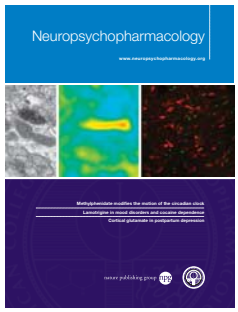

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The Role of the Endocannabinoid System and Genetic Variation in Adolescent Brain Development

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Abstract

During adolescence, both rodent and human studies have revealed dynamic changes in the developmental trajectories of corticolimbic structures, which are known to contribute to the regulation of fear and anxiety-related behaviors. The endocannabinoid (eCB) system critically regulates stress responsivity and anxiety throughout the lifespan. Emerging evidence suggests that during adolescence, changes in eCB signaling contribute to the maturation of local and corticolimbic circuit populations of neurons, such as mediating the balance between excitatory and inhibitory neurotransmission within the prefrontal cortex. This function of the eCB system facilitates efficient communication within and between brain regions and serves a central role in establishing complex and adaptive cognitive and behavioral processing. Although these peri-adolescent changes in eCB signaling promote brain development and plasticity, they also render this period a particularly sensitive one for environmental perturbations to these normative fluctuations in eCB signaling, such as stress, potentially leading to altered developmental trajectories of neural circuits governing emotional behaviors. In this review, we focus on the role of eCB signaling on the regulation of stress and anxiety-related behaviors both during and after adolescence. Moreover, we discuss the functional implications of human genetic variation in the eCB system for the risk for anxiety and consequences of stress across development and into adulthood.

Introduction

Endocannabinoid (eCB) signaling serves an integral role in brain development and regulating stress and anxiety throughout the lifespan. During adolescence, dynamic changes in eCB signaling parallel normative changes in corticolimbic circuitry and fear-related behaviors. Although the exact mechanisms by which eCB signaling shapes neurodevelopmental processes remains unknown, emerging evidence suggests that the eCB system regulates the activity of local and circuit populations of neurons and is particularly important for mediating the balance between excitatory and inhibitory neurotransmission during adolescence. This function of the eCB system facilitates the efficiency of communication within and between brain regions, including corticolimbic structures critical for the regulation of fear and anxiety.

To date, the majority of research into eCB signaling during adolescence has considered the long-term consequences of disrupting the normative activity of this system during development (Lee, Hill, & Lee, 2016; Rubino & Parolaro, 2016; Dow-Edwards & Silva, 2017). Here, we focus on the role of eCB signaling for the regulation of stress and anxiety-related behaviors both during and after adolescence. Throughout this review we also consider the importance of developmental timing and how the downstream effects of perturbations to eCB signaling after exposure to stress or exogenous cannabinoids change depending on developmental stage. This approach provides insight into how the eCB system changes across the lifespan as well as how it contributes to typical and atypical brain and behavioral development. Moreover, we discuss the functional implications of genetic variation in the eCB system for the consequences of stress and risk for anxiety across development and into adulthood.

The endocannabinoid system

The eCB system is largely composed of two inhibitory G-protein coupled receptors (GPCRs), CB1 and CB2, and two major endogenous ligands, N-arachidonoylethanolamine

(anandamide/AEA) and 2-arachidonoylglycerol (2-AG). In addition, eCB signaling is highly regulated by metabolic enzymes including fatty acid amide hydrolase (FAAH) and monoacylglyceride lipase (MAGL) which hydrolyze AEA and 2-AG, respectively (Figure 1). Together, dynamic interactions between these eCB system components play an important role in central nervous system development, synaptic plasticity, and the homeostatic maintenance of cognitive, behavioral, emotional, developmental, and physiological processes (Mechoulam & Parker, 2013; Lu & Mackie, 2016).

The precursors for AEA and 2-AG are present in the lipid membranes of post-synaptic neurons. Thus, AEA and 2-AG are synthesized 'on demand' and transferred in a retrograde manner across the synaptic cleft to bind to eCB receptors at the presynapse and regulate the release of other neurotransmitters, including glutamate, GABA, dopamine, serotonin, and acetylcholine (Figure 1; Howlett et al., 2002; Piomelli, 2003; Lovinger, 2008; Jutras-Aswad, DiNieri, Harkany, & Hurd, 2009; Katona & Freund, 2012). AEA and 2-AG have been implicated in mediating both long- and short-term synaptic plasticity, respectively (Mackie, 2006; Lu & Mackie, 2016), evident from notable differences in the pharmacokinetic properties of the two ligands. Specifically, AEA acts as a partial agonist for the CB1 receptor, binding with high affinity, but inducing poor intracellular signal transduction (Hillard, Wilkison, Edgemond, & Campbell, 1995). AEA activity appears to represent a tonic signal that gates and maintains steady-state (homeostatic) conditions (Hill & Tasker, 2012). Conversely, 2-AG has a relatively low binding affinity to the CB1 receptor, but produces a robust intracellular response (Hillard et al., 1995). 2-AG activity is highest in response to sustained depolarization and represents a stimulus-induced phasic signal involved in several forms of activity-dependent synaptic plasticity. Importantly, the signaling pathways mediated by AEA and 2-AG likely interact to regulate behavior (Blankman & Cravatt, 2013).

The CB1 receptors are one of the most abundant classes of GPCR, expressed on multiple neuronal populations throughout the brain, with a notable presence on GABAergic and

glutamatergic neurons (Marsicano & Lutz, 1999; Hill et al., 2007). In contrast, CB2 receptors are predominantly found in peripheral tissues, with presence in the central nervous system largely localized to microglia (Cabral, Raborn, Griffin, Dennis, & Marciano-Cabral, 2008). In this review, we will focus largely on the CB1 receptor as it is responsible for most eCB signaling in the brain (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). However, readers are directed to a comprehensive review of the eCB system for information on additional components of the eCB system beyond the scope of this review (Lu & Mackie, 2016). CB1 receptors primarily couple to Gi and Go classes of G-protein (Howlett et al., 2002). As a result, binding induces a local reduction in cyclic adenosine monophosphate (cAMP) and an affiliated decrease in cAMP-dependent protein kinase (PKA) activity (Vogel et al., 1993; Howlett et al., 2002). In turn, this leads to the activation of A-type potassium channels as well as the inhibition of voltage-gated calcium channels and disruption of the vesicle fusion process (Lovinger, 2008). Together, these downstream effects decrease the probability of neurotransmitter release from the presynaptic membrane, where the majority of CB1 receptors are localized. This mechanism contributes to the neuromodulatory capacity of the eCB system, making the system particularly well suited for the regulation of synaptic transmission in the brain and mediation of numerous forms of plasticity (Kano, Ohno-Shosaku, Hashimotodani, Uchigashima, & Watanabe, 2009; Castillo, Younts, Chávez, & Hashimotodani, 2012).

Notably, the resultant inhibition of neurotransmitter release provides feedforward inhibition of further postsynaptic synthesis of eCB ligands. At this point, AEA and 2-AG are rapidly removed by a membrane transport process involving the enzymatic breakdown by distinct hydrolyzing enzymes. Specifically, FAAH (Cravatt et al., 1996; Di Marzo, 2011; Fu et al., 2012) breaks down AEA into arachidonic acid and ethanolamine (Ahn, McKinney, & Cravatt, 2008). The breakdown of 2-AG involves at least eight participating enzymes; however, the majority of 2-AG degradation in the brain occurs via the activity of MAGL (Ueda, Tsuboi, Uyama, & Ohnishi, 2011), resulting in arachidonic acid and glycerol (Ueda et al., 2011). The

localization of these enzymes likely contributes to the nature of signaling by the eCB ligands. For example, FAAH is found post-synaptically, where its presence can contribute to feedback inhibition processes to maintain baseline synaptic regulation by eCB signaling (Häring, Guggenhuber, & Lutz, 2012). Conversely, MAGL is mostly co-localized with CB1 receptors pre-synaptically, where it contributes to the mediation of stimulus-induced 2-AG activity (Häring et al., 2012). Finally, inhibiting the enzymatic activity of FAAH and MAGL can prolong the activity of the eCB ligands (Gaetani et al., 2009). In recent years, targeting these enzymes has been used increasingly as a method of modulating eCB signaling. As will be discussed later in this review, FAAH in particular reflects an important avenue of future research, as disruptions to the normal activity of this enzyme have been linked to dramatic changes in both brain and behavioral measures (Sipe, Chiang, Gerber, Beutler, & Cravatt, 2002; Chiang, Gerber, Sipe, & Cravatt, 2004; Hariri et al., 2009; Gunduz-Cinar et al., 2013b; Dincheva et al., 2015).

Endocannabinoid system ontogeny

The presence of the eCB system has been detected from the earliest phases of ontogenetic development, when it plays an essential role in neuronal development and circuit connectivity in a number of species, including rodents and humans (Rodríguez de Fonseca, Ramos, Bonnin & Fernández-Ruiz, 1993; Berrendero et al., 1998; Williams, Walsh, & Doherty, 2003; Aguado et al., 2006; Berghuis et al., 2007; Harkany et al., 2007; Harkany, Keimpema, Barabás, & Mulder, 2008; Mulder et al., 2008; Fride et al., 2009; Zurolo et al., 2010; Maccarrone, Guzmán, Mackie, Doherty, & Harkany, 2014). In rodents, the ability of eCBs to regulate synaptic transmission emerges around postnatal day (PND) 10 and increases throughout development and into adulthood (Rodríguez de Fonseca et al. 1993; Zhu & Lovinger, 2010; Liang, Alger, & McCarthy, 2014). Dynamic alterations occur in the eCB system throughout early life and well into adolescence (Ellgren et al., 2008; Heng, Beverley, Steiner, &

Tseng, 2011; Lee & Gorzalka, 2012; Rubino et al., 2015), when overall enhanced levels of eCB signaling are observed (Figure 1).

Contributing to the heightened activity of the eCB system during adolescence are peaks in the expression of CB1 receptors as well as AEA and 2-AG (Figure 1). In rodents, the highest expression of CB1 receptors is observed at the onset of adolescence (~PND 30), particularly in prefrontal cortex (PFC) and striatum, with a subsequent decline approaching adulthood (~PND 70; Berrendero et al., 1998; Rodríguez de Fonseca et al., 1993; Ellgren et al., 2008; Schneider, 2008; Heng et al., 2011; Klugmann, Klippenstein, Leweke, Spanagel, & Schneider, 2011). There is currently limited evidence of how eCB signaling changes across development in humans. Although marked similarities have been observed in the developmental patterns of CB1 receptor expression between humans and rodents, expression within human PFC tissue peaks much earlier (prior to 5 years of age), after which levels gradually decrease until adulthood (Choi et al., 2012; Long, Lind, Webster, & Weickert, 2012). Overall, the available evidence indicates greater variation of the eCB system in early life relative to adulthood across a number of species.

With regard to the two major eCB ligands, AEA levels exhibit a progressive, though somewhat fluctuating, increase from early adolescence to adulthood, while 2-AG levels are high in both early and late adolescence with notably attenuated expression in mid-adolescence (Figure 1; Ellgren et al., 2008; Heng et al., 2011; Lee, Hill, Hillard, & Gorzalka, 2013; Rubino et al., 2015). Fluctuations in AEA expression (Figure 1) have been shown to be relatively consistent across development in corticolimbic regions with prominent eCB activity, including PFC, hippocampus, amygdala, and hypothalamus (Lee et al., 2013). However, AEA and 2-AG levels in nucleus accumbens and striatum appear to exhibit unique developmental trajectories (Ellgren et al., 2008). In rodents, complementary changes in FAAH activity are observed during adolescence, which may contribute to the observed fluctuations in AEA levels (Figure 1; Lee et al., 2013). In humans, overall increases across the lifespan have been observed in FAAH as

well as the AEA synthesizing enzyme N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD; Long et al., 2012). Peaks in FAAH expression during adolescence may indicate heightened levels of regulation over the timing of AEA availability during this period (Long et al., 2012). Less is known about the developmental trajectory of MAGL expression, but evidence in humans shows gradual decreases in MAGL from the first year of life onward, with more rapid decreases following the onset of adolescence (Long et al., 2012). A peak in expression of the 2-AG synthesizing enzyme diacylglycerol lipaseα (DAGLα) occurs during adolescence, which when coupled with co-occurring decreases in CB1 receptor expression may reflect an increase in the spatial control of 2-AG activity (Long et al., 2012).

Given the timing of puberty and changes in the eCB system, it is possible that pubertal changes in hormone secretion relate to fluctuations in eCB signaling during adolescence. Sex differences have been observed in the timing of fluctuations in eCB system expression, with CB1 receptor expression levels peaking earlier in females than males (Rodriguez de Fonseca et al., 1993). In both sexes, peak expression occurs just before the onset of puberty, which is ~PND 35 in female rodents and ~PND 40 in male rodents. Likewise, levels of AEA in hypothalamus have also been shown to peak immediately prior to puberty in female rodents (Wenger et al., 2002). Subsequently, eCB activity fluctuates throughout the estrous and menstrual cycles as well (Gonzalez et al., 2000; Bradshaw, Rimmerman, Krey, & Walker, 2006; El-Talatini, Taylor, & Konje, 2010). A close interaction between the endocannabinoid system and gonadal hormones (Murphy, Rodriguez, & Steger 1991; Rodriguez de Fonseca, Cebeira, Ramos, Martin, & Fernandez, 1994) might contribute to the major developmental changes occurring in the eCB system during pubertal maturation. The eCB system has been shown to modulate the release and activity of gonadal hormones (androgens, estrogen, and progesterone) and gonadotrophins (follicle-stimulating hormone and luteinizing hormone) in humans and rodents alike (Kolodny, Masters, Kolodner, & Toro, 1974; Dalterio, Bartke, & Burstein, 1977; Dalterio, Mayfield, & Barkte, 1983; Kumar & Chen, 1983; Rodriguez de Fonseca

et al., 1994; Wenger, Ledent, Csernus, & Gerendai, 2001; Tsutahara et al., 2011). Overall, eCB activity appears to primarily attenuate the release of gonadal hormones in order to maintain the correct physiological levels (Gorzalka & Dang, 2012). In turn, changes to gonadal hormone functioning can influence eCB signaling through a feedback loop involving the hypothalamus, pituitary, and limbic regions (MacCarrone et al., 2000, 2001; MacCarrone, Bari, Di Rienzo, Finazzi-Agro, & Rossi, 2003; Nguyen & Wagner, 2006; Gorzalka & Dang, 2012). Taken together, this evidence suggests that the eCB system may contribute to the timing of pubertal onset and play a role in a number of sex-specific behaviors that are regulated by gonadal hormones.

Endocannabinoid regulation of stress, fear, and anxiety

The eCB system has a well-established role in the regulation of fear and anxiety-related behavior in both humans and rodents (Riebe, Pamplona, Kamprath, & Wotjak, 2012; Ruehle, Rey, Remmers, & Lutz, 2012; Higuera-Matas, Ucha, & Ambrosio, 2015). Generally, enhanced eCB signaling is associated with reductions in conditioned fear and anxiety, whereas inhibiting the eCB system produces the opposite effect (Akirav, 2011; Gunduz-Cinar et al., 2013b; Bluett et al., 2014; Gray et al., 2015). These effects rely in large part on eCB signaling within corticolimbic structures such as PFC, amygdala, and hippocampus (Herkenham et al., 1991; Rubino & Parolaro, 2008; Hill, Karatsoreos, Hillard, & McEwen, 2010; Campolongo, Trezza, Ratano, Palmery, & Cuomo, 2011; Hill & Tasker, 2012; Lee & Gorzalka, 2012; Morena, Patel, Bains, & Hill, 2016). eCB signaling in these regions may be especially important during development, when corticolimbic regions and related emotional behaviors undergo dynamic changes and risk for anxiety disorders increases.

A sensitive window for corticolimbic circuitry and eCB signaling

Anxiety disorders are the most common disorders during adolescence (Kessler et al., 2005; Merikangas et al., 2010; Lee et al., 2014), which is a distinctive period for fear learning and corticolimbic circuitry. Corticolimbic regions exhibit substantial changes in their reactivity and connectivity throughout development (Moriceau & Sullivan, 2006; Hare et al., 2008; Gee et al., 2013; Gabard-Durnam et al., 2016; Pattwell et al., 2016; Wu et al., 2016). Consistent with these neurodevelopmental changes, adolescents exhibit unique patterns of anxiety-related behavior and fear learning in rodents and humans alike. Specifically during adolescence, extinction learning for cued fear memory is diminished across species (McCallum, Kim & Richardson, 2010; Pattwell et al., 2012). During this same sensitive period, expression of contextual fear is suppressed in rodents (Pattwell, Bath, Casey, Ninan, & Lee, 2011). These changes in fear learning during early adolescence correspond to a time of robust corticolimbic eCB changes (Heng et al., 2011).

The regulatory role of the eCB system has been shown to emerge early in life (Trezza, Cuomo, & Vanderschuren, 2008; Higuera-Matas et al., 2015). In early development, the highest CB1 receptor expression is observed in amygdala and hippocampus (Rodríguez de Fonseca et al., 1993), which remain prominent areas for eCB signaling in adulthood along with an increased eCB presence in cortex, striatum, and cerebellum (Herkenham et al., 1991; Mackie, 2005; Jutras-Aswad et al., 2009). Though on average CB1 receptor expression peaks during adolescence and attenuates by adulthood, the most robust developmental changes occur in prefrontal and limbic regions, whereas CB1 receptor declines are not observed until later in adolescence in sensorimotor areas (Heng et al., 2011). A similar developmental pattern is observed for the functionality of CB1 receptors in prefrontal and limbic regions, as evidenced by reductions in CB1 receptor-dependent inhibition of synaptic transmission in PFC during the transition from adolescence to adulthood (Heng et al., 2011).

These variations in the regional and temporal expression of components of the eCB system across adolescent development may contribute to the emergence of a sensitive window

during early adolescence when corticolimbic circuitry is particularly sensitive to perturbations of eCB signaling. Consistent with this notion, changes in the eCB system, as well as environmental modulation of the eCB system (e.g., via acute or chronic stressors) during this period, have been shown to produce long-term effects on stress responses in adulthood and may alter risk for later anxiety. Indeed, CB1 receptor antagonism during adolescence results in increased mobility, an active stress coping behavior, during a forced swim test when tested in adulthood (Lee, Hill, Hillard, & Gorzalka, 2015). Alongside changes in the behavioral stress response, adult mice who experienced CB1 receptor antagonism had reduced AEA in amygdala, increased AEA in hypothalamus, and upregulated cortical CB1 receptor expression (Lee et al., 2015). These rats also exhibited lower corticosterone levels in association with repeated restraint exposure (Lee et al., 2015), in contrast to an increase in circulating corticosterone levels following CB1 receptor antagonism in adult mice (Patel, Roelke, Rademacher, Cullinan, & Hillard, 2004), suggesting that alterations to the eCB system may have different effects on HPA (hypothalamus, anterior pituitary and adrenal cortex; see below) axis reactivity in adolescence versus adulthood. In addition, exposure to CB1 receptor agonists during adolescence produces enduring increases in anxiety-related behavior measured during a number of rodent behavioral tests (Rubino & Parolaro 2008; Schneider, Drews, & Koch, 2005; but see Wegener & Koch 2009; O'Shea, McGregor, & Mallet, 2006). Perturbations to the maturing system may disrupt the refinement or function of corticolimbic circuits, resulting in long-term behavioral changes. Whereas disruptions in eCB signaling during adolescence may be especially influential, evidence suggests that similar manipulations of the eCB system in adulthood are not as consequential in terms of their neurobiological, cognitive, and behavioral effects (Bambico, Nguyen, Katz, & Gobbi, 2010; Cass et al., 2014; Zamberletti et al., 2014).

Developmental timing and endocannabinoid regulation of the HPA axis

Normative maturation of the eCB system across adolescence parallels robust changes in patterns of HPA axis stress responding (Rubino et al., 2008; Lee & Gorzalka, 2012; Lee et al., 2015), indicating that eCB changes during development may contribute to developmentally specific patterns of stress reactivity and fear learning. The components of the HPA axis interact to produce and regulate the physiological response to a stressor. In the short term, activation of the neuroendocrine system in response to stressful stimuli is adaptive and necessary for an organism to respond appropriately to potential threat. However, chronically elevated levels of stress hormones may be harmful. Existing evidence implicates eCB activity in regulating basal HPA activity, as well as in the habituation of HPA activity as an organism learns that a stressor is no longer a threat (Hill & Tasker, 2012). In general, eCB signaling inhibits HPA axis activity, contributing to the maintenance of low basal levels of glucocorticoids and restricting HPA axis activity to acute stress (Gorzalka & Hill, 2009). While eCB activity suppresses the release of glucocorticoids, stress and HPA activation can also induce long-term changes in the eCB system, emphasizing the feedback-dependent regulatory role of the eCB system (Gorzalka & Hill, 2009).

HPA axis functioning changes across development, and the effects of stress on eCB signaling and corticolimbic circuitry vary depending on the timing of exposure. Although basal glucocorticoid levels tend to be comparable in adolescence and adulthood (Pignatelli, Xiao, Gouveia, Ferreira, & Vinson, 2006; Romeo & McEwen, 2006; Romeo et al., 2006), habituation of the HPA axis response to a stressor differs with age. For example, adolescent animals require roughly twice as much time to return to basal levels relative to adults following the same stressor (Romeo, Lee, Chhua, McPherson, & McEwen, 2004; Romeo, Lee, & McEwen, 2005), indicating a greater sensitivity of the HPA axis during adolescence relative to adulthood. In line with these findings, the timing of stress exposure appears to moderate the effects of stress on eCB signaling (Lee & Hill, 2013; Reich, Mihalik, Iskander, Seckler, & Weiss, 2013). Corticolimbic structures, particularly the basolateral amygdala (BLA), are central to this close relationship

between stress and eCB signaling. Indeed, CB1 receptor signaling in BLA has been shown to gate behavioral and neuroendocrine responses to stress- and fear-provoking stimuli (Hill, Miller, Carrier, Gorzalka, & Hillard, 2009; Gunduz-Cinar et al., 2013b; Bluett et al., 2014; Gray et al., 2015) with additional evidence suggesting that this regulation is mediated specifically by AEA in BLA (Hill & Tasker, 2012). Reductions in levels of AEA in the amygdala have been suggested to facilitate HPA axis responsivity and the generation of stress-related behaviors that can lead to anxiety (Gorzalka & Hill, 2009; Gray et al., 2015). Mechanistically, recent evidence suggests that corticotrophin-releasing hormone (CRH), which is upregulated in the amygdala and PFC in response to chronic corticosteroid or stress exposure, gates stress reactivity through modulation of the eCB system. Elevations in CRH evoke the rapid induction of FAAH, which in turn reduces both AEA and 2-AG levels in amygdala as well as attenuating prefrontal AEA expression (Gray et al., 2015, 2016). In this way, the data of Gray and colleagues (2015, 2016) elucidates an important link between eCB signaling and the effects of stress on anxiety.

Endocannabinoids and the development of corticolimbic circuitry

The eCB system plays a central role in the maturation of corticolimbic circuitry, supporting fundamental processes such as the balance of excitatory and inhibitory neurotransmission. Dynamic synchronized interactions between excitatory and inhibitory cortical signaling during adolescence are necessary for the selectivity of information processing, which influences how inputs from limbic regions to PFC manifests in adolescent fear responding and anxiety (O'Donnell, 2011; Uhlhaas & Singer, 2011). Information from afferents originating in limbic regions including hippocampus, amygdala and mediodorsal thalamus is integrated into PFC where it can be used to shape emotional and behavioral responding (Ishikawa & Nakamura, 2003; Sherwood et al., 2010). A subset of these glutamatergic afferents express CB1 receptors, providing a mechanism to enhance input selectivity to cortical neurons (Fortin & Levine, 2007). Furthermore, fine-tuning the selectivity of PFC pyramidal neuronal firing requires

inhibitory control from GABAergic interneurons, which is mediated by eCB signaling (Rao, Williams, & Goldman-Rakic, 2000; Trettel & Levine, 2002; Fortin, Trettel, & Levine, 2004; Bartos & Elgueta, 2012). In turn, activation of CB1 receptors expressed in glutamatergic neurons also regulates GABAergic signaling, which is critical to the developmental remodeling of local inhibitory circuits during adolescence (Fortin & Levine, 2007; Tseng, Chambers, & Lipska, 2009).

Across adolescence, marked increases in synaptic pruning further contribute to the increased efficiency and selectivity of information processing (Meyer, Ferres-Torres & Mas, 1978; Andersen, Thompson, Rutstein, Hostetter & Teicher, 2000; Nunez, Sodhi & Juraska, 2002; Teicher, Andersen & Hostetter, 1995; Rubia et al., 2006; Toga, Thompson & Sowell, 2006). In PFC, synaptic pruning occurs to a greater extent at excitatory than inhibitory synapses, resulting in a marked increase in prefrontal synaptic inhibition (Dow-Edwards & Silva, 2017). As a result, the balance of excitation and inhibition in individual neurons and within networks has been shown to differ greatly in adolescents compared to adults (Uhlhaas et al., 2009; Sturman & Moghaddam, 2011). Through continuous homeostatic regulation of both glutamatergic and GABAergic terminals, the eCB system makes critical contributions to the balance of excitation and inhibition as well as the strengthening and elimination of cortical excitatory synaptic connections (Marsicano et al., 2003; Katona & Freund, 2008; Bossong & Niesink, 2010; Lubman, Cheetham, & Yücel, 2015). By this mechanism, eCB signaling is believed to contribute to the synchronization of functional activation within and between neural networks, which facilitates the efficiency of information processing in PFC as well as the ability to encode a functional representation of behavioral and cognitive outputs (Freund, Katona, & Piomelli, 2003; Gerdeman & Lovinger, 2003; Buzsáki & Draguhn, 2004; Raver, Haughwout, & Keller, 2013; Sales-Carbonell et al., 2013).

The brain may be especially susceptible to disruptions in eCB signaling during adolescence. A recent study by Cass and colleagues (2014) showed marked decreases in the

inhibitory regulation of prefrontal neuronal activity in response to ventral hippocampal inputs in adult rodents that had undergone repeated pharmacological stimulation of the CB1 receptor during early adolescence (PND 35-40). The effect was age-dependent, with similar disinhibition seen in early and mid-adolescence (PND 40-45), but not late adolescence (PND 45-50) or adulthood. The authors attributed their findings in part to a downregulation of local GABAergic transmission in PFC based on the finding that delivery of the GABA-A α1 positive allosteric modulator indiplon during adulthood reversed the disinhibition caused by CB1 receptor stimulation during adolescence. While dynamic changes are occurring in prefrontal GABAergic transmission during adolescence, the brain appears to be particularly susceptible to factors that disrupt this maturational process. Interestingly, the resulting input processing patterns of adult animals exposed to CB1 receptor stimulation during early or mid-adolescence resemble those of a juvenile animal (Thomases et al., 2013), indicating that perturbations of eCB signaling during adolescence may in fact hinder maturation of PFC and its connections.

Similarly, perturbations of eCB signaling during adolescence have been suggested to impede the structural maturation of neuronal circuits in PFC (Renard et al., 2016). For example, CB1 receptor stimulation significantly alters dendritic arborization of pyramidal neurons in layer 2/3 in the medial PFC (Renard et al., 2016). Moreover, blocking eCB activity during adolescence prevented normal developmental decreases in postsynaptic density-95, GluN2A, and GluA2 (Rubino et al., 2015). Given the role of these proteins in the stabilization of excitatory synapses, it may be that eCB tone is required for the pruning of glutamatergic synapses to adult levels (Rubino et al., 2015).

Taken together, these findings highlight a critical role for eCB signaling in mediating the relative levels of excitatory and inhibitory transmission in PFC. By this mechanism, the eCB system is likely to influence the emergence of GABAergic regulation of prefrontal plasticity that has been shown to increase across adolescence (Caballero, Flores-Barrera, Cass, & Tseng, 2014). The establishment of eCB-mediated regulation of synaptic transmission during

adolescence sets the framework for continued cortical regulation throughout adulthood (Auclair, Otani, Soubrie, & Crepel, 2000). Notably, this regulatory role contributes not only to the maturation of prefrontal neurons and their output capacity, but also to the selective integration of inputs to PFC (Caballero, Granberg, & Tseng, 2016). Long-lasting cognitive and behavioral effects observed in both humans and rodents after perturbation of the eCB system during adolescence have been attributed in part to disruption of normative eCB signaling that regulates PFC maturation (Realini, Rubino, & Parolaro, 2009; Caballero & Tseng, 2012; Rubino et al., 2015). In these ways, the eCB system mediates maturational processes in the PFC and its inputs that comprise the corticolimbic circuitry involved in fear regulation, which may be especially important in the context of increased risk for anxiety during adolescence.

Effects of FAAH genetic variation on corticolimbic circuitry and anxiety

In recent years, cross-species translational research has provided powerful insight into the role of genetic variation in eCB signaling in both typical and atypical neurobiological and behavioral development. A common polymorphism in the human FAAH gene (C385A; rs324420) has been shown to regulate FAAH enzymatic activity. The variant FAAH A385 allele leads to reduced FAAH activity, resulting in increased levels of AEA (Chiang et al., 2004; Sipe et al., 2002). Consistent with the demonstrated role of the eCB system in anxiety, threat learning, and stress reactivity in rodents (Chhatwal, Davis, Maguschak, & Ressler, 2005; Gunduz-Cinar, Hill, McEwen, & Holmes, 2013a; Gray et al., 2015), the FAAH C385A polymorphism in humans has been associated with variation in neural and behavioral responding to threat and stress (Hariri et al., 2009; Gunduz-Cinar et al., 2013b; Dincheva et al., 2015). Carriers of the A385 allele exhibit reduced amygdala reactivity to threat (Hariri et al., 2009) as well as quicker habituation of amygdala reactivity to threat (Gunduz-Cinar et al., 2013b), possibly due to enhanced eCB signaling. Indeed, AEA activation of the CB1 receptor in BLA is integral to the regulation of behavioral and neuroendocrine responses to stress- and

fear-provoking stimuli (Hill et al., 2009; Hill & Tasker, 2012; Gunduz-Cinar et al., 2013b; Bluett et al., 2014; Gray et al., 2015). Thus, while exposure to stress typically results in the rapid mobilization of FAAH to deplete the signaling pool of AEA and increase neuronal excitability in the BLA (Gunduz-Cinar et al., 2013b), individuals expressing the variant FAAH A385 allele may sustain higher levels of AEA under stressful conditions.

The generation of a knock-in mouse that expresses the variant A allele of the FAAH polymorphism and recapitulates the molecular and biochemical phenotypes of human A allele carriers has allowed for examination of the precise mechanisms by which genetic variation in the eCB system influences anxiety (Dincheva et al., 2015; Figure 2). In both mice and humans during adulthood, carriers of the variant A allele exhibit stronger frontoamygdala functional connectivity (Dincheva et al., 2015), a pattern of connectivity that is associated with reduced anxiety and more effective emotion regulation (Hariri, Mattay, Tessitore, Fera, & Weinberger, 2003; Urry et al., 2006; Hare et al., 2008; Kim, Gee, Loucks, Davis, & Whalen, 2011; Burghy et al., 2012). Specifically, A allele carriers showed stronger resting-state functional connectivity between the amygdala and ventromedial PFC, and knock-in mice showed increased projections from infralimbic cortex (IL) to BLA. These effects were specific to this circuit, as there were no effects of genotype on connectivity between the amygdala and dorsal anterior cingulate cortex in humans or on projections from the prelimbic cortex to the BLA in mice. A allele carriers and knock-in mice demonstrated parallel effects of enhanced fear extinction and lower anxiety (Dincheva et al., 2015). The selective increase in connectivity within a circuit important for fear regulation suggests that genetic variation in the FAAH polymorphism may result in a gain of function (i.e., lower anxiety) by enhancing top-down control of the amygdala.

Given that anxiety disorders often emerge during adolescence, when eCB signaling is undergoing dynamic changes (Lee et al., 2016), we tested whether the effects of FAAH genetic variation differed as a function of developmental stage. Converging evidence across humans and mice demonstrated that the genotypic effects of the A allele on frontoamygdala circuitry and

anxiety emerge during adolescence (Gee et al., 2016). Adolescents and adults with the A allele displayed increased structural connectivity in the uncinate fasciculus, relative to non-A allele carriers, whereas there were no effects of genotype in children. Consistent with these findings in humans, knock-in mice had increased projections from IL to BLA relative to wild-type mice during adolescence and adulthood, but not during the preadolescent period (Figure 3). Similarly, genotypic effects on anxiety emerged during adolescence across mice and humans, such that A allele carriers had lower anxiety during adolescence and adulthood, but not prior to adolescence. Phenotypic expression of the FAAH polymorphism in humans may vary as a function of developmental changes in eCB signaling, gene expression, and normative maturation of frontoamygdala circuitry. CB1 expression, FAAH activity, and AEA levels are relatively steady during childhood but fluctuate significantly across adolescence (Wenger et al., 2002; Lee & Gorzalka, 2012; Lee et al., 2013), as frontoamygdala circuitry is also undergoing dynamic changes (Hare et al., 2008; Gee et al., 2013; Gabard-Durnam et al., 2016; Wu et al., 2016). During this period of developmental change and as AEA levels begin to wane, the system may be especially sensitive to effects of the A385 allele on FAAH expression. This novel gene by development interaction observed across species provides insight into how risk for anxiety and consequences of stress vary across development (Gee & Casey, 2015) and may contribute to efforts to tailor treatments for anxiety based on genetic variation and developmental stage.

Conclusions and future directions

Despite compelling evidence for the important role of the eCB system in brain development and regulating stress and anxiety-related behaviors, the precise mechanisms by which eCB signaling shapes adolescent brain development remain unknown. Delineating the trajectory of normative eCB signaling and how this goes awry following environmental stress will be critical for understanding risk for anxiety disorders during the unique period of adolescence.

Given the dynamic and interacting changes across many systems during development, and that the long-term effects of perturbations to normative eCB signaling will vary depending on the timing and duration of exposure, one of the biggest challenges for eCB research is isolating how perturbations in eCB signaling affect the brain during development. A number of recent advances are particularly promising for the rapidly evolving literature on the eCB system. For example, the development of PET ligands enabling the investigation of FAAH binding (Boileau et al., 2015; Boileau et al., 2016; Wang et al., 2016), and the assessment of circulating eCBs (Hill et al., 2009; Hill et al., 2013) are transforming the possibilities for studying eCB signaling in humans. While much of the research on genetic variation has focused on FAAH, there has also been expanded investigation into eCB-related genetic variation related to other eCB genes (e.g., Carey et al., 2015). Finally, examining the effects of perturbations to eCB signaling at specific developmental stages and their association with subsequent behaviors that are particularly important during adolescence (e.g., social behavior; Doenni et al., 2016) will provide critical insight into sensitive windows and their long-term effects on functioning.

The eCB system contributes to brain development and regulation of stress and anxiety across the lifespan, and dynamic changes in eCB signaling during adolescence may relate to changes in corticolimbic circuitry and risk for anxiety during this unique developmental period. Increases in eCB signaling specific to adolescence contribute to heightened levels of synaptic plasticity, which may allow adolescents to incorporate information from new experiences, constantly update their representations of the world, and respond in efficient and adaptive ways to salient aspects of their environment. Flexible interactions with the surrounding environment facilitate the acquisition of skills and experiences necessary for achieving independence, such as acquiring resources and engaging in social interactions necessary for survival (Spear, 2010). However, adolescence is also a particularly sensitive period for environmental influences of stress and increased risk for anxiety. Perturbations to the eCB system during this time may disrupt the refinement of cortical circuits and their interactions with limbic regions, resulting in

long-term consequences for emotional behavior and stress responding. Cross-species research on developmental changes in eCB signaling and individual differences in eCB-related genetic variation will be critical for understanding the complex role of the eCB system in shaping adolescent brain development and the regulation of stress reactivity and anxiety during a time of substantial change.

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Figure Captions

Figure 1. Corticolimbic endocannabinoid (eCB) signaling changes dynamically across rodent development. (A) Major pathways of endocannabinoid degradation. (B) Schematic of eCB signaling within a synapse. (C) Developmental trajectories of the components of the eCB system. CB1 receptor expression peaks with the onset of adolescence. 2-AG is highest around birth and may fluctuate throughout adolescence. AEA gradually increases during early life and fluctuates during adolescence. FAAH activity fluctuates in reciprocal fashion to AEA during adolescence (based on data from Berrendero et al., 1999; Ellgren et al., 2008; Fernandez-Ruiz et al., 2000; Heng et al., 2011; Lee et al., 2013; Rodriguez de Fonseca et al., 1993; Rubino et al., 2015; Wenger et al., 2002). Adapted from Lee, Hill, & Lee (2016), *Genes Brain and Behavior*.

Figure 2. Functional and structural connectivity between ventromedial prefrontal cortex (vmPFC) and amygdala in adult humans and mice with FAAH C385A. (a) fMRI functional connectivity compared between subgenual vmPFC (x, y, z=0, 40, −3) and bilateral amygdala in A-allele carriers (n=17) relative to C homozygotes (n=18). (b) Anterograde tracer (AAV2-eGFP; eGFP), targeted to IL, labelled afferents in BLA in FAAHA/A mice (n=4) and controls (FAAHC/C; n=4). Drawing illustrates anatomical boundaries. (c) Retrograde tracer (fluorogold; FG), targeted to IL, labelled BLA cell bodies in FAAHA/A mice (n=4) and controls (FAAHC/C; n=4). (Scale bars, 100 μm.) Means ± SEM. presented. *$p < 0.05$, ***$p < 0.001$. NS, not significant. Reprinted from Dincheva et al. (2015), *Nature Communications.*

Figure 3. Phenotypic differences in frontolimbic circuitry resulting from FAAH polymorphism emerge during adolescence in human and mouse. (A, Left) Posthoc analyses revealed a significant genotypic effect on uncinate fasciculus (UF) fractional anisotropy in participants 12 years of age and older [n=509; 249 females; $F(1,491) = 14.02$; $p = 0.0002$] but

not in those under 12 years of age [n=541; 259 females; $F(1,523) = 0.513$; $p = 0.474$]. (A, Right) Mask in Montreal Neurological Institute standard space where UF ascends from the temporal lobe used as the seed region for probabilistic tractography in humans (upper left); UF tract mask is derived from probabilistic tractography averaged across human participants (n=1,050). (B, Left) Consistent with the findings in humans, a significant genotype by age group interaction [$F(2,36) = 58.72$; $p < 0.0001$] in IL afferent fibers to BLA emerged, such that knock-in mice (AA: n=7 per age) had higher fiber density than WT mice (CC: n=7 per age) during adolescence at age P45 ($p < 0.0001$) and adulthood at P75 ($p < 0.0001$). (B, Right) Drawing of anatomical boundaries and anterograde tracer targeted to IL and labeling afferents in BLA. CeA, central amygdala; MeA, medial amygdala; PL, prelimbic; *$p < 0.05$. Reprinted from Gee et al. (2016), *PNAS.*