Review

The endocannabinoid system in the regulation of emotions throughout lifespan: a discussion on therapeutic perspectives

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Abstract

Alterations in emotion regulation processes may form the basis of psychopathologies. The endocannabinoid (eCB) system, composed of endogenous ligands, the enzymatic machinery in charge of their metabolism and the specific metabotropic receptors, has emerged as a major neuromodulatory system critically involved in the control of emotional homeostasis and stress responsiveness. Data from animal models indicate that the eCB system plays a key role in brain development, and is probably involved in the control of emotional states from early developmental stages. The present review summarizes the latest information on the role of the eCB system in emotionality and anxiety-related disorders throughout the lifespan. Putative therapeutic strategies based on the pharmacological modulation of this system will be discussed. Given the fact that the pharmacological modulation of the eCB system has recently arisen as a promising strategy in the management of anxiety and mood disorders, the potential efficacy of this pharmacological approach (i.e. blockers of the catabolic pathway) will be discussed, as well as pharmacological alternatives such as modulators of cannabinoid receptors other than the classical CB1 receptor, or administration of other plant-derived compounds (e.g. cannabidiol).

Keywords

Adolescent, anxiety, development, neonatal, stress, treatment

Introduction

Cannabis is mainly consumed for its euphoriant properties, which are usually accompanied by decreased anxiety levels and heightened sociability. However, dysphoric reactions, anxiety, panic and psychosis have also been reported. Recent developments in the cannabinoid field have rapidly increased our comprehension of the multifunctionality of the endocannabinoid (eCB) system, including its involvement in emotionality control and stress responsiveness. An active eCB system in the developing brain has been demonstrated, and the characterization of the temporal changes of this system during brain development seems to be critical for present and future emotional homeostasis of individuals. Changes in the system have been observed in response to a number of environmental stimuli, mostly stressful, thus suggesting the eCB system as a critical system engaged not only in the control of state anxiety, but also in the emotional adaptation to the changing environment. In turn, our better understanding of the eCB system functionality has opened new avenues for the development of novel pharmacological strategies in the management of mood and anxiety disorders. At present, enhancing eCB signalling by blockade of anandamide (AEA) degradation has emerged as a promising therapeutic tool with notable anxiolytic and antidepressant properties in rodents. Despite optimistic perspectives, the efficacy of these drugs is still to be proven in humans, and studies in developing animals should not be undervalued.

The present review summarizes the latest information on the role of the eCB system in emotionality and anxiety-related disorders based on the available evidence from animal studies. The role of this system in emotional regulation from early postnatal stages will also be emphasized. Further, putative therapeutic strategies based not only on AEA degradation blockade will be discussed.

The endocannabinoid system

The eCB system is composed of endogenous ligands (known as 'endocannabinoids'), of specific metabotropic receptors that are activated by these endogenous ligands, and of the enzymatic machinery in charge of their synthesis and deactivation. Endocannabinoids are endogenously

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produced phospholipid derivatives, released 'on demand', that exert biological activity through stimulation of cannabinoid receptors (Breivogel and Sim-Selley, 2009; Kano et al., 2009). So far, five endogenous ligands have been identified: N-arachidonoylethanolamine (anandamide, AEA), 2-arachidonoyl glycerol (2-AG), noladin, virodhamine and N-arachidonoyl dopamine, of which AEA and 2-AG are the most studied (Pertwee, 2008).

The synthetic pathway responsible for the generation of AEA from phospholipids was long believed to be mediated by Ca^{2+}-dependent phospholipase D activity and the conversion of N-acylated phosphatidylethanolamines (NAPE) by NAPE-hydrolysing phospholipase D (Okamoto et al., 2004). Recently, an alternative pathway via the synthesis of the AEA precursor glycerophospho-N-arachidonoylethanolamine (GP-NArE) from NAPEs by a/b-hydrolase 4 and the Mg^{2+}-dependent catalysed conversion of GP-NArE to AEA by phosphodiesterase 1 (GDE1) has been proposed (Simon and Cravatt, 2008). AEA activity at the synaptic cleft is limited by cellular uptake, through an AEA membrane transporter (AMT), followed by intracellular degradation into arachidonic acid mainly by the fatty acid amide hydrolase (FAAH) (Giang and Cravatt, 1997). FAAH activity has emerged as a critical factor in the control of AEA signalling, based upon the increased levels of AEA observed in transgenic mice lacking this enzyme ($FAAH^{-/-}$) (Cravatt et al., 2001). 2-AG is synthesized from diacylglycerol (DAG) by sn-1-diacylglycerol lipases. The synthesis of DAG by phospholipase C is regulated by Ca^{2+} availability, and is therefore the rate-limiting factor in the activity-dependent biosynthesis of 2-AG (Stella et al., 1997). Upon release, 2-AG is mainly hydrolysed by monoglyceride lipase (Dinh et al., 2002), and, to a lesser extent, by a/b-hydrolase 6 and 12 (Blankman et al., 2007) (for an update in the field, see Hanus and Mechoulam, 2010).

Endocannabinoids have been shown to modulate neurotransmission, mainly acting as retrograde transmitters (Marsicano and Lutz, 2006), and have been reported to be at the basis of a plethora of physiological processes, including pain, cognition, regulation of endocrine and metabolic function, emotionality and motivation processes (Hiley, 2009; Kunos et al., 2008; Lutz, 2007; Maccarrone, 2009; Moreira and Lutz, 2008; Pagotto et al., 2006). Until recently, only metabotropic receptors coupled to Gi/o proteins were considered as cannabinoid receptors, mainly type 1 and type 2 cannabinoid receptors (CB1R and CB2R). CB1Rs are widely expressed in the central nervous system, including limbic brain regions (Mackie, 2008). In contrast, CB2R has been classically considered as the 'peripheral cannabinoid receptor', given its abundant expression in the immune system. However, the expression of CB2Rs in brain microglia during neuroinflammation is now well accepted (Carlisle et al., 2002), and their presence in diverse central brain areas has been recently described. In the adult rodent brain, CB2R expression has been detected in a subset of brainstem neurons (Van Sickle et al., 2005), cerebellar neurons (Suarez et al., 2008) and neural stem cells in the subventricular zone (Goncalves et al., 2008). However, the presence of CB2R in the central nervous system still generates controversy (see Atwood and Mackie (2010) for a more detailed discussion). More recently, the orphan receptor GPR55, coupled to Gq/G12/13 proteins, has been shown to have affinity for AEA (Lauckner et al., 2008; Ryberg et al., 2007; Waldeck-Weiermair et al., 2008), and might constitute a novel cannabinoid receptor (Ross, 2009). GPR55 can be found in cerebellum, hippocampus, cortex, striatum, hypothalamus and brain stem, although its expression in the brain is generally lower than that of CB1R (Ryberg et al., 2007). The coupling of GPR55 to a signalling pathway that differs from that of CB1R and CB2R suggests the existence of a more delicate regulation of eCB signalling in these brain regions (Waldeck-Weiermair et al., 2008). Moreover, additional alternative targets of some eCB ligands are worthy of mention. In this regard, the transient receptor potential vanilloid type 1 (TRPV1) ion channel has been reported to mediate some AEA effects (Starowicz et al., 2007). Taken together, activity of endogenous cannabinoid ligands through activation of any of these receptors might have a significant role in the physiology of the eCB system, and thus have to be taken into consideration.

Development of the eCB system: neonatal period and adolescent age

Endocannabinoids and their receptors, mostly CB1, are present from early stages of gestation and have been demonstrated to be involved in neural development and structure maturation, including in the nigrostriatal pathway and prefrontal cortex (Anavi-Goffer and Mulder, 2009; Fride et al., 2009). Studies on prenatal development of the eCB system almost exclusively focus on the CB1R. In fact, CB1R mRNA can be detected as early as embryonic day 11 in a subset of cells in the neural tube (Buckley et al., 1998). Temporal fluctuations in CB1R expression during brain development were revealed by using in-situ hybridization in the forebrain, brainstem and cerebellum (Berrendero et al., 1998). In the rodent neocortex, CB1R mRNA expression reaches its maximum during gestation day 16.5, and gradually declines towards birth (Berghuis et al., 2005; Mulder et al., 2008). Furthermore, the CB1R protein can be detected in early neural progenitors (Aguado et al., 2005, 2006; Morozov et al., 2009) and in the axons and growth cones of developing cortical projection neurons (Mulder et al., 2008; Vitalis et al., 2008; Watson et al., 2008). Therefore, the eCB system may exhibit a functional role in brain development, probably by guiding distinct brain structures along development pathways. Moreover, at early developmental stages, the eCB system seems to influence the expression of key genes for neural development, and to participate in axonal growth and fasciculation and in the establishment of correct neuronal connectivity (Fernandez-Ruiz et al., 2004; Harkany et al., 2007; Vitalis et al., 2008; Watson et al., 2008).

During embryonic development, CB1R is highly expressed in zones of axon development such as the corpus callosum and the corticospinal pathway (Romero et al., 1997), thus providing evidence for a role of the eCBs in the establishment and organization of neuronal networks. In contrast, during

late gestation and early postnatal development, CB1R binding is markedly reduced in the pyramidal tract and internal capsule (Romero et al., 1997). In the developing rat brainstem, the CB1R mRNA level gradually decreases from gestational day 21 towards adulthood, while in the cerebellum, CB1R expression continuously increases, reaching maximum expression during adulthood (Berrendero et al., 1998). In the developing human brain, shifts in temporal expression from mid-gestation (week 20) to adulthood are also evident (Wang et al., 2003). [^{3}H]-CP55,940 binding and agonist-stimulated [^{35}S]-GTPgS autoradiography in the hippocampus, cerebellum white matter areas and, to a lesser extent, in the neocortex, are elevated during major developmental events that shape these structures and their connections (Mato et al., 2003). Notably, spatial and temporal distribution of CB1R expression were investigated using CP55,940 and WIN55,212-2, non-selective CB1R/CB2R agonists with an affinity for GPR55 (Henstridge et al., 2009; Lauckner et al., 2008; Oka et al., 2007; Ryberg et al., 2007). The nature of these results should therefore be carefully interpreted as an exclusive index of the CB1R expression pattern. WIN55,212-2-induced [^{35}S]-GTPgS binding in the presence of a CB1R antagonist (Romero et al., 1997) and in CB1R knock-out mice (Selley et al., 2001) implies the existence of another WIN55,212-2 binding receptor in the central nervous system. Despite arguments about the presence of CB2Rs in the brain of adult rats, during embryogenesis CB2R mRNA is undetectable in brain tissue, and is mainly expressed in the liver (Buckley et al., 1998; Shouman et al., 2006).

Levels of both 2-AG and AEA show temporal variation during brain development. Brain AEA levels gradually increase during postnatal development, reaching a maximum in adulthood, while 2-AG synthesis gradually increases during embryonic development, peaks immediately after birth and normalizes during postnatal development (Fride, 2008). The spatiotemporal dynamics of the key enzymes responsible for the synthesis and degradation of eCBs during brain ontogeny have only been partly unravelled. Diacylglycerol lipases (DAGLs) are present in the brain during early development, and expression is maintained throughout life (Berghuis et al., 2007; Bisogno et al., 2003). DAGLs are distributed to the developing axons of pyramidal neurons (Mulder et al., 2008), while in the adult brain DAGLa is mainly localized in dendrites (Katona et al., 2006). This indicates a different role of the eCB system in developing and mature neurons. Expression of the enzyme responsible for AEA synthesis, NAPE-PLD, is low during embryonic development, and can be detected in GABAergic and glutamatergic processes in the developing cortex (Berghuis et al., 2007). The expression of NAPE-PLD steadily increases after birth, coinciding with a marked increase in AEA levels (Morishita et al., 2005); however, the expression and distribution patterns of the newly identified AEA-synthesizing enzymes in the embryonic brain have not yet been identified. No information on the distribution of 2-AG-degrading enzymes during embryonic and early postnatal life is available, but the expression of the AEA-hydrolysing enzyme FAAH gradually increases during postnatal life in the rodent hippocampus (Morozov et al., 2004). This coincides with increased levels of AEA during postnatal life, and further confirms the role of FAAH in controlling AEA signalling.

Development of the eCB system continues during adolescence, the transition period between childhood and adulthood. Most of the psychobiological aspects of the transition from infancy into adulthood can be identified in most mammalian species, including rodents, whose adolescence is frequently considered as the age window between postnatal days (pnd) 30 and 45 (Spear, 2000). During adolescence dramatic hormonal and physical changes occur, with the adolescent brain undergoing notable plastic changes and important maturational rearrangements within major neurotransmitter systems. In this regard, auto-radiographic studies in forebrain areas of adolescent non-human primates reported an overproduction followed by a subsequent pruning among GABAergic, adrenergic, cholinergic, serotonergic and dopaminergic receptors (Lidow et al., 1991). Similarly, the eCB system continues rearrangement and maturational processes during adolescence. Brain AEA levels gradually increase during development, reaching adult levels at adolescence, while the expression of CB1Rs appears maximal among adolescents and drops thereafter towards adult levels (Belue et al., 1995; Rodriguez de Fonseca et al., 1993). More recently, distinct developmental trajectories in CB1R expression in cortical areas have been described for adolescent rats. The most pronounced and progressive decrease in CB1R expression was observed in medial prefrontal and other limbic/associative regions. In contrast, major changes in sensorimotor cortices occurred only after the end of adolescence (pnd 40). Electrophysiological measures of CB1 receptor function further confirmed the same developmental trajectory of CB1R maturation in the prefrontal cortex (Heng et al., 2011). These observations provide evidence for the eCB system to be at the peak of activity during adolescence, although maturation of limbic/associative vs. sensorimotor systems seems not to be synchronized. Discontinuities in eCB signalling during adolescence might be of great relevance for understanding the neurobiological mechanisms underlying the characteristic behavioural repertoire of youth, including sensation-seeking and proneness for risk behaviours (Laviola et al., 2003). In turn, these developmental discontinuities might help to better understand the effects of cannabis abuse during adolescence, when its use is a problem of major health concern see Figure 1.

Role of the eCB system in the regulation of emotionality along the lifespan

In the early 1960s, Δ^9-tetrahydrocannabinol (Δ^9-THC) was identified as the primary active ingredient responsible for the psychotropic effects of marijuana (Gaoni and Mechoulam, 1964). At present, marijuana (*Cannabis sativa*) is among the most widely used drugs in Western societies. It is mainly consumed for its euphoriant properties, which are usually accompanied by decreased anxiety and increased sociability. However, dysphoric reactions, anxiety, panic and psychosis have also been reported (Crippa et al., 2009). The same bidirectional profile has been described in rodents following cannabinoid agonist administration, with low doses producing anxiolytic-like responses while an opposite picture has

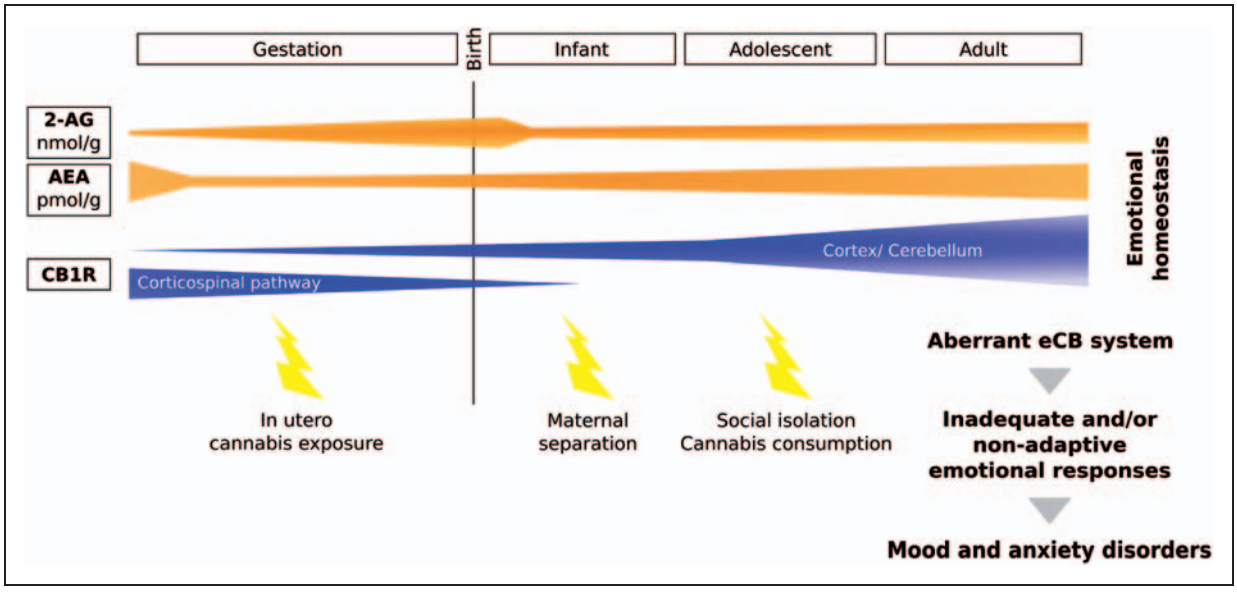

Figure 1. Temporal changes in the two major endocannabinoids (eCBs), 2-AG and AEA, and CB1R expression along the lifespan are indicated. Proposed development of the eCB system is based on review literature (Fride et al. 2009; Harkany et al. 2007). The fine-tuning of the eCB system is critical for adult emotional homeostasis. In turn, it is speculated that disruption of the normal development of the eCB system, at critical time windows (i.e. gestation, neonatal and infant life and/or adolescence), results in an aberrant eCB system organization that may result in inadequate and/or non-adaptive emotional responses, possibly followed by emergence of mood and anxiety symptoms.

been related to high doses (Moreira and Lutz, 2008; Viveros et al., 2005b). In the last decade, great efforts have been devoted to elucidating the specific role of the eCB system in emotional control and stress response, and recent developments have rapidly increased our comprehension of the role played by the eCB system in this field (Finn, 2010; Marco and Viveros, 2009; Moreira and Lutz, 2008; Viveros et al., 2005b), although there are still some questions to be resolved.

An increasing body of evidence points to the eCB system as a key system in the regulation of emotional homeostasis. CB1Rs are widely expressed within emotion-associated brain areas (Mackie, 2008), and genetic and pharmacological blockade of CB1Rs further support the role of the eCB system in the control of emotionality. Increased anxiety-like behaviours have been reported among CB1R knock-out mice exposed to paradigms of unconditioned anxiety (i.e. the light–dark box and the elevated plus maze); this altered behavioural phenotype was also accompanied by profound alterations in adrenocortical activity (Haller et al., 2002; Martin et al., 2002; Uriguen et al., 2004). Similarly, administration of a CB1R antagonist, rimonabant (SR141716A), also produced anxiogenic-like effects in rats (Arevalo et al., 2001; Navarro et al., 1997). Data from both genetic and pharmacological blockade of CB1Rs are controversial, since conflicting results have also been reported (Haller et al., 2004a, 2004b; Marsicano et al., 2002). However, such discrepancies might be due to differences in the basal emotional state of animals, since baseline trait levels of emotionality are critically influenced by both the genetic strain and environmental testing conditions (Clement et al., 2002; Marco et al., 2011b; Yilmazer-Hanke, 2008). Regardless of these discrepancies, currently available literature suggests the eCB system as a key system in the control of emotional states.

More recently, the behavioural phenotype of FAAH null-mutant ($FAAH^{-/-}$) mice has been evaluated. These animals, characterized by augmented circulating AEA levels, showed reduced anxiety levels in both the elevated plus maze and the open field, together with an antidepressant profile indicated by reduced immobility in both the forced swim and tail suspension tests (Bambico et al., 2010). This emotional profile, which was attenuated by rimonabant, a CB1R antagonist, further supports the role of the eCB system in the regulation of emotional behaviour. Endogenous cannabinoid ligands, acting through the stimulation of CB1R, may dampen emotional reactions to environmental conflict situations, thus enhancing anxiolytic-like responses in certain behavioural paradigms. Although the picture regarding eCBs and anxiety is rather complicated, individual emotionality seems to critically depend on endogenous basal tone, probably at certain brain regions key in emotion regulation such as the amygdala and hippocampus. Accordingly, the eCB system might be critically dependent on the environmental context (Marco et al., 2011b). The latter may well explain why, depending on the experimental context, the same cannabinoid compounds may tone down anxiety, cause no effects, or even prompt anxiogenic-like reactions.

CB2R expression has been detected in several brain regions (for a review, see Atwood and Mackie, 2010), and its participation in depression and drug addiction processes (Onaivi et al., 2008), as well as in psychotic disorders (Ishiguro et al., 2010) has been recently demonstrated. However, the participation of CB2R in emotional-related behaviour has been rarely investigated, although recently the over-expression of CB2R in mutant mice has been reported to reduce anxiety-like behaviours in different behavioural paradigms (Garci and Manzanares, 2011).

Therefore, there is an urgent need to further investigate the role of CB2R in emotional control and stress reactivity. However, in this regard, the extent of CB2R expression in neurons has remained controversial and a note of caution has arisen on the interpretation of currently available data on CB2R expression.

AEA is a ligand for not only cannabinoid receptors (CB1 and CB2) but also for peroxisome proliferator-activated nuclear receptor-alpha (PPARalpha) (O'Sullivan, 2007), for the orphan receptor GPR55 (Ryberg et al., 2007), and for the transient receptor potential vanilloid 1 (TRPV1) receptors (Marinelli et al., 2007; Van Der Stelt and Di Marzo, 2004). TRPV1s belong to a large family of calcium-permeable cation channels (Caterina et al., 1997) and can be activated by different environmental stimuli, and by endogenous cannabinoids such as AEA (Ross, 2003; Toth et al., 2003). TRPV1s are expressed in various brain regions related to anxiety (McGaraughty et al., 2003; Mezey et al., 2000), and recently their role in anxiety has been investigated. Systemic administration of capsazepine, a TRPV1 antagonist, induced anxiolytic-like effects in rats (Kasckow et al., 2004). More recently, TRPV1 knock-out mice have been shown to exhibit less fear-related responses than their wild-type littermates in behavioural paradigms of both innate and conditioned fear (Marsch et al., 2007). Accordingly, the dual FAAH/TRPV1 blocker N-arachidonoyl-serotonin is able to induce CB1-mediated anxiolytic-like effects more potently than selective blockers of FAAH or TRPV1, further suggesting opposite roles for CB1 and TRPV1 receptors (Micale et al., 2009). Therefore, the participation of TRPV1 receptors on the effects of AEA on emotionality cannot be excluded. Meanwhile, the participation of PPARalpha and GPR55 in such cannabinoid-induced effects is still to be elucidated.

Changes in eCB content in specific brain areas have been reported in response to a number of environmental stimuli (Gorzalka et al., 2008; Patel and Hillard, 2008). Following experimental conditions that resemble acute aversive and/or stressful stimuli, transient changes in brain eCB content have been reported. An increase in AEA levels in the periaqueductal grey matter was found in response to painful stimuli (Walker et al., 1999). Food deprivation in rats increased both AEA and 2-AG content within the nucleus accumbens, while within the hypothalamus only 2-AG levels were augmented (Kirkham et al., 2002). Similarly, in fear-conditioned animals the presentation of the conditioned stimulus, i.e. an acoustic tone previously associated with an electric footshock, produced a transient increase of AEA levels in the basolateral amygdala (Marsicano et al., 2002). In general, increases in eCB content, at least within limbic areas, occur in response to acute exposure to aversive or stressful stimuli. However, the opposite is observed when exposure to such a stimulus is prolonged. A single episode of immobilization restraint (30 min) reduces hypothalamic 2-AG and AEA contents within amygdala and hippocampus (Gorzalka et al., 2008; Patel et al., 2004, 2005; Rademacher et al., 2008). Based upon these observations, eCB changes were proposed to underlie emotional responses, and to participate in coping with arousal and/or stressful situations. In fact, several studies have proposed that the eCB system might be involved in the basis of recovery and/or adaptation processes to environmental changes, including (but not limited to) stressful situations (Hill et al., 2010; Hohmann et al., 2005; Rademacher et al., 2008). Changes in brain eCB signalling in the adaptive response to a diversity of environmental stimuli can be hypothesized. Remarkably, the eCB system is also involved in the modulation of rewarding behaviours such as food intake, sexual behaviour or social play (Fattore et al., 2010; Marco et al., 2011b). Therefore, the eCB system might participate in the complex machinery that regulates the evaluation of environmental inputs, and may allow both gratification and perception of pleasure as well as danger and fear response.

To date, data from developing animals in the field of emotional control are scarce. However, in recent years, interest in this topic has dramatically increased and several studies have suggested the putative involvement of the eCB system in the control of emotionality from early development stages (see Fride et al., 2009; Viveros et al., 2005a, for review). At early neonatal stages, the recording of ultrasonic vocalizations (USVs) has emerged as a suitable model to study the development of emotionality in rodent pups (Hofer et al., 2002; Shair, 2007). Rodent pups at neonatal stages emit USVs at frequencies between 30 and 90 kHz in response to isolation from their mother and littermates. USV emissions are sensitive to pharmacological modulation by anxiolytic and anxiogenic drugs, including cannabinoids (Fride, 2005; Kathuria et al., 2003; McGregor et al., 1996; Scattoni et al., 2009). Administration of the potent cannabinoid agonist CP55,940 markedly reduced the rate of isolation-induced USV emission, causing an almost complete inhibition of calls at the higher dose tested (McGregor et al., 1996). Blockade of AEA deactivation, by both a transport blocker (AM404) and an inhibitor of AEA hydrolysis (URB597), has been reported to reduce the number of USVs emitted by rat pups in isolation (Bortolato et al., 2006; Kathuria et al., 2003), thus indicating that these compounds may have a potential anxiolytic role at this early developmental stage, and in turn demonstrating the involvement of the eCB system in the control of emotional states at this neonatal age frame (see Table 1). Therefore, from early neonatal ages the eCB system might be actively controlling an animal's emotional status.

As mentioned above, adolescence is the transition period between childhood and adulthood; this is a stage characterized by psychological changes that affect the individual's sense of identity, their self-consciousness and their relationships with others. Most of the psychobiological aspects of the transition from infancy into adulthood can be identified in most mammalian species, including rodents (Spear, 2000). Among adolescents, affiliative and playful behaviours are prevalent, together with a notable trend for sensation-seeking and risk-taking (Adriani and Laviola, 2004; Kelley et al., 2004; La Greca et al., 2001, Laviola et al., 1999; Primus and Kellogg, 1989; Spear, 2000; Vanderschuren et al., 1997). The effects of administration of cannabinoid agonists on anxiety-related responses appear to be different in young versus adult animals although, surprisingly, acute effects of cannabinoid compounds on anxiety levels have been scarcely investigated. In a series of experiments performed on adolescent rats administered with CP55,940 and exposed to the elevated plus maze,

Table 1. Anxiety-related effects of direct (THC; CP55,940; WIN55,212-2) or indirect (URB597 and AM404) cannabinoid agonists in neonatal and adolescent rodents exposed to diverse behavioural paradigms

Species	Postnatal days (pnd)	Drug dose (mg/kg)	Time interval	Effects	References
NEONATAL PERIOD					
Wistar rats	10 pnd	URB597 (0.1)	30 min	Reduced rate of isolation-induced USV emission.	(Kathuria et al., 2003)
Long-Evans rats	11-13 pnd	CP55,940 (0.01–1)		Reduced rate of isolation-induced USV emission. The higher dose caused an almost complete inhibition of calls.	(McGregor et al., 1996)
Wistar rats		AM404 (1–5)	30 min	Reduced rate of isolation-induced USV emission.	(Bortolato et al., 2006)
ADOLESCENT PERIOD					
Wistar rats	38-43 pnd	URB597 (0.1)	30 min (for 6 days)	Decreased impulsivity levels in the intolerance-to-delay task in an animal model relevant for neuropsychiatry.	(Marco et al., 2007)
Wistar rats	44 pnd	CP55,940 (0.001–0.100)	30 min	No anxiety-related effects were observed in the elevated plus maze.	(Marco et al., 2006; Viveros et al., 2005a)
Wistar rats	26-28 pnd	URB597 (0.1 and 0.2)	2 h	Increased social play behaviour: pinning and pouncing frequencies.	(Trezza and Vanderschuren, 2008)
		WIN55,212-2 (0.3 and 1)	30 min	Decreased social play behaviour: pinning and pouncing frequencies.	
Wistar rats	26-28 pnd	AM404 (5)	30 min	Decreased social play behaviour: pinning and pouncing frequencies.	(Trezza and Vanderschuren, 2009)
		VDM11 (0.5 and 1)	15 min	Increased social play behaviour: pinning and pouncing frequencies.	

In all the experiments above-reported drugs were intraperitoneally administered. Only data from male animals are reported. USV, ultrasonic vocalizations.

male juvenile rats appear to be less sensitive to the anxiolytic-like effects of this cannabinoid compound, whereas, in contrast, females adolescent rats seemed particularly vulnerable to its anxiogenic-like effects (Marco et al., 2006). In parallel with the pro-social effects of marijuana among humans, recent studies in rodents have shown that social play behaviour in adolescence can be modulated by cannabinoid compounds. The direct CB1R agonist WIN55,212-2 reduced social play, whereas the indirect cannabinoid agonist URB597 enhanced this behaviour (Trezza and Vanderschuren, 2008). More recently, the effects of AEA transporter inhibitors on social play behaviour have also been evaluated. The prototypical AEA transporter inhibitor N-(4-hydroxyphenyl)-arachidonamide (AM404) reduced social play, whereas its more selective analogue N-arachidonoyl-(2-methyl-4-hydroxyphenyl)amine (VDM11) enhanced it (Trezza and Vanderschuren, 2009). These paradoxical effects on social play behaviour may depend on how the eCB system is stimulated. During social play, eCBs might be released in brain areas mediating this behaviour (Marco et al., 2011b), and thus the prevention of AEA hydrolysis (URB597) and AEA transport (VDM11) may enhance social play by magnifying eCB tone. In contrast, activation of cannabinoid receptors throughout the brain (WIN55,212-2) may inhibit sociability, perhaps by disrupting cognitive functions necessary to perform adequate social interactions (Egerton et al., 2006).

The eCB system plays an important role in the developing brain, participating in almost all aspects of neuron development. Endocannabinoids seem to have an active contribution in neurogenesis processes, in neuronal migration, specification and in the formation of adequate connections with neighbouring or distal neurons (Anavi-Goffer and Mulder, 2009; Fride, 2008; Harkany et al., 2007). Normal brain development, including eCB signalling, seems critical for the formation of functional neuronal networks and therefore for the appropriate functioning of individuals later in life. In this regard, the fine-tuning of the eCB system during brain development might also be critical for adult emotional homeostasis, thus allowing individuals to cope with everyday life events within the physiological range of arousal and emotionality. Aberrant eCB system brain development may possibly prompt inadequate and/or non-adaptive responses that in the long term might be followed by pathological states such as mood and anxiety disorders. Therefore, disruption of eCB signalling has a large impact on normal brain development.

On the one hand, the study of possible alterations on the eCB system during brain development becomes essential, as the structure of this system can profoundly interfere with the general maturation of the central nervous system. Early life events may critically impact the developing eCB system, and probably other brain networks (Marco et al., 2011a). The eCB system has been shown to be critically affected by developmental insults such as neonatal maternal deprivation (Llorente et al., 2008; Macrì and Laviola, 2004; Suarez et al., 2009, 2010) and rearing in social isolation (Malone et al., 2008; Robinson et al. 2010), and aberrant behaviours have often been reported after growing in adverse environments (Fone and Porkess, 2008; Marco et al., 2009a; Pryce

et al., 2005; Veenema, 2009). The reported possible association between an altered eCB system and behavioural abnormalities urges further investigation. Further investigation is needed to better understand the interactions occurring between the developing brain, highly susceptible to adverse environments, and the fine-tuning of the eCB system.

On the other hand, prenatal exposure to cannabis may have irreversible consequences on the formation of functional neuronal networks. In utero exposure to cannabinoid agonists induces notable neurofunctional alterations in the offspring. There is increasing evidence that humans exposed in utero to cannabis suffer from hyperactivity, cognitive impairments and altered emotionality. Both human longitudinal cohort studies and animal models strongly emphasize the long-term influence of prenatal cannabinoid exposure on the development of diverse neurotransmitters systems relevant to behaviour, and possibly related to neuropsychiatric disorders (for review see Campolongo et al., 2009; Fride et al., 2009; Jutras-Aswad et al., 2009). Exposure to cannabis during critical windows of brain development seems to have devastating consequences. Indeed, the pharmacological manipulation of the eCB system at early developmental stages can result in long-lasting neurobehavioural consequences (see, for example, Llorente et al., 2007). Not only are embryonic and prenatal stages of brain development critical for the long-lasting consequences of cannabinoids; adolescence has also emerged as an age period highly sensitive to the adverse outcomes of cannabis exposure. Chronic cannabinoid administration during adolescence has been reported to cause persistent behavioural alterations in emotionality (Biscaia et al., 2003; Macrì and Laviola, 2004; O'Shea et al., 2004), vulnerability to drug abuse (Ellgren et al., 2007) and impact on cognitive function (Schneider, 2008). Evidence from animal studies and recent longitudinal human studies has also suggested a potential association between regular heavy cannabis use during adolescence and psychotic episodes (see Di Forti et al., 2009; Hall and Degenhardt, 2008; Leweke and Koethe, 2008 for review). Therefore, given the potential therapeutic value of cannabinoid system modulators, a detailed investigation of possible adverse effects of these drugs on the central nervous system of immature individuals is warranted.

Cannabis-based therapeutic perspectives

Although significant advances have been made in the treatment of mood disorders during the past decades, around 30% of the population do not respond to current therapies, and the search for novel pharmacological approaches continues. Cannabinoids have been extensively proposed as an efficient tool for the management of mood and anxiety disorders (for review see Bosier et al., 2010; Mangieri and Piomelli, 2007; Pacher et al., 2006). Cannabis, as mentioned above, is mainly consumed for its euphoriant properties, which are usually accompanied by decreased anxiety and increased sociability, although anxiety and panic reactions are often reported (Crippa et al., 2009). This complex scenario observed among humans is replicated in animal models (see above, and Moreira and Lutz, 2008; Viveros et al., 2005b), thus offering a unique possibility for understanding the neurobiological basis for the role of the eCB system in anxiety. Cannabinoids are effective in chemotherapy-induced emesis, and nabilone, a synthetic THC, has been licensed for this use for several years. Cannabis is frequently used by patients with multiple sclerosis (MS) for muscle spasm and pain. Small clinical studies have confirmed the usefulness of THC as an analgesic. Currently, the synthetic cannabinoid agonist HU211 is undergoing trials as a protective agent after brain trauma. However, the therapeutic use of cannabinoids can also be accompanied by adverse reactions such as panic or anxiety attacks, which are worse in the elderly and in women, and less likely in children (Croxford 2003; Williamson and Evans, 2000).

In recent years, attention has moved from directly acting on cannabinoid receptors (i.e. by agonists such as THC) to indirectly preserving and enhancing the spatiotemporal specificity of eCB activity. In particular, a promising strategy exploits interference with deactivation processes (e.g. FAAH inhibitors), thus increasing eCB neurotransmission (Chevaleyre et al., 2006; Freund et al., 2003; Saario and Laitinen, 2007). These eCB enhancers are preferred to direct cannabinoid agonists, since they are thought to preserve the spatiotemporal specificity of eCB-mediated synaptic activity while limiting undesired side effects (Freund et al., 2003; Piomelli et al., 2006). Nowadays, a great variety of these compounds are already available for preclinical studies, although not all of them have been evaluated for their potential role in the regulation of emotionality in rodents. AEA elimination is prevented by transport inhibitors such as AM404 (Beltramo et al., 1997), UCM707 (Lopez-Rodriguez et al., 2003), OMDM-1 and OMDM-2 (Ortar et al., 2003), and VDM11 (De Petrocellis et al., 2000), as well as by inhibitors of AEA hydrolysis such as URB597 (De Petrocellis et al., 2000) and OL-135 (Boger et al., 2005). Transport inhibitors have been tested in different behavioural paradigms to evaluate their potential as anxiolytic drugs, and AM404 effectively reduced anxiety-like responses in the elevated plus maze (Bortolato et al., 2006; Braida et al., 2007; Patel and Hillard, 2006), the defensive withdrawal and the separation-induced USVs in rat pups (Bortolato et al., 2006) but not in the light–dark box (Rutkowska et al., 2006). To our knowledge, no experiment has investigated the anxiety-related effects of the remaining AEA transport inhibitors (i.e. UCM707, OMDM-1 and OMDM-2 and VDM11).

Notably, certain highly selective irreversible FAAH inhibitors, such as cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597) (Fegley et al., 2005), have been reported to modulate reduced self-control and impulsive behaviour (Marco et al., 2007) and to reduce anxiety- and depression-like signs in several animal models, without symptoms of cannabinoid intoxication and/or of abuse liability (see Table 2; for review see Bortolato et al., 2007; Gobbi et al., 2005; Kathuria et al., 2003; Marco et al., 2007; Piomelli et al., 2006; Vlachou et al., 2006). Surprisingly, the reversible FAAH inhibitor, 1-oxo-1-[5-(2-pyridyl)-2-yl]-7-phenylheptane (OL-135) has been mostly tested in pain-related models (Chang et al., 2006; Lichtman et al., 2004), and no anxiety-related effects were observed when evaluated in the elevated plus maze (Naidu et al., 2007).

These promising findings for FAAH inhibitors have attracted the attention of several companies, and a phase I clinical trial in order to evaluate the safety, tolerability,

Table 2. Emotional-related effects of the most extensively studied FAAH inhibitor, URB597, behavioural paradigms for the evaluation of anxiety-like drug activity in adult rodents

Paradigm	Animal	URB597 (mg/kg)	Time prior testing	Anxiety-related effect	Reference
Zero-maze	Wistar rats	0.1	30 min	↓	(Kathuria et al., 2003)
Ligh/Dark test	Sprague-Dawley rats	0.1 and 0.3 (1st and 2nd days)	40 min	↓	(Scherma et al., 2008)
		0.1 and 0.3 (3rd day)	40 min	—	
	C57 mice	1	2 h	—	(Moreira et al., 2008)
Elevated plus-maze	ICR mice	0.1 and 0.3	30 min	↓	(Patel and Hillard, 2006)
	C57 mice	0.1, 1 and 10	30 min	—	(Naidu et al., 2007)
	ICR mice	0.1, 1 and 10	30 min	—	
	ICR mice	0.1, 1 and 10	30 min	—	
			120 min	↓ (Modified EPM according to Patel and Hillard, 2006)	
	C57 mice	1	2 h	↓	(Moreira et al., 2008)
	C57 mice	0.1 and 0.5	30 min	—	(Micale et al., 2009)
		1		↓	
	Swiss mice	0.1 and 0.5 (7 days)		—	
		1 (7 days)		↓	

In all the experiments the above-reported drugs were intraperitoneally administered.
Paradigm: EPM, elevated plus maze. Anxiety-related effects: reduced anxiety levels (↓), anxiolytic-like effect; no effects (—); increased anxiety levels (↑), anxiogenic-like effect.

pharmacokinetics and pharmacodynamics of a FAAH inhibitor (F-04457845, Pfizer) has been just completed (http://ClinicalTrials.gov/show/NCT00836082), although in this case pain management was the main focus. However, this first step may open new avenues for the development of FAAH inhibitors directed to treat anxiety disorders related to pain conditions that may thereafter be extrapolated to other emotional-related psychopathologies. However, a note of caution should be sounded regarding possible FAAH inhibitors off-target. Some inhibitors, including URB597 and OL-135, have been recently reported to inhibit carboxylesterase activities in rat liver. Since carboxylesterases hydrolyse a variety of ester-containing drugs and pro-drugs, it has been speculated that certain FAAH inhibitors, by inhibiting carboxylesterases, might have drug–drug interactions with other medicines if developed as therapeutic agents (Zhang et al., 2007). Moreover, long-lasting consequences of targeting the eCB system should also be taken into consideration. In this regard, repeated administration of URB597 during adolescence, a developmental phase critical for its enhanced brain plasticity (see above), persistently modified CB1R binding in a region-dependent manner. Therefore, administration of modulators of the eCB system during active neurodevelopmental phases (i.e. adolescence) may have implications for the maturational end-point of the eCB system itself, which could lead to permanent alterations in neuronal brain circuits and behavioural responses (Marco et al., 2009b). Less extensively investigated, a few inhibitors of 2-AG hydrolysis have been reported such as URB602, a non-competitive and partially reversible inhibitor of monoacylglycerol lipase (Hohmann et al., 2005; King et al., 2007). Regarding these compounds, research has focused on their therapeutic potential for the treatment of inflammatory pain (Hohmann, 2007), while their possible participation in emotional control has been left aside.

Studies on the neurobiological background of anxiety indicate that the pathogenesis of anxiety may be related to the process of an extinction of aversive memories. It has been suggested that disruption of selective attention for emotional stimuli may confer the risk of mental disorders such as phobias and post-traumatic stress disorder (PTSD). Recent studies on molecular and cellular mechanisms responsible for individual fear extinction have suggested drugs stimulating endogenous cannabinoid system as novel and more effective forms of clinical treatment of anxiety disorders, including PTSD (Lehner et al., 2009; Lutz 2007). Marsicano et al. demonstrated that the endogenous cannabinoid system has a central function in extinction of aversive memories. Pharmacological and genetic disruption of CB1Rs strongly impaired short-term and long-term extinction in auditory fear-conditioning tests, in the absence of changes in memory acquisition and consolidation processes (Marsicano et al., 2002). Similarly, systemic administration of rimonabant, a CB1R antagonist, prior to extinction training in the fear-potentiated startle test led to a significant impairment in extinction (Chhatwal et al., 2005). While the administration of the CB1 agonist WIN 55,212-2 did not appear to affect extinction, administration of AM404 led to dose-dependent enhancements in extinction, together with a decreased shock-induced reinstatement of fear. In these experiments, the effects of AM404 were demonstrated to be mediated through CB1R, thus AM404 was acting to increase CB1 receptor activation during extinction training (Chhatwal et al., 2005). More recently, the eCB system has been proven to play a

facilitatory role in extinction by elevating brain AEA levels, through either genetic deletion or pharmacological inhibition of FAAH, namely OL-135 administration (Varvel et al., 2007). More recently, AM404 has been shown to induce anti-anxiogenic effects in the fear-potentiated plus maze test (Bitencourt et al., 2008). Depending on the test, the eCB system is required in the acquisition and/or extinction of memory. In particular, the activation of CB1 receptor-mediated signalling is centrally involved in the facilitation of behavioural adaptation after the acquisition of aversive memories, and targeting the eCB system (e.g. FAAH inhibitors) represents a promising pharmacological approach to treat psychopathologies hallmarked by an inability to extinguish maladaptive behaviours, such as PTSD. In line with this hypothesis, at present a clinical trial (phase IV) on the efficacy of Δ^9 treatment for the management of PTSD is going on. Adult subjects of both genders are being currently recruited, and the first results will be available by the end of 2010 (http://ClinicalTrials.gov/show/NCT00965809).

In the cannabis plant, phytocannabinoids other than THC are frequently found in relatively high concentrations. Among these, cannabidiol (CBD) is by far the most abundant and the most extensively studied one that does not produce psychotomimetic effects (Mechoulam and Hanus, 2002). CBD attenuates the psychotomimetic and anxiogenic effects of THC in humans (Karniol et al., 1974; Zuardi et al., 1982). Systemic CBD administration has been reported to induce anxiolytic-like effects in animal models (Guimaraes et al., 1990; Resstel et al., 2006). CBD also induced an anti-anxiogenic effect in the fear-potentiated plus maze test (Bitencourt et al., 2008), and more recently it diminished behavioural and autonomic stress-induced responses (Resstel et al., 2009). Therefore, CBD has been suggested as a novel pharmacological approach to reduce the anxiogenic effects of stress and promote the extinction of fear memories. Moreover, CBD has also been reported to induce antipsychotic- (Zuardi et al., 1991, 2006) and antidepressant-like (Zanelati et al., 2010) effects in rodents. To our knowledge, no CBD effects have been investigated within developing animals, either during the neonatal period or at adolescence. Despite the certain role played by CBD in emotionality, its mechanism of action is not completely understood. It has a low affinity for CB receptors (Petitet et al., 1998; Thomas et al., 1998) but can block the reuptake of AEA (Bisogno et al., 2001) and its metabolism (Mechoulam and Hanus, 2002). Moreover, CBD may possess agonistic properties at 5-HT1A receptors (Russo et al., 2005), and some CBD-induced behavioural effects have been demonstrated to be mediated by activation of these 5-HT1A receptors (Resstel et al., 2009; Zanelati et al., 2010).

Sometimes the mixture of diverse phytocannabinoids, for example THC and CBD, improves the therapeutic value of the mixture. In fact, patients taking the synthetic derivative nabilone for neurogenic pain actually preferred cannabis herb and reported that it relieved not only pain but also the associated depression and anxiety (Williamson and Evans, 2000). In this context, an oromucosal spray containing a combination of THC and CBD (Sativex®; GW Pharmaceuticals) has been developed. This innovative drug has been reported to be effective against neuropathic pain (Berman et al., 2004; Nurmikko et al., 2007) and seems to have a promising future in the management of spasticity caused by MS (Collin et al., 2007), and was devoid of undesired side effects related to anxiety. Nevertheless, more studies are needed to address this issue, given the crucial role of the eCB system in emotional homeostasis.

Concluding remarks and future perspectives

Integrating basic research on emotion regulation processes and clinical research on psychopathology is an important health issue. Recent developments have highlighted the important role of the eCB system in regulating emotional behaviours across lifespan. It is important to fully understand the normative development of the eCB system in emotional control in order to provide valuable insights in early-onset neuropsychiatric disorders. New challenges are to further refine our knowledge of the expression of eCB molecules in the brain, and their temporal expression and integration within basic physiological systems integrating emotional regulation over the lifetime. Investigation of the eCB system may also result in the discovery of novel biomarkers of vulnerability to such psychopathological states that may enable the early diagnosis or, at least, the identification of subjects with increased vulnerability. Also, the pharmaceutical industry faces the challenge of redirecting its strategies in order to develop new therapeutic approaches also suitable for the management of anxiety disorders among children and adolescents in the absence of enduring detrimental side effects.

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Conflict of interest

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