and 20 mg/kg ascending limb, 30 mg/kg descending limb · effects still observed after 4 h	[20]
	10 and 30 mg/kg	0 min before testing	Mouse (ICR)	OF (120 min, ? lux)	↑	· Not mediated through CB1 alone and not the result of inverse agonism alone	[5]
	3 and 10 mg/kg	0 min before testing	Mouse (C57BL/6?)	OF (60min, ? lux)	¾	· Treatment 20 min after habituation	[122]
	3 mg/kg	0 min before testing	Rat (Wistar)	OF (120 min, ? lux)	¾	· No effect on locomotion and short-term habituation to the test environment during 2nd exposure (1st exposure: 8-h habituation to the setup 24 h before)	[97]
	3 mg/kg	30 min before testing	Rat (Wistar)	EPM	¾		[97]
	3 mg/kg	30 min before testing	Rat (Wistar)	Holeboard (5 min, ? lux)	¾	· No effect on horizontal and vertical ambulation · Reduced exploration of the holes	[4]
	1 mg/kg	60 min before first exposure	Rat (Wistar)	OF (120 min, intervals; 350 lux)	¾	· No effect on locomotion · Potentiation of the effects of D2 receptor activation	[46]
	0.5 mg/kg	30 min before testing	Rat (Wistar)	OF (120 min, intervals, 350 lux)	¾	· No effect on locomotion · Antagonizes the effects of AM404	[47]
AM251	3, 10 and 30 mg/kg	0 min before testing	Mouse (129/SVE)	OF (30 min, ? lux)	¾		[112]
CB1 null-mutation			$CB1^{CL}$ Mice	OF1 (10 min, < 5 lux)	↑		[69, 125]
				OF2 (5 min, 500 lux, d1-d3)	↑	· Increased horizontal and vertical locomotion during the first exposure · Normal long-term habituation (d1-d3)	[69, 125]
				OF (30 min, ? lux)	¾		[123]
				OF (5 min, 500 lux)	¾	· No effect on horizontal locomotion · Vertical exploration ↓ in 1-month and (↓) in 4-month old mice	[75]
			$CB1^{AZ}$ Mice	OF (40 min, ? lux)	↓		[115]
			$CB1^{GM}$ Mice	OF (30 min, 0 lux)	¾		[76]
TRPV1 null -mutation			$TRPV1^{MC}$ Mice	?	¾	· Data not shown	[17]
			$TRPV1^{JD}$ Mice	OF (30 min, ? lux)	¾		[27]
				Holeboard (10 min, ? lux)			[27]

EPM – elevated plusmaze; OF – open field, collective term for test environments of different dimensions and characteristics; ↑ hyperactivity; ↓ hypoactivity; ¾ no effect; ? – detailed information missing. If not stated otherwise, drugs were administered intraperitoneally or subcutaneously.

in the basolateral amygdala [76]. This observation provides first evidence that synthesis and release of anandamide and 2-AG are dynamically regulated during cognitive processes.

In a recent study, blockade of CB1 by SR141716A reversed the amnesic effects of β-amyloid fragments in a passive avoidance task [81]. Although the specificity of this effect for β-amyloid fragments as compared to other peptides

Table 3.2. Role of Endocannabinoids in Locomotion: Inactivation of Endocannabinoid Uptake and Degradation by Pharmacological and Genetical Means

Interference	Dose range	When	Animal (Strain)	Task	Locomotion	Comment	Ref.
AM404	10 mg/kg	10 min before testing	Rat (Wistar)	OF (10 min, ? lux)	↓	· Assessed during the last 5 min of exposure · Trend towards reduced exploration	[49]
	10 mg/kg	0 min before testing	Rat (Wistar)	OF (120 min, intervals, 350 lux)	↓	· 30 and 60min after treatment · Blocked by SR141716A	[47]
	10 µg icv	0 min before testing	Rat (Wistar)	OF (120 min, intervals, 350 lux)	↓	· 60 and 120 min after treatment · Counteracts the effects of D2 receptor activation	[7]
	1 mg/kg	0 min before testing	Rat (SHR / WKY)	Làt-maze (30 min, ? lux)	↓	· Alleviation of hyperactivity in SHR without significant effects on normal motor behavior of progenitor WKY rats	[7]
UCM707	0.1, 1 and 10 mg/kg	10 min before testing	Rat (Wistar)	OF (10 min, ? lux)	¾	· No overt effect on locomotion during the last 5 min of exposure	[29]
URB532	5 and 10 mg/kg	30 min before testing	Rat (Wistar)	OF (20 min, ? lux)	↓	· Dose-dependently	[65]
FAAH null-mutation	Vehicle	15 min before testing	FAAH Mice	OF (5 min, ? lux)	¾		[22]

OF – open field, collective term for test environments of different dimensions and characteristics; ↑ hyperactivity; ↓ hypoactivity; ¾ no effect; ? – detailed information missing. If not stated otherwise, drugs were administered intraperitoneally or subcutaneously.

remains to be shown, this finding may have implications for the development of novel pharmacotherapeutic strategies for the treatment of patients suffering from Alzheimer's disease.

So far, the role of endocannabinoids in cognition was assessed by pharmacological and genetical inactivation of CB1. There are no reports about consequences of potentiation of endocannabinoid signaling.

The cellular mechanisms underlying the effects of endocannabinoids on early memory consolidation and memory extinction are still unknown. These processes are likely to involve an activation of selected kinases (e.g., [31, 124]) that have been implicated in both consolidation and extinction of aversive memories (for review see [28]). Moreover, the similar behavioral consequences of an inactivation of CB1 and an inhibition of phosphatases suggest a potential link between the endocannabinoid system and phosphatases. For instance, inhibition of protein phosphatase 1 facilitated recognition memory and prolonged memory retention [45], similarly to null-mutation of CB1. Moreover, inhibition of protein phosphatase 2B (calcineurin) impaired extinction of aversive memories [72], thus again resembling the phenotype of CB1-deficient mice. Last but not least, as a retrograde messenger, anandamide modifies neurotransmitter release (for review see [111]) and synaptic transmission (for reviews see [2, 131]) in different ways with potential implications for cognitive processes.

5. ROLE OF ENDOCANNABINOIDS IN EMOTIONALITY

Several studies addressed the question as to the involvement of endocannabinoids in innate fear and anxiety (Tables **5.1**-**5.3**). In Wistar rats, blockade of CB1 receptors by SR141716A caused an increase in anxiety-related behavior [4, 97, 109]. In contrast, lower doses of SR141716A, administered 60 rather than 30 min before testing, had no effects [65]. Data obtained in mice were more inconsistent. Administration of SR141716A either decreased [1, 53, 108] or increased [1] anxiety-related behavior, depending on the genetic background of the animals [1] and the test situation. Strikingly, SR141716A increased anxiety-related behavior also in CB1-deficient mice [53], indicating that the drug might mediate its effects on emotionality at least in part *via* a receptor different from CB1. This conclusion is supported by other studies, suggesting the existence of a so far unidentified 'CB3' receptor [41, 51, 52, 100].

Data obtained from mice with null-mutation of the CB1 gene were similarly inconsistent as those for SR141716A treatment (Table **5.2**). Even mice of the same line ($CB1^{-/-CL}$) showed either unaltered [69, 75] or increased [78, 123] anxiety-related behavior. Mutation of the CB1 gene had no effect on anxiety in another line ($CB1^{GM}$; [76]). The anxiogenic effect of CB1 ablation in $CB1^{-/-CL}$ could not be

Table 4.1. Role of Endocannabinoids in Cognition: Pharmacological Inactivation of CB1

Interference	Dose range	When	Animal (Strain)	Task	Memory	Comment	Ref.
SR141716A	0.1 – 3 mg/kg	0, 5, 15 and 90 min after first exposure	Rat (Wistar)	Juvenile recognition	↑ –	· Recognition of juvenile 2h after first encounter if treated at 0 or 5 min · No effect if treated at 15 or 90 min	[120]
	1 mg/kg	0 min after first exposure	Rat (Wistar)	Juvenile recognition	↑	· Disruption of retroactive interference	[120]
	0.03 – 0.1 mg/kg	0 min after first exposure	Rat (Wistar, 24 month)	Juvenile recognition	↑	· Restoration of juvenile recognition assessed 45 min after the first encounter	[120]
	0.1 – 1 mg/kg	0 min after first exposure	Mouse (CD1, 12 month)	Juvenile recognition	↑	· Restoration of juvenile recognition assessed 30 min after the first encounter	[120]
	1 mg/kg	20 min before acquisition	Rat (SD)	Radial maze	↑	· Increased reference memory assessed after a 6-h delay	[70]
	1 mg/kg	20 min after acquisition	Rat (SD)	Radial maze	¾	· No effect on reference memory assessed after a 6-h delay	[70]
	1 mg/kg	20 min before recall	Rat (SD)	Radial maze	¾	· No effect on reference memory assessed after a 6-h delay	[70]
	1 mg/kg	0 min after acquisition	Rat (SD)	Radial maze	↑	· Improved reference memory assessed after a 7-h delay · No effect on reference memory assessed after a 6-h delay	[132]
	3 mg/kg	30 min before acquisition	Mouse (C57BL/6JOlaHsd)	Fear conditioning	¾	· No effect on memory acquisition and consolidation of auditory-cued fear memory	[76]
	3 mg/kg	30 min before recall	Mouse (C57BL/6JOlaHsd)	Fear conditioning	↓	· Impaired short-term and long-term extinction of auditory-cued fear memory	[76]
	3 mg/kg	2 and 10 min after recall	Mouse (C57BL/6JOlaHsd)	Fear conditioning	¾	· No effect on long-term extinction of auditory-cued fear memory	[76]
	1 mg/kg	30 min before 2^{nd} retention test	Mouse (Swiss)	Passive avoidance	(↑)	· No effect on memory recall in control mice · Reversal of the amnesic effects of β-amyloid fragments	[81]
	1.5 mg/kg	20 min before training	Rat (LE)	DNMS	¾	· No effect on working memory at a dose sufficient to block effects of WIN55,212-2 on DNMS · No effect on neuronal firing in the hippocampus	[54]
	0.5 mg/kg	20 min before testing	Rat (SD)	T-maze	¾	· No effect on spontaneous alternation (working memory) at a dose sufficient to block effects of THC	[96]

DNMS – delayed-non matching-to-sample procedure; ↓ amnesic effects; ↑ promnesic effects; ¾ no effect. If not stated otherwise, drugs were administered intraperitoneally or subcutaneously.

reversed by benzodiazepine treatment [123], indicating that the impaired regulation of GABAergic transmission in $CB1^{-/-}_{CL}$ mice (for review see [40]) significantly contributes to the phenotype observed.

Potentiation of endocannabinoid signaling in Wistar rats by irreversible blockade of FAAH had opposite effects to blockade of CB1 receptors (Table **5.3**). Administration of URB532 and URB597 reduced anxiety-related behavior, an effect that could be blocked by SR141716A [65].

There are several reasons that might explain the inconsistent data on an involvement of endocannabinoids in anxiety. First, anxiety-related behavior seems to critically depend on the genetic background of the animals (e.g., [24, 50]). This includes strain differences in maternal care that might influence the emotionality of the offspring ([13, 14, 39]; for review see [83]). Unfortunately, a significant number of studies still fails to provide sufficient information about the rat or mouse strain tested [133], what hampers the interpretation and comparability of the data with respect to genetic background. Second, most of the behavioral paradigms used for the measurement of innate fear and anxiety are highly sensitive to the lab environment, housing conditionings and handling of the animals during the experiment [19, 21]. Other confounding factors are the time

Table 4.2. Role of Endocannabinoids in Cognition: Genetical Inactivation of CB1

Interference	Dose range	When	Animal	Task	Memory	Comment	Ref.
CB1 null-mutation			$CB1^{CL}$ Mice	Y-maze	↓	· Decrease in spontaneous alternation (impaired working memory)	[69]
				Object recognition	↑	· Prolonged recognition of a familiar object ($CB1^{+/+}$ < 24h; $CB1^{-/-}$ > 48h)	[104]
				Object recognition	↑	· Superior recognition memory in 1-month and 4-month old mice	[75]
				Active avoidance	↑	· Improved avoidance learning in the shuttle-box	[78]
			$CB1^{AZ}$ Mice	Watermaze	¾ ¾ ↓	· No differences in working memory task · Reference memory not affected · Impaired relearning of new platform position · Swimming problems, increased floating; >50% of $CB1^{-/-}$ had to be excluded during relearning because of "strange" swim strategies and seizures	[127]
			$CB1^{GM}$ Mice	Fear conditioning	↓	· Impaired short-term and long-term extinction of auditory-cued fear memory	[76]

↓ amnesic effects; ↑ promnesic effects; ¾ no effect

point of testing in respect to the circadian rhythm of the animals and the test conditions. In particular the illumination strength shifts the balance between spontaneous locomotion and anxiety-related inhibition or activation of

Table 5.1. Role of Endocannabinoids in Fear and Anxiety: Pharmacological Inactivation of CB1

Interference	Dose range	When	Animal	Task	Anxiety	Comment	Ref.
SR141716A	0.1 - 3 mg/kg	30 min before testing	Rat (Wistar)	Defensive Withdrawal (350 lux)	↑	· Dose-dependently (significant at 3 mg/kg) · Test performed under familiar conditions (10-min habituation to environment 24h before)	[97]
	3 mg/kg	30 min before testing	Rat (Wistar)	Defensive Withdrawal (350 lux)	↑		[109]
	3 mg/kg	30 min before testing	Rat (Wistar)	EPM (? lux)	↑	· Locomotion unaffected	[97]
	3 mg/kg	30 min before testing	Rat (Wistar)	EPM (? lux)	↑		[4]
	2 mg/kg	60 min before testing	Rat (Wistar)	EZM (? lux)	¾		[65]
	2 mg/kg	60 min before testing	Rat (Wistar, pups)	Isolation-induced ultrasound vocalization	¾		[65]
	0.1 – 10 mg/kg	30 min before testing	Mouse (Swiss)	EPM (85 lux)	(↓)	· No effect in naive mice (Trial 1) · Decreased anxiety in test-experienced mice (Trial 2) at 1 but not 3 mg/kg	[108]
	1 and 3 mg/kg	40 min before testing	Mouse ($CB1^{CL}$)	EPM (? lux)	↓	· Anxiolytic effects at 3 mg/kg in both $CB1^{+/+}$ and $CB1^{-/-}$ mice	[53]
	0.03 - 3 mg/kg	30 min before testing	Mouse (ICR)	LD (? lux)	↓	· Dose-dependently	[1]
	0.03 - 3 mg/kg	30 min before testing	Mouse (C57BL/6?)	LD (? lux)	↓	· Inverse U-shaped curve	[1]
	0.03 - 3 mg/kg	30 min before testing	Mouse (DBA/2?)	LD (? lux)	↑	· Dose-dependently	[1]

EPM – elevated plusmaze; EZM – elevated zero maze; LD – light-dark avoidance task; ↓ anxiolytic effects; ↑ anxiogenic effects; ¾ no effect; ? – detailed information missing. If not stated otherwise, drugs were administered intraperitoneally or subcutaneously.

Table 5.2. Role of Endocannabinoids in Fear and Anxiety: Genetical Inactivation of CB1

Interference	Dose range	When	Animal	Task	Anxiety	Comment	Ref.
CB1 null-mutation			$CB1^{CL}$ Mice	EPM (100 lux)	¾		[69]
				Novel Object (500 lux)	↓	· Increased exploration	[69]
				EPM (? lux)	↑		[53]
				LD (500 lux)	↑	· No effect on horizontal locomotion · Vertical exploration (↓)	[78]
				Resident-Intruder	(↑)	· Increased aggression	[78]
				LD (500 lux)	(¾)	· No overt effect on anxiety in 1- and 4-month old $CB1^{-/-}$	[75]
				LD (? lux)	↑	· No anxiolytic effects of bromazepane · Anxiolytic effects of buspirone at high doses only	[123]
				EPM (? lux)	↑		[123]
				Social interaction (? lux)	↑		[123]
				OF (? lux)	↑		[123]
			$CB1^{GM}$ Mice	EPM (10 lux)	¾		[76]

EPM – elevated plusmaze; LD – light-dark avoidance task; OF – open field; ↓ anxiolytic effects; ↑ anxiogenic effects; ¾ no effect; ? – detailed information missing. If not stated otherwise, drugs were administered intraperitoneally or subcutaneously.

locomotion. Finally, a variety of stressors have the general capacity to influence the behavioral performance of the animals (for reviews see [66, 129]). This point gains particular importance in light of a contribution of endocannabinoids to stress-related alterations in emotionality. As summarized in Table **5.4**, pharmacological blockade of CB1 and, to some extent, also genetic ablation of CB1 reduce passive coping strategies in forced swimming and tail suspension tests [112]. Interestingly, CB1-deficient mice of the same line as used for forced swimming ($CB1^{-/-AZ}$) showed severe alterations in their swimming behavior in the water-maze task. These alterations became evident during relearning, with the consequence that approximately 50 % of the $CB1^{-/-AZ}$ mice had to be excluded from the experiment because of 'strange' swim strategies and seizures [127]. It is conceivable that relocation of the originally learned platform position is particularly stressful for the animals. The resulting activation of the endocannabinoid system would ensure an adequate coping with the changed situation in $CB1^{+/+}$ but not $CB1^{-/-}$ mice. The role of endocannabinoids for the generation of stress-coping strategies is further complicated by their putative interaction with endogenous opioids [125].

Taken together these data suggest that endocannabinoids may alter the animals' emotionality depending on the aversiveness of the test situation. Once activated, endocannabinoids seem to counteract arousal [110], what may lead to a decrease in anxiety and the adoption of adequate stress-coping strategies. The endocannabinoid system could, thus, be regarded as a general protective system that prevents hyperexcitation not only at the neuronal [90] but also at the behavioral level.

6. SUMMARY

Recent studies revealed biological functions of endocannabinoids on cognition and emotionality that are different from the effects of exogenously administered cannabinoids. The contribution of endocannabinoids to

Table 5.3. Role of Endocannabinoids in Fear and Anxiety: Inactivation of Endocannabinoid Degradation

Interference	Dose range	When	Animal	Task	Anxiety	Comment	Ref.
URB532	0.1 - 10 mg/kg	30 min before testing	Rat (Wistar)	EZM (? lux)	↓	· Dose-dependently · Blocked by SR141716A	[65]
	1 – 10 mg/kg	30 min before testing	Rat (Wistar, pups)	Isolation-induced ultrasound vocalization	↓	· Blocked by SR141716A	[65]
URB597	0.05 – 0.1 mg/kg	30 min before testing	Rat (Wistar)	EZM (? lux)	↓	· Dose-dependently · Blocked by SR141716A	[65]
	0.05 – 0.1 mg/kg	30 min before testing	Rat (Wistar)	Isolation-induced ultrasound vocalization	↓	· Effective at 0.1 mg/kg · Blocked by SR141716A	[65]

EZM – elevated zero maze; LD – light-dark avoidance task; ↓ anxiolytic effects; ? – detailed information missing. If not stated otherwise, drugs were administered intraperitoneally or subcutaneously.

Table 5.4. Role of Endocannabinoids in Stress Coping: Pharmacological and Genetical Inactivation of CB1

Interference	Dose range	When	Animal	Task	Immobility	Comment	Ref.
SR141716A	0.3 - 3 mg/kg	30 min before testing	Mouse (Swiss)	FST	↓	· Decreased immobility at 3 mg/kg (U-shaped dose-response curve)	[112]
AM251	1 – 30 mg/kg	30 min before testing	Mouse (129/SVE)	TST	↓	· Decreased immobility at 10 mg/kg (U-shaped dose-response curve)	[112]
	1 and 10 mg/kg	30 min before testing	Mouse (C57BL/6Tac)	FST	↓	· Decreased immobility at 1 and 10 mg/kg	[112]
	10 mg/kg	30 min before testing	Mouse ($CB1^{AZ}$)[f]	FST	↓	· Decreased immobility in $CB1^{+/+}$ but not $CB1^{-/-}$	[112]
CB1 null-mutation			$CB1^{AZ}$ (C57BL/6N, F11)[f]	FST	(↓)	· Slightly less immobility in $CB1^{-/-}$ vs. $CB1^{+/+}$ mice · Mice were treated with vehicle 30 min before testing	[112]

FST – forced swim test; TST – tail suspension test. If not stated otherwise, drugs were administered intraperitoneally or subcutaneously; [f]-female mice

working-memory processes appears negligible. In contrast, the interaction of endocannabinoids with their CB1 receptors limits the retention of recognition and reference memory, most likely by interfering with early consolidation. It, furthermore, facilitates memory extinction in aversively-motivated learning tasks. Depending on the genetic background and the test situation, endocannabinoids show anxiolytic properties and enable the adoption of adequate stress-coping strategies. Future studies have to address the questions (1) as to the cellular and molecular correlates of the influence of endocannabinoids on memory consolidation, memory extinction and stress coping, (2) as to whether or not the involvement of endocannabinoids in extinction is specific for aversive test situations, and (3) as to the contribution of TRPV1 and endovanilloids to cognition and emotionality. The discovery of the physiological role of endocannabinoids together with the development of pharmacological compounds that interfere with endocannabinoid uptake and degradation [23, 38, 42] might open the avenue for novel pharmacotherapeutic strategies for the treatment of human psychiatric and neurological disorders such as generalized anxiety disorder, phobias, post-traumatic stress disorder, Alzheimer's disease and epileptic seizures [25, 101].

ACKNOWLEDGEMENTS

I thank Krisztina Monory, Anja Siegmund and Giovanni Marsicano (all MPI Munich) for their comments on this manuscript. Supported by a grant of the *VolkswagenStiftung* (I/78 562).

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